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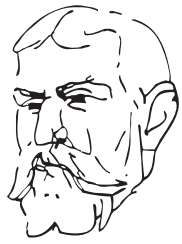

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ORIGINAL ARTICLE

CHARACTERIZATION OF STRUCTURAL DISORDERS OF THE LIVER DEPENDING ON THE DURATION OF SUBHEPATIC CHOLESTASIS IN PATIENTS OF DIFFERENT AGE GROUPS

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ABSTRACT

The aim: To study structural disorders of the liver depending on the duration of subhepatic cholestasis in patients of different age groups.

Materials and methods: 50 obstructive jaundice patients were subdivided into two groups. Group I (n = 25) consisted of young (18–44-year-old) and middle-aged patients (45–59-year-old), while the Group II (n = 25) included elderly (60–74-year-old) and senile patients (75–90-year-old).

Results: We performed morphological and morphometric studies of 50 liver biopsy specimens taken from patients of different age groups with different duration of obstructive jaundice: less than 7 days, 7-14 days, 14-21 days, 21-28 days, and over 28 days.

Conclusions: In patients of the Groups I and II, pathological hepatic changes in the early stages of mechanical jaundice were manifested in the form of hepatocyte dystrophy and hepatitis development. In the Group I patients, manifestations of steatohepatitis, fibrosis and initial signs of liver cirrhosis were noted in the late stages of subhepatic cholestasis. In addition to the above-mentioned changes, Group II patients, in the late stages of mechanical jaundice, presented signs of severe fibrosis and well-shaped liver cirrhosis. Taking into account the above morphological changes in the liver with different duration of subhepatic cholestasis, we consider reasonable to decompress bile ducts in patients of older age groups at earlier stages of mechanical jaundice compared to young and middle-aged patients, thus preventing post-decompression liver dysfunction and the subsequent development of biliary cirrhosis.

KEY WORDS: obstructive jaundice, liver biopsy, morphological i morphometric study of the liver

Wiad Lek. 2023;76(1):9-16

INTRODUCTION

Among all liver pathologies, obstructive jaundice is the most common one, which, according to the WHO, is observed in 10-15% of the world's population [1, 2]. In case of short-term obstructive jaundice, hepatocytes develop a relative adaptation to cholestasis accompanied by a decreased bile secretion. In case of long-term obstruction of bile ducts, post-decompression liver dysfunction often develops due to failure of adaptation mechanisms [3, 4] despite a complete restoration of bile passage. Therefore, the choice of bile duct decompression technique and timing primarily depends on the duration of obstructive jaundice [5, 6]. Elderly patients demonstrated more pronounced changes in the liver associated with comorbidity [7]. The most reliable method of diagnosing structural changes in the liver is histological examination, which allows to ensure timely and comprehensive treatment [8]. In our opinion, the study of structural disorders of the liver with different duration of obstructive jaundice in patients of different age groups is still a pressing problem.

THE AIM

To study structural disorders of the liver depending on the duration of subhepatic cholestasis in patients of different age groups.

MATERIALS AND METHODS

To achieve the goal, we performed morphological and morphometric studies of 50 liver biopsy specimens taken from patients of different age groups with different duration of subhepatic cholestasis. Biopsy material was collected intraoperatively by microresection of the liver and puncture biopsy. The material was fixed in 10% neutral formalin solution (pH - 7.4) for 48 hours, followed by treatment with alcohol of increasing concentration and poured into paraffin. Resulted paraffin blocks were cut into serial semi-thin 5 μm slides, which were stained with hematoxylin/eosin and Van Gieson`s picrofuchsin in order to determine a degree of fibrotic changes in liver tissue and with Sudan III to detect fatty degeneration of hepatocytes. The microscopic

Table I. The relative volumes of structural components of the liver, Group I (M ± m)

Structural components	Duration of subhepatic cholestasis				
	less than 7 days	7-14 days	14-21 days	21-28 days	over 28 days
Hepatocytes, %	68.34±2.03	65.48±2.08	60.35±2.12	52.45±2.17	43.87±2.19
Bile ducts and cholangioles, %	7.01±1.06	6.7±1.08	6.4±1.05	6.02±1.03	5.86±1.02
Connective tissue, %	4.82±0.87	6.32±1.27	10.46±1.37	16.68±1.43	21.94±1.48
Vasculars, %	24.08±1.74	23.15±1.86	22.14±1.78	21.66±1.76	17.36±1.72
Stromal-parenchymal index	0.34±0.008	0.42±0.03	0.59±0.01	1.68±0.04	2.21±0.06

Table II. The relative volumes of structural components of the liver, Group II (M ± m)

Structural components	Duration of subhepatic cholestasis				
	less than 7 days	7-14 days	14-21 days	21-28 days	over 28 days
Hepatocytes, %	65.45±2.1	59.37±2.13*	52.64±2.17*	48.38±2.2*	39.92±2.24*
Bile ducts and cholangioles, %	6.24±1.02	5.82±1.05	5.24±1.03	5.02±1.02	4.29±1.01*
Connective tissue, %	6.11±0.98	8.75±1.25*	14.12±1.42*	19.47±1.57*	24.62±1.59*
Vasculars, %	21.70±1.64	20.66±1.72	19.82±1.76	16.14±1.73*	12.13±1.73*
Stromal-parenchymal index	0.42±0.01	0.98±0.02*	1.24±0.03*	1.89±0.046*	2.68±0.075*

Note: * – $p < 0.05$ – statistically significant difference in relation to the data of the Group I.

structure of the liver parenchyma was studied using a light microscope OLIMPUS BX41 at 100x, 200x and 400x magnification. Morphometric parameters of structural changes were determined using a computer software (Quick Foto Micro 2.3).

The patients were distributed by age according to WHO recommendations. During the study, 50 patients with obstructive jaundice were assigned to two groups. Group I (n = 25) consisted of young (18–44-year-old) and middle-aged patients (45–59-year-old), while the Group II (n = 25) included elderly (60–74-year-old) and senile patients (75–90-year-old). Each study group was subdivided into five subgroups of patients with different duration of obstructive jaundice: less than 7 days, 7-14 days, 14-21 days, 21-28 days, and over 28 days. Five liver biopsy material units were studied in each subgroup.

The research was conducted in compliance with the major principles of GCP guidelines (1996), Council of Europe Convention on Human Rights and Biomedicine (1997), World Medical Association Declaration of Helsinki on ethical principles for medical research involving human subjects (1964-2000) and Order of Ministry of Health of Ukraine № 281 of November 1, 2000, being approved by the Committee on Bioethics of the National Pirogov Memorial Medical University, Vinnytsia (Minutes No. 30 dated 10.12.2018).

The obtained data were statistically processed using descriptive statistic methods involving Microsoft Office Excel 2010 spreadsheet. As quantitative indicators, we calculated sample mean, standard deviation, and mean error. In case of normal distribution of quantitative indicators, we used Student's t-test for their comparison. The difference between the analyzed indicators was considered statistically significant at a significance level of 0.05 (error probability 5% ($p < 0.05$).

RESULTS

Histological study of liver biopsy slides of Group I patients with a duration of subhepatic cholestasis up to 7 days was characterized by the pronounced plethora of central veins, sinusoidal capillaries, and portal veins, centrilobular cholestasis accompanied by a blockade of stellate reticuloendotheliocytes with bile pigments. The venous lumens were significantly dilatated. Parenchyma was characterized by an unclear division into lobes. Most hepatic tubules had signs of discomplexation. Isolated hepatocytes were imbibed with bile. Some of them were in a state of necrosis; mitotically active binucleated hepatocytes were identified. In some cases, the hepatic cells showed signs of small- and large droplets obesity. Morphologically, relative volume of hepatocytes was 68.34 ± 2.03%. The relative volume of connective tissue was 4.82 ± 0.87%, vascular volume

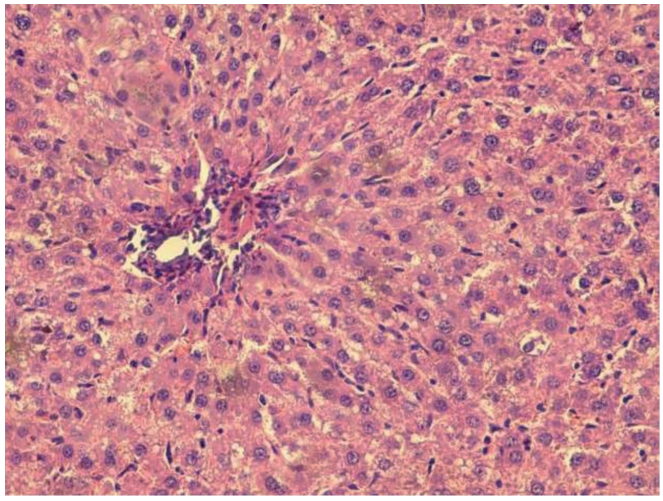

Fig. 1. Group I. Liver tissue subhepatic cholestasis less than 7 days. Hematoxylin-eosin staining, x400

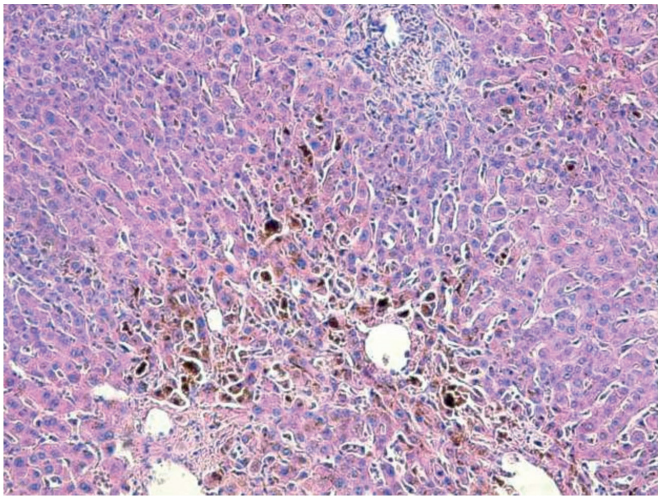

Fig. 2. Group I. Liver tissue with subhepatic cholestasis from 7 to 14 days. Hematoxylin-eosin staining, x200

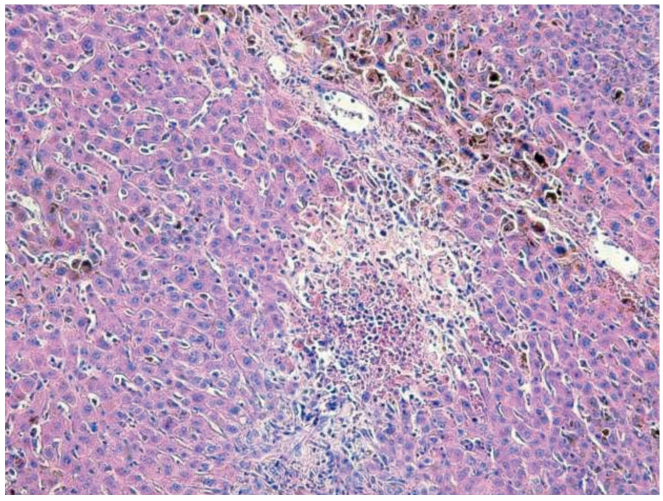

Fig. 3. Group I. Liver tissue with subhepatic cholestasis from 14 to 21 days. Hematoxylin-eosin staining, x200

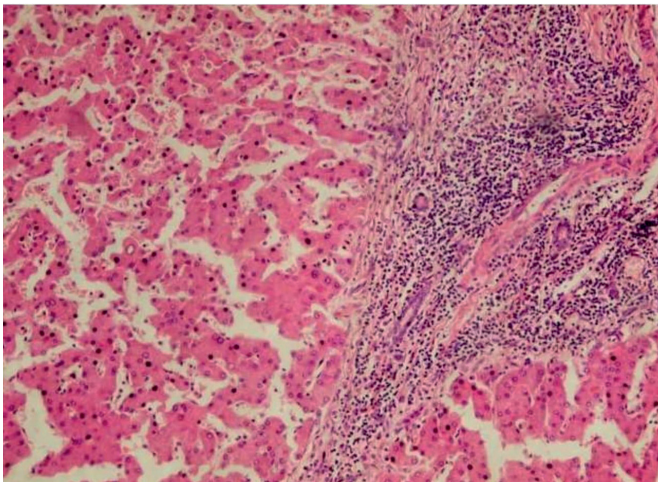

Fig. 4. Group I. Liver tissue with subhepatic cholestasis from 21 to 28 days. Hematoxylin-eosin staining, x200

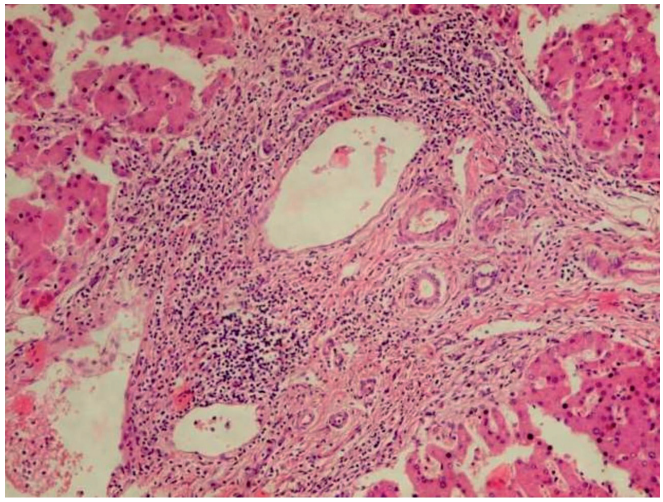

Fig. 5. Group I. Liver tissue with subhepatic cholestasis over 28 days. Hematoxylin-eosin staining, x200

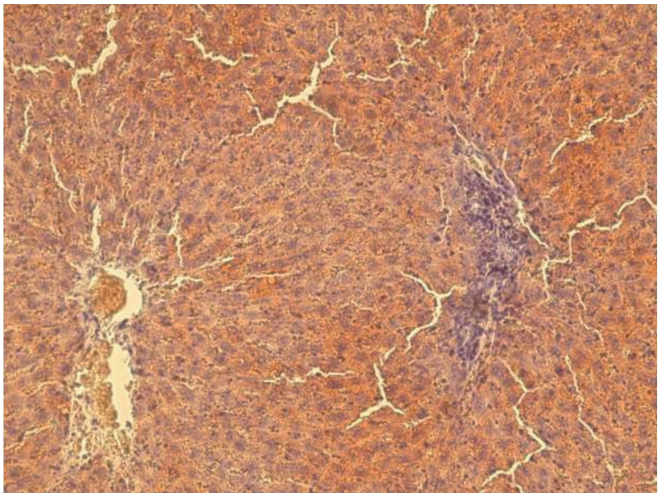

Fig. 6. Group II. Liver tissue with subhepatic cholestasis less then 7 days. Sudan III staining, x400

- 24.08 ± 1.74%, the stromal-parenchymal index - 0.34 ± 0.008. The relative volume of bile ducts and cholangioles was 7.01 ± 1.06% (Table I). These changes were associated with dilatation, edema, and dissection of portal tracts. No fibrous changes of the liver parenchyma were determined in this period (Fig. 1).

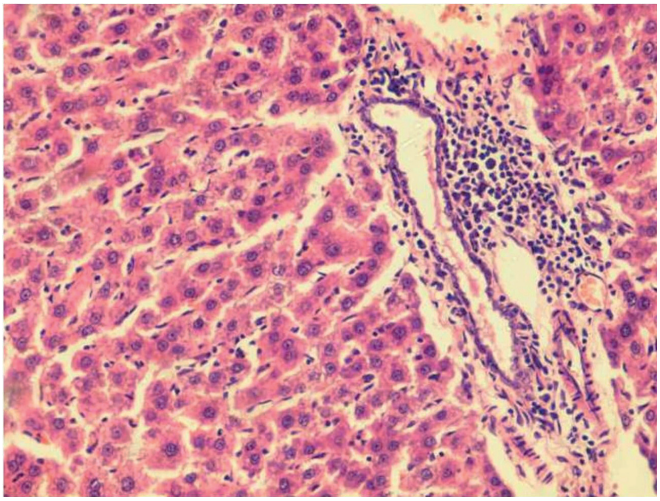

Fig. 7. Group II. Liver tissue with subhepatic cholestasis from 7 to 14 days. Sudan III staining, x400

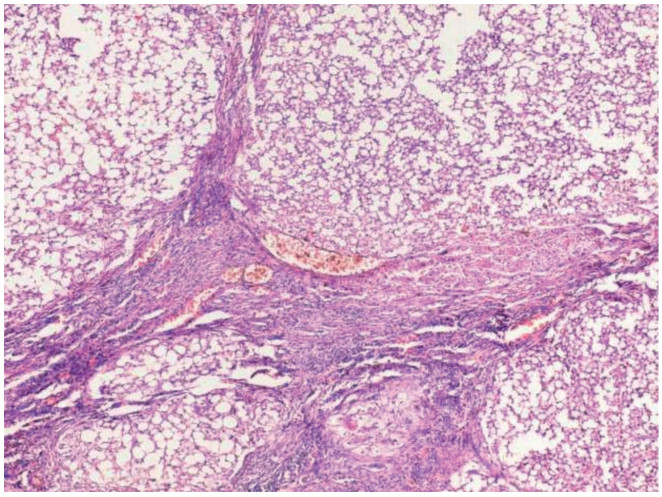

Fig. 9. Group II. Liver tissue with subhepatic cholestasis from 21 to 28 days and over 28 days. Hematoxylin-eosin staining, x100

Histological examination of liver tissue taken from Group I patients with obstructive jaundice lasting from 7 to 14 days also demonstrated the expressed venous plethora and even more pronounced centrilobular cholestasis accompanied by a blockade of stellate reticuloendotheliocytes with bile pigments. Most hepatocytes were imbibed with bile. Some of them were in a state of necrosis; pseudoglandular structures around bile thrombi were observed. In this period, a proliferation of active fibroblasts around the bile duct dilatations was observed. Sclerotic changes around the vessels were insignificant and manifested as a weak growth of soft fibrous tissue around some central veins. Morphologically, relative volume of hepatocytes was 65.48 ± 2.08%. The relative volume of connective tissue was 6.32 ± 1.27%, vascular volume - 23.15 ± 1.86%, stromal-parenchymal index - 0.42 ± 0.03. The relative volume of bile ducts and cholangioles was 6.7 ± 1.08% (Table I). In some cases, an inflammatory cell

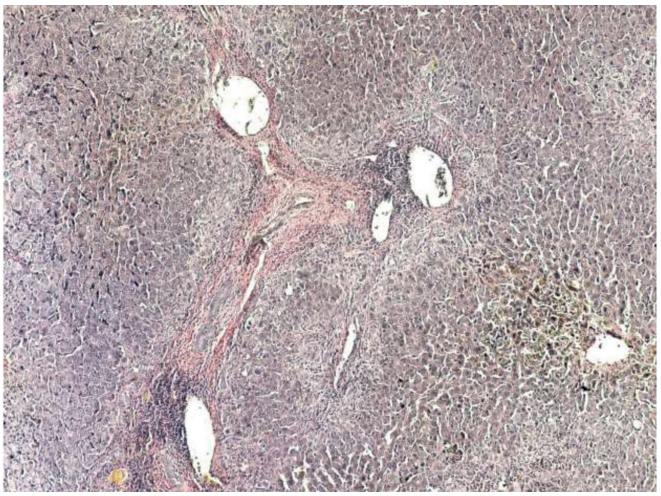

Fig. 8. Group II. Liver tissue with subhepatic cholestasis from 14 to 21 days. Van Gieson`s picrofuchsin staining, x100

infiltrate consisting of mononuclear cells, histiocytes and segmental leukocytes was present (Fig. 2).

Histological examination of liver tissue taken from Group I patients with a subhepatic cholestasis lasting from 14 to 21 days revealed pronounced centrilobular cholestasis associated with the blockade of stellate reticuloendotheliocytes by bile pigments. Pseudoglandular structures around biliary thrombi, growth of fine fibrous tissue around some central veins, and fuchsinophilia of portal tracts were also noted. Morphologically relative volume of hepatocytes was 60.35 ± 2.12%, connective tissue - 10.46 ± 1.37%, vascular volume - 22.14 ± 1.78%, stromal-parenchymal index - 0.59 ± 0.01. The relative volume of bile ducts and cholangioles was 6.4 ± 1.05% (Table I). This period of obstructive jaundice was characterized by presence of inflammatory cell infiltrate from mononuclear cells, segmental leukocytes and histiocytes (Fig. 3).

Morphologically, histological examination of liver biopsy material taken from patients of Group I with obstructive jaundice lasting from 21 to 28 days presented a progression of cellular and portal disorders. Bile discharge and deposition of bile thrombi in the intercellular space took place. Groups of hepatic cells were subject to necrosis and lysis (biliary infarction), associated with reactive inflammation developed in the form of clusters of segmented leukocytes and macrophages. Morphologically, the relative volume of hepatocytes was 52.45 ± 2.17%. Stroma collapse, its fibrosis with moderate infiltration of lymphohistiocytic elements, proliferation of bile ducts was observed. Relative volume of connective tissue was 16.68 ± 1.43%, vascular volume – 21.66 ± 1.76%, stromal-parenchymal index - 1.68 ± 0.04. The bile ducts were dilated, tortuous, their lumen was filled with bile cylinders and bile microliths. Morphologically, relative volume of bile ducts

and cholangioles was 6.02 ± 1.03% (Table I). The largest number of bile cylinders was found in the center of lobules, in liver cells, less often in the cytoplasm of stellate reticuloendotheliocytes. Bile droplets and grains were observed in the cytoplasm (Fig. 4).

Morphologically, the histological examination of liver tissue taken from Group I patients with subhepatic cholestasis lasting more than 28 days showed further progression of parenchymal and portal disorders. These changes contributed to forming fibrous connective tissue septa. Bile passed into the surrounding tissue from damaged bile ducts, forming bile basins with perifocal polymorphic infiltration of inflammatory cells. The spread of fibrosis foci and liver parenchyma necrosis was observed. Morphologically, relative volume of bile ducts and cholangioles was 5.86 ± 1.02%. The relative volume of hepatocytes tended to further decrease and was equal to 43.87 ± 2.19%. An inflammatory granulomatous reaction developed in the portal tracts. The process was also characterized by expressed periductal fibrosis in the form of concentric collagen layers around bile ducts. Relative volume of connective tissue was 21.94 ± 1.48%, vascular volume - 17.36 ± 1.72%, stromal-parenchymal index - 2.21 ± 0.06 (Table I). These changes indicated the development of severe fibrosis and the onset of liver cirrhosis (Fig. 5).

Histological examination of liver biopsy slides taken from Group II patients with obstructive jaundice lasting less than 7 days revealed a noted granular and small vacuolar dystrophy of hepatocytes. The latter was determined in centrilobular located hepatocytes. Also, we observed a discomplexation of hepatic tubules, foci of collapsed hepatic tissue with significant dilatation of sinusoidal spaces, proliferation and activation of stellar reticuloendothelial cells. Another characteristic feature was a combination of hydropic and fatty liver dystrophy. The latter was small droplets and focal one. Heteromorphism of hepatocytes, nuclear polymorphism, polychromia, and manifestation of apoptosis in some cells also were observed. Morphologically, relative volume of hepatocytes was 65.45 ± 2.1%. The relative volume of connective tissue was 6.11 ± 0.98%, vascular volume - 21.70 ± 1.64%, stromal-parenchymal index - 0.42 ± 0.01. These values did not statistically significantly exceed the corresponding values shown of the study Group I with a duration of obstructive jaundice less than 7 days ($p > 0.05$). Morphologically, the relative volume of bile ducts and cholangioles was 6.24 ± 1.02% (Table II), which also did not statistically significantly differ from the corresponding readings of the study Group I ($p > 0.05$) (Fig. 6).

Group II patients with a duration of subhepatic cholestasis from 7 to 14 days was pronounced changes of alternative, inflammatory and reparative-sclerotic nature in the structural components of the liver. The phenomena of intracellular and intraductal cholestasis were building up in all parts of lobules with the formation of bile clots in dilated bile ducts. Signs of cholangitis persisted, but lymphocytes and histiocytes began to dominate in the inflammatory infiltration material, and the concentration of polymorphonuclear leukocytes decreased (signs of chronic inflammatory process). We detected numerous small hepatocyte necrosis foci with infiltration of collapsed stroma by neutrophils, lymphocytes and histiocytes. Most hepatocytes had signs of protein and fatty degeneration. Inflammatory infiltrate cells were represented by lymphocytes detected in both portal tracts and sinusoids in the form of lymphocyte chains. In addition, sclerotic changes were detected in central veins and vessels of portal zones. Portal areas presented a build-up of periductal fibrosis, lymphohistiocytic infiltration, a significant number of fibroblasts, and formation of thin connective tissue septa in some cases, which wedged in the interparticle stroma and parenchyma. Morphologically, relative volume of bile ducts and cholangioles was 5.82 ± 1.05% and vascular volume - 20.66 ± 1.72%. These indicators did not significantly exceed the corresponding readings of the study Group I with a duration of subhepatic cholestasis from 7 to 14 days ($p > 0.05$). Morphologically, the relative volume of hepatocytes was 59.37 ± 2.13%, connective tissue - 8.75 ± 1.25%, and stromal-parenchymal index - 0.98 ± 0.02 (Table II), which statistically significantly exceeded the corresponding figure of the study Group I ($p < 0.05$) (Fig. 7).

Histological examination of liver tissue taken from patients of the Group II with obstructive jaundice lasting from 14 to 21 days revealed more pronounced changes of inflammatory and reparative-sclerotic nature in all structural components of the liver, accompanied by signs of widespread intracellular and intraductal cholestasis with the formation of bile clots. The most pronounced changes manifested in the central parts of the lobules, accompanied by an outflow of bile beyond the limits and the formation of bile basins, which led to the emergence of large focal necrosis of the parenchyma. Almost all cases associated with hepatocytes in the state of hydropic dystrophy, which were located mainly in the central parts of liver lobules, less often in the peripheral ones. Adipose hepatosis was also noted. Hepatocytes had different degrees of fatty degeneration, manifested within the range of small- to large-droplet steatosis. As a rule, small lipid droplets were found in the cytoplasm of centrilobular hepatocytes. Heteromorphism of hepatocytes, nuclear polymorphism, polychromia, and manifestation of apoptosis were also

observed. The pronounced dystrophic changes in liver tissue associated with a progression of periportal and centrolobular perivenular fibrosis, as well as pericellular sclerosis sites. In some cases, we detected the cirrhosis signs presented as thin fibrous septa wedged in liver lobules, central veins connected with the vessels of the portal tract, associated with the emergence of small false lobules consisting of proliferating hepatocytes in the condition of fatty and protein dystrophy, and the absence of radial orientation of hepatic cords. Fibrous septa and stroma of dilated sclerosed portal tracts had signs of focal inflammatory infiltration, consisting mainly of lymphocytes and macrophages. Morphologically, relative volume of bile ducts and cholangioles was 5.24 ± 1.03, vascular volume - 19.82 ± 1.76%, which did not statistically significantly differ from the corresponding readings of the study Group I with a duration of obstructive jaundice from 14 to 21 days ($p > 0.05$). Relative volume of hepatocytes was 52.64 ± 2.17%, connective tissue - 14.12 ± 1.42%. These readings statistically significantly exceeded the corresponding figures of the study Group I ($p < 0.05$). Stromal-parenchymal index was 1.24 ± 0.03 (Table II), which was also statistically significantly higher than the figures of the study Group I with a duration of subhepatic cholestasis from 14 to 21 days ($p < 0.05$) (Fig. 8).

Patients of the study Group II with a duration of subhepatic cholestasis from 21 to 28 days and over 28 days, presented large focal necrosis of liver parenchyma and the development of a pronounced periductal sclerotic process. The formation of fibrous septa in combination with nodular regeneration of the parenchyma led to a failure of hepatic histoarchitectonics with subsequent development of the liver cirrhosis. Hepatocytes were in a state of hydropic and fatty dystrophy. The expressed sclerotic processes of the stroma in the form of periportal and centrolobular perivenular fibrosis and pericellular sclerosis were also noted. Cirrhosis of the liver occurred in the vast majority of cases. Patients with obstructive jaundice lasting from 21 to 28 days presented morphologically relative volume of bile ducts and cholangioles equal to 5.02 ± 1.02%, which did not statistically significantly differ from the corresponding figure of the study Group I with a subhepatic cholestasis lasting from 21 to 28 days ($p > 0.05$). Relative volume of hepatocytes was 48.38 ± 2.2%, connective tissue - 19.47 ± 1.57%, and vascular volume - 16.14 ± 1.73%. These indicators statistically significantly exceeded the corresponding readings of the study Group I ($p < 0.05$). Stromal-parenchymal index was equal to 1.89 ± 0.046 (Table II), which was also statistically significantly higher than in the study Group I with a duration of obstructive jaundice from 21 to 28 days ($p < 0.05$). Morphologically, relative volume of bile ducts and cholangioles in patients with a duration of subhepatic cholestasis over 28 days was 4.29 ± 1.01%, which statistically significantly differed from the corresponding figure of the study Group I ($p < 0.05$). Relative volume of hepatocytes was 39.92 ± 2.24%, connective tissue - 24.62 ± 1.59%, vascular volume - 12.13 ± 1.73%, stromal-parenchymal index - 2.68 ± 0.075 (Table II), which also statistically significantly exceeded the corresponding figures of the study Group I with a duration of subhepatic cholestasis over 28 days ($p < 0.05$). These changes suggest a damage of hepatic histoarchitectonics with the development of severe cirrhosis of the liver (Fig. 9).

DISCUSSION

The comparative study of hepatic morphological changes showed that the severity of structural disorders in patients with subhepatic cholestasis depended primarily on the disease duration. At early stages of mechanical jaundice (first 2 weeks), were morphological changes presented as bilirubinostasis with hepatocytes and canaliculi, and signs of protein and fat dystrophy associated with the development of biliary hepatitis [9-11]. At later stages of subhepatic cholestasis (more than 2 weeks), were the bile duct epithelium proliferation and periportal fibrosis. The mechanical jaundice with the duration more than 1-3 months in addition to dystrophic and necrobiotic changes, there were a pronounced fibrosis of the portal stroma and biliary cirrhosis of the liver [9, 10, 12]. In these studies, structural changes in the liver with different durations of subhepatic cholestasis were studied. However, hepatic morphological changes in mechanical jaundice patients of different age groups were not taken into account.

In the Group I patients at early terms of subhepatic cholestasis (up to 7 days, 7 to 14 days), morphological changes in the liver mainly consisted in:
- intraductal cholestasis;
- moderate polymorphic cellular inflammatory infiltration of the stroma.

At later terms of obstructive jaundice (from 14 to 21 days; from 21 to 28 days), changes in the liver parenchyma in patients of the Group I were associated with the development and progression of:
- intraductal and intracellular cholestasis;
- reactive stromal hepatitis;
- dystrophy of hepatocytes;
- small focal necrosis of the liver parenchyma;
- initial fibrosis.

At the latest terms of subhepatic cholestasis (over 28 days), morphological hepatic changes in the Group I patients consisted in:

- multiple diffuse extra- and intracellular bilirubin deposits;
- severe dystrophy of hepatocytes;
- large focal necrosis of the liver parenchyma;
- development of severe fibrosis and onset of liver cirrhosis.

Patients of the Group II with obstructive jaundice lasting up to 7 days, presented the following changes in the liver:
- intracellular and intraductal cholestasis;
- stromal hepatitis;
- combination of protein and fatty liver dystrophy.

In patients of the Group II with subhepatic cholestasis lasting from 7 to 14 days and from 14 to 21 days, changes were associated with the development and progression of:
- intracellular and intraductal cholestasis associated with the formation of bile clots;
- chronic inflammation;
- severe dystrophy of hepatocytes;
- small and large focal necrosis of the liver parenchyma;
- severe fibrosis and onset of cirrhosis of the liver.

Patients of the Group II with obstructive jaundice lasting 21 to 28 days and over 28 days presented the following morphological changes in the liver:
- large focal necrosis of the liver parenchyma;
- severe periductal and periportal fibrosis;
- failure of hepatic histoarchitectonics and the development of severe liver cirrhosis.

CONCLUSIONS

1. In patients of the Groups I and II, pathological hepatic changes in the early stages of mechanical jaundice were manifested in the form of hepatocyte dystrophy and hepatitis development.
2. In the Group I patients, manifestations of steatohepatitis, fibrosis and initial signs of liver cirrhosis were noted in the late stages of subhepatic cholestasis. In addition to the above-mentioned changes, Group II patients, in the late stages of mechanical jaundice, presented signs of severe fibrosis and well-shaped liver cirrhosis.
3. Taking into account the above morphological changes in the liver with different duration of subhepatic cholestasis, we consider reasonable to decompress bile ducts in patients of older age groups at earlier stages of mechanical jaundice compared to young and middle-aged patients, thus preventing post-decompression liver dysfunction and the subsequent development of biliary cirrhosis.

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ORIGINAL ARTICLE

EFFICACY OF PRIMARY REHABILITATION MEASURES ASSOCIATED WITH THE DEVELOPMENT OF RECURRENT BRONCHIAL OBSTRUCTION SYNDROME IN YOUNG CHILDREN WITH RESPIRATORY DISORDERS IN NEONATAL PERIOD

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ABSTRACT

The aim: To improve primary prophylactic measures associated with the development and progression of recurrent bronchial obstruction syndrome in young children, who had suffered respiratory disorders in neonatal period.

Materials and methods: Algorithm of primary prophylactic measures implied adequate balanced nutrition, sanation of living conditions, restriction of contact with infectious agents, sanation of chronic foci of infection, systematic training and general fitness. The investigation included 160 young children (1 day – 3 years of age). The basic group (n=80) involved children, who had experienced respiratory disorders in neonatal period and received appropriate respiratory therapy (artificial ventilation and / or spontaneous breathing with continuous positive airway pressure and supply of free oxygen), control group – children, who did not have respiratory disorders and respiratory therapy (n=80).

Results: Conducted investigation throughout 12-month monitoring enabled to record the development of recurrent bronchial obstruction syndrome in 43 children (respectively, 30 – 37.50% patients of the basic group versus 13 – 16.25% of control group; p 0.05), could not be obtained.

Conclusions: Comparative analysis within groups did not show a reliable difference in the development of recurrent bronchial obstruction syndrome in children (p>0.05), which can be explained by partial following of doctor's recommendations. There is the need in further study of the issue involving more patients for a longer period of monitoring.

KEY WORDS: respiratory disorders, young children, neonatal period, bronchial obstruction syndrome, primary rehabilitation measures, efficacy

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INTRODUCTION

In the recent years, respiratory pathology remains an urgent problem in clinical pediatrics, which has not only medical, but also social meaning [1-7]. Statistic data prove that among total pediatric morbidity, diseases of respiratory organs constitute half of all disorders [7]. Respiratory disorders are most commonly recorded in premature children (30 – 80% depending on gestational age); however, they also occur in full-term children (5 – 10%) [3].

Frequently, respiratory pathology starts from the first days of a child's life, which is largely because nowadays children, who in neonatal period required continuous respiratory maintenance (artificial ventilation, spontaneous breathing with continuous positive airway pressure, oxygen therapy) for different reasons, survive [2, 4]. In the recent decades it has been proven that not only diseases of the respiratory system in neonatal period, but continuous respiratory therapy itself (in particular, artificial ventilation) often result in the formation of recurrent and chronic bronchopulmonary pathology in the future [5]. Moreover, there are data that in early age of each third child, who is on artificial ventilation in neonatal period, recurrent and chronic bronchopulmonary diseases (bronchial asthma (BA), bronchopulmonary dysplasia (BPD), recurrent pneumonia, recurrent bronchitis) occur, development of which is closely associated with the pathology of neonatal period [3, 6].

THE AIM

To improve primary prophylactic measures aimed at the development and progression of recurrent bronchial obstruction syndrome (BOS) in young children, who had suffered respiratory disorders in neonatal period.

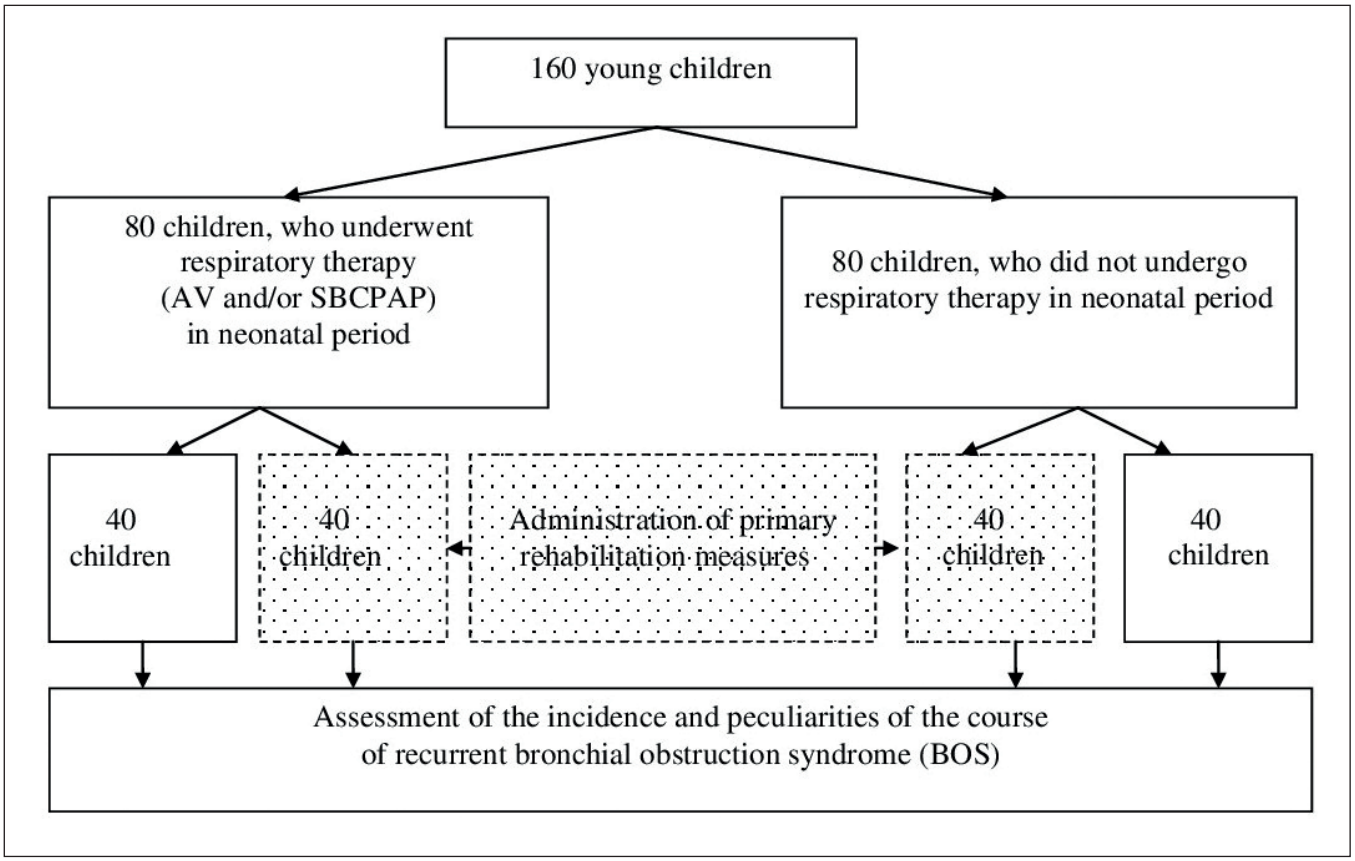

Fig. 1. A scheme of organization of the investigation

MATERIALS AND METHODS

General clinical (study of anamnesis, data of objective examination and dynamic monitoring); modern statistic analysis. In the presence of clinical indications, total blood count, radiological examination of the thoracic organs was administered; bronchoscopy, echocardiography, USS of the thymus, and Mantoux test were performed in some clinical cases, doubtful for diagnosis. If required, children were examined by an otolaryngologist, neurologist and other specialists.

Overall, 160 young children (1 day – 3 years), who were treated in departments of reanimation and intensive therapy for neonates, pathology of newborns and premature babies, pediatric and allergological departments of Communal Nonprofit Enterprise "City Children's Clinical Hospital of Lviv" were involved in the research.

The basic group included children, who had experienced respiratory disorders in neonatal period and received proper respiratory therapy – artificial lung ventilation (ALV) and/or spontaneous breathing with continuous positive airway pressure (CPAP) and supply of free oxygen (n=80), control – children, who did not have respiratory disorders and respiratory therapy (n=80).

Administration of primary rehabilitation measures was performed randomly in 40 patients of the basic group and 40 – in control group. In the rest of the patients (40 in each group), a suggested complex of measures was not used.

The scheme of organization of the investigation is presented in fig. 1.

STATISTICAL ANALYSES

Categorical (qualitative) signs were presented as a number of patients and in their percentage. Shapiro-Wilk criterion has been applied to check the normality of distribution. Quantitative data with normal distribution character have been presented as M±SD, where M is a mean value, SD – standard deviation.

In order to check the significance of difference between groups of categorical (qualitative) signs we have applied the tables of frequency (tables IV) and a Pearson χ^2 criterion. A two-sided t-test for unrelated groups has been used to check the significant differences between groups of quantitative data with normal distribution. To check the significant differences between related groups (e. g. indexes during different visits) we have applied a t-test for related groups. A difference between groups was considered significant in case of $p < 0,05$.

All of statistical calculations were performed using software RStudio v. 1.1.442 and R Commander v.2.4-4.

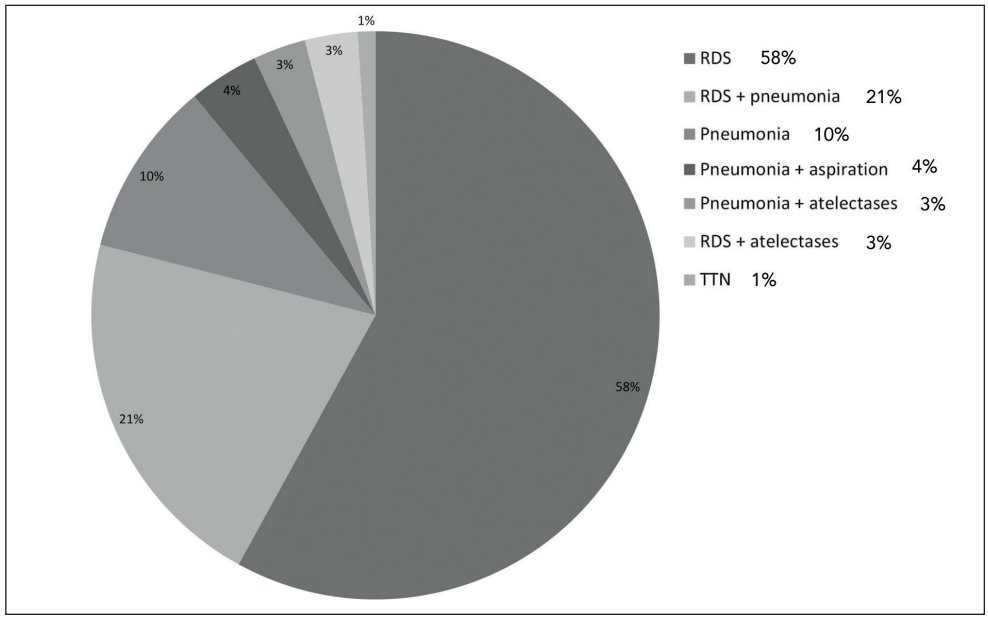

Fig. 2. Structure of the diseases, which were the cause for conduction of respiratory therapy

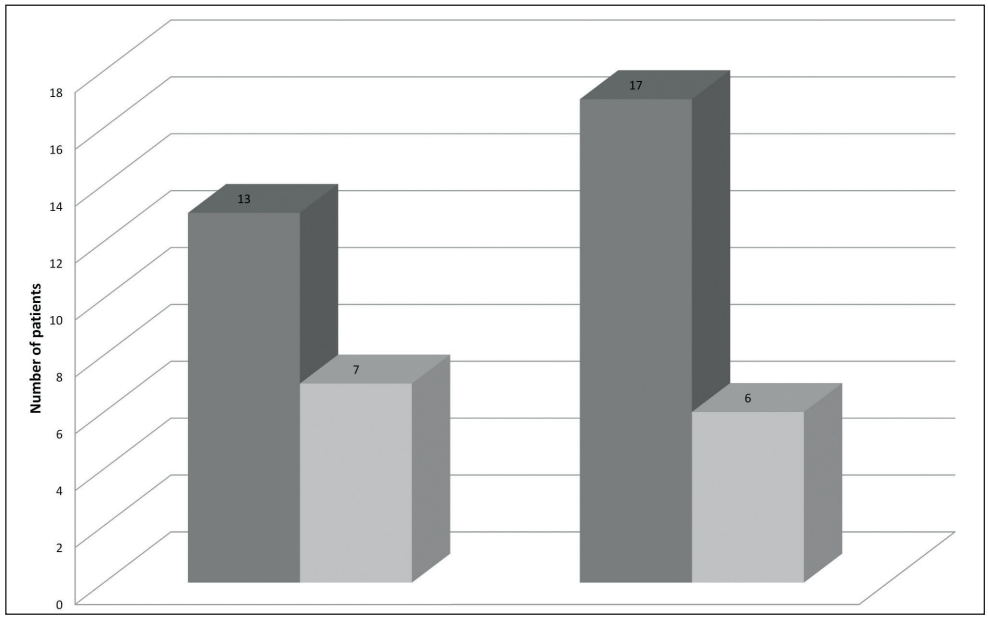

Fig. 3. Verification of nosological forms of recurrent bronchial obstruction syndrome in children

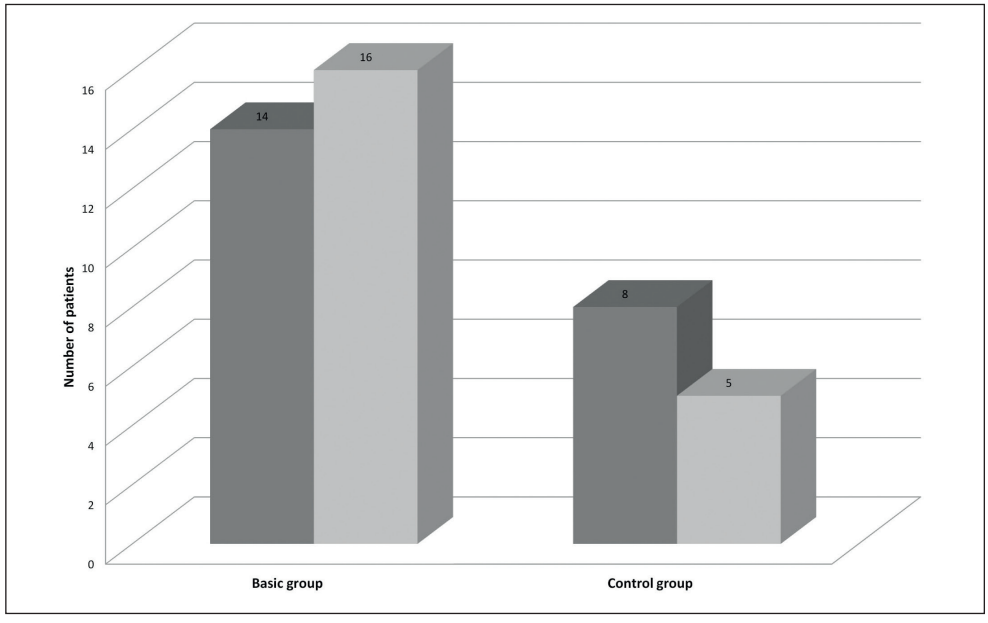

Fig. 4. Development of recurrent bronchial obstruction syndrome in children with the administration of primary rehabilitation measures

Table I. Distribution of children of the basic group by gender and age at the start of the investigation

Gender	Age at the time of investigation, weeks						Total	
	0 – 1		1 – 2		2 – 3			
	absolute	%	absolute	%	absolute	%	absolute	%
Girls	17	37	12	54.5	5	41.7	34	42.5
Boys	29	63	10	45.5	7	58.3	46	57.5
Total	46	100.0	22	100.0	12	100.0	80	100.0

Table II. Distribution of children in control group by gender and age at the start of the investigation

Gender	Age at the time of investigation, weeks						Total	
	0 – 1		1 – 2		2 – 3			
	absolute	%	absolute	%	absolute	%	absolute	%
Girls	11	28.9	15	55.6	7	46.7	32	40
Boys	27	71.1	12	44.4	8	53.3	48	60
Total	38	100.0	27	100.0	15	100.0	80	100.0

Table III. Variations of respiratory therapy applied in children of the basic group

Type of respiratory therapy	Number of patients	
	absolute	%
Artificial ventilation + spontaneous breathing with continuous positive airway pressure + supply of free oxygen	50	62.5
Spontaneous breathing with continuous positive airway pressure + supply of free oxygen	30	37.5
Total	80	100

Table IV. Distribution of patients in the basic group depending on the duration of conducted respiratory therapy

Duration of respiratory therapy (hours)	Number of patients	
	absolute	%
below 72	5	6.25
72 – 120	15	18.75
120 – 150	10	12.5
over 150	50	62.5

Table V. Distribution of children by age at the time of manifestation of bronchial obstruction syndrome

Group	Age at the time of investigation, weeks						No development of BOS		Total	
	< 12		12 – 24		24 – 36					
	absolute	%	absolute	%	absolute	%	absolute	%	absolute	%
Basic	23	28.75	17	21.25	5	6.25	35	43.75	80	100
Control	16	20	9	11.25	4	5	51	63.75	80	100

RESULTS

Distribution of children by gender and age at the start of the investigation is presented in tables (I and II).

Formed groups reliably did not differ by percentage of boys (46 [57.5%] in the basic group versus 48 [60%] in control group; p>0.05), mean body mass at birth (2025.2±79.3 g in basic group versus 2226.5±64.1 g in control group; p>0.05) and mean gestational age (respectively, 33.1±0.4 weeks versus 34.6±0.3 weeks; p>0.05).

Percentage of premature children was approximately the same in the groups (71 [88.8%] versus 69 [86.3%]; p>0.05) and patients born in multiple pregnancy (respectively, 13 [16.3%] versus 10 [12.5%]; p>0.05.

Endogenous surfactant was administered to 16.3% (13) of children in the basic group. This medicine was not administered to children of control group (p<0.01).

Antenatal steroid prophylaxis (p<0.05) was conducted for 9 [11.3%] parturient women of the basic group and 4 [5%] women from control group.

Variations of respiratory therapy, applied in children of the basic, are presented in table III.

According to the data of world literature, the primary cause for conduction of respiratory therapy in newborns is respiratory distress syndrome (RDS). Our analysis proved such statistic pattern. In particular, in the structure of nosologies, which were the reason for conduction of respiratory therapy, percentage of children with RDS constituted 58%, 21% – RDS + pneumonia, 10% – pneumonia. Other causes accounted for 10%, among them being aspiration + pneumonia, pneumonia + atelectases, RDS + atelectasis, transient tachypnea of the newborn (TTN) (fig. 2).

Duration of conducted respiratory therapy among patients of the basic group is presented in table IV.

Our analysis enabled to detect single development of bronchial obstruction in children under 3 years of age in 9 (11.25%) patients of the basic group and 11 (13.75%) – in control group (p>0.05). Simultaneously, two exacerbations were observed in 6 (7.5%) children of the basic group and 5 (6.25%) – control group (p>0.05). In these cases, BOS was referred to as acute obstructive bronchitis. Conduced investigations enabled to record the development of recurrent BOS (3 and more episodes of bronchial obstruction) in 30 (37.50%) patients of the basic group and 13 (16.25%) – control group (p<0.05). Thus, at least one episode of BOS occurred in 56.26% of patients in the basic group and 36.25% – in control group. Among them, recurrent episodes were recorded in 37.61% of the basic group and 22.5% – control group.

In 10 children of the basic group, who had undergone continuous respiratory therapy, the development of BPD was observed. Moreover, under 3 years of age, transformation of the disease to BA was observed in 3 (30%), to obstructive bronchitis – in 2 (20%) cases, 2 (20%) individuals suffered from frequent acute respiratory viral infections, 1 (10%) – recurrent laryngotracheitis, and convalescence occurred in 2 (20%) patients. Thus, the development of recurrent bronchial obstruction syndrome (bronchial asthma and obstructive bronchitis) was observed in 50% young patients, who had been diagnosed with BPD in neonatal period.

Analyzing anamnestic data of children in both groups, special attention was paid to determination of age, at which first episodes of bronchial obstruction were manifested in a child. Distribution of children by age at the time of manifestation of BOS is presented in table V.

Conducted analysis of atopia development in children enabled to reveal that the development of atopic dermatitis was the most common in children of the basic and control groups (29.3±4.4 % of children in the basic group and 27.8±3.7 % in control group; p>0.05). There were also recorded allergic rhinosinusitis (in patients of the basic group – 9.3±3.3 % versus 64±2.1 % in control group; p>0.05), severe urticaria (respectively, 4.1±1.2 % versus 5.6±1.8 % in control group; p>0.05), rarely – insect sting allergy and reactions to medicines (p>0.05). Based on investigated factors that influence the development of recurrent BOS in children with respiratory disorders in neonatal period, we have elaborated a complex of primary rehabilitation measures, aimed at prevention of the development of recurrent bronchial obstruction syndrome in such children. The suggested complex included the following measures.

1. Adequate balanced nutrition, which implied a choice of substantial food rich in calories with optimal amount of proteins, fats, carbohydrates and vitamins. Maximally continuous maintenance of breastfeeding was encouraged. If a child developed symptoms of allergy and breastfeeding was impossible, hypoallergenic formula and / or hypoallergenic diet (individual according to nutrition diary) was administered.
2. Sanation of living conditions that was implemented by avoiding contact with everyday allergens – daily humid cleaning and airing of the rooms, elimination of dust collectors (soft toys, cabinets with books, carpets), avoidance of a child's contact with epidermal allergens.
3. Restriction of contact with infectious agents – sanation of chronic foci in a child and family members, early anti-epidemic measures and limited attendance of organized children's groups.
4. Systematic hardening of the body. At first, a child was administered series of hardening in the air, then – in water. The process of body hardening started only under conditions of complete wellbeing of a child in warm season following the principle of gradual increase in load.
5. General physical therapy, which included exercises on a fitball and massage of the chest. A specialist performed the massage twice a year with a course of 14 days. Aqueous procedures and swimming were performed with gradual decrease in water temperature (approximately by one degree every two weeks), the incidence and duration of which was determined by the age and mood of a child.

To assess the efficacy of primary prophylactic measures, patients were examined in a clinic three times: at the start and in 12 months ± 14 days from the moment of administration of primary prophylactic measures.

During a visit, complaints were recorded and routine systematic examination was performed. In the presence of clinical indications, total blood count and X-ray examination of the thoracic organs was administered, and Mantoux test was performed in some clinical cases, doubtful for diagnosis. If required, children were examined by an otolaryngologist, neurologist and other specialists.

Parents controlled the symptoms of the disease. Final assessment of efficacy of rehabilitation measures was performed during scheduled visits to a clinic based on anamnestic data and objective examination of a patient.

During each visit, an individual conversation with a child's parents about the importance of following doctor's recommendations was conducted.

Comparison of clinical efficacy of primary prophylactic complex was performed within the group (compared with patients who did not receive it) and with control group.

The conducted investigations throughout 12-month monitoring enabled to record the development of recurrent BOS in 43 children (respectively, 30 – 37.50 % of patients in the basic group versus 13 – 16.25 % in control group; $p<0.05$). The analysis of the record of nosological forms in patients of the basic group, conducted within the group, showed that recurrent episodes of obstructive bronchitis were diagnosed in 13 (43.33 %), bronchial asthma – in 17 (56.67 %) children (fig. 3).

Thus, the development of recurrent bronchial obstruction syndrome was much more common among the patients, who had experienced respiratory disorders in neonatal period and received respiratory therapy compared to the patients, who had not experienced such problems ($p < 0.05$), and was mostly caused by BA.

However, we did not manage to obtain the results, which could confirm the efficacy of suggested primary rehabilitation measures, since comparative analysis within groups did not show a reliable difference in the development of recurrent BOS ($p>0.05$), fig. 4.

DISCUSSION

Nowadays the respiratory pathology remains a topical issue in clinical paediatrics, which has not only medical but also social significance [2]. Statistics confirm that respiratory diseases account for half of all diseases among the overall paediatric incidence rate [3]. Respiratory disorders are most often registered in premature infants (30-80% depending on gestational age), but they also occur in full-term infants (5-10%) [7]. Moreover, in recent years, respiratory pathology differs in many respects from those that doctors dealt with even 10 years ago, which is largely due to changes in the pathomorphosis of diseases [3, 5]. Professional literature contains information about the connection between conducted respiratory therapy in neonatal period and further formation of recurrent and chronic bronchopulmonary pathology, which is often accompanied by the development of bronchial obstruction syndrome (BOS) [3].

Respiratory pathology often occurs from the first days of a child's life, which is mostly because at present children who needed long-term respiratory support for various reasons in the neonatal period: ALV, CPAP, oxygen therapy more and more often survive. In recent years, it has been proven that not only diseases of the respiratory system of the newborns, but also long-term respiratory therapy (including ALV) often lead to the development of recurrent and chronic bronchopulmonary pathology in the future [2]. In particular, there is evidence that at an early age, every third child who underwent ALV in the neonatal period, develops recurrent and chronic bronchopulmonary diseases (BA, BPD, recurrent pneumonia, recurrent bronchitis), the development of which is closely related to the neonatal pathology [6].

To date, a link between ALV in the neonatal period and the subsequent development of airway hyperreactivity, which is clinically manifested by recurrent BOS has been proven [3]. Early examinations are necessary for children with recurrent BOS to establish the causes of disease relapse. Collection of genealogical, medical, biological, social, hygienic and epidemiological anamnesis, complete analysis of clinical symptoms and assessment of dynamics of the disease are very important [8].

There are several debatable issues in the organization of adequate monitoring and treatment of BOS in young children with respiratory disorders in the neonatal period. In particular, the specific contribution of factors that are decisive in its development (respiratory disorders, aggressiveness of respiratory therapy, immaturity of the child's lungs, genetic predisposition, adverse environmental effects) is discussed [4]. It is important to find possible ways to prevent the development of recurrent or chronic bronchopulmonary pathology.

A detailed and in-depth analysis of this set of issues will help to study the features of recurrent BOS in children who have suffered from respiratory disorders in the neonatal period and develop a preventive strategy for its development, which will prevent disease or improve prognosis, reduce chronic morbidity and disabil-

ity. The above situation has determined the relevance and purpose of our study.

Primary prevention (first level) involves working with children at risk of the development of the disease. It should begin long before the baby is born and include educational work with the pregnant woman and her family.

Secondary prevention (second level) is performed after the first signs of the disease appear to prevent the formation of recurrent and chronic pathology.

In the literature, there is tertiary prevention (third level), which involves working with sick children and is aimed at preventing the development of severe cases of recurrent BOS and their complications [2, 7].

One of the main directions of the preventive strategy of recurrent BOS is the selection of a balanced diet. The main task in the nutrition of infants is to ensure breastfeeding.

It is not always possible to organize primary preventive measures before the birth of a child. Opportunities expand significantly after the birth of a child. The answers to the question of whether breastfeeding can prevent the development of obstructions are full of contradictions. The lack of convincing data on the preventive effect of breastfeeding is because children for ethical reasons can not be artificially randomized to breastfeeding or artificial feeding. [1].

First of all, those foods that can provoke an exacerbation of recurrent BOS in the baby should be excluded from the diet of the nursing mother. It should be noted that the anamnestic data, mother's observations while keeping a food diary, elimination and food challenge tests can identify causative allergens in the vast majority of patients. On this basis, it is possible to choose an elimination diet for each patient individually. A properly elaborated diet not only limits the intake of allergens but also has a non-specific hyposensitizing effect and improves the condition of the digestive system, which, in turn, increases immunological tolerance to food allergens [5].

If for various reasons, breastfeeding is not possible, the selection of artificial feeding should be approached carefully. It is clear that formulas containing whey protein or casein hydrolyzate are much less sensitizing to the child's body, and a high degree of protein hydrolysis leads to almost complete loss of the ability to cause an allergic reaction. At present, there are a large number of formulas on the market with partial or complete whey protein or casein hydrolyzate.

Developing an individual diet for children over 1 year is a difficult task. Such food is difficult to choose, even more, difficult to implement. Children become more independent, begin to attend organized children's groups, which significantly complicates the organization of dietary nutrition. However, certain rules must be followed when choosing a diet for such children. First of all, before receiving the results of allergy tests, it is recommended to prescribe an empirically selected diet, which excludes causative (selected on the basis of a food diary) allergens. It is inadmissible to indulge in the wide exclusion of all known obligate allergens from the diet, it is necessary to identify the "culprit" allergen individually and at the same time provide its equivalent replacement to maintain the completeness of the child's diet. The organization of nutrition of such patients involves limiting the consumption of foods with high sensitizing activity [3].

It is possible to transfer the child to a usual food without essential restrictions in 10-12 months after the total disappearance of manifestations of recurrent BOS. However, foods that should be excluded should be introduced gradually, alternately, slowly [1].

Along with a balanced diet in the treatment of children with recurrent BOS, it is important to properly organize the life of the child and care for him / her. First of all, exposure to inhalation and contact allergens should be eliminated or limited. The room where the child lives should be frequently ventilated and wet cleaned twice a day. The number of dust collectors (carpets, upholstered furniture) in the apartment should be sharply limited. Pets are not allowed in the apartment. It is necessary to limit the child's contact with irritants (use of natural wool clothing, irritating hygiene products). The stimulus may be high or low temperature [4].

Limiting contact with infectious factors is the elimination of chronic foci of infection, timely anti-epidemic measures, limited attendance of organized children's groups and places with large crowds (public transport, market, playground) [9].

Coldwater treatment is recommended in the settings of the overall health of the baby in the warm season. First, the child is prescribed sessions of cold air treatment, and then – cold water with adherence to the principle of gradual increase in load [2].

Strengthening exercises include exercise ball and chest massage. Massage is performed by a specialist twice a year [4].

Unfortunately, despite the whole range of preventive measures used today, the rate of recurrent BOS remains high. It is possible that soon some preventive principles will be improved or changed. It comes as a natural result because scientists continue their active search. Probably our immediate prospect and greatest hope are to conduct immunomodulation with Th1 immunoadjuvants, DNA vaccines and cytokine-related antigens (interleukin-12 or interferon-gamma) [10].

The leading role in the development of atopic reactions is played by the balance between Th-1 and Th-2 cells. It is proven that children with the recurrent BOS usually have more Th-2 cells, what results in the excessive antibody production [7]. The factors stimulating the formation of Th-1-response mediators at the same time reduce the atopic tendency that is often present in the mechanism of the recurrent BOS [6].

Thus, prevention in young children who had respiratory disorders in the neonatal period is one of the most basic measures of paediatrics.

Currently it has been proven that the cause of insufficient efficacy of any therapeutic or preventive measures is, primarily, partial following of all recommendations. Not always, patients and their close relatives properly accept information from health care providers. Thus, we paid special attention to individual training of parents, which was conducted during each visit to a clinic. We tried to form correct notion about the essence of bronchial obstruction syndrome, risk factors of its occurrence and conditions of progressing in each child. Such work can promote the formation of parents' active position in further treatment of their child.

CONCLUSIONS

1. The development of recurrent bronchial obstruction syndrome occurred much more frequently among the patients, who had experienced respiratory disorders in neonatal period and received respiratory therapy compared to the patients, who had not suffered such problems ($p < 0.05$), and was basically caused by bronchial asthma (56.67 %).
2. The efficacy of suggested primary rehabilitation measures could not be proven (comparative analysis within groups did not show a reliable difference in the development of recurrent bronchial obstruction syndrome; $p>0.05$).
3. The likely cause of insufficient efficacy of any therapeutic or preventive measures is partial following of doctor's recommendations, thus, it is necessary to form an active position of parents in the process of a child's treatment.
4. The efficacy of primary rehabilitation measures concerning the development of recurrent bronchial obstruction syndrome in young children with respiratory disorders in neonatal period requires further study involving many patients throughout a continuous monitoring period.

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Ethical Committee or Institutional Animal Care and Use Committee Approval: Danylo Halytsky Lviv National Medical University 20/12/2010 № 10; Nonprofit Communal Enterprise "City Children's Clinical Hospital of Lviv"; 16.Nov.2018 № 6. We are grateful to the children and their parents for participating in the study.

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ORIGINAL ARTICLE

INTERLEUKIN-6 AND NKG2D AS PROGNOSTIC FACTORS IN IRAQI FEMALES WITH PITUITARY GAND ADENOMA: A LONGITUDINAL STUDY

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ABSTRACT

The aim: To assess the role of circulating IL-6 & NKG2D in the prognosis of pituitary adenoma.

Materials and methods: Thirty female with new diagnosis of prolactinoma (pituitary gland adenoma) were enrolled in the study. ELISA test was used to evaluate the level of IL6 and NKG2D. ELISA tests were conducted before the initiation of treatment and six months later.

Results: There are significant differences in mean levels of IL-6 and NKG2D, and the anatomical type (tumor size) (-418.7 & 418.9, $p<0.001$) of anatomical tumor (-373.72 & -373.920, $p=0.001$). There is a significant difference between the two immunological markers (IL-6 & NKG2D) (-0.305; $p<0.001$). The IL-6 markers significantly decreased in means on follow up (-197.8; *p*-value≤ 0.0001) while the reverse occur in NKG2D, which increased in levels post-treatment compared with baseline measurement. The high expression of IL-6 positively correlated with the risk of macroadenoma (>10 microns) and poor resonse to treatment and vice versa ($p<0.024$). High expression of NKG2D significantly ($p<0.005$) correlated with good prognosis and increased chance for tumor response to medicine and shrinkage in size compared with low concentration.

Conclusions: The higher the level of IL-6, the larger the size of adenoma (macroadenoma) and the poorer the response to treatment. The higher the level of NKG2D indidcate a better prognosis, therefore, IL-6 and NKG2D correlate negatively in prolactinoma patients.

KEY WORDS: Interlukin-6, Natural Killer Group2 D, Prolactinoma, Microadenoma, Macroadenoma

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INTRODUCTION

The pituitary adenoma is a benign tumor that arises from the anterior lobe of the gland. Based on tumor size, there are two types of pituitary adenoma; microadenoma (less than 10 mm) and macroadenoma (more than 10 mm). Pituitary adenoma also can be classified according to the origin of the cellular component and secretion of hormones such as prolactin, growth hormone, and adrenocorticotropin-secreting adenoma [1, 2]. Typically, patients with pituitary adenoma are presenting with many symptoms including hyperprolactinemia, sterility, menstrual irregularity, amenorrhea, galactorrhea, and pituitary space-occupying lesions. In addition, severe cases may have more aggressive features such as headaches, vision loss, and cranial nerve palsies. Prolactinoma represents 5/100,000 of adenoma tumors [3, 4]. Although surgical resection, chemotherapy and radiotherapy are the main lines of treatments in pituitary adenoma. Surgical option is feasible inonly 66 to 78% of the cases, and the radiation may affect healthy tissue and cause hypopituitarism and cerebrospinal fluid leakage [5, 6]. Therefore, the researches are evolving to detect new markers and treatment methods for pituitary adenoma. The pathogenetic mechanism ofpituitary adenoma may involve several factors including epigenetics, genes, and tumor microenvironment (TME). The TME is a unique environment consisting of tumor cells, immune system cells, and extracellular matrix that affects tumor growth, proliferation, andangiogenesis [6-8]. Current researches have focused on immune cells, chemokines and cytokines that affecttumour progress and invasiveness [9, 10]. The immune cells in tumor microenvironments recognize transformed cells,bindtheir ligand and activate, recruit, and secrete cytokines that kill and control tumor growth [11], for instance, natural killer (NK) cells are activated in the early immune response against intracellular antigens and tumor cells [10]. These cells fight tumors and have cytotoxic activity against them without prior sensitization or immunization, in addition, they produce different types of

cytokines like INF-γ, perforin and granzyme, resulting in the subsequent activation of the adaptive immune system [11]. NK cells classified according to surface receptors into two essential subsets CD56dimCD16+ comprised 90% of the circulating cells whereas the other type CD56bright CD16- represent only 5% in the circulation [8]**.** Natural killer cells have numerous activator and inhibitor receptors. One of the essential activator receptors is NKG2D. It is expressed on all types of natural killer cells that activated after binding of the activator receptors and ligand such as major histocompatibility complex class I chain-related genes A and B (MICA and MICB) [12]. Moreover, IL-6 is an essential inflammatory cytokine secreted by several myeloid and lymphocytes cells. IL-6 play a role in the tumor microenvironment, and the correlation between IL-6 and pituitary adenoma were investigated by few studies [13-15]. The first study examined the IL-6 on rat pituitary glands tissues and concluded that IL-6 expressed lower in healthy tissue compared to pituitary adenoma tissue [16]. Another study concluded that IL-6 mRNA highly produced in pituitary gland adenoma cell culture [17]. Furthermore, Ma et al. studied the activator receptors NKG2D in prolactinoma and concluded that the level of NKG2D activity receptors correlated negatively with tumor progress [18]. The researchers study the impact of IL-6 in rats and the human pituitary gland tissue but not in the serum [19], hence, the present study was designed to examine the level of cytokine in the circulation. This research focused on the IL-6 cytokine and NKG2D activator receptors on natural killer cells (NK) as an immunological prognostic markers in pituitary adenoma to indicate the possibility that immune system molecules may be helpful in prognostic prediction and targeted clinical therapy.

THE AIM

This study aims to assess the role of circulating IL-6 and NKG2D protein in pituitary adenomaas prognostic parameters by defining their pre and post treatment levels and detect the correlation between them.

MATERIALS AND METHODS

In the current longitudinal study, thirty females with newly diagnosed prolactinoma in Murjan hospital Babil, Iraq, between April 2020 to May 2021, were enrolled. These patients mainly complain of irregular menstrual cycles, amenorrhea, hair loss, weight gain, pregnancy loss, breast discharge, and infertility. The pituitary adenomas were categorized according to the Tumors classification system 2017 [4]. Brain MRI revealed that 23 of the tumors weremicroadenoma (<10 mm) while only 7 cases weremacroadenoma (>10 mm). In addition, the blood tests revealed high levels of prolactin hormone, hyperandrogenemia, and increased serum insulin. In contrast, other hormones are their standard range, and the ultrasound scans indicated that adrenal glands, ovaries, and uterus are free from any mass or cyst. Demographic data were collected and questioners were asked regarding smoking, alcohol, and drug-addiction, marital status, family history of a prolactinoma or hormonal abnormality and family history for cancer. Body mass index (BMI) was calculated according to the criteria [20, 21]. Patients who suffer from any other major illness were excluded from the study. All the enrolled patients were treated with cabergoline 10 mg twice weekly. IL-6 and NKG2D levels were measured at the diagnosis and at six months after treatment.

METHOD OF BLOOD SAMPLES COLLECTION AND PROCESSING

Five millilitre of blood samples were taken from patients using disposable syringes under aseptic technique. Firstly, the blood was obtained through veinous puncture and, after that, the sample was transferred to a sterile plain tube, centrifuged at 2500 rpm for 10 minutes, and the serum samples were divided into several Eppendorf tubes and immediately frozen at –20°C then used for IL-6 and human NKG2D tests. All samples were obtained at 8 am on the second day of the menstrual cycle. The patients fast for 12 hours before the test. ELISA technique was used, In vitro gene by Thermo Fisher Scientific/Frederick/USA, ELISA kits were used to investigate NKG2D protein in serum, and Invitrogen by Thermo Fisher Scientific Bender Medsystems GmbH/ Vienna/Austria used to analyze interleukin-6.

STATISTICAL ANALYSIS

After complete data collecton, statistical analyses were done by using SPSS version 23 (Statistical Package for Social Sciences) and GraphPad prism. Horizontal bars indicate the mean difference among times for IL-6 and NKG2D. A one-way ANOVA will calculate *p*-values. A personal correlation test examined the association between IL6, NKG2D, anatomical tumor type (size), and tumor status (increased, shrinkage, or same size). The study used Kaplan-Meier and log-rank tests to calculate survival rate analysis. In addition, a receiver operating characteristic (ROC) for sensitivity and specificity test was utilized.

ETHICAL APPROVAL

The study has beed granted ethical approval by the research ethics committee of Al-farahidi university/ faculty of medical techniques.

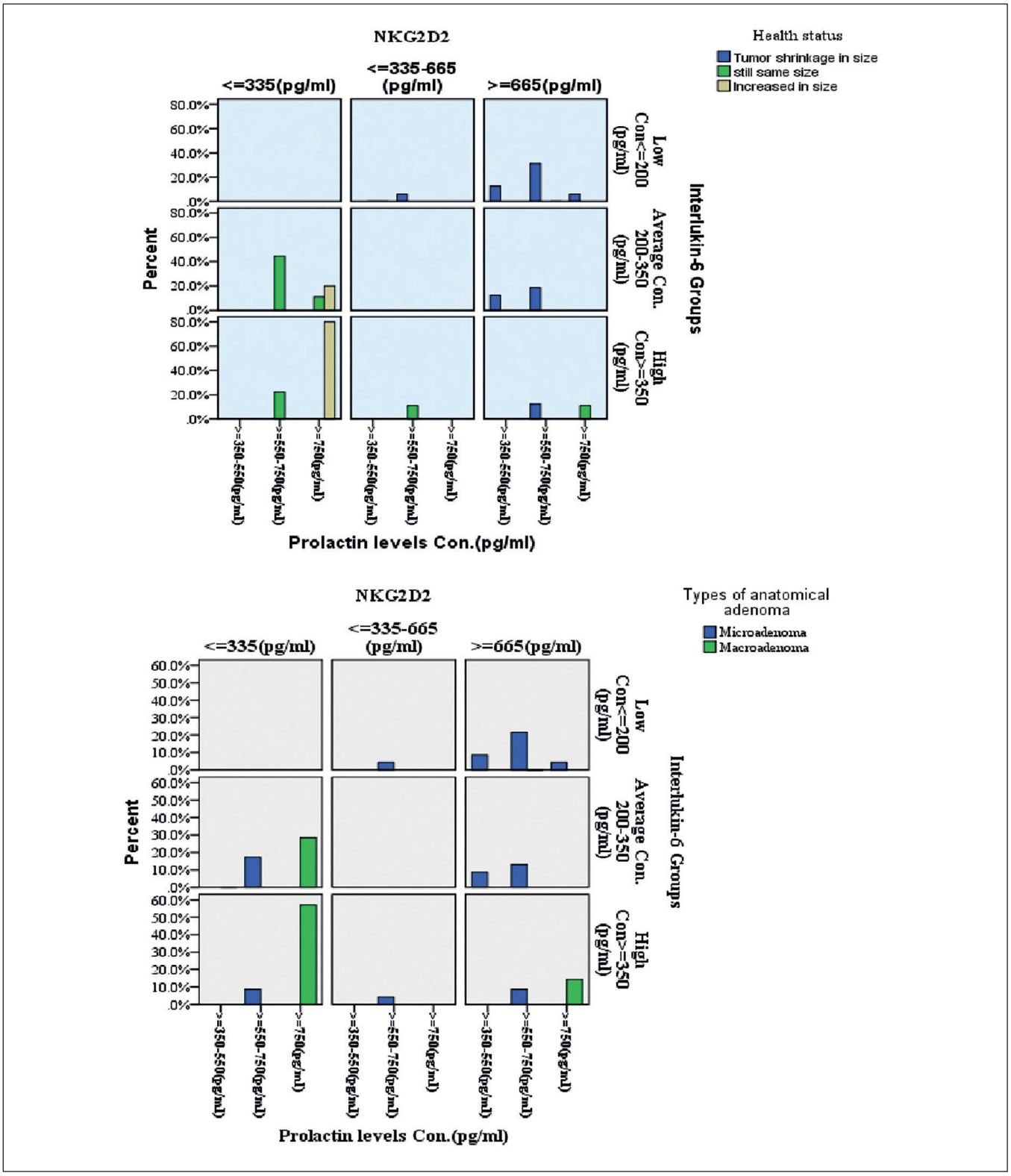

Fig. 1. The correlation between immunological variables among tumor size and types of tumor anatomy.

RESULTS

CLINICAL CHARACTERISTIC OF CASES

The study does not revealed a significant difference between the levels of IL-6 and NKG2D with demographical properties. On the other hand, there are significant differences in mean levels of IL-6 and NKG2D, and anatomical type (tumor size) $p<0.001$. In addition, there is a substantial difference between the two immunological markers (IL-6 and NKG2D) levels $p<0.001$. The level of IL-6 increased while NKG2D were decreased (Table I).

Table I. The mean of two selected (IL-6&NKG2D) outcome measurements by demographic property.

Dunnett's multiple comparisons tests	Mean 1	Mean 2	Mean Diff.	95.00% CI of diff.	Adjusted p-value
IL-6 vs. Family history for tumors	1.967	1.567	0.4000	-58.55 to 59.35	>0.9999
IL-6 vs. Family history for hormonal abnormality	1.967	1.414	0.5529	-58.90 to 60.01	>0.9999
IL-6 vs. Smoking	1.967	1.655	0.3115	-59.15 to 59.77	>0.9999
IL-6 vs. Anatomical types	1.967	375.69	-373.72	-450.18 to -218.73	<0.001
IL-6 vs. Tumor status a	1.967	420.7	-418.7	-466.0 to -333.1	<0.001
IL-6 vs. Age	1.967	29.20	-27.23	-86.18 to 31.72	0.8198
IL-6 vs. BMI	1.967	32.90	-30.93	-89.88 to 28.02	0.6921
IL-6 vs. Marital	1.967	1.367	0.6000	-58.35 to 59.55	>0.9999
IL-6 vs. PRL	1.967	710.6	-708.6	-767.6 to -649.6	<0.0001
NKG2D vs. PRL	1.767	710.6	-708.8	-758.3 to -659.3	<0.0001
NKG2D vs. IL-6	1.767	306.7	-305.0	-354.5 to -255.4	<0.0001
NKG2D vs. Family history for tumors	1.767	1.567	0.2000	-49.32 to 49.72	>0.9999
NKG2D vs. Family history for hormonal abnormality	1.767	1.414	0.3529	-49.60 to 50.30	>0.9999
NKG2D vs. Smoking	1.767	1.655	0.1115	-49.84 to 50.06	>0.9999
NKG2D vs. Anatomical	1.767	375.69	-373.920	-441.87 to -212.03	<0.001
NKG2D vs. Tumor size	1.767	420.7	-418.9	-481.1 to -347.6	<0.001

Table II. The correlation between an immunological variable with anatomical (tumor size) and status.

Variables	Anatomical type	IL-6	Tumor status
IL-6	r=0.321, p=0.08	r=1	r=0.752, p<0.001
NKG2D	r=-0.570, p<0.001	r=-0.716, p<0.001	r=-0.886, p<0.001

p-value: significant <0.05; r: personal correlation; PRL prolactin hormone

Table III. Changing in immunological marker after six months post-treatment.

Variables	First measurement (pre treatment period)	Second measurement (post six months of therapy)	Changes after six months	95% confidence interval
		IL-6(pg/ml)		
Mean	335.8	138.0	-197.8	-239.0 to-156.5
		range =0.6137 *p*-value=<0.0001		
		NKG2D(pg/ml)		
Mean	365.7	632.7	267.0	267.0 ± 42.00
		range= 0.4107 *p*-value=<0.0001		

N: number of samples (30), p: p-value (<0.05 significant) Con: concentration; pg/ml: picograms per militliler

Table IV. The area under curve (AUC) for the following study variables.

Test result variable(s)	Area	Std. Error	*P*-value[a]	Asymptotic 95% Confidence Interval	
				Lower Bound	Upper Bound
NKG2D, IL-6 levels by Tumor Status					
NKG2A Con.(pg/ml)	.824	.075	.024	.678	.970
Interlukin-6 Con. (pg/ml)	.716	.089	.033	.542	.890
NKG2D, IL-6 levels by Tumor size(anatomical type)					
NKG2A Con.(pg/ml)	.745	.092	.053	.565	.926
Interlukin-6 Con. (pg/ml)	.792	.081	.021	.634	.950
IL-6 level by NKG2D2					
NKG2D2	.556	.136	.697	.289	.823

a. Null hypothesis: true area = 0.5; Con: concentration; pg/ml: picograms per milliliter

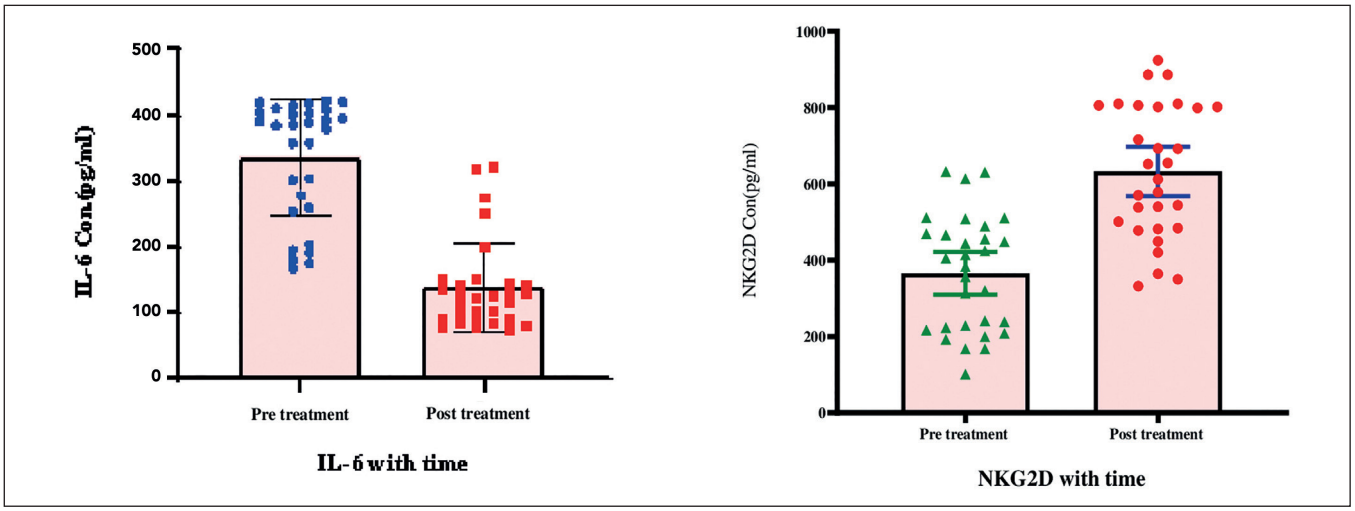

Fig. 2. A Dot diagram with its 95% confidence interval with time (baseline, after 6 months of follow up post-treatment).

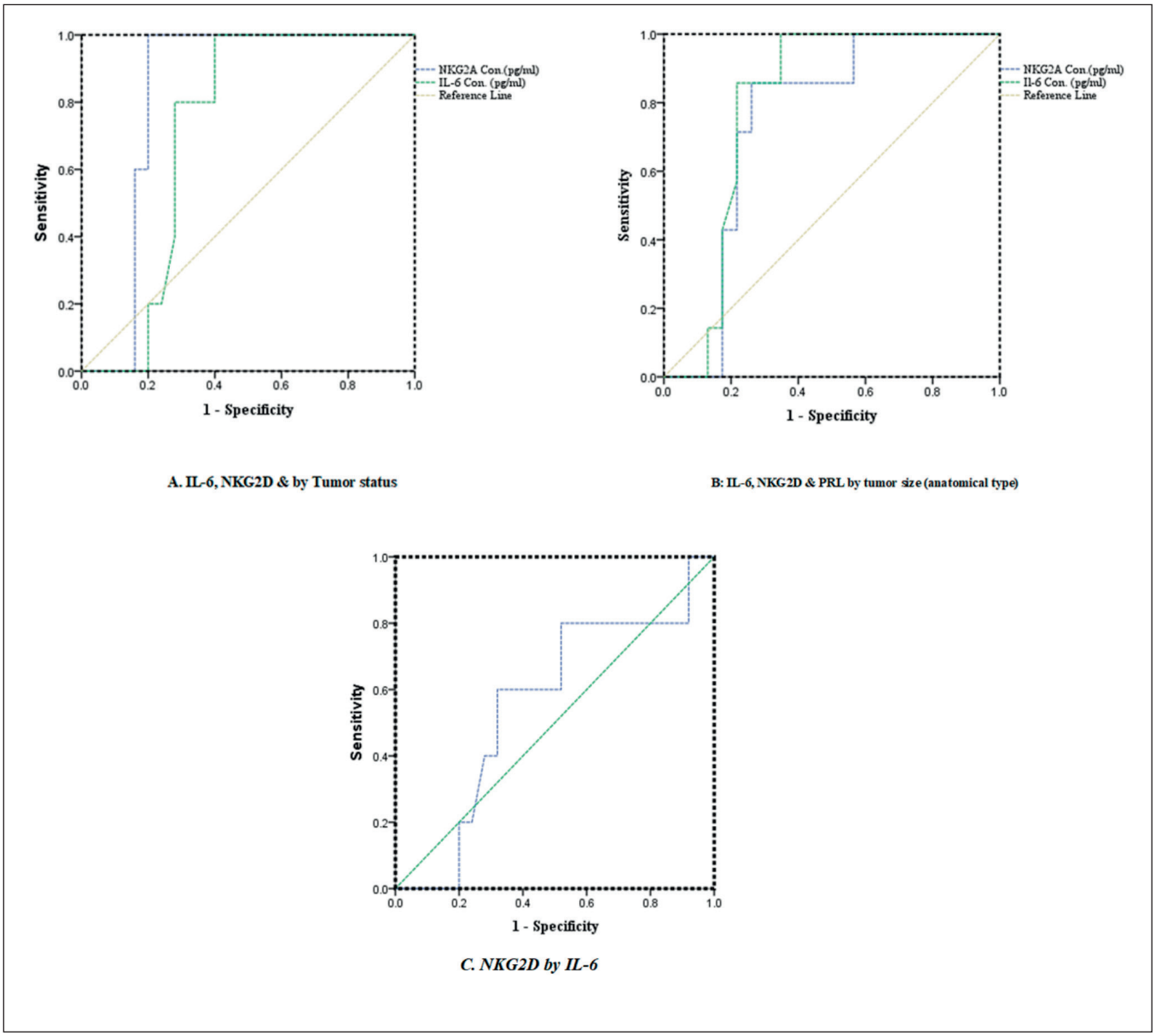

Fig. 3. A ROC Curve showing the trade-off between Sensitivity (rate of true positive test results) and 1-Specificity (rate of false-positive test results): A. IL-6 and NKG2D by tumor status; B. IL-6,, NKG2D & PRL by tumore size (anatomical type); C. NKG2D by IL- 6.

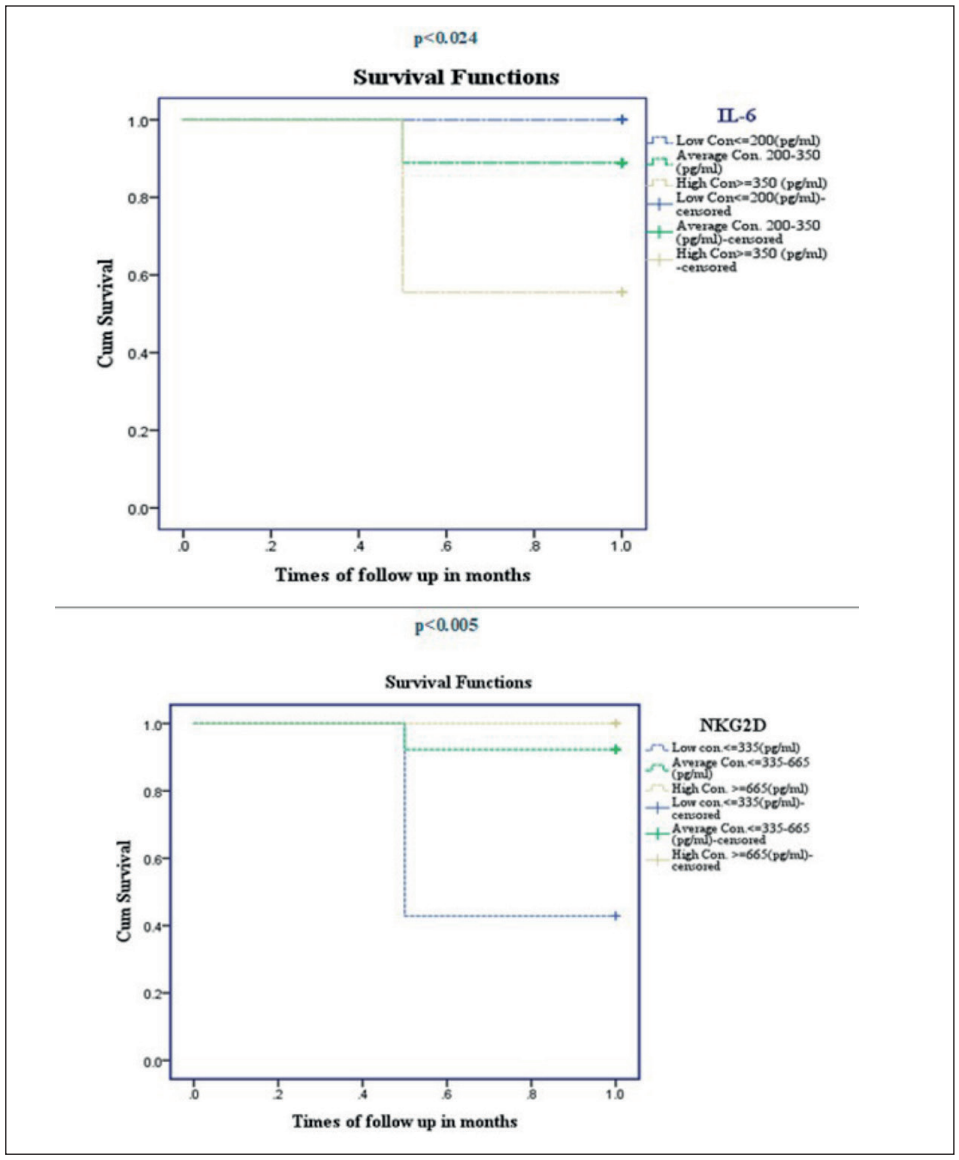

Fig. 4. A Survival Curve comparing the cumulative survival rates during 6 months of follow-up by IL-6 & NKG2D categories at baseline.

The correlation between IL-6 and NKG2D with anatomical type and status of tumor

To investigate the correlation between immunological markers, tumor size and the level of prolactin, the study used personal correlation. There is a moderate positive correlation between the level of IL-6 and anatomical type (r=0.321; *p*=0.08). The study finds strong negative correlation between NKG2D and anatomical classification (r=0.570; *p*=0.001). There are positive very strong correlate between IL-6, tumor status (r=0.752 and 0.834, *p*=0.001) with tumer size and negative very strong correlate between NKG2D with IL-6, anatomical type and tumor size (r= -0.761,-0.570 and 0.886 *p*=0.001) respectively (Table II and Fig. 1).

THE DIFFERENCE IN MEASUREMENT OF IL-6 AND NKG2D AT PRE- AND POST-TREATMENT FOR SIX MONTHS

The study measured the levels of the immunological variables (IL-6 and NKG2D) twice, pre- and six months after treatment points. IL-6 marker mean levels significantly decreased on follow up (-197.8; *p*-value≤0.0001), while the contrary occur in NKG2D, which increased in levels after treatment compared with baseline readings (Table III and Fig. 2).

THE VALIDITY OF THE TEST FOR IL-6 AND NKG2D

The two quantitative measurements were tested for their ability to predict a prolactinoma (micro or macro) and tumor status (shrinkage, same, increased in size). A low-level NKG2D expects a very good test (AUC area ≥0.8), while the high level of IL-6 appears a good test (AUC>0.7) to predict both tumor size and tumor status (Table IV and Fig. 3).

The study assessed the optimum cut-off value associated with the best performance and ability to differentiate between small and large size tumors and predict the tumor

progression after treatment. Levels associated with high possibility of giant size tumor and poor prognosis was ≤488.50 pg/ml for NKG2D and ≥394.0 pg/ml for IL-6. At these thresholds, NKG2D reports a sensitivity of 100% for the tumor status,and 70% for the tumor size or anatomical type and specificity of 80% for the tumor status, and 78% for anatomical or tumor size. On the other hands, the IL-6carries an 80% and 85% sensitivity for the tumor statusand tumor size respectively. IL6 specificity is 72% for the tumor status and 78% for tumor size. Testing positive at this optimum cut-off value (obtaining an NKG2D concentration ≤488.50 pg/ml and ≥394.0 pg/ml for IL-6) would establish a possible diagnosis of large tumors and poor prognosis prolactinoma. In IL-6, when used as a predictive marker for NKG2D, as shown in Tables IV and V, the optimum cut-off value for NKG2D (associated with the best performance and ability to differentiate between low levels of NKG2D cases those with high concentration) was ≤390.00 pg/ml. At this cut-off value, the test is 60% sensitivity and 60% specificity for high NKG2D concentration (AUC=0.556).

IL-6 AND NKG2D AND RESPONSE RATE AFTER TREATMENT

The high expression of IL-6 strongly correlates with a risk of macroadenoma(>10 mm) and poor response to treatment and vice versa (p<0.024). Moreover, the high expression of NKG2D significantly correlates (p<0.005) with good prognosis and increased chance for tumor response and shrinkage compared with low concentration (Fig. 4).

DISCUSSION

The study investigates the role of two immunological markers (IL-6 & NKG2D) as part of tumor microenvironment in the prognosis of pituitary gland adenoma and treatment response, which help to predict patients with a high likelihood of improvement without multiple relapses. The laboratory results show that the IL-6 level is significantly elevated in large size tumors compared to small ones, p=0.001. In addition they revealed a positive correlation between them. The result can predict that tumours in patients with high IL-6 exhibit a high resistance to treatment and increased in size after six months of follow-up. IL-6 is a proinflammatory cytokine that plays an essential role in circulation and tumor microenvironment. The result corresponds with the study of Velkeniers et al. which found that the expression of IL-6 in normal tissue of the pituitary gland is low compared with prolactinoma tissue [22]. Additioally, the results revealed a rise in prolactin hormones in conjunction with IL-6 (p<0.0001), therefore, IL-6 may induce the growth of pituitary adenomas and stimulate prolactin hormone production [23]. The study reports that the concentration of NKG2D receptor decreased in macroadenoma tumors (p=0.001). This finding concurs with Ma et al. who concluded that the NKG2D receptors down regulates on NK cells in prolactinoma patients [18]. NKG2D activating receptors in NK cell binds with MHC- I, releasing perforin granzyme and INF-γ and other cytokines that control tumor growth [24]. The reduction in NKG2D activity receptors may indicate a decrease in NK activity, leading to tumor invasion and progression [25], moreover, significant decreasein NKG2D biomarker is accompanied by a rise in the prolactin hormone (p<0.0001) that may be explained the inhibition of immune cells like NK & CD8T-cell, or downregulation of NKG2D ligand expressed by tumor [26-27]. This result agrees with Strobel et al. [27] who conclude that high level of prolactin inhibites the activity of NK in patients with recurrent pregnancy loss. The study demonstrates that IL-6 is significantly elevated with a decline in NKG2D (p<0.0001). The result showed a strong negative correlate between NKG2D and IL-6, anatomical type, and tumor size (r = -0.761,-0.570 & 0.886 p=0.001), respectively, this result agrees with the study of Xu et al. who found that IL-6 affects the toxicity of NK cells by downregulation of NKG2D receptors in gastration resistant prostate cancer [28], in addition, the results correspond with Xu et al., who found that IL-6-expressing tumor cells are more resistant to activators NK cells than IL-6-knockdown tumor cells. Moreover, the JAK-Stat3 is the most critical IL-6 downstream signalling that modulates PD-L1/NKG2D ligand levels in CRPC cells [29]. The ROC curve analysis for IL-6 revealed that serum IL-6 levels is an excellent test to predict patients with macroadenoma and tumor size progression status after six months of follow up, the results consistent with the study of Xu, et al., who found that the level of IL-6 has 72% sensitivity, 74% specificity, and (AUC=0.79) in colorectal cancer [30]. Similarly, the NKG2D test manifest a good beneficial effect in the prediction of both tumor size and tumor status, this result agrees with Zhao et al. and Huergo-Zapico et al. [31, 32]. Additionally, IL-6 consider a satisfactory test when cut-off value ≤ 390.00pg/ml andcorrelates with high NKG2D concentration (AUC=0.556) which concert with the result of Kjærgaard, et al., which found that NK cell expression of NKG2D lower with the high level of IL-6 in patients with septic compared with the non-septic patients [33]. This study uniquely examined the difference in IL-6 and NKG2D pre and post-treatment after follow-up period for six months. The results show a significantly decreased level of IL-6 while an increase in mean levels of NKG2D in patients with microadenoma tumor and good response for treatment (p<0.0001). The high expression of NKG2D receptors assist to anticipate those who will respnd with reduction in tumor size after six months of treatment (p<0.001), reflecting the excellent survival rate. The result agrees with the study of Ma et al. and Huergo-Zapico L, et al. [18, 32], however, regarding IL-6, the survival and response rate increase with the lower level of IL-6 (p<0.0001) the results corresponding withVelkeniers et al and Xu et al findings [17, 28].

CONCLUSION

The higher the level of IL-6, the larger the size of adenoma (macroadenoma) and the poorer the response to treatment. The higher the level of NKG2D indidcate a better prognosis, therefore, IL-6 and NKG2D correlate negatively in prolactinoma patients.

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ORIGINAL ARTICLE

FEATURES OF THE DAILY PROFILE OF ARTERIAL BLOOD PRESSURE IN PATIENTS WITH RHEUMATOID ARTHRITIS IN COMBINATION WITH ARTERIAL HYPERTENSION

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ABSTRACT

The aim: To determine the features of daily shifts in blood pressure (BP), the influence of the presence of rheumatoid arthritis (RA) on BP control and identify factors that affect BP among patients with RA in combination with resistant hypertension (RH).

Materials and methods: All material for writing this scientific work were the results of a comprehensive survey of 201 people with RH and RA, hypertension (H) and RA, RA without H, H without RA and relatively healthy individuals. A laboratory study was performed: rheumatoid factor, C-reactive protein (CRP), K+ serum, and creatinine levels. All patients underwent office BP measurement and ambulatory BP monitoring for 24 hours. Statistical processing of the study results was carried out using "IBM SPSS Statistics 22".

Results: Among patients with RA in combination with RH non-dippers (38.7%) are the most common type of BP profile. Patients with RH in combination with RA are characterized by an increase in BP more at night ($p < 0.003$), which corresponds to the high frequency of night-peackers (17.7%). The presence of RA determines worse control of diastolic BP ($p < 0.01$) and more vascular overload on organs and systems during the night ($p < 0.05$).

Conclusions: An increase in BP in patients with RA in combination with RH is more significant at night, characterized by poorer BP control and greater vascular load at night indicating the need for tighter control of BP during sleep. Non-dippers are most often detected among patients with RA in combination with RH, which is prognostically unfavorable for the development of nocturnal "vascular accidents".

KEY WORDS: rheumatoid arthritis, resistant hypertension, ambulatory blood pressure monitoring

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INTRODUCTION

Various epidemiological studies have been conducted, but the reason for the increase in the prevalence of hypertension (H) in patients with rheumatoid arthritis (RA) is still unclear. A direct link between the prevalence of H and inflammation was not detected, but the incidence of H in patients with RA ranges from 52-73% [1-3]. It cannot be ruled out that systemic inflammation plays an important role in the development of H in RA, as the level of C-reactive protein (CRP) in RA varies and this marker may not cover long-term cumulative inflammatory load. The studied populations were well targeted by traditional disease-modifying and immunobiological drugs on endothelial function, arterial stiffness and blood pressure (BP) [4-6]. In addition, certain biological drugs have a beneficial effect on endothelial function and arterial stiffness [5] and, thus, can prevent an increase in the incidence of H. Given the contribution of H in increased risk of premature death from cardiovascular disease (CVD) and insufficient assessment of the impact of these factors in patients with RA are particularly relevant predictors of this comorbidity [7]. The problem of resistant hypertension (RH) attracts special attention, because the development of RH is significantly faster with long-term use of drugs such as glucocorticosteroids (GCs) and nonsteroidal anti-inflammatory drugs (NSAIDs) [8].

THE AIM

The aim of this study is to determine the features of daily shifts in BP, the influence of the presence of RA on BP control and identify factors that affect BP among patients with RA in combination with RH.

MATERIALS AND METHODS

The study was conducted on the basis of the rheumatological and therapeutic departments of the Kyiv City Clinical Hospital No.3, which were the clinical base of the Department of Propedeutics of Internal Medicine No.2 of Bogomolets National Medical University. The

study included a retrospective cohort study, that was based on the medical history data of 560 patients with rheumatoid arthritis or arterial hypertension, who underwent observation in the clinic during 2017-2020. All patients were residents of Kyiv and Kyiv region.

Inclusion criteria for groups 1-4 were following: age 45-59 and 60-74 years, patients with hypertension of the II stage, 1 and 2 degrees and did not receive antihypertensive therapy or received monotherapy with ACE inhibitors or ARBs (except group 3), RA (except group 4), CKD not higher than II stage (GFR 60-89 ml/ min./.73 m^2), LVEF more than 40%, K+ blood serum from 3.0 to 5.0 mmol/l, informed consent to participate in the study. Exclusion criteria were next: 3rd stage hypertension, CKD III-V stages, acute kidney damage in anamnesis, endocrine pathology (diabetes mellitus, Addison's disease, etc.), clinical signs of hypovolemia, office: systolic blood pressure < 115 mm Hg or diastolic < 55 mm Hg, atrial fibrillation and flutter, A-V block II-III on ECG, CHF III-IV according to NYHA, decreased left ventricular ejection fraction (<40%) or valvular heart disease, acute myocardial infarction or other cardiovascular events (Q-myocardial infarction, non-Q-myocardial infarction, unstable angina, myocardial revascularization, stroke, TIA) in the anamnesis, alcohol abuse, drug addiction or mental disorders, infectious diseases, active chronic diarrhea, oncological and hematological diseases, active phases of diseases of the gastrointestinal tract and liver, gout, K+ blood serum > 5.0 mmol/l, Na+ blood serum < 130 mmol/l, current therapy with spironolactone or another mineralocorticoid receptor blocker, use of thiazide or loop diuretics within 6 weeks before the start of the study, inability to provide informed consent for participation in research. Group 5 (the control group) consisted of healthy volunteers of the appropriate age and sex without a previous history of arthritis of any genesis or a stable increase in BP.

201 people were examined in total: 1 group - patients with RH and RA (n = 62), 2 group - patients with H and RA (n = 39), 3 group - patients with RA without H (n = 41), group 4 - patients with H without RA (n = 37), group 5 - healthy individuals (n = 22). All patients with H received the same type of combined antihypertensive therapy ((ARB or ACE inhibitors) + diuretic (hydrochlorothiazide 25 mg or indapamide 1.25 mg) + CCB (amlodipine 10 mg)) and rosuvastatin 20 mg, RA patients - basic therapy with methotrexate 15 mg/week.

Criteria for inclusion in the study: age from 45 to 74 years (middle and old age, according to the WHO classification, 1968); reliable diagnosis of RA and basic therapy according to ACR / EULAR criteria (2010, 2015), glomerular filtration rate (GFR) more than 60 ml/ min/1.73^2, K^+ serum level from 3.0 to 5.0 mmol/l.

A survey of patients with RA and H was conducted, which took into account the duration of both RA and H, specifying the basic therapy in both cases, the duration of GCs use. A laboratory study was performed to determine the content of creatinine, K^+, rheumatoid factor, and CRP levels; GFR was calculated using the formula CKD-EPI, RA activity was measured according to DAS28.

All patients underwent office BP measurement and ambulatory BP monitoring (ABPM) for 24 hours. Office BP was measured according to European Society of Hypertension criteria. ABPM measurements were performed on a non-dominant arm using an automatic ABPM50 monitor, Hubei Province, China. The following parameters were analyzed: average values of systolic BP, diastolic BP per day in the active (day) and passive (night) periods (average day and night (dn), average day (d), average night (n) values for SBP, DBP). The circadian rhythm of BP was calculated according to the night–day ratio (NDR) of SBP and DBP. After assessing NDR of BP, the following groups of patients and types of daily charts of BP curves were identified: Dippers - people with normal nocturnal BP (NDR = 10-20%); Non-dippers - people with insufficient registered BP decrease at night (NDR <10%); Over-dippers - people with considerable decrease of BP at night (NDR > 20%); Night-peackers - people without a recorded increase in BP at night (NDR is negative).

All groups of patients are comparable in age, sex, levels of the glucose and smoker status; groups of patients with RA are comparable in RA variant, duration of RA, RA activity by CRP level, and DAS28-CRP scale, which corresponds to high disease activity in both cases, the need for the use of NSAIDs and GCs; groups of patients with H are comparable in the duration of H (see Table I for details).

The null hypothesis was formed: the dynamics of the daily profile in patients with RA in combination with RH does not differ from the daily changes in BP in people with RH and RA, H and RA, RA without H, H without RA and relatively healthy individuals.

Statistical processing of the obtained results was performed using "IBM SPSS Statistics. Version 22". Under the condition of normal distribution of the studied trait, parametric statistical methods were used in the sample: for descriptive statistics the mean value of the indicator (M), standard deviation (σ), standard error (SE), 95% confidence interval for the mean (95% CI) were determined. The median values (Me), 25 and 75 quartiles (Q25 - Q75) as well as a percentage (%) were used. Comparison of groups on qualitative binary data was performed using Pearson's $\chi 2$-test (corrected by Yates), Fisher's exact test. Odds ratio (OR) and the Pearson correlation coefficient were used for the measurement of relationship between variables.

Table I. General clinical characteristics of the examined groups of patients

	Group 1 (n=62)	Group 2 (n=39)	Group 3 (n=41)	Group 4 (n=37)	Group 5 (n=22)
Age, years, M ± σ	62.9 ± 9.0	61.9 ± 7.0	59.1±8.5	60.6±9.6	50.1±4.94
Women, abs. (%)	52 (83.9)	30 (76.9)	37 (90.2)	31 (83.8)	16 (72.7)
Seropositive RA,n (%)	51 (82.3)	29 (74.4)	31 (75.6)	-	-
CRP, mg / l, Me (25% -75%)	6.9 (2.9-17.0)	6.7 (3.8-23.9)	6.0 (2.6-24.1)	-	-
Rheumatoid factor, U / l, Me (25% -75%)	51.0 (13.1-126.6)ǂ	48.2 (10.4-130.4)	24.1 (11.6-75.4)	-	-
DAS28-CRP, M ± σ	5.4 ± 1.0	5.6 ± 1.0	5.3±1.1	-	-
Duration of RA, years, M ± σ	9.2 ± 8.0	8.7 ± 7.1	8.6±9.2	-	-
Duration of H, years, M ± σ	10.8 ± 7.2	7.0 ± 3.8	-	9.2±6.1	-
Took NSAIDs, abs. (%)	50 (80.6)	33 (84.6)	31 (75.6)	-	-
Took GCs, abs. (%)	21 (33.9)	11 (28.2)	15 (36.6)	-	-
Smoking, abs. (%)	7 (11.3)	5 (12.8)	7 (17.1)	5 (12.2)	3 (13.6)
Body mass index, kg/m2, Me (25%-75%)	29.9 (27.7-31.6)ǂ†	28.6 (24.9-33.7)	23.4 (21.3-26.3)	32.4 (28.9-36.1)	23.8 (21.4-26.4)
Glucose, mmol/l, Me (25%-75%)	4.7 (4.2-5.3)	4.7 (4.4-5.2)	4.7 (4.3-5.0)	4.8 (4.3-5.2)	4.7 (4.3-4.9)

Notes: ǂ - significance of differences with group 4 (p <0.05); † - significance of differences with group 5 (p <0.05).

Table II. Distribution of patients of experimental groups by circadian profile, n (%)

	Group 1 (n=62)	Group 2 (n=39)	Group 3 (n=41)	Group 4 (n=37)	Group 5 (n=22)
Dippers, abs., (%)	22 (35.5)*#ǂ †	18 (46.2) †	20 (48.8) †	18 (48.6) †	22 (100.0)
Non-dippers, abs., (%)	24 (38.7) †	16 (41.0) †	13 (31.7) ǂ †	12 (32.4) †	0
Over-dippers, abs., (%)	5 (8.1)* #ǂ†	1 (2.6) #ǂ†	6 (14.6) ǂ †	6 (16.2) †	0
Night-peackers, abs., (%)	11 (17.7)*#ǂ †	4 (10.3) #ǂ†	2 (4.9) ǂ †	1 (2.7) †	0

Notes: * - p <0.05 compared to group 2, # - p <0.05 compared to group 3, ǂ - p <0.05 compared to group 4, † - p <0.05 compared to group 1 compared to group 1 group 5.

RESULTS

The incidence of H in our study is 71.1% (n = 142) among patients with RA. RH is diagnosed in 61.4% (n = 62) cases of patients with RA in combination with H, which is 6 times higher than in the general population [9]. Unfortunately, there is no data in the literature on the frequency of RH in patients with RA, so this study is quite relevant. Office SBP is higher in patients of group 1 (141.1±8.0 mm Hg) than in patients of groups 2, 3, 4 and 5 by 10.1 mm Hg, 29.3 mm Hg, 9.5 mm Hg and 32.0 mm Hg, respectively (all p <0.01), office DBP (83.7±5.5 mm Hg), in turn, by 12.2 mm Hg and 16.6 mm Hg higher in patients of groups 1 than in patients of groups 3 and 5 (p <0.001), office PBP (57.3±7.8 mm Hg) is higher by 6.7 mm Hg, and 16.9 mm Hg, by 6.1 mm Hg and 15.3 mm Hg in patients of group 1 compared with group 2, 3, 4 and 5, respectively (all p <0.05).

After the analysis of the circadian profile of BP (NDR) in group 1, non-dippers are most often determined, and the prevalence of them does not differ from groups 2, 3 and 4 (p>0.05), on second place in frequency - dippers, which in 1.3 times less often than in groups 2 ($\chi^2 = 4.3$, p <0.05), and 1.4 times less than in groups 3 and 4 ($\chi^2 = 4.7$, p <0.05). Among patients of group 1 the highest frequency of the most unfavorable circadian profile (night-peackers) is found, which is 1.7 times higher than in group 2 ($\chi^2 = 3.9$, p <0.05), 2.4 times higher than in group 3 ($\chi^2 = 4.2$, p <0.05) and 6.6 times than in group 4 ($\chi^2 = 5.1$, p <0.05). The high frequency of non-dippers detection in patients with RA and RH may indicate an increase in cardiovascular risk of this category of patients [10]. It should be noted that the increase in CRP levels and high RA activity by DAS28-CRP are closely associated with the development of night-peackers (OR = 1.32, 95% CI 1.02-2.98, p = 0.005 and OR = 1.38, 95% CI 1.02-3.03, p = 0.004). In the case of NSAIDs and GCs usage, it increases the chances of non-dippers and night-peackers (OR = 1.48, 95% CI 1.06-3.24, p = 0.003 and OR = 1.45, 95% CI 1.04-2.99, p = 0.03). The share of over-dippers` detection in group 1 is 3.1 times higher than in group 2 ($\chi^2 = 5.2$, p <0.05), 2.3 times lower than in group 3 ($\chi^2 = 3.8$, p <0.05), and 2 times than in group 4 ($\chi^2 = 4.4$, p <0.05), in group 5 - 100.0% detection of dippers (see Table II).

We found that patients of group 1 compared with group 2 have higher SBPdn (141.5 (135.7-146.0) mm Hg) by 9.2 mm Hg (p = 0.003), DBPdn (83.4 (78.1-86.6) mm Hg) by 5.4 mm Hg (p = 0.04); SBPd (139.1 (131.9-147.0) mm Hg) by 16.1 mm Hg (p = 0.002); DBPd (84.4 (72.2-86.6) mm Hg) by 9.7 mm Hg (p = 0.04) after analyzing the parame-

ters of ABPM. Compared with group 3, patients in group 1 have higher SBPdn by 28.0 mm Hg (p <0.001), DBPdn by 16.7 mm Hg (p <0.001); SBPd by 23.3 mm Hg (p <0.001), DBPd by 15.2 mm Hg (p <0.001), SBPn by 41.4 mm Hg (p <0.001), DBPn by 22.0 mm Hg (p <0.001). SBP in our patients with RA in combination with H correspond to other studies [11]. When comparing groups 1 and 4, we found that in group 1 SBPdn higher by 10.8 mm Hg (p = 0.002), DBPdn by 7.5 mm Hg (p = 0.008), SBPn by 20.6 mm Hg (p>0.001), DBPn by 6.7 mm Hg (p = 0.009) than in group 4. Summarizing the data obtained, it should be noted that patients with RA and RH have a higher level of SBP and DBP than patients with RA and H and patients with H. These changes are due to higher BP at night, which together with the higher frequency of night-peackers characterizes this category of patients as those at higher risk of severe cardiovascular events, which confirms the importance of monitoring and proper BP managementat night [12-14].

The results of the correlation analysis turned out to be interesting. In group 1 female have higher DBPdn (r = 0.29, p <0.05), with increasing with age. The values of DBPn (r = 0.33, p <0.01), PBPdn (r = 0.31, p <0.05), PBPd (r = 0.31, p <0.05), and PBPn (r = 0.32, p <0.05) are elevated. With a longer duration of RA, a higher number of night-peackers is registered (r = 0.30, p <0.05); patients on NSAIDs use have higher rates of office SBP (r = 0.27, p <0.05) and DBP (r = 0.33, p <0.01). With increasing levels of IL-6, are detected higher values of SBPdn (r = 0.27, p <0.05), DBPn (r = 0.41, p <0.01), PBPn (r = 0.31, p <0,05) and lower levels of SBP and DBP NDR (respectively r = -0.35 and r = -0.32, p <0.05).

DISCUSSION

After comparing our data with the results of a cross-sectional analysis of ABPM, obtained from 26,170 patients, we determined that in patients of group 1 the frequency of dippers is 1.3 times lower than in the general population, with the quantity of non-dippers + night-peackers 56.4% versus 51.3% of group 2, 36.6% of group 3 (p = 0.008) and 35.1% of group 4 (p = 0.006), which shows a significantly worse prognosis of cardiovascular catastrophes in patients with both RA and RH, and RA and H compared with patients with RA and H separately and with the general population [15]. It may also be associated with endothelial and immunological changes leading to premature atherosclerosis, as the presence of macrophages and lymphocytes. Tumor necrosis factor alpha, interleukin (IL) -6, -1 and other factors such as T- and B- cells synthesize in the atheroma [16, 17]. In addition, changes in hemodynamics may also cause higher morbidity and mortality from CVD in these patients (episodes of coronary ischemia during sleep, increased risk of arrhythmia, orthostatic hypotension at night, cerebral ischemia with hypoactive delirium and increased risk of stroke, especially lacunar) [18].

NDR of BP is a physiological response of the body, but a significant increase in BP is the risk of developing hypertensive disorders of target organs and cardiovascular events [19]. Morning increase in SBP is one of the manifestations of changes in the autonomic nervous system, when SBP is increased to a certain value due to sympathetic activity is physiological. At the same time as the activity of the sympathetic nervous system elevates, the levels of blood cortisol and procoagulative activity what leads to the activation of the renin-angiotensin-aldosterone system. Activation of all these factors and a significant increase in SBP (more than 34 mm Hg compared to SBP during sleep) are the cause of increased risk of stroke, myocardial infarction, arrhythmias and sudden cardiac arrest during this period. In our study, SBP NDR in patients of group 1 is lower 1.7 times than in group 2 (p = 0.006), 2.1 times than in group 3 (p = 0.005) and 1.5 times than in group 4 (p = 0.008); DBP NDR in group 1 is 1.4 times lower than in group 3 (p = 0.001). Our data correspond to the analysis of 29 patients with RA and H [18].

Patients with RA are characterized by significant frequency, very low awareness and poorer control of H [20] that leads to the development of adverse cardiovascular events. According to Bartels et al., despite doctors' awareness of the high cardiovascular risk in patients with RA, 22% of the 2,677 outpatient visits to the clinic did not include BP measurements, and 47% were not accompanied by BP management, only 31% cases were accompanied by advice on BP control, of which less than 10% of visits were properly documented and provided the right recommendations [21]. According to other data, by daily monitoring of BP in patients with RA, "masked" H is detected with a frequency of 10-30%, both due to lack of adequate BP control and by increasing BP at night [22]. In addition, the frequency and features of RH in patients with RA have not been studied in depth, which is an important problem for management in these patients.

It was found that 79% and 66% had H (in automatic BP measurement) after analyzing the data of 62 patients with RA according to the criteria of ACC / AHA and ESC / ESH, respectively, according to Bartolini and colleagues [20]. Panoulas et al. studied 400 patients (mean age 63 years) with RA and the prevalence of H in that cohort was 70.5% (282 people). Among those, 60% knew about H and received treatment, 40% were not diagnosed, and only 22% had optimally controlled BP. Multivariate regression analysis showed that the frequency of H was positively correlated with the age of patients (1.054, p = 0.001), body mass index (1.06, p = 0.038) and the use of GCs (2.39, p = 0.045) [1]. At the same time, H can increase morbidity and

mortality from cardiovascular diseases (CVD) in patients with RA who develop H decades earlier than in the general population.

Not only H does increase the overall risk, but also the above-mentioned deterioration of endothelial function and premature atherosclerosis (high levels of CRP and IL-6 are positively correlated with CVD in RA) and other traditional (hyperlipoproteinemia, smoking, diabetes) and non-traditional risk factors (abnormal revascularization function of endothelial progenitor cells in damaged peripheral vessels, gene polymorphism, elevated lipoprotein A, decrease in the level of high-density lipoproteins, insufficient compliance of the arterial wall compared with healthy subjects) [23]. We should not forget about the relationship between RA and H due to common mediators of inflammation, changes in lipoprotein spectrum, immune response, but the features of cardiovascular comorbidity in RA remain unknown [18, 24].

Unfortunately, studies of RH in patients with autoimmune diseases have not attracted the attention of a large number of researchers. The first publications on the relationship between CRP levels and cardiovascular risk in patients with RH appeared in 2016, when data were published that patients with RH have above average CRP levels (3.8 mg / l), there is a twice as high risk of cardiovascular events and a higher risk of cardiovascular death among them [25]. New evidence appeared later that proinflammatory agents such as IL-6, IL-1β, IL-10, and tumor necrosis factor-α may play a significant role in the development of RH by not only increasing the BP but also elevating arterial stiffness, the development of endothelial dysfunction, the occurrence of oxidative stress and target organs damage [26, 27]. It should be noted that such a picture of slightly elevated BP is confirmed in another Czech study and correlated with the age of patients [8]. The high value of BP may correspond to the increase in the thickness of the intima-media complex and the myocardial mass of the left ventricle and characterizes both direct load on blood vessels and mechanical pressure on atherosclerotic plaques [28]. Moreover, important regulatory factors of BP are the sympathetic nervous and renin-angiotensin-aldosterone systems [27].

CONCLUSIONS

Among patients with RA in combination with RH the most common type of the violation of the daily profile of BP is non-dippers (38.7%). These patients are characterized by an increase in BP more at night ($p < 0.003$), that corresponds to an increase in the proportion of night-peackers (17.7%). The presence of RA determines worse control of diastolic BP ($p < 0.01$) and more vascular overload on organs and systems at night ($p < 0.05$). In patients with RA and RH worse BP control is determined by female gender, old age, longer duration and higher RA activity, NSAIDs intake ($p < 0.05$).

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ORIGINAL ARTICLE

ANALYSIS THE METABOLIC STATUS OF PATIENTS WITH CORONARY ARTERY DISEASE AND NONALCOHOLIC FATTY LIVER DISEASE DEPENDING ON BODY MASS INDEX

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ABSTRACT

The aim: To analyze the metabolic status of patients with coronary artery disease and nonalcoholic fatty liver disease depending on body mass index.

Materials and methods: The cohort of patients included 107 people with coronary artery disease (CAD), nonalcoholic fatty liver disease (NAFLD) and overweight (n=56) or obesity (n=51). In all patients glucose, insulin, HbA1c, HOMA-IR, hsCRP, transaminases, creatinine, urea, uric acid, lipid profile, anthropometric parameters and ultrasound elastography were measured.

Results: During the analysis of serum lipid spectrum in patients with obesity: lower levels of HDL and higher TG concentration compared with patients who had overweight. The insulin level was almost twice as high as in patients with overweight and the HOMA-IR index was 3.49 (2.13;5.78), where as in patients with overweight it was 1.85 (1.28;3,01), $p<0.01$. In patients with coronary artery disease and overweight, the of hsCRP was 1.92 (1.18;2.98) mg/l and was significantly different from the hsCRP level in obese patients, which was 3.15 (2.64;3.66) mg/l, p=0,004.

Conclusions: In patients with coronary artery disease, non-alcoholic fatty liver disease and obesity, the metabolic profile was characterized by a more unfavorable lipid spectrum: lower levels of HDL and higher triglicerid concentration. Carbohydrate metabolism in obese patients included disorders such us impared glucose tolerance, hyperinsulinemia and insulin resistance. There was also a correlation between body mass index with insulin and glycated hemoglobin. Higher concentration hsCRP in obese compared with patients with overweight was observed. This confirms the role of obesity in the patogenesis of coronary artery disease, non-alcoholic fatty liver disease and systemic inflammation.

KEY WORDS: insulin resistance, body mass index, nonalcoholic fatty liver disease, coronary artery disease, high sensitivity C-reactive protein

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INTRODUCTION

Rapid globalization, urbanization, an aging society, and an increasing number of chronic diseases are posing new challenges for today's health care and economy. The progressive increase in the number of people with overweight or obesity makes it possible to speak of it as a pandemic of non-communicable origin. However, the number of patients with type 2 diabetes mellitus (T2DM) is increasing, which should be considered not only as an endocrine disease but also as a disease affecting the heart and blood vessels. Leading rolls in the pathogenesis of cardiometabolic complications are played by hyperglycemia, hyperinsulinemia and insulin resistance (IR), which lead to oxidative stress, endothelial dysfunction, activation of systemic inflammation and dyslipidemia [1].

Non-alcoholic fatty liver disease (NAFLD) is increasing globally and is the leading cause of chronic liver disease in developed countries. NAFLD often leads to poor quality of life, disability and death due to progression of steatosis, non-alcoholic steatohepatitis, fibrosis, cirrhosis, liver failure, and hepatocellular carcinoma. The prevalence of NAFLD in different countries ranges from 14-40 %. NAFLD is an integral part of the metabolic syndrome because the risk factors for it are dyslipidemia, IR or T2DM and obesity [2]. Moreover, it is the abdominal type of distribution of adipose tissue that often leads to the formation of NAFLD. NAFLD can be considered as a predictor of the development of an atherosclerotic process with which it has a common pathogenetic mechanisms - endothelial dysfunction, IR, oxidative stress. The pathogenesis of NAFLD is based on a change in the profile of hormones-regulators of fat metabolism of leptin and adiponectin. As a results, IR decreases the sensitivity of muscle and adipose tissue to insulin, leading to hyperglycemia or hyperinsulinemia. The latter increases lipolysis in adipose tissue, increases the level of free fatty acids (FFA) and slows down the rate of their β-oxidation in the mitochondria. The acceleration of the transport of FFA and their insufficient oxidation

leads to the accumulation of excess Triglycerides in the cytoplasm of hepatocytes and their secretion of a large number of very low density lipoproteins, which causes liver steatosis. Excessive FFA on the background of steatosis increases lipid peroxidation, promotes oxidative stress, increases the synthesis of TNF-α, IL-6,high sensitivity C-reative protein (hsCRP). These factors contribute to the activation of the inflammatory process, apoptosis, cytolysis, dystrophy and liver fibrosis, that is, the development of nonalcoholic steatohepatitis [3].

The traditional concept that positions adipose tissue as an energy depot was refuted when scientists demonstrated the link between pro-inflammatory cytokines and obesity. In obesity, the balance of pro- and anti-inflammatory adipokines changes towards the pro-inflammatory due to the increase in the volume of visceral adipose tissue and changes in its metabolism. Visceral fat is much more active in the endocrine plane than the subcutaneous, it is the secretion of factors that systematically affect the body's immune, metabolic and endocrine processes and cause damage to the endothelium, activation of leukocytes, impaired blood clotting, and the involvement of the coagulation system and impact on the complement system. An additional mechanism that contributes to inflammation associated with obesity is changes in the gut microbiota and increased intestinal wall permeability, which facilitates the passage of lipopolysaccharide complexes into the bloodstream, which leads to the development of chronic endotoxemia and increase the production of inflammatory factors. Components of metabolic syndrom generate chronic systemic low-grade inflammation, "metaflamation", which interferes with adipose tissue homeostasis [4, 5].

THE AIM

The aim of article is analyze the metabolic status of patients with coronary artery disease and nonalcoholic fatty liver disease depending on body mass index.

MATERIALS AND METHODS

Clinical trial was conducted with accorandce to the Declaration of Helsinki, The Convention for the Protection of Human Rights and Biomedicine, Legislation of Ukraine and agreed by commission on ethics of research, experimental development and scientific works of Danylo Halytsky Lviv National Medical University: Protocol No. 3 of March 25, 2019. All patients signed an informed consent before the study.

The patients enrolled in this study had coronary artery disease (CAD), NAFLD and overweight or obesity. The cohort of patients included 107 people (women – 26 (24,3 %), men – (81 (75,7 %). The average patients age was 60,3 (58,8;61,7) years. All patients were divided in two groups depending on BMI: 1stgroup 25-29.9 kg/m^2 (26,9 (25.5;28.0) kg/m^2), 2nd group >30kg/m^2(33.3 (31.9;35.9 kg/m^2). The degree and type of obesity were determined by WHO criteria and IDF (2015). Diagnosis of CAD was considered verified by the results of coronarography and/or the presence of myocardial infarction (MI) in anamnesis more than three months before. 102 (95,3%) patients had a history of MI and/or myocardial revascularization procedures. Treatment of patients with CAD was administered according to unified clinical protocol "Stable coronary artery disease" approved by the Ministry of Health of Ukraine № 152,dated 02.03.2016 (with amendments 23.09.2016 № 994) and Guidelines for the Management of Dyslipidaemias (ESC/EAS 2016).

Physical examination and anthropometric parameters was also conducted. Fasting glucose and serum glucose at 2 hours after drinking the glucose solution, insulin, glycated hemoglobin (HbA1c), HOMA-IR, lipids, hsCRP, ALT, AST, ALP, GGTP, creatinine, urea, uric acid were measured for all patients. The level of $HbA1_C$ in the whole blood was determined by turbidimetric assay method, using test system «HemoglobinA1c-direct» BioSystems (Spain). Insulin and hsCRP in serum was determined on chemiluminescent immunoassay analyzer «Immulite 2000» (Siemens, Germany) using a proper reagent (Immulite2000Insulin and Immulite 2000hsCRP, USA). Biochemical indices and lipids were performed by generally accepted methods on automatic analyzer «BioSystems» (Spain) using original set of reagents.

Insulin resistance index was calculated by the formula:

HOMA-IR=fasting insulin (μIU/ml)*fasting serum glucose (mmol/l)/22.5.

Patients with HOMA-IR>2.77 was insulin resistance (IR).

Elastography is used as an alternative to liver biopsy to assess liver stiffness owing to its accuracy, non-invasiveness and easy acceptance among patients. With the increasing prevalence of NAFLD worldwide, elastography is the most appropriate non invasive to assess fibrosis, NASH, and non-NASH NAFLD. A Toshiba Aplio 300 ultrasound device was used (Toshiba Medical Systems, Tokyo, Japan) .

The results are given as mean values with statistical error. The values with normal distribution are presented as confidence interval (95 %); and the values, where distribution significantly differ from normal, are presented as interval of 25 % and 75 % percentiles. Comparison of groups was performed by means of Mann-Whitney U-test. Categorical data were presented as proportions and analyzed using the Chi-square test. Spearman's rho correlation tests were used to report the associations between variables. The results were considered statistically reliable at $p<0.05$.

Table I. Data of lipid, glucose metabolism and liver and kidney function in patients with CAD, NAFLD and overweight (1stgroup) and patients with CAD, NAFLD and obesity (2nd group)

Baseline Characteristic	1st group, n=56	2nd group, n=51	P
HDL, mmol/l	1,25 (1,00;1,45)[2]	1,16 (1,08;1,24)[2]	0,04
LDL, mmol/l	2,73 (2,47;2,99)[1]	2,44 (1,96;3,26)[2]	0,07
Cholesterol, mmol/l	4,88 (4,54;5,22)[2]	4,31 (3,78;5,39)[2]	0,16
Triglycerides, mmol/l	1,37 (1,00;2,21)[2]	1,71 (1,43;1,99)[1]	0,01
ALT, U/l	24,65 (15,13;35,55)[2]	23,50 (15,80;42,15)[2]	0,28
AST, U/l	28,74 (26,19;31,29)[1]	25,20 (20,35;31,35)[2]	0,21
ALP, U/l	87,65(79,44;95,86) [1]	78,73 (78,22;84,52)[2]	0,14
GGTP, U/l	36,50(24,45;59,08)[2]	44,90 (27,70;64,75)[2]	0,09
Creatinine, mcmol/l	87,27 (83,09;91,45)[1]	89,91(89,40;93,44)[2]	0,11
Urea, mmol/l	5,71 (5,27;6,15)[1]	5,48 (4,97;5,86)[2]	0,19
Uric Acid, mcmol/l	379,17 (352,60;405,74)[1]	385,00 (308,50;456,00)[2]	0,34
Glucose, mmol/l	6,00 (5,50;6,42)[2]	6,11 (5,65;6,70)[2]	0,06
Glucose 2 ,mmol/l	7,52 (7,18;7,78)[2]	8,09 (7,71;8,47)[1]	0,03
HbA1, %	5,08 (4,90;5,26)[1]	5,30 (4,70;5,90)[1]	0,11
Insulin, μIU/ml	7,14 (4,56;11,33)[2]	13,00 (7,78;20,15)[2]	< 0,01
HOMA-IR	1,85 (1,28;3,01)[2]	3,49 (2,13;5,78)[2]	< 0,01

[1] – values with normal distribution, M (M-CI;M+CI).

[2] – values, where distribution significantly differ from normal, Me (25%;75%).

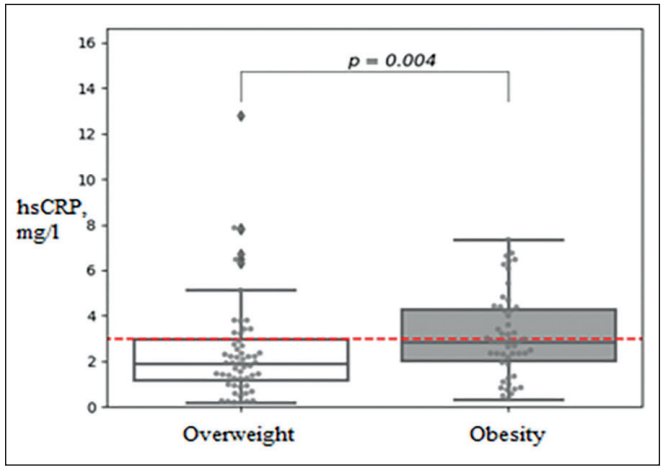

Fig. 1. Concentration of hsCRP in patients with CAD and NAFLD depending on the presence of obesity, p =0.004

RESULTS

During the analysis of serum lipid spectrum in patients with obesity: lower levels of HDL (1.16 (1.08;1.24) mmol/l versus 1.25 (1.00;1.45)) mmol/l, p<0.05) and higher TG concentration (1.71 (1.43;1.99) mmol/l vs 1.37 (1.00;2.21) mmol/l, p<0.05) compared with patients who had overweight. LDL levels were not significantly different between the groups, as they were more dependent on the statin dose taken by patients to correct dyslipidemia. The levels of liver enzymes, creatinine, urea, and uric acid were not significantly different between the groups.

Indicators of the carbohydrate spectrum also had great features in patients with coronary artery disease and obesity, the level of glycemia 2 hours after loading was significantly higher than in patients with overweight (8.09 (7.71; 8.47) mmol/l vs 7.52 (7.18; 7.78) mmol/l, p=0.03), the insulin level was almost twice as high as in patients with overweight (13.00 (7.78;20.15) μIU/ml versus 7.14 (4.56;11.33) μIU/ml, p<0.01), and the HOMA-IR index was 3.49 (2.13;5.78), where as in patients with overweight it was 1.85 (1.28;3,01), p < 0.01. Fasting glucose and HbA1c concentrations did not differ significantly between these groups. The proportion of patients with impaired glucose tolerance among patients with CAD, NAFLD and overweight was 21.4 % (12 patients), among patients with CAD, NAFLD and obesity - 33.3 % (17 patients), with no significant difference between the groups also (p=0.17). Among patients with coronary artery disease with obesity, the proportion of patients with hyperinsulinemia was significantly higher than among patients with BMI and was 52.9 % (27 people) versus 21.4 % (12 people) (p<0.01). The proportion of patients with HOMA-IR-confirmed IR was 58.8 % of patients with obesity (39 patients) and 30.4 % of patients with overweight (17 patients) and differed significantly between groups (p<0.01) (Table I).

In order to evaluate systemic inflammation, which is considered one of the pathogenetic mechanisms of atherosclerosis and cardiovascular risk factor, the concentration of hsCRP in the serum of patients was determined. The

proportion of persons with this marker increased in the BMI group was 25.0 %, where as in the obese group it was 43.1 %, p<0.05. The median and 25 % and 75 % percentiles of hsCRP in patients with CAD and overweight were 1.98 (1.18;2.98) mg/l, respectively, while in obese patients with CAD - 3,15 (2.64; 3.66) mg/l, the difference between the medians was statistically reliable (p<0.01). The clinical significance of hsCRP as a cardiovascular risk factor has a cut-off of 3.0 mg/l, and the upper quartile in the group with overweight was within this range. In contrast, for obese patients, the upper quartile exceeded the cut-off (Fig. 1).

We carried out a study to determine the value of liver stiffness measurement based on ultrasound elastography, in patients with NAFLD. In the first group the stage of fibrosis was F0- 93%, F1- 7%, in the second group the value was F0- 71%, F1,F2 - 29%.

In the study of correlations between BMI and lipid, carbohydrate metabolism, and hsCRP levels, there were significant associations with insulin levels (r=0.35, p<0.05), glycated hemoglobin levels (r=0.22, p<0.05) and hsCRP level (r=0.41, p<0.05). The lack of association with the lipid spectrum is evidently due to the effects of statin therapy in these patients.

DISCUSSION

Pandemic of obesity has led to an increase in research in this area. Accordingly, there is a new understanding of the mechanisms of development of CAD, NAFLD in obesity. In patients with cardiovascular pathology, widespread atherosclerosis, dyslipoproteinemia in 90% of cases fatty infiltration of the liver with elements of fibrosis occurs, which, according to the authors, is a pre-stage of steatohepatitis [1]. NAFLD is one of the most common chronic diseases associated with the accumulation of intrahepatic triglycerides.

Many scientific studies indicate that insulin resistance is characteristic of NAFLD, even without obesity. However, NAFLD alone cannot be considered a cause of insulin resistance, but rather a consequence.

A common pathogenetic mechanism of coronary heart disease and NAFLD is atherogenic dyslipidemia, which is found in 20-80%. A high correlation between total calcium index and lipid metabolism in patients with stable angina with NAFLD has been established. This indicates the direct involvement of disorders of lipid metabolism and systemic inflammation in the processes of atherogenesis of patients with NAFLD [5, 6].

Patient's age and long-term disease lead to deepening of lipid metabolism disorders in patients with comorbid pathology. The main cause of death of such patients are cardiovascular diseases [3]. "Non-lipid" risk factors for atherosclerosis include arterial hypertension, impaired carbohydrate metabolism, obesity, hypodynamia, smoking, and the like. Therefore, prevention should be aimed at correcting these risk factors as well.

Today, the issue of digestive organ dysfunction, in particular the liver and intestines, plays an important role in the development of dyslipidemia. Qualitative and quantitative changes of blood lipids are associated with inhibition of the activity of the reticuloendothelial system of the liver, enterohepatic circulation of bile acids, and impaired co-operation in the system. In the formation of insulin resistance in obesity plays an important role adipose tissue synthesizing, the effects of which affect the formation of dysmetabolic processes, oxidative stress, leading to disorders of the cardiovascular syste.

The Ajmal M.R. et al. study indicates that NAFLD is very common in patients with cardiovascular disease (69.2%) and is significantly associated with metabolic syndrome and its individual components. The levels of hsCRP and TNF-α were significantly higher in patients with NAFLD and showed an upward trend with increasing visceral fat [4, 7]. Kim J. et al. in retrospective study proved that the concomitant presence of NAFLD and systemic inflammation as assessed by hsCRP increases the risk of coronary artery calcification (non-invasive surrogate marker of atherosclerosis) development over four years [8].

Our study had some limitations: 1) the patients who participated in the study at the time of the examination received atorvastatin, β-blockers and diuretics at different doses for cardiovascular pathology, which could affect the metabolic profile; 2) the study did not have a control group with normal body weight, which could affect the interpretation of the indicators.

CONCLUSIONS

In patients with coronary artery disease, non-alcoholic fatty liver disease and obesity, the metabolic profile was characterized by a more unfavorable lipid spectrum: lower levels of HDL and higher triglicerid concentration. Carbohydrate metabolism in obese patients included disorders such us impared glucose tolerance (postprandial glycemia 8,09 (7.71;8.47) mmol/l vs 7.52 (7.18;7.78) mmol/l, p=0.03), hyperinsulinemia (serum fasting insulin 13.00 (7.78;20.15) μIU/ml vs 7.14 (4.56;11.33) μIU/ml, p<0.01) and insulin resistance (HOMA-IR 3.49 (2.13;5.78) vs 7.14 (4.56;11.33) μIU/ml, p<0.01). There was also a correlation between body mass index with insulin and glycated hemoglobin. Higher concentration hsCRP in obese compared with patients with overweight was observed (hsCRP 3.15 (2.64; 3.66) mg/l vs 1.98 (1.18; 2.98) mg/l, p<0.01) and significant associations BMI with hsCRP (r=0.41, p<0.05). This confirms the role of obesity in the patogenesis of coronary artery disease, non-alcoholic fatty liver disease and systemic inflammation.

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ORIGINAL ARTICLE

ANTIBIOTIC RESISTANCE OF *SALMONELLA TYPHI* CARRIER ASSOCIATED WITH GALL BLADDER CHRONIC INFECTION IN AL-NAJAF PROVINCE

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ABSTRACT

The aim: This study was undertaken to identify antibiotic resistance of *Salmonella Typhi* in specimens of gall bladder tissue after cholecystectomy.

Materials and methods: *Salmonella Typhi* identification from the isolates have been depended on morphology of the colony and biochemical tests as a first step in identification while final identification has been achieved by the automated VITEK-2 compact system then PCR technique**.**

Results: Depending to finding via the VITEK tests and PCR technique and thirty-five *Salmonella Typhi* sample have been obtained. This research shown that about 35 (70%) positive result contains, 12 (34.3%) isolates was positive from stool and 23(65.7%) from gall bladder tissue. The results revealed difference in *S. Typhi* resistance to some antibiotics, where *S. Typhi* has wide-ranging sensitivity: 35 (100%) to Cefepime, Cefixime and Ciprofloxacin and revealed great sensitivity 22 (62.8%) to Ampicillin. *S. Typhi* isolates proved extremely resistant 19 (54.2%) and 25 (71.4%) to Trimethoprim/Sulphamethoxazole and Chloramphenicol respectively. Increment in the rate of *Salmonella* that has multidrug resistance to chloramphenicol, ampicillin, furazolidonecotrimoxazole, streptomycin, and tetracycline is a developing problem and worldwide worry matte.

Conclusions: Resistant forms of *Salmonella* enteric serotype Typhi were detected with increment in the rate of multidrug resistance to chloramphenicol, ampicillin, and tetracycline so currently, Cefepime, Cefixime and Ciprofloxacin and revealed great sensitivity and have become the mainstay of treatment. Challenging difficult which rises in this study is the extend of Multidrug resistant strain (MDR) of *S. Typhi*.

KEY WORDS: *Salmonella Typhi*, fliC-d, antibiotic resistance

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INTRODUCTION

Salmonella enterica serotype *typhi* is produced typhoid fever that restricted to infected people only, and the infected rate of this illness is assessed about 21 million, causing in approximately 200,000 deaths globally each year thus its remnants a main reason of mortality and morbidity [1]. It has the capability to effect chronic asymptomatic infection that chiefly continuing in the gallbladder [2]. The gallbladder is a small organ situated at the right upper quadrant of abdomen, under the liver. Bile is composed from cholesterol, bile salts, water, fats and bilirubin pigment. Its main function is bile storage that made in the hepatocytes then the bile discharge to the second part of small bowel for digestion [3]. *S. typhi* produce infection and proliferation that associated to its capability for creation of biofilm in the gallbladder of human (typhoid carriers) by this way it can escape the immune system without presentation any symptoms. The only reservoir for *S. typhi* is human and is spread by contaminated food or water [3]. Because *S. typhi* is a man-particular bacterium, those carriers consider a serious reservoir for additional spread of illness by shedding of bacteria in feces, which is an intermittent and sporadic occurrence [4]. Principally in regions of great endemicity, about 80-90% of chronic infested carriers have gallstone so carrier form is connected to the gallstones [5]. Multiple drug resistance (MDR) has converted a major risk to the typhoid treatment and further infectious sicknesses. Since the 1970s, this risk has augmented in *Salmonella enterica serovars typhi*, *S. typhi* able to exhibit MDR determinants where as holding the capability to efficiently spread and persists

Table I. Detection of DNA content by agarose gel electrophoresis.

Target genes	Primer sequence (5'-3')		Thermal cycling condition	PCR product size
FliC-d	F	ACTCAGGCTTCCCGTAACGC	5 min of denaturation at 95°C (1 cycle), then by 35cycles of amplification; for each of heat denaturation at 94 °C for 40 s, primer annealing at 55.5°C for 30 s and DNA extension at 72 °C for 40 s then one cycle for final extension at 72°C for 5 m minutes. The reaction was finished by cooling at 4°C.	763
	R	GGCTAGTATTGTCCTTATCGG		

in the human [6]. Here we talk about the *S. typhi* MDR phenotype evolution in Najaf with consider choices for management.

On the surfaces of cholesterol type of gallstones, *S. typhi* able to syntheses biofilms in human carriers and on the gallbladder epithelium of mouse carriers and mouse gallbladders [7]. Formation of biofilm was verified by persistence mechanism and prolonged gallbladder colonization [8]. Next to antibiotics have been introduce in the typhoid fever treatment in the middle of last century, there were increasing in the rate of resistant bacteria [6].

THE AIM

The aim of the present study to detect the multidrug resistance *S. Typhi* among patients with typhoid fever *via* the subsequent goals:

- *S. Typhi* Isolation and identification through using of present conventional techniques, VITEK system and molecular technique *via fliC-d (flagella)* gene
- Antibiogram testing and MIC test of *S. Typhi* isolates by the computerized VITEK-2 compact system.

MATERIALS AND METHODS

COLLECTION OF SPECIMENS

A cross-sectional study was directed in 50 specimens that have been gathered from ill persons complaining from cholecystitis attending to AL-Furat General Hospital and AL-Sadder Medical City during the time between September 2017 to December 2018, sample was including (stool and gallbladder tissue) [9]. Patients had signs of cholelithiasis and cholecystitis such as include pain, fever, right upper quadrant mass, tenderness and other signs from primary positive cultures, Single colonies has been isolated and identified agreeing with standards [10]. All specimens have been cultured on the XLD, Salmonella-Shigella agar MacConkey and blood agar plates, incubated under 37°C and aerobically condition for about 18 - 24 hour, then, the bacteria were identified by morphological, biochemical test. The Proofing has been set byVITEK-2 Compact system and finally by PCR technique. The PCR assay has been achieved to distinguish the flagella *FliC-d* gene for validation the proofing of *Salmonella Typhi* (Table I) [11].

RESULTS

Almost all isolates of *S. Typhi* have been show, that carrying FliC-d gene, which is distinctive for *S. Typhi*: as 35 (70%) positive result include that about 23 (65.7%) isolated samples was positive from tissue of gallbladder and 12 (34.3%) from feces (Fig. 1).

ANTIBIOGRAM TESTING BY VITEK-2 COMPACT

Table II showed the antibiotic sensitivity screening test to a different antimicrobial drugs: as high percent of the bacteria were resistant to the conventional 1st class of antimicrobial drugs, in this research we choose to manage typhoid fever Ampicillin 20% Trimethoprim/ Sulphamethoxazole 60%, Trimethoprim 50%, Chloramphenicol 65.71% and Gentamycin 100%.

The present study confirmed the findings about major of the strains were high rate of sensitivity to Meropenem, Cefixime, ceftriaxone, ciprofloxacin, Cefepime and Impenem.

A challenging difficult which rises in this study is the extant of multidrug resistant strain (MDR) of *S. Typhi*. Multidrug resistant (MDR) strain phenotype was distinct for isolates, that are resistant to minimum of 3 classes of antibiotics, by present definition of MDR: 19 of 35 (54.28%) of isolates were confirmed as MDR - 10 (28.57%) of them were resistant to four classes of antibiotics and the remaining 9 (25.71%) of them were resistance to three classes of antibiotics (Table III), and 16 of 35 (45.71%) of isolates were non MDR.

DISCUSSION

As isolates of *S. Typhi*, that carrying FliC-d gene which was 35 (70%) positive result include that 23 (65.7%) about isolated samples were positive from tissue of gallbladder and 12 (34.3%) from feces. This result is associated with Holmes I. [14], which detect that out of eighty 80 assumed of typhoid fever, flagella gene fliC-d has been

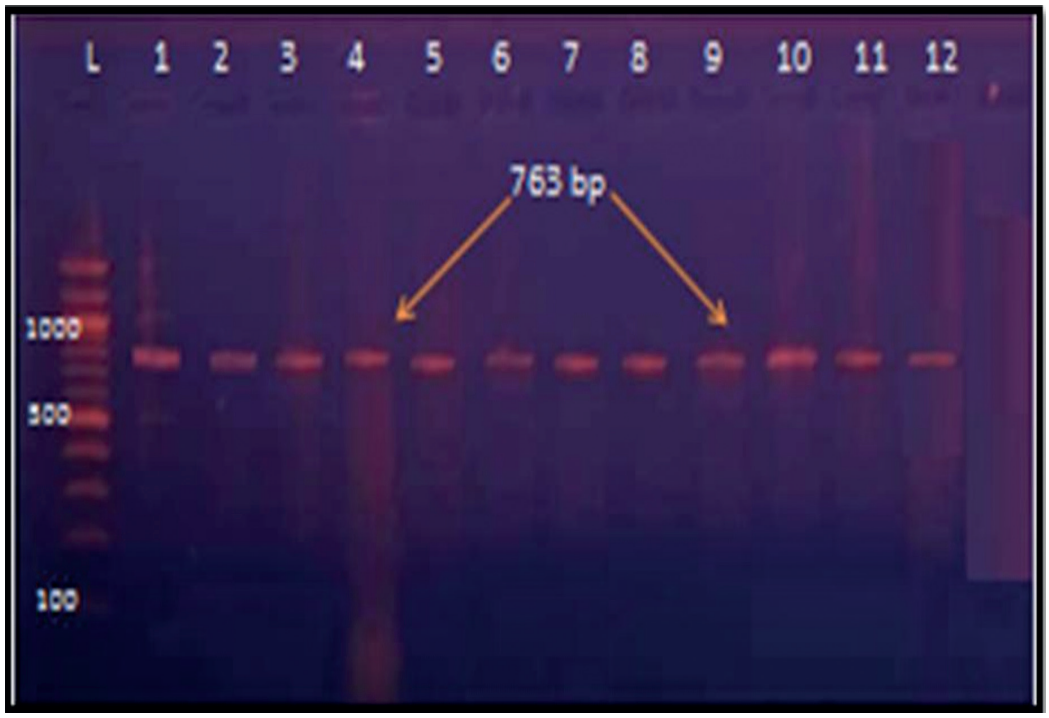

Fig. 1. Ethidium bromide-stained agarose gel electrophoresis of PCR amplification products of isolates of *S. Typhi* which amplified with FliC-d gene primers with product 763 bp for 1 hr. at 80 volt/cm (antibiogram testing by VITEK-2 Compact).

distinguished by polymerase chain reaction in about 56 (70%) cases, that corresponds with what was founded in other study done in Bangladesh as positive isolate by PCR was 88.7% of typhoid fever supposed cases. The PCR positivity rate is (65% – 71.9%) in variant research [14]. Research showed high percent of the *S. Typhi* were resistant to the conventional 1st class of antimicrobial choose to manage typhoid fever in which Ampicillin 20%, Trimethoprim/Sulphamethoxazole 60%, Trimethoprim 50%, Chloramphenicol 65.71% and Gentamycin 100%. These basic findings are consistent with research by Mengo D. et al [15] showing that high resistant to Chloramphenicol 74% ampicillin 75% in Kenya. Overall these findings nearly Chloramphenicol are in accordance with findings reported by Mutai W.C. et al [16], that show notify large percentage (72%) of resistance to ampicillin and chloramphenicol. A similar results was found in Samwa by Njum A.A. et al [17] research demonstration that highly resistant to Gentamycin (70.22%), ampicillin (27.57%) and Trimethoprim (70%) and in research in Tunis by Kalai W. et al [18] that showed recistance for tetracycline (62.9%), ampicillin (84%) and Trimethoprim/Sulphamethoxazole (37%). Opposing to the current study on *S. Typhi* antibiogram, the bacterium is sensitive completely to those antimicrobials that had not been tried for long period to typhoid fever management [19]. A research in Nepal stated an increment in rate of susceptibility to the Cotrimoxazole (98.8%), ampicillin (97.6%) and Chloramphenicol (98.8%) [20]. Resistance strains of *S. Typhi* to Chloramphenicol are by the help of two ways: transfer Chloramphenicol by specific efflux proteins and enzymatic inactivation by type A or type B chloramphenicol acetyltransferase [21]. The present study confirmed the findings about major of the strains were high rate of sensitivity to Meropenem, Cefixime, Ceftriaxone, Ciprofloxacin, Cefepime and Impenem. This is reliable with what has been stated in preceding study by Njum A.A. et al [17] in Smawa, that illustrate that S.Typhi are sensitive for Cefepime and Ceftriaxone in 100%, and by Alcaine S.D. et al. that sencivity to Ceftriaxone and Cefixime is 100% in India [22]. A challenging difficult which rises in this study is the extant of multidrug resistant strain (MDR) of *S. Typhi*. Multidrug resistant strain phenotype was distinct for isolates that are resistant to minimum of 3 classes of antibiotics, by present definition of MDR in 19/35 (54.28%) of isolates and 16/35 (45.71%) of isolates were non MDR. The increment in the MDR *S. Typhi* to Ampicillin, Chloramphenicol, Cotrimoxazole, Tetracycline, Furazolidone and Streptomycin is a developing problem and worldwide worry matter [8, 17]. The emergence of resistance of antimicrobial agent in *S. Typhi*, have same dangerous issues as for other microorganisms [23]. MDR is learned from animals by food chain. Additionally, abandoned practice of antibiotics in veterinary medicine is a main effector in appearance of *S.Typhi* resistance this antibiotic therefore, may be considered as a substitute in the typhoid fever management bearing in mind its little resistance extents. Research in India, Nigeria, and Germany discovered the existence of CTM-X gene group of extended-spectrum β-lactamase resistance (ESBL) that confers resistance to ceftriaxone [16]. With recent reports from different countries recording resistance to ceftriaxone, routine screening of such isolates is significant.

Table II. Antibiotic testing of *S. Typhi* isolated with the automated VITEK-2 compact system by using AST-XN05 and AST-N222 cards.

No	Antibiotic classes	Antibiotic disk	Amount of isolate exhibited					
			Sensitive / Susceptible		Intermediate		Resistant	
			No	[%]	No	[%]	No	[%]
1	*Ampicillin*	AMP	21	60	7	20	7	20
2	*Piperacillin*	PRL	21	60	0	0	14	40
3	*Cefixime*	CFM	35	100	0	0	0	0
4	*Ceftriaxone*	CTR	35	100	0	0	0	0
5	*Cefepime*	FEP	35	100	0	0	0	0
6	*Meropenem*	MEM	35	100	0	0	0	0
7	*Imipenem*	IMP,	35	100	0	0	0	0
8	*Aztreoname*	ATM	31	88.57	4	11.43	0	0
9	*Amikacin*	AK	0	0	0	0	35	100
10	*Gentamycin*	CN	0	0	0	0	35	100
11	*Ciprofloxacin*	CIP	35	100	0	0	0	0
12	*Tetracycline*	Tet	35	100	0	0	0	0
13	*Trimethoprim*	TRI	17	48.57	18	51.43	0	50
14	*Trimethoprim / Sulphamethoxazole*	SXT	14	40	0	0	21	60
15	*Chloramphenicol*	C	12	34.29	0	0	23	65.71

Table III. The prevalence of Multidrug resistant strain (MDR) among 35 *S.Typhi* isolates.

Symbol of isolates (MDR)	Resistance of antibiotic	Resistance antibiotic classes	No. of resistant antibiotic classes
S1,S5,S6,S21	Amp, PRL, AK, CN, TRI, SXT, C	*Penicillin's, Aminoglycosides, Folate pathway inhibitors, Phosphonic acid*	4
S2,S7	Amp, PRL, AK, CN, SXT, C	*Penicillin's, Aminoglycosides, Folate pathway inhibitors, Phosphonic acid*	4
S20,S23,S28	PRL, AK, CN, TRI, SXT, C	*Penicillin's, Aminoglycosides, Folate pathway inhibitors, Phosphonic acid*	4
S24	PRL, AK, CN, TRI, C	*Penicillin's, Aminoglycosides, Folate pathway inhibitors, Phosphonic acid*	4
S3,S11,S12,S17,S18,S19	AK, CN, TRI, SXT, C	*Aminoglycosides, Folate pathway inhibitors, Phosphonic acid*	3
S13,S22	PRL, AK, CN, SXT, C	*Aminoglycosides, Folate pathway inhibitors, Phosphonic acid*	3
S27	AK, CN, TRI, SXT, C	*Aminoglycosides, Folate pathway inhibitors, Phosphonic acid*	3

CONCLUSION

Outcomes from this research direct that there is an important difference in resistance between *S. Typhi* isolates to the variant antibiotic drug suggested for management. *S. Typhi* strains which are MDR are till now high nevertheless constant checking of responsively to the original first-line antibiotics is essential ever since the MDR strains have latterly revealed amplified susceptibility. Furthermore, there is an appearance of strains with intermediate resistant and susceptibility to ciprofloxacin, therefore, ciprofloxacin usage for typhoid fever management requires routine observation to avoid more extent of those strains. Resistant forms of Salmonella enterica serovar typhi were detected with increment in the rate of multidrug resistance to Chloramphenicol, Ampicillin and Tetracycline. Currently, Cefepime, Cefixime and Ciprofloxacin and revealed great sensitivity and have become the mainstay of treatment. Challenging difficult which rises in this study is the extend of Multidrug resistant strain (MDR) of *S. Typhi*.

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ORIGINAL ARTICLE

THE OPINION OF MEDICAL YOUTH ON VACCINATION FROM CORONAVIRUS DISEASE COVID-19

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ABSTRACT

The aim: To determine the opinion of 6th year students and interns in the specialty "General Practice - Family Medicine" on vaccination against COVID-19.

Materials and methods: We conducted an anonymous online survey of 268 sixth-year students, interns of the first and second year of study in the specialty GP/FM. Research design: 1.Creating a pilot version of the questionnaire based on a literary search. 2.Approbation and discussion of the questionnaire in the focus group. 3.Online surveys of respondents and statistical processing of the data.

Results: The questionnaire was completed by 188 students, 48 interns in 1 year of study and 32 interns in 2 years of study. Among interns in 1st and 2nd year of study, the vaccination rate was 95.8% and 93.8%, respectively, among students - 71.3%, which is twice as much as among the general population. 30% did not receive the vaccine they considered most effective, but were vaccinated with the one that was available.

Conclusions: The level of vaccination against COVID-19 in future doctors was 78.3%. The most significant reasons for refusing COVID-19 vaccination were past illness (COVID-19) - 24%, fear of vaccination - 24%, uncertainty about the effectiveness of immunoprophylaxis - 17.2%. Reasons that stimulated vaccination: the desire to protect against severe COVID-19 - 62.8%, the need to work in the medical field - 49.5%, the desire to protect others from the risks of COVID-19 infection - 38%.

KEY WORDS: vaccination, COVID-19, interns

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INTRODUCTION

On March 11, 2020, the World Health Organization (WHO) announced the beginning of the pandemic of COVID-19 coronavirus infection. In Ukraine, COVID-19 was first confirmed on March 3, 2020, and one year later, the number of registered cases was 1.4 million and the mortality rate was about 28,000 [1], which increased to 100,000 cases by February 2022 [2]. According to Johns Hopkins University, as of June 29, 2021, there have been over 3.9 million deaths among more than 182 million cases of coronavirus infection worldwide [3].

COVID-19 vaccine prophylaxis, according to the WHO, is the most effective way to control infection and mortality.

The results of the 2019 Wellcome Trust survey on COVID-19 vaccine prophylaxis in Ukraine showed that 29% of Ukrainians considered vaccination safe and 50% considered it effective [4,5]. According to research of the sociological group "Rating", 55% of Ukrainians agreed to be vaccinated on condition of free receipt of the vaccine, while 35% were willing to pay the cost themselves [6].

Ukraine, as a state, became a member of the WHO global vaccine alliance initiative (GAVI-Alliance) on January 30, 2021, which ensured access to vaccines for the population [7, 8]. However, government efforts to vaccinate against COVID-19 encountered pronounced skepticism and resistance from Ukrainians.

As of February 2, 2022, Ukraine had 30,027,933 first and second doses of the vaccine against coronavirus, with 33% of the adult population fully vaccinated. In particular, the first dose was received by 15364681, booster more than 300 thousand people (information is dynamically updated) [9]. By comparison, in neighboring Poland 57% of the adult population has already been fully vaccinated, and in France, Great Britain, Belgium, and Germany the figure exceeded 70%.

Since November 2021, in Ukraine there is a valid order about the list of professions whose employees are subject to mandatory preventive vaccinations. [10]

Considering the polarity of opinions in the world and Ukraine about COVID-19 vaccine prophylaxis, it is reasonable and urgent to study the question about the reasons that guide Ukrainians in their decision to get vaccinated.

THE AIM

Objective of the study: assess vaccination status of 6th year students and interns in the specialty "General

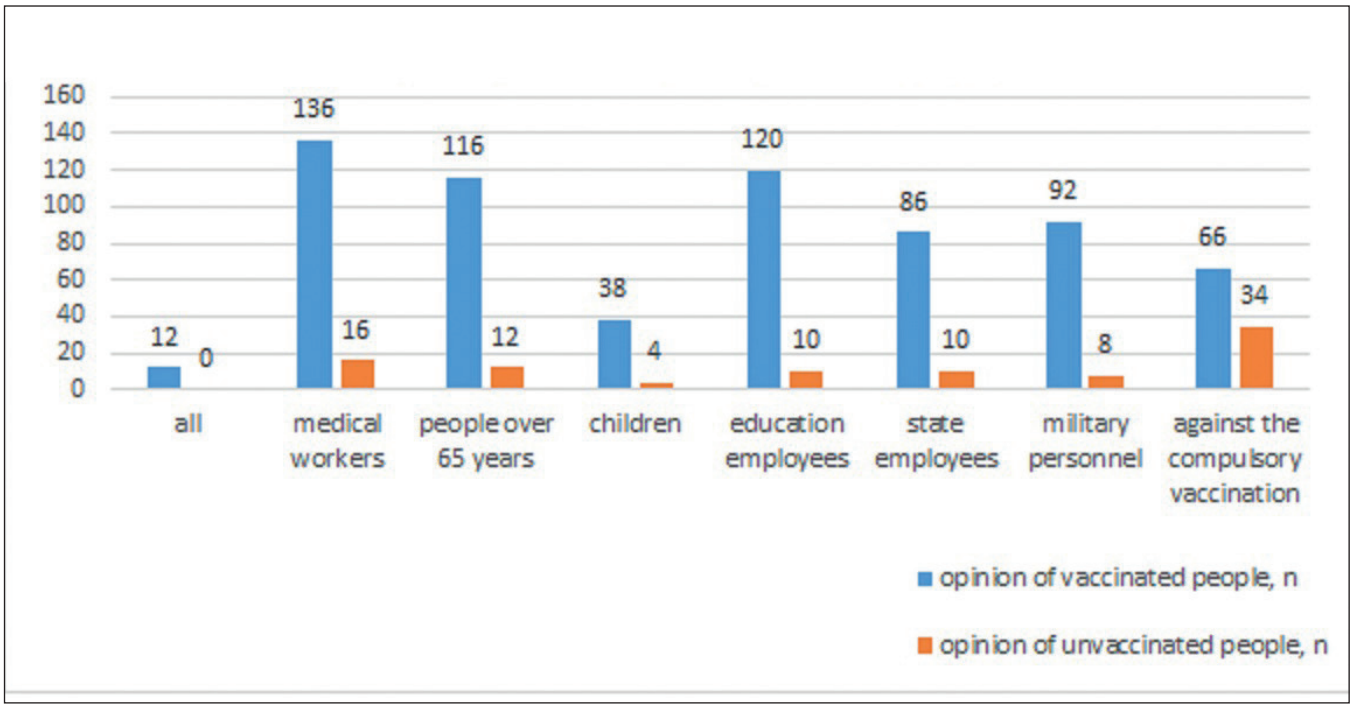

Fig. 1. Population groups who must be vaccinated compulsory according to respondents

Practice - Family Medicine" against coronavirus disease COVID-19, identify the reasons for refusal and motives for vaccination and their opinions on cohorts for mandatory vaccination.

MATERIALS AND METHODS

We conducted an anonymous online questionnaire survey of 268 6-year students, first- and second-year interns in the specialty "General Practice - Family Medicine". According to the results of the analysis among the study groups, the mean age among women was 22 (22; 24) and the mean age among men was 22 (22; 24). Among 6th year students, the age of men was 22 (22; 23), and among women 22 (22; 22). Among the interns of the first year, the age of men was 23 (23; 24), and the age of women was 23 (23; 24). Among the interns of the second year of study, the age of men was 26.5 (24.5; 27), and for women 25 (24; 28). Quantitative characteristics of respondents depending on the status of education are presented in Table I.

Our study included all interns of the first and second year of study in the specialty "General Practice - Family Medicine". The 6th year students were selected as follows: in each group of students, which was formed by the dean's office at the beginning of their studies, every second person from the list was involved in the survey. After evaluating the answers, we found that the level of refusal of respondents to complete the questionnaire was 2%. The questionnaire was carried out by means of the author's questionnaire during the period from 01.11 till 15.11.2021. The study included several stages:

1. Creation of a pilot version of the questionnaire based on a literature search
2. Testing and discussion of the questionnaire in a focus group with amendments (the focus group consisted of 5 teachers and 10 of the 6th year students, who were trained at the time and agreed to participate voluntarily)
3. Online survey of respondents and statistical processing of the data obtained

The questionnaire was based on the free version of the Google Forms tool and contained 8 closed-ended questions and 2 with open-ended and expanded response options. At the end of the questionnaire each of the respondents had the opportunity to leave a comment on the questions or wishes. The survey was conducted online.

Characteristics of the main group: the selected cohort of respondents included random participants and accounted for at least 30% of the total number on the course, so the sample can be considered representative of the universities. On average, respondents spent about 12 minutes completing the questionnaire. Links to the questionnaire were distributed online with an abridged version thanks to the free service https://cutt.ly and social messengers Viber, Telegram.

Statistical processing of the obtained data included: checking the normality of data distribution using the Kolmogorov-Smirnov test (with Lilliefors correction); determining the mean values (median with lower and

Table I. The structure of the distribution of respondents by gender and status of education

	Gender		Total, n (%)
	Women, n (%)	Men, n (%)	
6th year students n (%)	138 (73,4%)	50 (26,6%)	188 (100,0%)
1st year interns n (%)	44 (91,7%)	4 (8,3%)	48 (100,0%)
2nd year interns n (%)	24 (75,0%)	8 (25,0%)	32 (100,0%)
Total, n (%)	206 (76,9%)	62 (23,1%)	268 (100,0%)

Table II. Average age among respondents depending on educational status

	K-S test	p	Age, years
6th year students, year	0.382733	<0.0001	22 (22;22)
1st year interns, year	0.370753	<0.0001	23 (23;24)
2nd year interns, year	0.198233	0.093	26 (±2.32)
General	0,298092	<0.001	22 (22;23)

Table II. The structure of the distribution of respondents by vaccination status and education status

Group	Status of education	Vaccinated, n (%)	Unvaccinated, n (%)	Total, n (%)	P-value	95% CI
1	6th year students n (%)	134 (71,3%)	54 (28,7%)	188 (100%)	p1-2=0.004 p1-3=0.070	0,157; 1,038 -0,028; 1,014
2	1st year interns n (%)	46 (95,8%)	2 (4,2%)	48 (100%)	p1-2=0.004 p2-3=1.000	-1,038; -0,157 -0,726; 0,518;
3	2nd year interns n (%)	30 (93,8%)	2 (6,25%)	32 (100%)	p3-1=0.070 p3-2=1.000	-1,014; 0,028 -0,518; 0,726

Table IV The structure of the distribution of respondents by social position

	Vaccinated (n=210)	Unvaccinated (n=58)	the level of statistically significant difference
Discourage others to vaccinate from COVID-19	4,8% (n=10)*	6,9% (n=4)*	$\chi2$ =0.418 p=0.518
Not discourage others to vaccinate from COVID-19	95,2 % (n=200)	93,1% (n=54)	
Recommend to others to be vaccinated with COVID-19	82,9% (n=174)	34,5% (n=20)	$\chi2$=53.208 p<0,001
Not recommend to others to be vaccinated with COVID-19	17,1% (n=36)	65,5% (n=38)	

*All were exclusively 6th year students

upper quartiles); and determining the significant difference between relative values using the Mann-Whitney (U) test, Pearson Chi-square test ($\chi2$). The difference was considered significant at $p<0.05$.

Statistical analysis was performed using the licensed software Statistica v.6.1 (Statsoft Inc., USA, license NoAGAR909E415822FA).

The actual study was conducted in accordance with the World Medical Association's Declaration of Helsinki "Ethical Principles for Medical Research Involving Human Subjects" (revision of 10.2013, adopted at the 64th General Assembly, Fortaleza, Brazil)

RESULTS

Verification of the normality of the age indicator for the general sample the Kolmogorov-Smirnov test (with Lilliefors Significance Correction) K-S test 0,298092 p-value <0.001. The results of the verification of age indicators for normality among groups of different educational status are presented in Table II.

No statistical difference between male and female age was found U=1634 (p-value=0.832).

In the group of respondents who participated in the questionnaire, the proportion of persons who had had COVID-19 was 55.2% (n=148) and 44.8% (n=120) who

had not had the disease. The vaccination rate among those who had COVID-19 was 77% (n=114).

Of the 268 persons interviewed, 78.3% (n=210) were vaccinated, of which 70.1% (n=188) had a complete course of vaccination and 8.2% (n=22) had one dose. Unvaccinated persons accounted for 21.6% (n=58).

According to our data, 60.6% of the 6th year students (n=114) were vaccinated with both doses of vaccines (n=188), 24.5% of the 1st year interns (n=46), 14.9% of the 2nd year interns (n=28). Among those vaccinated with a single dose of the vaccine (n=22) the rate of the 6th year students was 90,9% (n=20), and of the 2nd year interns - 9,1% (n=2). In the unvaccinated group (n=58): 93% were year 6 students (n=54), 3.5% were 1st year interns (n=2), 3.5% were 2nd year interns (n=2). According to our survey 64 people, who accounted for 30%, did not receive the vaccine, that they considered the most effective.

In accordance with the study design, in the next step, all vaccinated individuals who had completed the full course and received only one dose of vaccination were combined into one group numbering 210 people "vaccinated at the time of this study".

The mean age among the vaccinated individuals was 23 (22; 24), which was not different from the unvaccinated individuals 22 (22; 23) (U=1239, p=0.102); this fact allowed us to state that the groups of vaccinated and unvaccinated individuals were not different in age and were representative.

After a one-way analysis of variance ANOVA of vaccination status indicators depending on the status of education, a significant difference was found F = 6.949312 p-value = 0.00135. The results of Post-hoc analysis of ANOVA with Bonferoni correction are given in Table III. More detailed analysis of the obtained data was confirmed by $\chi 2 = 12.741$ p-value <0.001, which confirms the difference between the group of 6th year students and interns of the 2nd year of study. That is, the level of immunization among 6th year students was lower than among 1st year interns, but it did not differ between the groups of 6th year students and 2nd year interns or between interns of different years of training.

At the third stage of the actual study, an analysis of the reasons for refusal of vaccination among the respondents was carried out according to the design. Thus, among unvaccinated persons (n=58) the proportion of reasons for refusal was structured by rating as follows:

1. having already been sick - 24% (n=14)
2. afraid of getting vaccinated - 24% (n=14)
3. consider the vaccine ineffective - 17.2% (n=10)
4. Their option (total n=8, 13.8%): "weak body at the moment" - 1.7% (n=1), "weak immunity at the moment" - 1.7% (n=1), "dissatisfaction with organization of process at vaccination sites and lack of screening before vaccination, fear of infection at vaccination sites" - 3.4% (n=2), "delayed process" - 3.4% (n=2), "presence of chronic diseases that may contribute to complications" - 3.4% (n=2)
5. vaccine is dangerous - 10.3% (n=6)
6. categorically against any vaccination - 10.3% (n=6)
7. complying with all hygiene rules, so I won't get sick - 6.8% (n=4)
8. pregnancy - 6.8% (n=4)
9. parents against - 3.4% (n=2)
10. religious beliefs - 3.4% (n=2)
11. against methods used by the state to increase the number of vaccinated persons - 3.4% (n=2)

We also analyzed the main reasons that motivated the respondents to get vaccinated. The answers of the respondents were distributed as follows:

1. desire to protect themselves from a severe current- 62.8% (n=132)
2. need for work (it is necessary for my work) - 49.5% (n=104)
3. desire to protect myself from getting sick - 47.6% (n=100)

Comment: according to the research, vaccination does not protect against the infection with coronavirus disease, which may be a sign of insufficient awareness of the effects of vaccination, and subsequently disappointment, which allows us to state a low level of knowledge of the respondents on the essence of vaccine prophylaxis.

4. desire to protect others - 38% (n=80).
5. I was forced to do it - 14.2% (n=30)
6. severely ill with COVID-19 - 2.8% (n=6)
7. travel without interference - 2.8% (n=6)
8. television, internet, and outdoor advertising - 0.9% (n=2)
9. desire to develop a strong collective immunity - 0.9% (n=2)

The group of people who chose the variant "they made me get vaccinated" - 30 people, among them 14 people chose exclusively the variant "they made me get vaccinated" (46,7%), 12 people chose "they made me get vaccinated" and "necessity for work" (40%), four people in spite of this variant chose also socially approved reasons such as: desire to protect themselves from disease, desire to protect themselves from heavy current and desire to protect others (13,3%).

Analysis of answers to the two questions "Have you advised someone to be vaccinated against COVID-19?" and "Have you refused to vaccinate someone against COVID-19?" revealed some features of social position of the presented respondents.

The number of 6th year students who recommended vaccination was 62.9% (n=122), 22.6% (n=44) of 1st year interns, and 14.4% (n=28) of 2nd year interns. Based on the data of our study, vaccinated individuals were more likely to recommend vaccination to others than those

who were not vaccinated. Among the unvaccinated, there were no people who both refused and recommended vaccination at the same time.

According to the analysis of respondents' responses in relation to who the unvaccinated people thought should be vaccinated, the following groups of the population were identified: children - 6.8% (n=4), medical workers - 27.5% (n=16), people over 65 years - 20.6% (n=12), education employees - 17.2% (n=10), State employees - 17.2% (n=10), military personnel - 13.7% (n=8), and 58.6% (n=34) were against the vaccination of COVID-19.

In the opinion of the vaccinated people, the following groups of the population are subject to mandatory vaccination: all 5.7% (n=12), medical workers 64.7% (n=136), persons over 65 years of age 55.2% (n=116), children 18% (n=38), education employees 57% (n=120), civil servants 41% (n=86), military personnel 43% (n=92). COVID-19 vaccination was opposed by 31.4% (n=66). Each respondent could choose any number of population groups to be vaccinated.

DISCUSSION

Disagreements regarding the vaccine received and the most effective, according to the respondents, were recorded in 30% (n=64) of cases. Which, in our opinion, can be related to a powerful advertising campaign implemented by pharmaceutical business workers who paid little attention to the consequences of defending their interests. This created dissatisfaction with the range of available vaccines, slowed the vaccination process in general and undermined confidence in the health care system. In the future, to overcome this, our state had to spend certain resources on real clarification that all registered vaccines were effective.

When analyzing the level of immunization among respondents, depending on their status of education, it was determined that the highest level of vaccination is determined among interns.

The question of «why» medical interns had a higher proportion of vaccinations than 6th year students remains debatable. In our opinion, this may be due to the fact that, in accordance with the laws of our country, workers in the field of providing medical services had to be vaccinated according to the national vaccination calendar, including the vaccine against the coronavirus disease COVID-19. This is evidenced by the fact that the second most common reason for wanting to get vaccinated was «necessity for work». That is, one of the necessary levers on the way to the prevention of infectious diseases remains a strict legislative policy. [10] It may also be due to the fact that interns feel more responsible for their own health, because they are in constant contact with patients. The awareness of young doctors that the safety and survival of medical workers is a key factor in overcoming the global pandemic and minimizing its consequences. [11] Therefore, we consider it necessary to convey to students the concept of the value of the life and health of a medical worker in the first place, who should be a model for the population, especially in primary medical care.[11]

The survey revealed an interesting fact that one-third of vaccinated respondents and two-thirds of non-vaccinated respondents were against mandatory vaccination introduced by the state in response to the initial low level of vaccination coverage. That is, it should be remembered that restrictive measures may cause discontent among some population groups, which will increase resistance.

CONCLUSIONS

The rate of vaccination against COVID-19 among future physicians was 78.3%, which at the time of the study was quite high in Ukraine.

The most significant ranked reasons for refusing COVID-19 vaccination among future physicians were the presence of a preexisting disease (COVID-19) - 24%, fear of vaccination - 24%, and uncertainty about the effectiveness of immunoprophylaxis - 17.2%.

Among the reasons motivating young adults to vaccinate were the desire to protect themselves from the severe course of COVID-19 - 62.8%, the need to work in the medical field - 49.5%, and the desire to protect others from the risks of COVID-19 infection - 38%.

Future physicians' social attitudes were characterized by negative attitudes toward forced vaccination (one in three of those surveyed), and a probably higher percentage among vaccinated individuals regarding recommending vaccination to others - 82.9% versus 34.5% among unvaccinated individuals who also recommended vaccination to others.

Regardless of their own vaccination status, prospective physicians felt that health care workers, those over 65 years of age, and educators were subject to mandatory vaccination.

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ORIGINAL ARTICLE

SPECIFIC FEATURES OF THE ORAL MICROBIOME IN YOUNG CHILDREN WITH LARYNGOPHARYNGEAL REFLUX AND ITS ROLE THE DEVELOPMENT OF RECURRENT RESPIRATORY DISEASES

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ABSTRACT

The aim: To examine the composition of the oral microbiome in young children with laryngopharyngeal reflux (LPR) and its role the development of recurrent respiratory diseases.

Materials and methods: There were examined 38 children with physiological gastroesophageal reflux (GER), 18 children with LPR who had a medical history of recurrent bronchitis and 17 healthy children (control group). The study included the collection of anamnesis, objective examination. The qualitative and quantitative microbial composition of the upper respiratory tract was performed obtained by oropharyngeal deep swab. Salivary pepsin level and IL-8 were determined by enzyme-linked immunosorbent assay.

Results: This research showed significant alterations in the oral microbiome of patients with GER and LPR as compared to healthy control. We found that gram-negative microbiota such as *Klebsiella pneumoniae, Escherichia coli, Proteus vulgaris*, *Proteus spp.* and *Candida albicans* were identified in children with GER and LPR compared to the healthy control. At the same time, the amount of such a representative of the normal microbiome as *Streptococcus viridans* in children with LPR was sharply reduced. There were established a much higher mean salivary pepsin level of the patients with LPR than in the GER and control group. We found the association between high pepsin levels, saliva IL-8 levels and frequency of respiratory pathology in children with LPR.

Conclusions: Our study confirms that increased levels of pepsin in saliva are a risk factor for recurrent respiratory diseases in children with LPR.

KEY WORDS: GER, LPR, oral microbiome, children

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INTRODUCTION

Gastroesophageal reflux (GER) is a common and normal physiological process in children whatever their age. Gastroesophageal reflux is generally defined as retrograde passage of gastric contents into the esophagus with or without regurgitation/vomiting [1]. Laryngopharyngeal reflux (LPR) is the result of the reflux of gastric contents into the laryngopharynx mucosa [2]. LPR can lead to upper respiratory tract pathologies via three mechanisms. The most important mechanism is a direct noxious effect of gastric contents on the mucosa, causing its swelling, mucus hypersecretion, ciliary dyskinesia, and stimulation of the secretion of inflammatory mediators. Another mechanism consists of triggering a vagus nerve response supported by excessive vagal reactivity, which is observed in patients with LPR compared with healthy people. The third hypothesis postulates an association between LPR with changes of oral microbiome [3, 4].

Several studies show that acid does not damage the mucosa by itself. These studies state that pepsin plays the key role in mucosal injury, which can explain the mechanism of injury in the laryngeal mucosa in non-acidic reflux [5]. Pepsin is considered to be the most aggressive protease in the gastroduodenal refluxate. Pepsin is undetectable in the laryngeal mucosa of healthy individuals [6]. Pepsin is refluxed to the extraesophageal areas where it adheres to the epithelium [7]. Pepsin can be found in many different tissue samples such as laryngeal mucosa, paranasal sinus mucosa, saliva, middle ear effusion, tracheal secretions and bronchoalveolar lavage fluid [8]. Pepsin remains active up to pH 6.5. Then, it is inactivated but is still stable and can be reactivated if the pH drops. The most recent studies show that pepsin can also be reactivated within the acidic intracellular environment after receptor mediated uptake of pepsin by laryngeal epithelial cells, even if the pH in the throat is up to 7.4.

Moreover, the laryngeal epithelial cells are susceptible to pepsin even in a non-acidic environment because pepsin stimulates the expression of many proinflammatory cytokines and receptors, such as IL6, IL8, TNF-α and others [4, 9].

IL-8 is a multifunctional cytokine that participates in in both acute inflammation and chronic inflammatory injury associated with LPR [10]. The main function of IL-8 is to attract neutrophils and activate them. Activation of neutrophils by IL-8 results in development of enzymes involved in tissue degeneration and development of lesions. IL-8 plays an important role in immunity of the oral cavity [11].

The saliva is the main regulator of the total number of microorganisms in the oral cavity. Changes in its physical and chemical properties as a result of LPR may contribute to oral dysbiosis [2]. Poor oral health has long been recognized as a clinical risk factor for developing lung infections. There are no data about influence of LPR on the composition of the oral microbiome in young children and its role the development of recurrent respiratory pathology.

THE AIM

The aim of this study was to examine the composition of the oral microbiome in young children with LPR and its role the development of recurrent respiratory diseases.

MATERIALS AND METHODS

The study was pilot in nature, and the article discusses results obtained during the pilot stage.

In accordance with the aim of the study, we performed a clinical and laboratory examination of 73 children aged from 3 months to 7 years, who were subsequently subdivided into the 2 study groups. First group included thirty-eight children with physiological GER (mean age - 6.8 ± 0.9 months). Second group included eighteen children with LPR who had a medical history of recurrent bronchitis (mean age - 4.6±0.04 years). Seventeen clinically healthy children (mean age – 5.7±0.3 months) constituted the control group. Signed informed consent was obtained from all participants before the study.

All the study subjects were screened for the criteria of exclusion: acute inflammatory diseases of the gastrointestinal tract, its congenital pathology (pylorostenosis, esophageal atresia, congenital diaphragmatic hernia), chronic hereditary and congenital bronchopulmonary diseases (bronchial asthma, cystic fibrosis, primary ciliary dyskinesia, congenital malformations of bronchus and lungs), severe organic lesions of the central nervous system, accompanied by dysphagia, children had taken antibiotics in the past 1 month.

Anamnestic data for each study subject were collected using a semi-structured questionnaire and interviews with parents, as well as through analysis of the children's medical records. Clinical-anamnestic criteria and laboratory-instrumental methods (including fibroesophagogastroscopy) were used for diagnostic GER.

Salivary pepsin levels were determined for the diagnosis of LPR. All parents were instructed to provide three saliva samples of 1 ml volume: in children of the 1st group – the first on waking prior to eating, immediately after regurgitation and 1 hour after the meal; in children of the 2nd and control group – the first on waking prior to eating, half an hour before the meal and 1 hour after the meal. We used an enzyme-linked immunosorbent assay (Human Pepsin ELISA Kit, Elabscience, USA) for quantitative determination of the studied salivary pepsin levels. The sensitivity of the method is 37.50 pg/ml, the detection range – 62.50-4000 pg/ml. Specificity of the method: this kit recognizes human PP in samples.

Salivary IL-8 was measured by enzyme-linked immunosorbent assay kit (Human IL-8 ELISA Kit, Elabscience, USA). The sensitivity of the method is 4.69 pg/mL, the detection range – 7.81-500 pg/ml.

The study of qualitative and quantitative microbial composition of the upper respiratory tract was performed obtained by oropharyngeal deep swab according to the standard method. The collection of biomaterials was carried out in the morning on an empty stomach, after using the toilet in the oral cavity, into a disposable sterile sealed container. Clinical samples were delivered to the laboratory within an hour after the sampling of the material and inoculated on ready-made nutrient media made at the factory.

Statistical data processing was performed using standard statistical analysis software Statistica for Windows v. 6.1. Shapiro-Wilks test for normality was run to evaluate the distribution of quantitative variables. Numerical data were expressed as medians (Me) and the interquartile range (IQR, [Q25; Q75]) or as mean ± standard error according to their parametric distribution. The Mann-Whitney U-test was used to evaluate the differences between the independent groups for quantitative values, and Pearson's χ^2 test was run to compare the qualitative characteristics in the study groups. Statistical significance was defined as $p<0.05$.

This study was conducted according to the declaration of Helsinki on Biomedical Research Involving Human Subjects. Ethical approval for the research protocol was granted by the Commission on Biomedical Ethics of the Dnipro State Medical University.

RESULTS

The GER ang LPR occurrence in boys and girls was similar. There were 52.6 % of girls and 47.4 % of boys in 1st group and 44.4 % of girls and 55.6 % of boys in 2nd group.

The regurgitation incidence in children of the 1st group was more often before 3 months of age (73.7 % vs. 44.4 %, $\chi^2 = 4.55$; $p<0.05$), and in children of the 2nd group – after 6 months of age (33.3 % vs. 5.3 %, $\chi^2 = 7.86$; $p<0.01$) (Table I).

Children of the 2nd group were more likely to be born prematurely than children of the 1st group (55.6 % vs. 21.1 %, $\chi^2 = 6.67$; OR=4.7 [1.4-15.8]; $p<0.05$) and more often were artificial feeding in the first year of life (66.7

Table I. Clinical and anamnestic characteristics of children of 1st and 2 nd groups, n (%)

Characteristics	1 group (n=38)	2 group (n=18)	χ^2	p	OR [95%CI]
Age, M±m	6.8 ± 0.9 months	4.6 ± 0.04 years			
Threatened abortion	14 (36.8)	5 (27.8)	0.45	p>0.05	0.7 [0.2-2.2]
Toxicosis	16 (42.1)	7 (38.9)	0.05	p>0.05	0.9 [0.3-2.8]
Urogenital pathology in mother	4 (10.5)	2 (11.1)	0.00	p>0.05	1.1 [0.2-6.4]
Fetal hypoxia	6 (15.8)	4 (22.2)	0.34	p>0.05	1.5 [0.4-6.3]
Delivery was:					
natural	20 (52.6)	8 (44.4)	0.33	p>0.05	0.7 [0.2-2.2]
C-section	18 (47.4)	10 (55.6)	0.33	p>0.05	1.4 [0.5-4.3]
Child was born:					
full-term	26 (68.4)	8 (44.4)	2.94	p>0.05	0.4 [0.1-1.2]
preterm	8 (21.1)	10 (55.6)	6.67	p<0.05	4.7 [1.4-15.8]
post-term	4 (10.5)	-	2.04	p>0.05	-
Feeding was:					
natural	8 (21.1)	2 (11.1)	0.82	p>0.05	0.5 [0.1-2.5]
artificial	14 (36.8)	12 (66.7)	4.37	p<0.05	3.4 [1.1-11.2]
partial breastfeeding	16 (42.1)	4 (22.2)	2.10	p>0.05	0.4 [0.1-1.4]
Violation of the feeding technique	16 (66.7)	3 (16.7)	11.79	p<0.01	0.3 [0.1-1.1]
Regurgitation starts:					
up to 3 months	28 (73.6)	6 (33.3)	8.34	p<0.01	0.2 [0.1-0.6]
from 3 to 6 months	8 (21.1)	4 (22.2)	0.01	p>0.05	1.1 [0.3-4.2]
after 6 months	2 (5.3)	8 (44.5)	12.78	p<0.01	14.4 [2.6-78.9]
Family history of GERD	8 (21.1)	10 (55.6)	6.67	p<0.05	4.7 [1.4-15.8]
Acute laryngitis	5 (13.2)	7 (38.9)	4.80	p<0.05	4.2 [1.1-16.0]
Wheezing during 1 year of life	7 (18.4)	11 (61.1)	10.21	p<0.01	7.0 [2.0-24.4]

Table II. Oral microbiota in children of 1st, 2nd and control group

Oral microbiota	1 group (n=38)	Control (n=17)	p level	2 group (n=18)	Control (n=17)	p level
Gram-positive, n (%)	22 (57.9)	17 (100.0)	p<0.01	16 (88.9)	17 (100.0)	p>0.05
Gram-negative, n (%)	10 (26.3)	0.0	p<0.05	8 (44.5)	0.0	p<0.01
Mixed, n (%)	8 (21.1)	0.0	p<0.05	10 (55.6)	0.0	p<0.001

Table III. Salivary pepsin values of the two groups

Pepsin, pg/ml	1st group (n=38)	2nd group (n=18)	Control (n=17)
M±m	456.8±56.9*	672.0±60.6**	28.5±11.6
Min-Max	139.4-1183.8	432.6-1467.5	0.0-141.7
Me	398.1	560.4	0.0
25-75%	255.3-608.3	514.0-863.9	0.0-28.7

* - difference between 1st and control group, $p<0.05$

** - difference between 2nd and control group, $p<0.05$

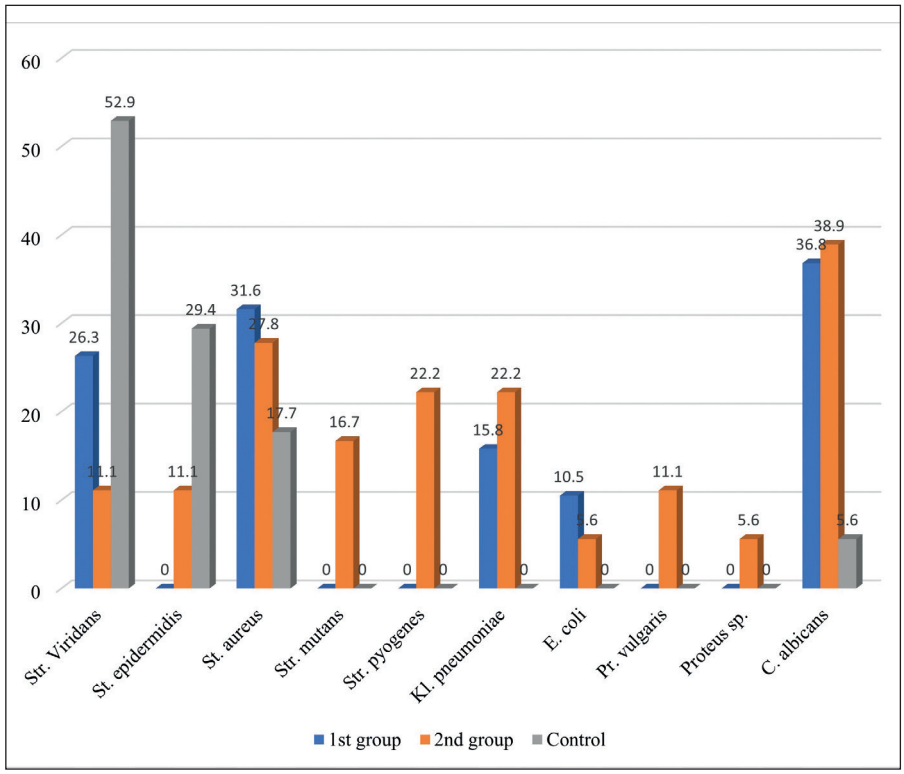

Fig. 1. Oral microbiome in children of 1st, 2nd and control group.

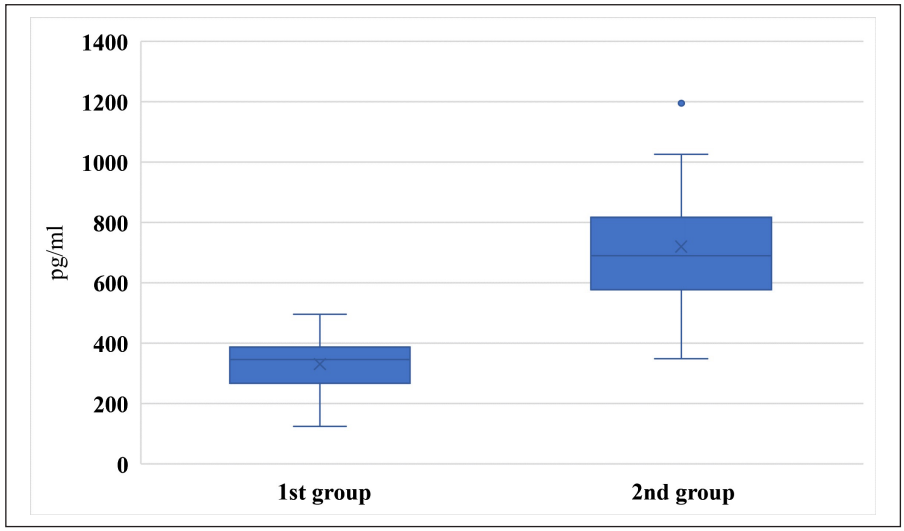

Fig. 2. The average level of saliva IL-8 in patients of the 1st and 2nd groups

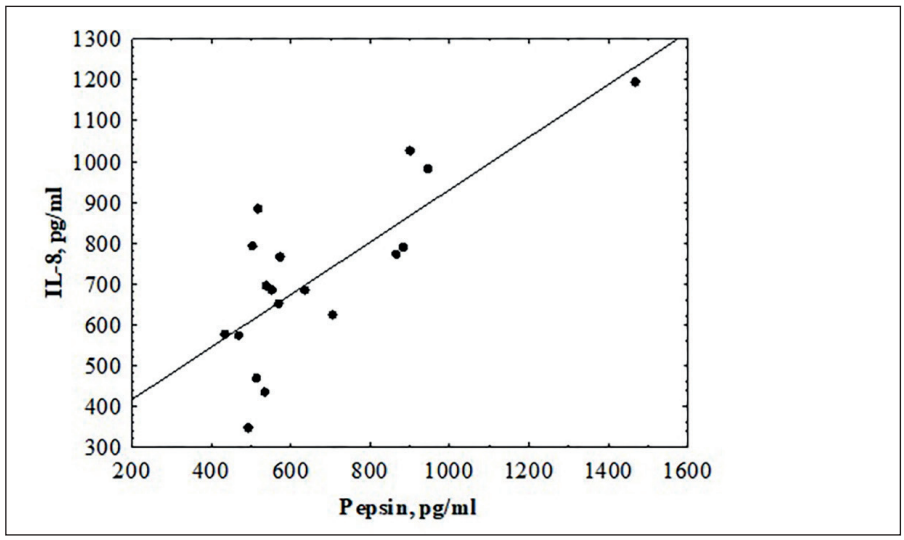

Fig. 3. Correlation between pepsin level and saliva IL-8 in children of 2nd group

% vs. 36.8 %, $\chi^2 = 4.37$; OR=3.4 [1.1-11.2]; $p<0.05$). No significant correlations were found between the severity of the regurgitation incidence and the nature of breastfeeding in both the 1st and 2nd groups.

Violations in feeding technique were more common in children of the 1st group (66.7 % vs. 16.7 %, $\chi^2 = 11.79$; $p<0.01$), among them the most common was aerophagia - in 33.3 %, as well as feeding the child in a supine position - at 16.7 %.

Family history of GERD was observed significantly more often in children of the 2nd group (55.6 % vs. 21.1 %, $\chi^2 = 6.67$; OR=4.7 [1.4-15.8]; $p<0.05$).

Respiratory pathology during the first year of life occurred more often in children of the 2nd group and was characterized by acute laryngitis (38.9 % vs. 13.2 %, $\chi^2 = 4.80$; $p<0.05$) and wheezing (61.1 % vs. 18.4 %, $\chi^2 = 10.21$; $p<0.01$). The children of the 2nd group after 1 year had recurrent acute bronchitis and laryngitis, the average frequency of which was 3.6±0.2 per year.

Microbiological examination (Table II) showed that in children of the 1st and 2nd groups in comparison with the control was dominated by gram-negative and mixed microbiota.

Gram-negative microbiota was identified in 26.3 % of samples in children of 1st group and in 44.5 % of samples in children of 2nd group and was not detected in any samples in the control group.

There was no significant difference between the representatives of the gram-negative microbiota in children of the 1st and 2nd groups. *Klebsiella pneumoniae* (15.8 %) and *Escherichia coli* (10.5 %) were found among the representatives of gram-negative microbiota in children of the 1 st group (Fig 1). *Klebsiella pneumoniae* (22.2 %), *Proteus vulgaris* (11.1%) and *Proteus spp.* (5.6%) and *Escherichia coli* (5.6 %) were found in children of the 2 nd group.

The mixed microbiota was identified in 21.1 % of samples in children of 1st group and in 55.6 % of samples in children of 2nd group and was not detected in any samples in the control group.

Staphylococcus aureus was the most common among representatives of gram-positive opportunistic pathogens. It was detected in 31.6 % of samples in children of the 1st group, in 27.8 % of samples in children of the 2nd group and in 17.7 % of samples in children of the control group, but no significant difference was found.

The representative of the normal microbiota *Streptococcus viridans* was significantly less frequently identified in children of the 2nd group (11.1 %), compared with the 1st (26.3 %) and control (52.9 %) groups.

Candida albicans was significantly more often identified in children of the 1st (36.8 %) and 2nd groups (38.9 %) compared to the control group (5.6 %).

There were analyzed 178 salivary samples in children for the presence of pepsin. The test was positive in 102 (89.5 %) of 114 samples in the 1st group, in 48 (83.3 %) of 54 samples in the 2nd group and only in 10 (19.6 %) samples of 51 in the control group. The average daily level of pepsin (Table III was significantly higher in children of the 2nd group than in children of the 1st and control groups (672.0±60.6 pg/ml vs. 456.8±56.9 pg/ml and 28.5±11.6 pg/ml, $p<0.05$).

Since saliva IL-8 is a cytokine that play an important role in pathogenesis of inflammatory and autoimmune diseases, based on the positive relationship between IL-8 and neutrophils in patients suffering from pulmonary diseases, the aim of this study was also to investigate the level of saliva IL-8. The level of saliva IL-8 was significantly higher in children of the 2nd group compared to the 1st one (720.1±50.1 pg/ml vs. 331.2±22.6 pg/ml, $p<0.05$) (Figure 2).

The presented study shows the positive correlation between saliva IL-8 and pepsin level in children of 2nd group ($r=0.78$, $p<0.05$) (Figure 3). The relationship of this salivary cytokine with pepsin levels can cause inflammation and the development of lesions of mucosal lesions.

We also found an association of IL-8 levels with the frequency of acute bronchitis in children of 2nd group ($r=0.73$, $p<0.05$).

In children of the 1st group, the level of saliva IL-8 did not correlate with the pepsin level ($r=0.43$, $p>0.05$).

DISCUSSION

LPR is one of the most common and important disorders of upper airway inflammation. In contrast to GER, LPR can cause chronic laryngeal damage with as few as three reflux episodes per week. Laryngeal mucosal barriers to reflux are significantly weaker than gastroesophageal barriers. Studies implicate pepsin exposure in the damage of laryngeal tissues. Pepsin can accumulate in laryngeal tissue after exposure via receptor-mediated endocytosis. It is postulated that pepsin is activated in the acidic intravesicular environment after endocytosis. In addition, pepsin causes intracellular damage to mitochondria in cultured hypopharyngeal and nasal epithelial cells and changes the expression of genes expressed in stress and toxicity that may correlate to the mechanism of nonacidic reflux injury in LPR [12]. Moreover, the epithelial cells of the throat are susceptible to pepsin even in a non-acidic environment because pepsin stimulates the expression of many proinflammatory cytokines [4]. Cytokines regulate many aspects of the immune response, therefore, along with other factors, they will be useful tools for diagnosing and monitoring the oral cavity, and saliva can be used as diagnostic

material to measure biomarkers released during disease onset and progression [11].

The results of our study established a much higher mean level of saliva pepsin of the patients with LPR than in the GER and control group. As pepsin is excreted only in the gastrointestinal tract below the level of the pharynx, all these results indicate greater retrograde movement of the gastric contents to the level of the upper aerodigestive tract in patients with LPR than in the control group. We also found the positive correlation between pepsin level, saliva IL-8 and frequency of respiratory pathology in children with LPR. IL-8 plays an important role in immunity of the oral cavity. IL-8 is a suspected mediator of reflux-induced esophagitis and exposure of hypopharyngeal cells to pepsin at pH 7 has been shown to induce production of the neutrophil chemoattractant IL-8 in vitro, suggesting a potential role for IL-8 in reflux-mediated inflammation. IL-8 is also elevated in airway secretions in acute severe asthma and observations of negative correlation between sputum IL-8 and forced expiratory volume (FEV1) have been reported in patients with severe asthma [13]. McNally P. et all found an association of high pepsin level and higher IL-8 concentrations in BAL fluid in children with cystic fibrosis (CF). These data suggest that GER is common in children with CF and aspiration of gastric contents is associated with more pronounced lung inflammation [14].

There is a hypothesis that salivary enzymes, inflammatory molecules and peripheral mononuclear cells present in saliva may modify the respiratory epithelium and promote colonization by respiratory pathogens. Oral microbiome and its balance play a major role in an individual's general homeostasis. Any disruption leads to an increase in certain bacterial species, especially Gram-negative ones, associated with the massive production of pro-inflammatory cytokines, which causes or maintains chronic low-grade inflammation [15].

Our research showed significant alterations in the oral microbiome of patients with GER and LPR as compared to healthy control. We found that gram-negative microbiota such as *Klebsiella pneumoniae, Escherichia coli, Proteus vulgaris* and *Proteus spp.* and *Candida albicans* were identified in children with GER and LPR compared to the healthy control. At the same time, we found that in children with LPR, the amount of such a representative of the normal microbiome as *Streptococcus viridans* is sharply reduced. The differences in oral microbiome in patients with GER and LPR as compared to healthy control may be a result of changes in saliva physical and chemical properties and may have effects on airway conditions. Oral bacteria that colonize the oropharynx may be aspirated in the lower respiratory tract, particularly in individuals at high risk of infection such as patients with dysphagia and can lead to recurrent respiratory pathology.

CONCLUSIONS

Our study confirmed that the oral microbiome has features in children with GER and LPR. In young children with physiological GER there is a significant decrease in the normal microflora (*Streptococcus viridans*) compared to the control, as well as the appearance of colonies of gram-negative microbiota. The features of the microbiota in older children with LPR are the almost absence of normal microflora and the dominance of gram-negative opportunistic pathogens (*Klebsiella pneumoniae, Proteus vulgaris and Proteus spp.*) or mixed flora.

The study was established a much higher mean salivary pepsin level of the patients with LPR than in the GER and control group. We found the positive correlation between pepsin level, saliva IL-8 and frequency of respiratory pathology in children with LPR.

Thus, our study confirms that high salivary pepsin levels lead to changes in the oral microbiome, which is a risk factor for recurrent respiratory diseases in children with LPR. The potential identification of both specific enzymatic patterns and microbiome alterations may lead to the development of more individualized treatment plans.

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ORIGINAL ARTICLE

CORRELATION BETWEEN HEPCIDIN AND PROCALCITONIN AND THEIR DIAGNOSTIC ROLE IN PATIENTS WITH COVID-19

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ABSTRACT

The aim: The purpose of this study is to find out the association between procalcitonin and hepcidin in patients with COVID-19, in addition to their role as diagnostic markers.

Materials and methods: A total of 75 patients infected with coronavirus were included in the current study, their age is ranging between 20 to 78 years. Those patients was hospitalized in Al-Sadr Teaching Hospital in Najaf, in Iraq. This study also included 50 healthy subjects which are volunteers and considered as a (control group). Biomarker (procalcitonin and hepcidin) measurements were achieved by electrochemiluminescent immunoassay (ECLIA) in the Elecsys immunoassay system.

Results: The present study showed a significant increase the serum cencentration of hepcidin and procalcitonin in patients with COVID-19 as compared tohealthy subjects. There was a highly significant increasing($p < 0.01$) in hepcidin and PCT level in patients with severe infection comparing to other catgaries.The current study also revealed that the sensitivity values of the markers were: 0.88%, 0.85 for procalcitonin and hepcidin respectively, which indicate high diagnostic power.

Conclusions: Serum levels of hepcidin and procalcitonin are increased as inflammatory markers in COVID-19 patients with relatively high sensitivity. It seems that these imflammatory markers obviously elevate in the severe cases COVID-19dusease.

KEY WORDS: Covid-19, hepcidin, procalcitonin, imflammatory markers

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INTRODUCTION

The novel coronavirus called "severe acute respiratory syndrome coronavirus 2" (SARS-CoV-2) was appeared in the end of 2019, when it was identified in China [1]. The pandemic status of COVID-19 then distributed extensively overall the world [2]. Both versions of this virus get in to the host cell through their common receptor [3]. The clinical signs and symptoms of coronavirus disease very greatly, including severe, moderate and mild forms. Therefore, the treatment of patients is corresponding to the severity of the clinical maninfestation. Of this clinical biomarker, serum procalcitonin (PCT) has been considered as a significant marker that increase significantly in patients with severe COVID–19 [4, 5]. This disease is not only restricted to the lungs, but the transport of the virus into the bloodstream has resulted in the invasion of almost all the body systems, including the liver, heart, kidney, brain, skin, eyes and intestine [6]. PCT is a protein that composed of 116 aminoacids and is the calcitonin peptide precursor, it is present in small concentrations in the blood stream (≤0.1 ng/mL) and acts as an proinflammatory marker of bacterial and viral infections. It was observed that serum procalcitonin concentrations to be very elevated (6–53 ng/mL) in clients with strong infections that are caused by bacteria comparing to clients with local mild infections caused by bacteria and viruses (0.1–1.5 ng/mL) [7, 8]. There are increasing evidences that in severly sick patients, there are many markers of strong inflammatory responses, which are composed of high serum levels of procalcitonin (PCT), C-reactive protein (CRP), hyperhepcidinemiaand D-dimer. These results suggested a possible critical role of a cytokine surge in the pathophysiology of COVID-19 [9-12]. PCT is a none of the important inflammatory biomarker, it also plays a crucial role in the development of inflammatory responses. Elevated serum cencentrations of PCT may be due to a cascade of reactions during acute inflammation; PCT concentration ranging between 0.05 and 1.00 ng/mL and used in the identification of

chronic inflammatory with decreased severity [13]. During the clinical manifestation of Covid-19 infection interleukin-6 (IL-6) and PCT increase both drastically [14]. Hyperhepcidinemia is resulted from the strong inflammatory response that is caused by infection, and is correlated with attendence to the COVID-19 isolation unit and high death rate and is considered as an evidence to detect patients with high–risk to direct the therapeutic implementation to manage inflammatory responses [15].

THE AIM

The objective of this study is to find out the association between procalcitonin and hepcidin in COVID-19 patients, in addition to their role as diagnostic markers related to acute inflammatory response.

MATERIAL AND METHODS

STUDY SAMPLE

A total of 75 patients with confirmed diagnosis of COVID-19 have been included in this study, their age is ranging between 20 to 78 years. Those patients was hospitalized in Al-Sadr Teaching Hospital in Najaf, in Iraq. This study also included 50 healthy volunteers used as a control. In the control group, male and female were 28 and 22 respectively, but, in COVID-19 patients, gender distribution was 42 male and 33 female.

MEASUREMENT OF BIOMARKERS (PCT AND HEPCIDIN)

All the patients were classified as "mild", "moderate", and "severe" based on the clinical maninfestation with the assistance of specialized physicians, diagnostic tests, and scanning computed tomography (CT). Moderate and severe cases were identified according to the pneumonia manifestations on CT imaging and had a (%SpO_2) of less than 40%. Severe levels were diagnosed as they require mechanical ventilation because of respiratory failure. The healthy voluntreers (control group) consists of 50 healthy people who have not shown previous and signs and symptoms of COVID-19. The study was conducted in the isolation unit at Al-Sadr Teaching Hospital in Najaf in Iraq. Biomarker (procalcitonin and hepcidin) measurements were achieved by electrochemiluminescent immunoassay (ECLIA) in the Elecsys immunoassay system. All biomarker parameters were measured in both patients and the control group (PCT, kit reference range: less than 0.1 ng/mL), (hepcidin, kit reference values: 1.23 – 36.46 ng/mL). An increase in deviation from normal values may be at risk for infection COVID-19. Statistical analysis was done by SPSS program (version 24) in which both descriptive (frequency and percentage) and inferential statistics (independent t-test and

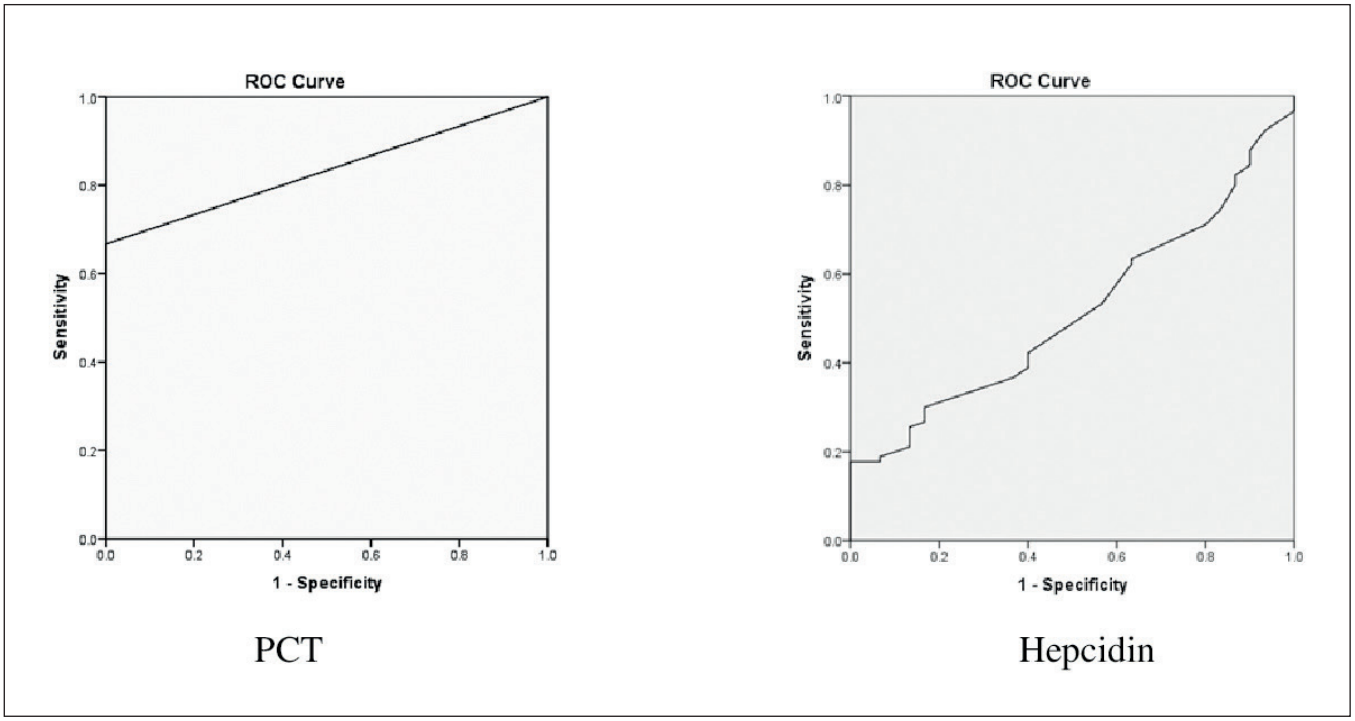

Fig. 1. The areas under the curve (AUC) of the biomarkers for the diagnosis of Covid-19 for PCT (A) and hepcidin (B).

Table I. Differences in procalcitonin and hepcidin between patients with COVID-19 and healthy control subjects.

Statistics	Control group, n = 50	Patients group, n = 75	p
PCT [ng/mL]			
Mean	0.043	1.33	<0.01 HS
SD	0.011	0.34	
Hepcidin [ng/mL]			
Mean	3.23	77.45	<0.01 HS
SD	1.5	6.2	

HS: High Sigmnificant at p<0.01

Table II. ANOVA table for the differences in procalcitonin and hepcidin in patients subgroups classified according to disease severity.

Subgroups	Mild n = 32	Moderate n = 38	Severe n = 24	F-Test p-value
PCT(ng/L)	1.24±0.12	1.36±0.13	1.56±0.16	14.56 (0.01) HS
Hepcidin	22.87±4.13	67.42±8.13	101.65±12.53	23.55 (0.006) HS

HS: High Sigmnificant at p<0.01

Table III. Differences in procalcitonin and hepcidin between male and female patients.

Biomarkers	Female n = 42	Male n = 33	Independent T-Test p-value
PCT(ng/mL)	1.35±0.15	1.39±0.17	1.56 (0.22) NS
Hepcidin(ng/mL)	47.66±6.77	88.69±9.51	9.78 (0.01) HS

NS: Non-Sigmnificant at p>0.05; HS: High Sigmnificant at p<0.01

Table IV. The areas under the curve (AUC) of PCT and hepcidinfor the diagnosis of Covid-19.

Characteristic	PCT	Hepcidin
AUC	0.81	0.74
Sig.	0.01	0.05
Cut-off Point	0.043	3.23
Sensitivity [%]	0.88	0.85
Specificity [%]	0.63	0.61
PPV	0.83	0.63
NPV	0.73	0.53

NPV: Negative Predictive Value; PPV: Positive Predictive Value ;

F-test) have been used. Pearson correlation coefficient (r) was utilized to assess correlation between markers. To identify the power of diagnosis of procalcitonin and hepcidin, the estimating of areas under the curve (AUC) of these biomarkers for the diagnosis of Covid-19 has been applied in the current study.

RESULTS

The current study included 75 hospitalized clients with COVID-19, in which 23 cases were mild, 38 cases moderate and 24 cases were severe. They were diagnosed by the doctor based on the patient's condition, computerized tomography scanning the blood test, and the degree saturation of oxygen.

The findings of the current study showed a significant elevation (p <0.01) that was observed in the PCT is levels in patients with COVID-19 (1.33 ng/mL) as compared with control group (0.043 ng/mL) and as it is shown in table I. In this study a significant increase (p <0.01) in serum levels of hepcidin was seen in patients with COVID-19 (77.45 ng/mL) comparing to control group (3.23 ng/mL), as also shown in table I.

The PCT levelwas significantly increased (p<0.05) in the severe subgroup (0.56±0.16**)** mg/L as compared with mild and moderate cases (table II.).

The serum hepcidin level was significantly (p<0.05) increased in the male group (88.69±9.51) mg/L as compared to female (47.66±6.77). The was no significant difference in the concentration of PCT between the two genders (table III).

To identify the diagnostic power of procalcitonin and hepcidin, the areas under the curve (AUC) of these biomarkers for the diagnosis of Covid-19 have been shown in table IV.. This table reveals that the sensitivity values of the markers were: 0.88% and 0.85% for procal-

citonin and hepcidin respectively, which indicate high diagnostic power. However, that the specificity values of the markers were: 0.63% and 0.61% for procalcitonin and hepcidin respectively (Fig.1).

DISCUSSION

The present study showed a significant elevation of the serum level of hepcidin and procalcitonin in patients with COVID-19 comparing to control group. These results come in agreement with studies that indicated COVID-19 status as an inflammatory conditiond results in high levels of hepcidin and PCT [16-20]. The increaed levels of PCT might be correlated with the increased production of cytokines during inflammation in patients with severe COVID–19 status. PCT synthesis is activated by interleukins which are released by destruction of patientslung tissue infected with COVID–19. Accordingly, a high level of PCT may be a critical proinflammatory predictive marker for diagnosis of disease and its prognosis in patients with mild COVID–19 [21]. There was a highly significant increase in hepcidin level among patients according to severity; the level was high in severe cases as explained in Table II. These results correspond with the studies [19, 20]. Many recent researches have shown the effect of hyperhepcidinemia in the assessment of the prognosis of disease in patients with COVID-19 and the difference was highly significant ($p<0.01$) [22, 23]. The inflammatory responses modulate iron metabolism. Hepcidin is an inflammatory marker that is recently identified and modulates iron intake via control of the digestive tract and iron liberation, which are both significant passage ways participated in the control of iron concentration for homeastasis. Hepcidin concentrations are increased in inflammatory responses that are caused by infections [24]. The suggested mode of action for hepcidin production and elevation by pro-inflammatory interleukins such as interleukin-1β (IL-1β), interleukin 6 (IL-6) and tumor necrosis factor-alpha (TNF-α). This cytokine activation resulted in increased inflammation that results in cellular damage and the secretion of hepcidin [25, 26]. It was found that patients with hyperhepcidinemia were to have high death rates, but here is an argument about this increase is due to consequence of the inflammation or because of the pathogenesis the virus [27]. Hepcidin levels have been observed to rise as a result of a cytokine storm, and it has also been seen in severe COVID-19 patients. Many inflammatory cytokines are rapidly produced during the cytokine storm in COVID-19, including IL-6, TNF-, IL-1, IL-12, and IFN-, which activate hepatocytes, Kupffer cells, and macrophages to manufacture hepcidin. Hyperhepcidinemia syndrome is an unregulated and defective immune response linked to macrophage activity. Notably, hepcidin is not only the outcome of excessive inflammation, but it also plays a harmful function in the inflammation process by stimulating the development of numerous pro-inflammatory mediators through its interaction with T-cell immunoglobulin and mucin domain 2 (TIM-2) [23]. As a result, the innate immune system will reduce iron bioavailability in order to inhibit virus multiplication during acute infection. The concentrations of the liver-derived iron hormone hepcidin – the key regulator of iron homeostasis – could increase and block the activity of the transporter ferroportin, which carries iron out of the cells, and thus reducing the quantity of iron absorbed from the diet, causing cellular sequestration of iron, through interleukin-6 and Toll-like-receptor-4 dependent pathways (i.e., principally in hepatocytes, enterocytes, and macrophages). Increased intracellular iron sequestration leads to an increase in cytosolic hepcidin, which polymerizes and stores iron to minimize free radical damage caused by iron [22]. Increased levels of hepcidin due to cytokine storm and secondary hem phagocytic lymphohistiocytosis were recorded in patients with severe COVID-19 [23]. The current study has an agreement with some prevcious studies that have shown a significant elevation in hepcidin in male comparing to female, this difference may be attributied to the hormonal changes between the two sexes that affect iron storage and metabolism [28, 29]. The sensitivity values of the markers were: 0.88%, 0.85 for procalcitonin and hepcidin respectively, which indicate high diagnostic power. This table reveals that the sensitivity values of the markers were: 0.88% and 0.85% for procalcitonin and hepcidin respectively, which indicate high diagnostic power. This result may come in accordance with Zhou et al. which found that hepcidin more than 32.7 ng/mL indicated the severity of COVID-19 and predicted (56.5% for sensitivity, 97.3% for specificity) [19]. To the best kneledge of the researchers, the is no previous study that identified specificity and sensitivity of PCT in COVID-19 patients. The limitation of the current study may be in the small sample size.

CONCLUSIONS

This research has recorded an association between serum hepcidin levels and Procalcitonin levels in COVID-19 patients, as well as an important link between increased hepcidin and PCT levels and COVID-19 severity. The results can be used as atinflammatory biomarker with a relatively high sensitivity to improve the diagnosis of COVID-19 disease

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ORIGINAL ARTICLE

ON ISSUE OF HIGH-QUALITY STOMATOLOGICAL SERVICE IN UKRAINE

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ABSTRACT

The aim: To detect the main problems regarding available high-quality stomatological service in Ukraine and define their main solutions.

Materials and methods: The authors used general scientific methods of synthesis, generalization, scientific data interpreting, systemic approach method, medical statistic method, and analysis of the activity of state and private institutions dealing with stomatological service in Ukraine. The paper is based on the materials of a representative selective study of Ukrainian households, held by the State Committee of Statistics of Ukraine to study people's self-estimation of their health and the availability of certain medical services.

Results: Most citizens of Ukraine (60-80%) are treated in the state/public healthcare sector. Though, during the last century, a decrease in dental visits per citizen in the state and public institutions has been noted, as well as a decrease of all medical service types' volume, offered in the mentioned institutions. In Ukraine the observed trends are represented as the decrease in the network institutions number, insufficient budgeting of state/public medical institutions, prevailing commercial characteristics of stomatological service and people's low income, which leads to decreased affordability, and quality of medical service, thus negatively affecting people's health.

Conclusions: The fundamental studies of the quality assessment show that the medical service requires strong structure, process quality, and result quality. The quality of medical service organization is extremely important and it should be maintained high on all levels of management and treatment processes, regarding the conditions of medical process and resources of medical organizations. Medical service should be patient-centered. To solve the problem, the entire state system of quality management is required in Ukraine.

KEY WORDS: Stomatological service quality, state/public stomatological institutions

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INTRODUCTION

The main task of state healthcare policy is to answer the people's need for high-quality accessible and affordable medical care. Provision of high-quality and accessible/affordable medical service is a most important healthcare issue, management of which presents with difficulties.

According to professor V.D. Vagner, the main characteristics of the stomatological service quality are its safety, clinical and economic efficiency, as well as its timely character; while its criteria are: adherence to standards, clinical course without complications, and satisfaction of the patient with its results [1].

The authors consider that high-quality medical service is a timely medical service, provided by qualified medical personnel, which corresponds to the medical service standards. The accessibility/affordability and quality of medical service make up the main components of modern medical science.

The medical service quality is affected by various cause-effect factors. Professor A.L. Lindenbraten defines the following factors: the resource availability in the institution providing medical service; institutions and medical personnel motivation for optimizing their activity outcomes and the behavior of medical service customers. He regards medical staff as that with an important role, as the medical personnel must be high-qualified, which can be reached through improved quality of the basic and further continuous medical education as well as registering faults and errors of medical staff [4].

N.B. Pavlov et al. [7] consider that the stomatological service quality is affected by the prevention and treatment methods, materials and technologies, and the scientific support of the service. Of great importance are also management combined with professional standards, raising qualification and standard documents. Increased medical service expenditures make up an important condition of its improved quality.

The results of special epidemiological studies evidence about a rather high level of stomatological morbidity in Ukraine, which significantly exceeds that one in Europe. The incidence of dental caries of temporary teeth in 6-year-old children is 87.9% (in European Union countries 20%), with the "carious, filled and extracted teeth" rate of 4.6; while the incidence of the permanent teeth caries in 12-year-old children is 72.3%, with the "carious, filled and extracted teeth" rate of 2.75 (in European Union countries 1.5) [6]. In 2016 eighty-eight percent of Ukrainians were diagnosed with tooth caries, 86-90%-with parodontal diseases, 60-75% – with dental-jaw disorders, and 75-85% required dental prosthetics [2]. Poor stomatological health level detected in Ukrainians stipulates for high relevance of the need for stomatological service increased availability and improved quality.

Poor stomatological health, presumably that of children, badly affects their overall health throughout their life. The President of the Association of Ukrainian stomatologists I.P. Mazur states that "stomatology is not luxury, but the method of preserving people's health" [5]. Thus, providing availability and high quality of stomatological service should be regarded not only as a medical, but significant medical-social and social-economic problem in Ukraine, which needs urgent attention.

The highest achievements in decreasing stomatological morbidity can be reached owing to the prevention of stomatological diseases. This needs the purposeful financing of the problem from the state and municipal budgets [2].

THE AIM

The purpose of the study is to detect the main problems regarding the provision of available high-quality stomatological service in Ukraine and define the main solutions.

MATERIALS AND METHODS

The authors used general scientific methods of synthesis, generalization, scientific data interpreting, systemic approach method, medical statistic method, and analysis of the activity of state and private institutions dealing with stomatological service in Ukraine. The paper is based on the materials of a representative selective study of Ukrainian households, held by the State Committee of Statistics of Ukraine to study people's self-estimation of their health and availability/affordability of certain medical services. The performance analysis of state(public) and private stomatological institutions, as well as the extent of certain stomatological services delivered to the people of Ukraine is based on the reports № 20 «Reports of legal bodies, regardless of the registered law state, and physical body-entrepreneur, dealing with medical practice" from 2008, 2012, 2017, 2018, 2019 and 2020, represented in the statistical reference books of the Center of medical statistics of Ministry of Health of Ukraine [9-11]. The analysis is based on statistical frequency distribution, tabular summary, generalization, and comparison. The availability data of stomatological service were analyzed by the representative selective studies of households, held by the State Committee of statistics of Ukraine in 2020, published in the statistical reference book "People Self-estimation of health and availability of certain types of medical service" [8]. These selective surveys of households regarding their health and availability of certain types of medical service make up a theme module of permanent study of the households life conditions. The selective combination of the studied households represents all population of Ukraine.

RESULTS

Nowadays, there are no definite criteria for evaluation of the medical service quality (including the stomatological one), which can be related to diagnosis, treatment, prevention, and disease outcomes. The issue needs analysis of individual factors by experts, thorough medical documentation analysis, and evaluation by the medical society. Though, a logical connection between the notions of "Stomatological service quality" and "Optimizing stomatological service" is evident. Due to optimization, the resilience of basic medical institutional characteristics increases, as well as the quality of provided services, their correspondence to the customers' requirements, environmental requirements and social responsibility; and medical personnel labor safety and health safety also.

The thorough studies of the healthcare service quality were held by A. Donabedian [13]. In the late 60ies of the previous century, he was the first to suggest the systemic classification of the quality analysis methods, which was later called the "Donabedian triad" (structure-processes-results) and it allowed to estimate the quality criteria.

The Donabedian triad is still relevant nowadays:

- structure quality is represented with the personnel qualification, availability and condition of the equipment as well as its rational use;
- process quality means that the medical service quality was appropriate when the treatment and diagnosis were appropriate to the patient's condition,

Table I. Dynamic pattern of the stomatological services volume, offered to the Ukrainians in state/public stomatological institutions*

Value	2008	2012	2017	2018	2019	2020
Mean average of stomatological visits made by 1 citizen	1.1	1	0.8	0.8	0.7	0.4
Mean average of stomatological visits made by 1 adult	1	0.9	0.7	0.7	0.6	0.3
Mean average of stomatological visits made by 1 child	1.7	1.6	1.3	1.2	1.1	0.5
Specific gravity of regular stomatological check-ups among the adults (in %)	22.1	21.3	17.2	15.5	13.4	6.6
Specific gravity of stomatological sanations a mong the adults (in %)	22.1	23.6	18.7	17.3	15.1	7.6
Specific gravity of regular stomatological check-ups among the children (in %)	70.9	69.5	56.4	51.7	43.1	17.2
Specific gravity of stomatological sanations among the children (in %)	70.9	41.9	33.8	30.9	25.9	12.6
Number of stomatological operations in outpatient departments (per 10 000 people)	85,6	79,8	62,2	59,1	56,42	41,2
Number of children who completed orthodontic treatment (per 10 000 children)	66.1	61.6	58.8	53.4	50.5	26.3
Number of adults with made dental prostheses (per 100 people)	1.4	1.2	0.8	0.8	0.7	0.5

*The table is based in the data of the Center of medical statistics of Ministry of Health of Ukraine.

Table II. Distribution of the services provided to the adult Ukrainians between the state/public and private stomatological institutions (in%)*

Type of stomatological institution	2008	2012	2017	2018	2019	2020
Stomatological visits (in %)						
State	87.3	84.4	79.8	77.3	73.4	63.1
Private	12.7	15.6	20.3	22.7	26.6	36.9
Totally	100	100	100	100	100	100
Stomatological sanations, during regular check-ups and patients' referrals (in%)						
State	86.6	83.1	78.0	74.5	70.0	56.5
Private	13.5	16.9	22.0	25.5	30.0	43.5
Totally	100	100	100	100	100	100
Dental caries treatment (in %)						
State	83.4	79.5	74.2	71.5	65.3	51.4
Private	16.6	20.5	25.8	28.5	34.7	48.6
Totally	100	100	100	100	100	100
Oral mucosa diseases treatment course (in %)						
State	83.0	80.7	73.0	71.3	58.24	41.41
Private	17.0	19.3	27.0	28.7	41.76	58.59
Totally	100	100	100	100	100	100
Stomatological operations (in%)						
State	83.0	80.7	73.0	81.2	70.7	73.1
Private	17.0	19.3	27.0	18.1	28.4	26.0
Totally	100	100	100	100	100	100
Stomatological prosthetics (in%)						
State	70.0	58.2	47.7	43.0	40.4	32.5
Private	30.0	41.8	52.3	57.0	59.6	67.5
Totally	100	100	100	100	100	100

*The table contains the data resulting from the individual calculations by the authors, based on the information from the Center of medical statistics of Ministry of Health of Ukraine.

this is the basic principle of the process approach to the quality provision system;
– result quality is a component of medical service that describes the relation between the achieved results with those which were possible to achieve.

It is worth mentioning that the subjective evaluation of the patient is affected by the criteria of a service organization, which are: availability, timely character, safety, succession and persistence, efficiency, patient-centered character as well as scientific-technical characteristics.

Regarding the medical service quality, three main tasks should be recalled: evaluation, provision and management. The evaluation, expertise, audit, and supervision make up the initial stage of the quality assessment activity. If it receives no further continuation, without recommendations on improvement, and assessment of the process management applied technologies, the activity seems to be almost unproductive.

Quality provision is mainly the issue of appropriate resource supply, professional competence and training of medical personnel, application of appropriate technologies.

A crucial component of the medical service quality is its availability (with accessibility and affordability). According to some selective studies of the household, held by the State Committee of Statistics of Ukraine in 2020 (38105 respondents were questioned, considered representatively as the whole population of Ukraine), the share of households in which any member couldn't receive medical service, buy drugs or medical supplies during last 12 months made up 19.2%.

Furthermore, 41.2% of respondents, among those who stayed without medical service, mentioned poor availability of stomatological service, and almost one-third of them – missed dental prosthetics (29.6%).

Among all questioned respondents, the share of those who couldn't make a dental visit in 2020 made up 7.9%, have prosthetics - 5.7%. So, in 2020 about 13.6% of respondents, or one in seven surveyed people couldn't obtain stomatological service, upon the necessity. The main cause of stomatological service poor accessibility (affordability), as 95.1% of respondents consider, was its extremely high cost.

The issue of affordability of stomatological service for most people of Ukraine has been registered, while we note the presence of numerous state (public) stomatological institutions in the country. In 2020 there were 1355 state and public institutions in Ukraine, providing stomatological service, with 168 specialized stomatological clinics, and 1187 general medical institutions with stomatological rooms in their structure. Recently, the number of specialized stomatological policlinics has considerably decreased due to re-organization of policlinics into departments of central regional or district hospitals. The significant decrease in the number of institutions with stomatological departments (rooms) has been mainly predisposed to the rural outpatient departments, which were joined, as the structural units, to the Centers of primary medical service or other institutions, considered before as separate institutions.

Recently private stomatological service has significantly expanded. In 2020 stomatological service in Ukraine was provided by 617 stomatological policlinics and 4467 stomatological private practices. The relation between state stomatologists and private specialists in 2020 was 59.7% to 40.3%.

The authors revealed that, despite the presence of a vast stomatological institutions network within the Ministry of Health of Ukraine, during 2008-2020 a significant decrease in state and public stomatological services volume was noted. In particular, the number of dental visits per one Ukrainian decreased from 1.1 in 2008 to 0.4 in 2020, among adults from 1.0 to 0.3 visits, and among children from 1.7 to 0.5 visits respectively. During that period the volume of the preventive services offered by state and public stomatological institutions has significantly decreased. So, the specific gravity of regular check-ups among adults has decreased from 22.1% in 2008 to 6.6% in 2020, and among children, respectively, from 70.9% to 17.2%; the specific gravity of sanations fell from 22.1% to 7,6 % among adults and from 70,9% to12,6% among the children (See table I).

The significant decrease in surgical, prosthetic, and orthodontic stomatological services in state (public) institutions should be also mentioned. In 2020, compared to 2008, the number of stomatological operations decreased twice (from 85.6 to 41.2 per 10 000 people), the number of children who received orthodontic treatment by 2.5 times (from 66.1 to 26.3 per 10 000 children), and the number of adults after stomatological prosthetics by 2.8 times (from 1.4 to 0.5 per 100 people).

The COVID-10 epidemic represents the cause of an abrupt decline in dental visits and total stomatological service volume in 2020. Though, the trend for such decrease appeared even before the epidemics, which indirectly evidences about decreased availability of stomatological service, caused by reduction of stomatological practices, deficiency of state budgeting for the stomatological service as well as decrease of expenses for free prosthetics and raised prices for orthodontic and other positions.

In Ukraine, the unsatisfactory quality of stomatological service in state (public) institutions is the basic cause of patient churn to private clinics.

The results of the performed analysis of stomatological services distribution between private and state

stomatological institutions (See table II) have shown that in 2020 private clinics dealt with 36.9% of all adult dental visits, 67.5% stomatological prosthetics cases, 48.6% of caries treatment cases, 43.5% cases of regular dental sanations and sanations after stomatological appointments, 26.0 % of out-patient stomatological operations, 58.6% of oral mucosa treatment referrals. A steady trend for increase of the private stomatological practice share within overall stomatological health service was observed in period of 2008-2020. Private stomatological institutions fortified their role in the prosthetic stomatological service branch.

The medical service quality considerably depends on the budgeting of health care institutions. In European countries, healthcare budgeting equals from 6-7% to 12% of Gross National Product. In Ukraine the state budgeting makes up less than 3%, and, consequently, this impedes with predisposition for the medical institutions high service quality [3].

DISCUSSION

Nowadays issues of quality and safety of medical service are regulated by strict requirements, which need modern facilities, implementation of advanced treatment methods, adoption of the legal-standard principles and financial support of the treatment and diagnostics.

The Organization Orders of Ministry of Health of Ukraine play significant role in providing effective stomatological institutions function as well as high-quality medical service. It is also important to monitor how the Orders are implemented in practice.

Professor A.V. Stepanenko [3] considers that the Order of Ministry of Health of Ukraine issued on 28.09.2012 №752 «On the sequence of medical service quality control" includes most world -famous methods of such control: external and internal, medical personnel self-assessment, expert assessment, clinical audit, quality indicators system monitoring and certification. Though, in real practice,and the procedure of clinical audit is performed only in certain Ukrainian healthcare institutions, which is, first of all, related to low awareness about its mechanisms, poor understanding of indicator roles in local protocols(recently the Ministry of Health of Ukraine cancelled this paragraph), as well as the low motivation of medical teams.

This is confirmed by Andrey Guk, vice-head of State Institution "Institute of neurosurgery after academician A.P. Romodanov of NAMS of Ukraine", who writes that "unfortunately, there is only one acting Order №752 of Ministry of Health of Ukraine issued on 28.09.2012 «On the order of medical service quality control" , which contains only general approach theses, emphasizes clinical-expert commission importance and doesn't stipulate for the prevention» [3].

The Western Europe stomatological clinics are characterized by widely implemented guaranteed quality standards, which were accepted according to the ISO 9001 and 9002, with obligatory medical insurance system alongside. For example, the an obligatory medical insurance covers full spectrum of stomatological prosthetic service. In Switzerland the stomatological prosthetic service is financed by the hospital coverage. Medical service in Canada is presumably covered by state financing and the medical insurance system [12].

The perfection of the stomatological service quality management is related to implementation of the management progressive technologies within 4 basic aspects: «medical service», «personnel», «patients» and «finances».

All countries face the challenge of poor availability, affordability and quality of medical service, but each country is characterized by its own peculiarities, and Ukraine must find and adopt effective methods of the medical service internal and external quality control perfection.

To conclude, emphasis should be put on the fact that under conditions of the modern healthcare system development, medical service quality should be high, with its control on all levels of management and treatment process. The medical service quality control should monitor such components as the medical service conditions, medical institutions processes and resources, as medical service should be patient-centered.

CONCLUSIONS

1. Legislative and standard basic principles, regulating the issue of providing medical services in Ukraine, as well as their safety, require modernization, in order to update the documents concerning the sequence of stomatological service, clinical recommendations, and standards.
2. Quality and safety of medical stomatological service cannot be provided without accepted decisions of the system effective functioning key issues: sufficient budgeting of the stomatological state/public institutions, renovation of their facilities, and actualization of the personnel management.
3. There is a strong need for the independent state system of medical service management and control in Ukraine, which needs generalization of the European Union countries' experience and adapting the best of those practices.

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ORIGINAL ARTICLE

ASSOCIATION OF SOME IMMUNOLOGICAL BIOMARKERS WITH RHEUMATOID ARTHRITIS PATIENTS IN THI-QAR PROVINCE

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ABSTRACT

The aim: The aim of this research is to evaluate some immunological biomarkers in cases of Rheumatoid arthritis and to verify their correlation with activity of disease among the population of Thi-Qar province.

Matherials and methods: This study included 45 cases of rheumatoid arthritis and 45 healthy subjects. All cases underwent complete history taking, thorough clinical examination, and laboratory tests including erythrocyte sedimentation rate (ESR), C-reactive protein (CRP), Anti-citrulline antibody (Anti-CCP) and rheumatoid factor (RF). IL-17and TNF-α blood level was measured by Enzyme Linked Immunosorbent Assay (ELISA) method. DAS-28 (Disease activity score 28) was evaluated.

Results: Serum levels TNF-α was higher in Rheumatoid arthritis patients (424.3±19.46 pg/ml) than in healthy individuals (112.7±4.73 pg/ml), and IL-17 blood levels were higher in Rheumatoid arthritis patients (233.5±241.4 pg/ml) than the healthy individuals group (47.24±49.7 pg/ml). There was significant association found among IL-17, DAS-28, CRP and hemoglobin levels.

Conclusions: In conclusion, IL-17 blood levels were significantly increased in peoples with rheumatoid arthritis than in healthy individuals. Its significant relationship with DAS-28 suggested that the level of IL-17 in serum could be important immunological biomarker for activity of disease in disease of Rheumatoid arthritis.

KEY WORDS: Rheumatoid arthritis, autoimmune disease, cytokines, biomarkers

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INTRODUCTION

Rheumatoid arthritis (RA) is a chronic inflammatory autoimmune illness with multifactorial a causes. Environmental gene interaction has been shown to take place critical vital role in developments of rheumatoid arthritis, like (HLA-DRB1), lifestyle, smoking, hormones, microbiota in the mouth and gut [1, 2]. Mainly lesion it is focused in the cartilage, bone, and synovial tissue. Inflammation is the main source for joint deformity with reduced joint movement in rheumatoid arthritis peoples [3]. Diagnosis of rheumatoid arthritis standards are chiefly established on clinical and laboratory manifestations examinations as well as study of imaging. Laboratory tests consist of antibodies and inflammatory marker like Anti-CCP, RF, ESR, CRP, and others. At present, numerous infections and anti-inflammatory cytokines, which act an essential crucial function, has been found roles in Rheumatoid arthritis, such as TNF-α, IL-6, IL-17 and IL-10, which it also plays important roles in human immune regulation [4-7]. Several cytokines are expressed; they have functional role in the synovial tissue. TNF-α, Interleukin-1β, IL-17, may be useful prognostic factors for Rheumatoid arthritis [8]. Interleukin-17 (IL-17), which was previously called CTLA-8 (cytotoxic T-lymphocyte-associated protein-8) was cloned, dubbed IL-17 in 1995, its receptor was cloned and recognized as a cytokine, formed by T cells, that affects fibroblast cells, endothelial and epithelial cells [9]. IL-17A (Interleukin-17A) is a cytokine of pro-inflammatory that take place a function in development of many autoimmune and inflammatory illnesses [10]. *In vitro* and *in vivo* experiments have revealed the effect of IL-17 on different types of cells, demonstrating its role in the primary stimulation and late chronic phases of a variety of illnesses: for example, IL-17A operates on keratinocytes to cause the expression of numerous Chemokines resulting in the recruitment of immune cells in psoriasis [11]. Moreover, the most common chronic inflammatory illness is rheumatoid arthritis [12]. IL-17A causes synovitis and degeneration of joint by working locally on osteoblast and synovial cells [13, 14]. Damage of structural in rheumatoid arthritis consists of erosion of bone and destruction of cartilage [15]. IL-17 causes increase production of carboxyterminal pep-

tides in synovial Rheumatoid arthritis, which is influence reverse when anti-IL-17 antibody is added [16]. Keeping this up, C-propertied of collagen type I, who represents the manufacture of collagen of type I as part of the repair process, it inhibits when IL-17 is added to synovial Rheumatoid arthritis [17]. All these findings together indicate that IL-17 enhances degradation of cartilage at the expense of synthesis of cartilage. TNF-α is major cytokine which mainly generated via macrophages [18]. TNF-alpha works as a powerful trigger of other pro-inflammatory cytokines and chemicals thereby, increasing response of inflammatory [19]. TNF-α enhances activation of osteoclast, synovial fibroblasts and chondrocytes, which secretes enzymes of tissue-damaging MMPs (matrix metalloproteinase), when combined with additional mediators of proinflammatory [20, 21]. The MMPs it degrades components of the extracellular matrix, resulting in the degradation of cartilage and bone it starts too early in the path of Rheumatoid arthritis [22]. Thus, all of these TNF-α activities promote synovium, increasing angiogenesis, promoting resorption of bone and cartilage [18], suppressing of Regulatory T cells, as well as enhancement of pain [23, 24]. TNF-α as well intensifies stimulation and differentiation of osteoclast [25]. A study found that inflammation intensity was accurately measured in Rheumatoid arthritis peoples via TNF-α in pretreatment and post-treatment stages [26]. DAS-28 is a statistically derived index that includes number of tender joints, joint swelling, ESR, and overall disease activity [27].

THE AIM

The purpose of this research is to estimate some immunological biomarkers in cases of rheumatoid arthritis and to verify their correlation to activity of disease among the population of Thi-Qar province.

MATERIALS AND METHODS

STUDY SUBJECT

This study was conducted on 45 patients with rheumatoid arthritis (11 male and 34 female). Their ages ranged between (30 - >70) years and they attended to Imam Al-Hussein Teaching Hospital in Thi-Qar province from July 2021 to December 2021. The blood parameters and immunological parameter such as erythrocyte sedimentation rate (ESR), C-reactive protein (CRP), Anti-citrulline antibody (Anti-CCP), rheumatoid factor (RF), serum levels of IL-17, DAS-28 (disease activity score 28) and TNF-α then was measured in both patient and healthy individuals group. 45 healthy individuals were selected to participate as a normal group for age- and gender-matched comparison of patients. ACR (American College of Rheumatology) guidelines 2010 and EULAR (European League against Rheumatology) criteria of classification for rheumatoid arthritis was used to diagnose the patients [28]. Diagnosis is based on these clinical examinations under the supervision of physicians.

METHODS

All cases underwent complete history taking, thorough clinical examination, and laboratory tests including complete blood count. The erythrocyte sedimentation rate (ESR) was recorded using the Westergren mm/hr method. The reading of the first hour is taken, CRP (C-reactive protein) by using latex agglutination assay, RF (Rheumatoid factor) (QUANTA ELISA Lite TM RF IgM), anti-CCP (cyclic citrullinated peptide antibody), using

Table I. Age distribution of patients with Rheumatoid arthritis.

Age (years)	Number	[%]
30-39	12	26.67
40-49	7	15.56
50-59	8	17.78
60-69	12	26.67
>70	6	13.33
Total	45	100%

Table II. Distribution of patients with Rheumatoid arthritis according to gender, residency, BMI, smoking and occupation.

Demographic and clinical enrollment	Number	[%]
Gender		
Female	34	75.56
Male	11	24.44
Residency		
Urban	29	64.44
Rural	16	35.56
BMI		
Normal weight	25	55.56
Over weight	6	13.33
Obesity	12	26.67
Over obesity	2	4.44
Smoking		
Yeas	8	17.78
No	37	82.22
Occupation		
Employed	19	42.22
Unemployed	26	57.78

Table III. Concentration of Interleukin-17 (IL-17) and Tumor Necrosis Factor alpha (TNF-α) for patients with Rheumatoid arthritis and healthy individuals.

Groups	Patients	Healthy individuals	p-value
IL-17 (pg/ml) Mean ± SD	241. 4±233. 5	49. 7±47.24	0.001
TNF- α (pg/ml) Mean ± SD	19. 46±424. 3	4. 73±112.7	0.001

Table IV. Blood and immunological parameters of rheumatoid arthritis patients.

Parameters (Mean ± SD)	Patients from rheumatoid arthritis group
Anti CCP (u/ml)	140.2±138.1
CRP (mg/l)	8.64±14.13
RF (Iu/ml)	89.31±78.99
ESR (mm/h)	24.57±41.34
Hemoglobin (g/dl)	1.25±10.9
DAS28	1.22±4.23

Table V. Association between IL-17 and some parameters in rheumatoid arthritis patients.

Parameters	IL-17		
	r	p-value	Result
Anti CCP	0.089	0.597	No significant positive correlation
CRP	0.972	0.001	Significant positive correlation
Rf	-0.041	0.791	No significant negative correlation
ESR	0.251	0.205	No significant positive correlation
Hemoglobin	-0.531	0.02	Significant negative correlation
DAS28	0.813	0.001	Significant positive correlation

Table VI. Assessment of laboratory features among rheumatoid arthritis patients in relation to their disease activity.

Parameter (Mean ± SD)	Remission Activity N: 9	Mild Activity N: 11	Moderate Activity N:16	Sever Activity N:9	Kruskall Wallis test	p-value
IL-17	7.32±34.3	32.2± 88.1	128.2±253.7	112.1±601.1	17.24	0.001*
Anti CCP	153±124	122.1±97.1	89.9± 163.1	224± 187	2.325	0.843**
RF	201.1±79.2	142.1± 92.1	109.5± 87.1	94.4± 54.2	0.299	0.942**
CRP	3.98± 7.11	8.24± 9.85	4.19± 12.4	7.21± 28.1	9.479	0.02*
ESR	29.2± 24	7.55± 27.3	29.4± 41.6	28.9± 47.3	1.901	0.521**
Hemoglobin	0.83± 10.2	0.92± 12.6	1.61± 11.4	1.22± 11.4	0.723#	0.472**

#: by ANOVA test; *: Significant; **: Non-significant

a CCP immuno can assay kit which is an ELISA (enzyme-linked immunosorbent assay). DAS-28 (disease activity score of 28) was used to measure disease activity of RA [27]. Serum interleukin-17 level was measured for Rheumatoid arthritis cases and healthy individuals by ELISA kit following the manufacturer's directions (Human Interleukin17) IL-17 ELISA Kit, (Mybiosource, USA). TNF-α level was measured by ELISA kit following the manufacturer's directions (Human Tumor Necrosis Factor α) TNF-α ELISA Kit (Cusabio, China).

STATISTICAL ANALYSIS

Data were analyzed on the Statistical Package for the Social Sciences (SPSS), version 23. The data are presented as the mean standard deviation (SD). The Kruskal-Wallis test was used. Spearman correlation coefficients were used. The results are significance at $p< 0.05$.

RESULTS

This study involved 45 patients with rheumatoid arthritis and 45 healthy individuals as controls. Patients' ages ranged between 30 - >70 years, and the age distribution of patients with Rheumatoid arthritis is shown in table I.

The current study found that the disease was recorded more in urban than in rural areas at percentage 64.44%, according to the BMI normal weight had a high incidence of rheumatoid arthritis 55.56%, obesity was the second rate 26.67%, a small percentage of smokers

were enrolled in the current study 17.78%.The disease was record in unemployed patients with a high rate 57.78% (Table II).

It was recorded that a significant difference in the level of IL-17 among the studied groups. IL-17 level's was significantly higher in the group of patients (233.5±241.4) than the healthy individuals group (47.24±49.7) with a p-value <0.001 and the level of TNF- α were significantly higher in the patients (424.3±19.46) than the healthy individuals (112.7±4.73) (Table III).

Regarding immunological parameters, the mean anti-CCP antibody in the patient group was 138.1±140.2 u/ml, and the mean of CRP was 14.13±8.64 mg/L, with regard to rheumatoid factor, the mean was 78.99±89.31 Iu/mL. The mean of ESR levels were 41.34±24.57 mm/hr, the mean of hemoglobin level was 10.9±1.25 g/dL, and the mean of DAS-28 was 4.23±1.22 (Table IV).

It has been recorded, that there is a significant positive relationship between the levels of IL-17 and CRP, also, there was a significant negative correlation between IL-17 and hemoglobin, and there was significant positive correlation between the level of IL-17 and DAS-28 (Table V).

There is a significant difference among the studied rheumatoid arthritis groups in the IL-17 and CRP levels, which were highest among those, who developed severe disease when compared with others. However, the difference was non-significant between the subgroups in the levels of anti-CCP, RF, ESR and hemoglobin (Table VI).

DISCUSSION

RA (Rheumatoid arthritis) is characterized by T-cells and macrophages infiltration in the joints, the synovium hypertrophy, numerous cytokines involvement, and gradually degradation for articular cartilage and bone [29, 30]. Results of the current study has shown that female patients were more than male with significant increase, these findings address international researches that showed that females were extra likely to have the illness than males, and this perhaps due to the variance in activity of hormonal between males and females as well as respect for behavior and lifestyle [31]. The disease recorded in urban areas often than in rural areas, and it may be due to the urban lifestyle in which stress, noise and air pollution are critical factors in decreasing of human immunity and spreading of diseases. In the current study, the age groups (30-39) and (60-69) were more than the other groups (more than in 12% for each), and this result comes in partially agreement with Widdifield et al. [32] who discovered that the occurrence of illnesses rises with age in both males and females. This difference perhaps due to the difference between populations in the world that affects the health awareness, environment and behavior [33]. Regarding BMI, the patients with normal weight had a high incidence of rheumatoid arthritis (55.56%), followed category with obesity by with rate of 26.67%. Certain researchers have indicated a correlation between rheumatoid arthritis and the obesity, and according to Qin et al., [34] there is a link between BMI and rheumatoid arthritis, mainly in females. Others, however, differ with Qin and reported similar results with the current research: Lu et al. [35] found that there was no significant correlation among BMI and Rheumatoid arthritis when few cases of Rheumatoid arthritis were identified after the age of 55, while 83% of rheumatoid arthritis cases were identified at or before the age of 55. The illness was also recorded in unemployed patients at a high ratio, this is due to poor lifestyle of patients' and low activity [36]. Small ratio of smokers appeared in the current research, as it is well known, that the influence of smoking on human health has been proved to cause immunosuppression and its negative impact on human health. Certain blood markers and immunological markers were studied in the current research of patients and compared to standard rate of these markers, and with healthy individuals, in this study, mean of anti-CCP was 138.1±140.2 u/ml, mean of CRP was 14.13±8.64 mg/L, regarding rheumatoid factor the mean was 78.99±89.31 Iu/mL, mean ESR was 41.34±24.57 mm/h, mean hemoglobin was 10.9±1.25 g/dL, and the DAS28 mean was found to be 4.23±1.22. These results were matched with the study of Metawi et al. [37] who found approximately the same value for these markers. The current study indicated a significant difference in the level of TNF-alpha in the serum of rheumatoid arthritis patients and healthy individuals. The findings of the current study are compatible with that obtained by Theagarajan et al. [38] who showed that the patients with rheumatoid arthritis had a significantly higher level of TNF-α when compared to a healthy control. In numerous cases, TNF-α appears at the inflammatory site, as well as in the circulatory blood, and is presumably responsible for the changes that occur in systemic inflammation [39]. Various studies have demonstrated an elevated level of TNF-α in the blood of RA patients [40]. IL-7 and TNF-α they are types of cytokines that function against human regulatory T-cells inhibitory activity. TNF-α in high level have been found in the synovial fluid and in the blood of Rheumatoid arthritis peoples, and thus this may be one of the factors that lead to the malfunction of regulatory T cells [41]. The current study showed that there is a significant difference among the studied groups in the IL-17 level. The level of IL-17 was significantly higher in rheumatoid arthritis peo-

ple's (233.5±241.4 pg/ml) than the healthy individual's group (47.24±49.7 gal/ml). This was in agreement with Dhaouadi et al. [42] who reported that blood levels of IL-17A were high significantly in Tunisian rheumatoid arthritis cases than in healthy subjects. Also, our results match those of Metawi et al. [37] who found dangerously high levels of IL-17A in patients with rheumatoid arthritis compared to healthy individuals also Melis et al. [43] who showed significant differences in levels of IL-17 in rheumatoid arthritis patients and healthy individuals. A meta-analysis of Lee and Bae [44] recorded this correlation between rheumatoid arthritis and elevated circulating IL-17 level. This study showed a high positive correlation between IL-17 and CRP, a significant negative link between IL-17 and hemoglobin (anemia of chronic disease).Anemia is more typically caused by a decrease in red blood cells formation in the bone marrow in rheumatic diseases caused by persistent inflammation, and increased formation of hepcidin resulting in disruption of iron metabolism. Anemia of chronic disease is usually euchromatic, euchromatic. Nevertheless, this can be linked microcytic anemia with chronic disease too [45]. These results were matched with the study of Al-Saadani et al. [46] who documented a significant positive relationship among serum levels of IL-17, ESR, CRP, and TNF-α. The present study revealed a significant positive relationship among IL-17 level with DAS-28. The findings of this research were in agreement with findings of Al-Saadani et al. [46] who reported that serum IL-17 levels were significantly associated with disease activity using DAS-28. Disease activity score of 28 (DAS-28) is a statistically generated indicator that includes the number of tender joints, the number of swollen joints, ESR and global activity of disease [27]. They demonstrated an essential vital role to serum IL-17 in pathogenesis of the characteristic destructive and inflammatory pattern of RA. In this study, in terms of disease activity, 11 rheumatoid arthritis cases (24.44%) had mild disease activity, and 16 rheumatoid arthritis cases (35.56%) had moderate disease activity, 9 rheumatoid arthritis cases (20%) had severe disease activity, the remaining 9 cases (20%) showed complete remission. There was a significant difference among the studied subgroups in IL-17 levels, which were among the highest among those with severe disease when compared to other levels. In addition, the difference between the subgroups was significant with respect to CRP which was also one of the highest among those with severe disease when compared with the others. However, the difference was non-significant between the subgroups in the levels of RF, anti-CCP, ESR, and hemoglobin. Pavlovic et al. [47], also had a similar finding, they reported that mean IL-17A blood levels in rheumatoid arthritis cases largely corresponded to activity of disease and severity, this perhaps explains the helpfulness of IL-17A level in the blood in determining activity.

CONCLUSIONS

The serum level of IL-17 was significantly increased in patients with Rheumatoid arthritis than in healthy individuals. Its significant relationship with DAS-28 suggested that the level of IL-17 in serum could be important biomarker for activity of disease in Rheumatoid arthritis.

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ORIGINAL ARTICLE

PREVALENCE OF GINGIVITIS IN CHILDREN WITH AUTISM SPECTRUM DISORDERS (ASD)

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ABSTRACT

The aim: To evaluate and compare the prevalence of gingivitis according to the PMA index in children aged 5 to 6 years in preschool with ASD and without disorders (Kyiv, Ukraine).

Materials and methods: Oral assessment was conduct on 69 children with ASD and 23 children without ASD aged 5 to 6 years. To determine the periodontal status it was used the papillary-marginal-alveolar index – PMA (according to Schour, Massler, in the modification of Parma).

Results: Children with ASD (18.84%) were 3.7 times less likely to have a clinically healthy periodontium than children without disorders (69.57%). The average PMA index among the main group was 6.8 times higher (15.31 ± 1.49%) than in the control group (2.25. ± 0.73%), but this difference was not statistically confirmed ($p > 0.05$). The most common pathology of periodontal tissues was chronic catarrhal gingivitis. 49.28% of children in main group with ASD had mild catarrhal gingivitis, while just 30.47% of children in control group without ASD had this pathology. Catarrhal moderate gingivitis was diagnosed in 31.88% of children from main group, symptoms of moderate gingivitis were not detected in the control group without disorders.

Conclusions: ASD children 5-6 years old may have major risk of developing such periodontal lesions as mild and moderate gingivitis. Further investigations need to be done to clarify prevalence of other oral pathologies in ASD individuals to understand the effect of the disorder on oral health.

KEY WORDS: gingivitis, autism spectrum disorders, Children, prevalence of periodontal disease, PMA index

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INTRODUCTION

Studies of recent years indicate a high incidence of major dental diseases in children. Periodontal disease ranks second in frequency of detection after dental caries according to the results of the Global Bank of Dental Data of the WHO. The prevalence and severity of gingivitis increase with age, with the first signs of the disease appear in children under 5 years [1].

It has been observed the increases in the number of children with autism spectrum disorders (ASD) annually in Ukraine (ranging from 28.2 to 35.7% every year). 7,491 children with disorders were registered in 2017 [2]. The growth in recent years is not so much because the actual increase in the number of children with ASD, but due to implementation the modern methods of diagnostics in the clinical practice. The use of the second version of the clinical protocol «Program-targeted care for children with ASD» (implemented by order of the Ministry of Health № 341 dated 15.06.2015 [3]) provides valid diagnostic techniques in order to obtain reliable results and improve the clinical diagnostics.

ASD are an umbrella term that includes (for ICD-10): childhood autism (F84.0); atypical autism (F84.1); Rett syndrome (F84.2); other childhood disintegration disorder (F84.3); Asperger's syndrome (F84.5) [4]. The prevalence of ASD per 10,000 children in the world is from 30 to 60 children (according to the WHO) and from 0.7 to 72.6 (according to 36 meta-analyzes) [5]. The probability of autism in boys is much higher compared to girls and continues to vary among certain racial/ethnic groups and communities [6]. Over the next decade, approximately 50,000 adolescents will enter adulthood each year [7]. It is likely that dentists will treat patients with ASD in their daily practice, so understanding the specifics of the disorder is important for planning effective treatment. A literature review of 22 studies about ASD children in Saudi Arabia showed a high prevalence of gingival and periodontal diseases [8]. It was reported that thirty-one percent (31%) of children with ASD had gum disease when examining periodontal status in the Eastern region of Saudi Arabia [9]. Assessment of periodontal health (according to the CPITN index) in 32 autistic and 48 non-autistic boys and girls aged 8 to 12 years (mean 9.7 ± 1.2 and 9.9 ± 1.1 years, respectively) (Praitai, Bangkok, Thailand, on the continent of Asia) found that children with ASD had

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significantly worse periodontal status than non-autistic children (P <0.05) [10].

Children with ASD have the complex of disorders that includes reduced saliva due to prescription drugs, unhealthy eating habits, poor oral hygiene and harmful oral habits like bruxism. They can increase the risk of periodontal disease in ASD children. Periodontal disease can lead to difficulty eating and speaking, mouth pain, sleep disorders and low self-esteem, resulting in a negative impact on the person's health and quality of life [11]. Oral status was assessed and compared in 144 children with ASD and 228 children with typical developmental ages 3 to 16 (Shanghai, China). Halitosis (p <0.001), bad oral habits, including mouth breathing (p <0.001) and biting objects (p <0.05) were more common in children with ASD than in children with typical development [12].

Although the fact that ASD is one of the most severe childhood psychoneurological disorders, the prevalence of periodontal disease in these children is insufficiently studied [13,14]. Children with ASD are not given enough attention due to specific autistic behavioral characteristics. The assessment of dental status in 483 children with ASD (Chennai, Tamil Nadu, India) showed that the gingivitis prevalence in children with mixed dentition was 50.0%, and with secondary dentition - in 48.96 % [15]. A survey of 61 children with ASD aged 6 to 16 years and 61 healthy children (Ajman, United Arab Emirates) found that 97.0% (59/61) of children with disorders, had gingivitis [16]. Mild gingivitis (according to the GI) was diagnosed in 46.3% of children in the study of 149 children with ASD aged 7 to 14 years (KwaZulu-Natal, South Africa) [17].

There are contradictory research results in the literature. The oral health of 347 preschool children from 19 special care centers (Hong Kong, China) with and without autism spectrum disorders was assessed and compared. Comprehensive oral health screening was performed among 74.1% (257) of children with ASD. The mean age of the children was 59 ± 10 months (32 to 77 months) and 84.4% were boys. Children with ASD had better periodontal status than children without disorders (p <0.001) [18].

The pronounced persistent social maladaptation and disability that accompanies ASD significantly complicates clinical examination. An examination of the oral cavity in 39 children with ASD and 16 children with other developmental disabilities (Southern Illinois, USA, on the North American continent) showed that 62% had gingivitis. In particular, older children with ASD who lived in boarding schools had manifestations of various forms of gingivitis more often. [19].

There is insufficient information on the prevalence of periodontal disease in children with ASD in Ukraine [7]. The periodontal status of children with ASD need to be investigated in connection with the growing prevalence of disorders in Ukrainian children.

THE AIM

The aim of our study was to evaluate and compare the prevalence of gingivitis according to the PMA index in children aged 5 to 6 years in preschool with ASD and without disorders (Kyiv, Ukraine).

MATELIALS AND METHODS

The children for the research were recruited from the educational and correctional preschool facility «Child with a future» of Solomyansky and in preschool Darnytskyi districts of Kyiv. Age 5-6 years is key to the study of dental morbidity according to WHO methods [20]. The ASD diagnosis was obtained from medical record for inpatient care or a advisory opinion of the chief child psychiatrist of the Ministry of Health of Ukraine. Parents of 82 children out of 112 children with ASD aged 5-6 years agreed to have their children participate in the study by signing a written informed consent form (response rate: 73.2%). They were assured that their identity remained anonymous. The inclusion criteria were: parents' consent, age between 5 and 6 years old, and an ASD level 1 or 2. The exclusion criteria were: the presence of developmental disorders, lack of cooperation.

The severity of autism (ASD level) was assessed by a psychologist. These levels included level one (mild ASD), level two (moderate ASD), and level three (severe ASD). Children with ASD level three were excluded from the study. Finally, 69 children were included in the study. The control group consisted of 23 unrelated children with typical development without disorders. The studied indicators had a slight variance and it was decided not to increase the control group to 69 children. Children examination were condacted in the «Children's Consultative and Diagnostic Center of Darnytskyi District of Kyiv» and in the Department of Pediatric Therapeutic Dentistry of the Private Higher Educational Institution «Kyiv Medical University». The examination was done under natural light using an intra-oral examination mirror. To determine the periodontal status it was used the papillary-marginal-alveolar index – PMA (according to Schour, Massler, in the modification of Parma).

The results were statistically analyzed. Qualitative variables are reported as number and percentage. Data

Table I. RMA index in children aged 5-6 years of the main and control groups

Observation groups	Age, months*	Number of children, n	Sex**				PMA index, %
			boys		girls		
			abs	%	abs	%	
main group	65,52	69	58	84.06	11	15.94	15.31±1.49
control group	65,48	23	12	52.17	11	47.83	2.25±0.73
The value of the difference (p)	p=0.639; p>0.05		p<0.05***		p<0.05***		p=0.427; p>0.05

Note. * Calculated using the t-test; ** Calculated using the chi-square test; *** Differences significant at $p < 0.05$.

Table II. Criteria for assessing the severity of gingivitis according to the PMA index

Index value, %	Degree of severity gingivitis
up to 25%	mild
25 - 50%	moderate
over 51%	severe

Table III. The severity of gingivitis according to the PMA index in the examined children aged 5-6 years with ASD and without (M ± m)

The condition of periodontal status	Main group (with ASD), n = 69		Control group (without disorders), n= 23		The value of the difference (p)
	Number of children, abs	The PMA index, %	Number of children, abs	The PMA index, %	
Intact periodontium	13	0	16	0	-
Mild gingivitis	34	11.18±0.69	7	7.38±0.19	0.775; p>0.05
Moderate gingivitis	22	30.76±0.57	0	-	-
Severe gingivitis	0	-	0	-	-
Average	69	15.31±1.49	23	2.25±0.73	0.427; p>0.05
The value of the difference (p_1)		0.973; p_1>0.05		-	

Note. The degree of reliability of discrepancies p was determined in relation to children with autism and without; p_1 - for children with ASD, with mild to moderate gingivitis.

Table IV. Gingivitis prevalence in the examined children aged 5-6 years with and without ASD

Periodontal status	Main group (with ASD), n = 69		Control group (without disorders), n= 23	
	Number of children, abs	Number of children, %	Number of children, abs	Number of children, %
Intact periodontium	13	18.84	16	69.57
Mild gingivitis	34	49.28	7	30.43
Moderate gingivitis	22	31.88	0	0
Severe gingivitis	0	0	0	0
General	69	100	23	100

were expressed as means ± standard deviations of the evaluated parameters. The Student paired t-test was used to compare intragroup and intergroup measurements. A level of significance of $p < 0.05$ was used for all statistical comparisons.

RESULTS

The mean age of the 69 examined children with ASD was 65.52 months. Boys (58 - 84.06%) predominated in the main group of children with ASD; there were 5.3 times less girls (11 - 15.94%). The distribution by sex in the control group without disorders was uniform, the mean age was 65.48 months. A successful clinical examination was achieved on the first attempt for 19 (27.5%) children with ASD and 23 (100%) for children without disorders.

The PMA index was used to determine the activity of the inflammatory process in periodontal tissues. The PMA index in children of the main group was slightly higher than in the control group (Table I).

The average PMA index in main group was 15.31±1.49%, while the PMA index in the control group was much lower and was 2.25±0.73% (p>0.05).

The table II shows the criteria for assessing the severity of gingivitis according to the PMA index.

The examined children were divided into 4 groups based on the anamnesis, clinical data and PMA index: with intact periodontium, with mild, moderate and severe gingivitis (Table III).

As can be seen from table II, 34 children with autism spectrum disorders had a mild degree of chronic catarrhal gingivitis and the average PMA index was 11.18±0.69%, while 7 children without disorders had mild degree of gingivitis and the average PMA index was 7.38±0.19% (p>0.05). The moderate severity of gingivitis was found in 22 children with ASD, the PMA index was 30.76±0.57%. The pathological process in most children most frequently was localized in the area of the frontal upper and lower teeth.

Gingivitis prevalence was studied in the examined children (Table IV).

13 children (18.84%) with autism spectrum disorders had a clinically intact periodontioum, while 23 children (69.57%) without disorders had the intact periodontium. Accordingly, 81.16% of children with ASD and in 30.43% of children without disorders had gingivitis.

49.28% of children with ASD (34 children) had edema, hyperemic interdental papillae and bleeding on probing (grade 1 - point) during the clinical examination that indicates the presence of mild catarrhal gingivitis. 7 children without disorders child (30.43%) had mild catarrhal gingivitis.

Parents of 22 (31.88%) children with ASD complained of bleeding during brushed or ate solid food in their children. Edema, hyperemia of interdental papillae and the gum marginal edge, bleeding on probing (grade 2 - line) were found during objective examination. It indicates that 31.88% (22 children) had moderate chronic catarrhal gingivitis. No children from control group without disorders had moderate gingivitis.

Severe gingivitis was not observed in either the main or control groups.

DISCUSSION

The cooperation level of children with ASD during oral examination was mostly rated as positive and definitely positive by the examiner in the present study, which was in line with the results of some studies [11, 21] but in contrast to some other studies [22]. This could be not only because of the exclusion criteria of the present study excluding children with severe ASD, but also to the absence of autistic children who do not attend preschool kindergarden and were homeschooled or attended private centers; these children usually have severe ASD or worse educational and behavioral conditions.

We examined 92 children aged 5-6 years. 69 children were in the main group with ASD and 23 children were in the control group without disorders. Children with ASD (18.84%) were 3.7 times less likely to have a clinically healthy periodontium than children without disorders (69.57%). 81.16% of children with ASD and 30.43% of children without disorders had gingival desease in primary dentition. In different studies, prevalence of gingivitis in children with ASD may vary. In one study is reported that gingivitis prevalence with mixed dentition was 50.0%, and with secondary dentition was 48.96% [15]; in another study gingivitis prevalence in children with ASD achived 97.0%[16]. Manifestations of various forms of gingivitis were more common in older children with ASD (62%) who lived in boarding schools [23]. The high heterogeneity of the data in the literature may be correlated with the diversity of the population screened, especially different age (from 6 (7) to 14 (18) years old).

The results of primary screening of periodontal status showed that the inflammation in the gums was evidenced by the PMA index. The average PMA index among the main group was 6.8 times higher (15.31 ± 1.49%) than in the control group (2.25. ± 0.73%), but this difference was not statistically confirmed (p> 0.05).

The most common pathology of periodontal tissues was chronic catarrhal gingivitis. 49.28% of children in main group with ASD had mild catarrhal gingivitis, while just 30.47% of children in control group without ASD had this pathology. Similar prevalence was shown in study [17], where mild gingivitis was diagnosed in 46.3% of children. The PMA index among the examined children with ASD were 11.18 ± 0.69%, in children without disorders - 7.38 ± 0.19 (p> 0.05).

Catarrhal moderate gingivitis was diagnosed in 31.88% of children from main group, symptoms of moderate gingivitis were not detected in the control group without disorders. The PMA index were 30.76 ± 0.57% among the examined ASD children with a moderate gingivitis.

The major limitation of this study was that the gender distribution in the control group (was equal) whereas the boys to girls ratio in the main group was 5.3: 1. This gender difference might have an impact on our study results, although it is consistent with national statistics for ASD of 4–5:1 [6]. Another limitation was no associations between the condition of the periodontal tissues and type of autistic symptoms as we did not collect data by the severity of ASD. Other limitations are the relatively small number of participants involved in the study and unequal size of the groups.

CONCLUSIONS

The prevalence of gingival disease in preschool children with ASD can be considered higher than in non-autistic individuals. The average PMA index was statistically higher in ASD children compared to children without ASD. Correspondingly, half assessed individuals with ASD presented symptoms of mild chronic catarrhal gingivitis, while just every third children without ASD was dignosed with this pathology. One third of ASD children had catarrhal moderate gingivitis, however children without disorders symptoms did not have moderate gingivitis.

ASD children 5-6 years old may have major risk of developing such periodontal lesions as mild and moderate gingivitis. Further investigations need to be done to clarify prevalence of other oral pathologies in ASD individuals to understand the effect of the disorder on oral health.

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ORIGINAL ARTICLE

INFLUENCE OF THERAPEUTIC GYMNASTICS ON BODY WEIGHT COMPOSITION, ANTHROPOMETRIC PARAMETERS AND QUALITY OF LIFE OF WOMEN WITH OBESITY IN THE CONDITIONS OF QUARANTINE RESTRICTIONS IN THE CONTEXT OF THE COVID-19 PANDEMIC

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ABSTRACT

The aim: To determine the effectiveness of application of special therapeutic physical exercises to improve the indicators of body weight, anthropometric parameters and quality of life of obese women in the conditions of quarantine restrictions.

Materials and methods: We examined 10 women aged 37 ± 5 years with obesity of various degrees, which was determined by the body mass index (BMI, kg/m2). All women for two months were involved in specially designed therapeutic exercises in the remote mode. Evaluation of the effectiveness of therapeutic exercises use was performed using the survey method to determine the quality of life of obese women according to a short version of the questionnaire "The world health organization quality of life (WHOQOL-BREF)"; anthropometric measurements of body parts sizes; bioimpedance analysis of body weight composition; statistical methods of data processing.

Results: The effect of therapeutic gymnastics according to the proposed programme on the component composition of body weight of obese women was proved, which indicates a decrease in total body weight, reduction of body fat, increase in total body water and muscle component of body weight. It is shown that under the influence of corrective physical exercises the proportions of the body of women changed, as evidenced by the dynamics of the circumferences of all measured parts of the body of obese women. The improvement of women's quality of life in all indicators was identified.

Conclusions: The use of special complexes of physical exercises, aimed at correction of the body weight of obese women,showed its significant effectiveness and led to the expected result.

KEY WORDS: physical exercises, body mass index, physical rehabilitatio

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INTRODUCTION

The problem of obesity in the population today is very acute in all countries and, in particular, in Ukraine. According to official WHO statistics (STEPS study, 2019), the prevalence of overweight in Ukraine is 59.0%, which is one of the highest rates among the countries of Eastern Europe and Central Asia. Almost a quarter of the population of Ukraine suffers from obesity (24.8%), and among women this indicator is much higher (29.8%) than among men (22.1%) [1]. At the same time, there is a negative trend of steady growth of overweight and obese people around the world [2-4]. More than 1.9 billion adults worldwide are overweight or obese, and the prevalence of obesity is increasing. Obesity affects endothelial function through obesity-related complications such as hypertension, dyslipidemia, diabetes, metabolic syndrome, and obstructive sleep apnea syndrome [1-4].

In quarantine conditions due to COVID-19, the problem of obesity is taking shape owing to forced stay at home and severe limitations of physical activity, chronic stress due to the pandemic, closure of gyms and other sports and health facilities and disruption in general habitual active lifestyle [5-9].

Overweight and obesity are not only an aesthetic problem for person, but also a significant risk factor for severe somatic diseases, primarily cardiovascular system [10], such as hypertension and myocardial infarction on the background of general atherosclerosis of the blood vessels. Overweight negatively affects the state of the musculoskeletal system, namely, is a compression factor in the degeneration of intervertebral discs in the lumbar area of the spine, is a factor in significant mechanical overload of the joints of the lower limbs and, as a result, leads to coxarthrosis, gonarthrosis and osteochondrosis. In addition, overweight

is a significant risk factor for type 2 diabetes and some types of cancers [10-12].

Obesity and overweight can cause not only a deterioration in physical condition of person, but also serious mental disorders such as acute or chronic depression due to chronic stress on the background of constant dissatisfaction with their appearance, psychological complexes and barriers, constant internal discomfort and anxiety, bad mood, low self-esteem, etc. In the social aspect, obesity can be a cause of discrimination and inability to get a desired job in the speciality [13-17].

All of those things are far from complete reflection, health problems at the three levels of life of overweight or obese people lead to a significant deterioration in human quality of life [15].

Another important problem with obesity is that it is too difficult to treat and stabilize the achieved results [16]. Most patients undergoing comprehensive therapy for obesity return to their previous size within 5 years [8]. Thus, obesity, as a severe psychosomatic disease with a chronic course and frequent relapses, is a complex medical, psychological and social problem that requires finding solutions.

These aspects of obesity indicate that its solution requires not episodic symptomatic treatment, but a comprehensive and individual approach to application of conservative treatment and rehabilitation, designed and planned for a long time, as well as the patient's willingness to rethink their lifestyles and habits, development of new behaviour model and its adoption. First of all, it concerns the change of human eating habits, normalization of sleep and increase of the level of physical activity [17]. Therefore, the problem of overweight and obesity requires a multidisciplinary approach to its solution.

Due to the fact that this article examines the problem of obesity in women and ways to solve it in quarantine conditions and, consequently, motor limitations, the main focus is on the impact of special therapeutic exercises on the component composition of body weight, body size and, as a consequence, on indicators of women's quality of life.

THE AIM

The objective of the work is to determine the effectiveness of special therapeutic physical exercises to improve body weight, anthropometric parameters and quality of life of obese women in conditions of quarantine restrictions.

According to the objective of work the following tasks are formulated:

1. Analyse the problem of overweight and obesity in the population of the world and Ukraine, in particular, and its worsening in the conditions of long-term quarantine restrictions in conjunction with the COVID-19 pandemic.
2. Conduct an initial examination of obese women in order to form homogeneous age groups of women with various degrees of obesity for the exercises in therapeutic gymnastics.
3. To make the plan, the programme and complexes of special medical gymnastics for women with obesity.
4. Remake control examinations of obese women to determine the effectiveness of therapeutic exercises.

MATERIALS AND METHODS

10 middle-aged women (37±5 years) with obesity of various degree took part in research of influence efficiency of special physical exercises of medical gymnastics directed on weight loss (3 women – I degree, 5 women – II degree, 2 women – III degree).

The degree of obesity was determined by the body mass index (BMI, kg/m^2), which is the most recognized diagnostic criterion for obesity, which was calculated from the indicators of height and body weight of the studied women. BMI was assessed according to the generally accepted WHO scale: 18,5-24,9 kg/m^2 – normal weight; 25,0-29,9 kg/m^2 – overweight (pre-obesity); 30,0-34,9 kg/m^2 – obesity of the I degree; 35,0-40,0 kg/m^2 – obesity of the II degree; more than 40,0 kg/m^2 – obesity of the III degree (morbid obesity).

The selection of the contingent of obese women for gymnastics was based on the results of women's self-diagnostics of their own anthropometric parameters and bioimpedance analysis of body weight (BIA), as well as questionnaires on the quality of life of women.

Anthropometric parameters included coverage indicators of some parts of the body, which women determined at home on their own with a centimetre tape once a week according to a clearly defined algorithm and provided recommendations on measurement techniques. The following anthropometric indicators were determined: the girth of the abdomen at the level of the bellybutton in a moment of the pause between inhalation and exhalation; hips girth at the most protruding points of the buttocks, hip girth under the buttocks and shoulder girth at the widest point.

Bioimpedance analysis of body weight composition was also performed by women on their own weekly using weights-analysers of component body weight composition, which were available in all women under research at home. The study of body composition was performed in the morning on an empty stomach, after emptying the bladder and intestines.

The following indicators of body weight composition were taken into account: body weight in kg, total

fat content in%; total water content in%; total muscle mass in kg; basic metabolism in kcal; bone (mineral) mass content in kg; the level of visceral fat. The scale analyser automatically saves body composition data on an SD card built into the device, which allows data to be downloaded to a computer.

Quality of life indicators were determined from a short version of the questionnaire recommended by the WHO as a tool for use in research or clinical tests "The world health organization of quality of life (WHOQOL-BREF)" [13]. All questions of the questionnaire were entered into the electronic Google form and the survey was conducted remotely at a convenient time for each woman before the start of the course of therapeutic gymnastics and after 2 months of training. The results of the survey in Google form were processed automatically.

After processing and analysis of all the results of the study of women, a programme of therapeutic gymnastics (TG) was developed, a set of special therapeutic exercises were implemented in the classes.

The TG programme was implemented in 2 stages: the first stage – introductory (2 weeks); the second stage – the main (7 weeks), which in turn was divided into two periods – the first period in the gentle-training motor mode (3 weeks) and the second period in the training motor mode (4 weeks). Therapeutic gymnastics was performed three times a week remotely for 2 months using the ZOOM platform. Each lesson at the introductory stage lasted 40 minutes and 60 minutes – at the main stage. The lesson consisted of three parts: preparatory – 5-10 minutes; main – 30-40 minutes and final – 5-10 minutes.

The sets of exercises were composed in accordance with the level of physical form of the studied women, namely for the entry level. The introductory stage included mainly general developmental exercises for all muscle groups in combination with strength and flexibility exercises to prepare the musculoskeletal system and cardiovascular system for more intense physical activity, balance exercises to training of the vestibular apparatus. Exercises at the introductory stage were performed with incomplete amplitude, at a medium pace, from the starting positions, standing, sitting, lying down. At the main stage of the TG programme, physical exercises were aimed at improvement and normalization of metabolism, in particular, fat metabolism and reducing excess body weight. At this stage, in addition to general developmental exercises, exercises from fitness systems of stretching, Pilates and yoga were used, strength exercises using your own weight, cushioning straps and dumbbells in combination with breathing exercises, relaxation and recovery exercises were used; elements of sports-oriented aerobics, which used simple series of movements, jumping, and running on the spot. Classes at the main stage were conducted with full range of motion, at a high pace with an increased number of repetitions of each exercise.

After two months of TG classes remotely, the last control measurement of coverage indicators and indicators of body mass composition was performed using the same methods and in the same conditions, and a survey was conducted on the quality of life of obese women.

All the received research material was processed with the help of computer programme of mathematical statistics "BioStat". The following indicators were calculated: M – average mathematical value, ± m – mathematical value error. Significance of differences between indicators was determined by one-sample Wilcoxon's t-test. The difference was considered significant at values $p \leq 0.05$.

RESULTS

First of all, the indicators of body weight and body mass index were analysed, on the basis of which all studied women were divided into three groups according to the degree of obesity. All subsequent calculations of the average values of anthropometry, BIA and women's surveys and their subsequent analysis in the dynamics of time were carried out, depending on the degree of obesity, i.e., separately for each group of women. Changes in body mass and body mass index of women during two months of TG classes according to the proposed programme are presented in table I.

The data in the table show that the greatest changes were in weight and BMI in women with III degree of obesity, their weight decreased by 5,3 kg during two months of regular TG exercises, and the degree of obesity changed by one position below, as evidenced by changes in BMI indicator. Less significant changes were in the group of women with second-degree obesity, who lost 2,9 kg within 2 months. The least pronounced, but statistically significant changes were observed in the group of women with obesity of the first degree, whose body weight decreased by only 2.7 kg, which also significantly affected their average BMI.

In order to understand due to which component of body weight composition weight loss was performed, an analysis of the dynamics of the components of the body composition of the studied women was conducted, the results of which are presented in table II.

As can be seen from the table, the fat component of body weight in all studied women at the beginning of TG was significantly increased and decreased within two months to a greater extent in women with obesity of I degree, and the least one – in women with III degree of obesity. The percentage of total body water at the begin-

Table I. Dynamics of body weight indicators and body mass index of obese women during 2 months of therapeutic exercises

Indicators / Groups of women	Body weight, kg		BMI, kg / m²	
	At the beginning of TG classes	At the end of 2 months of TG classes	At the beginning of TG classes	At the end of 2 months of TG classes
Obesity of the I degree	89,9±2,6	87,2±1,9*	32,3±0,9	31,5±0,8*
Obesity of the II degree	105,0±4,5	102,1±2,8*	38,1±1,0	37,1±0,5*
Obesity of the III degree	115,4±3,5	110,1±2,7**	40,1±1,0	39,0±0,6**

* – the reliability of differences between indicators at the level of p≤0,05;
** – the reliability of differences between indicators at the level p≤0,01.

Table II. Dynamics of indicators of the component composition of body weight of obese women during 2 months of therapeutic gymnastics exercises

Indicators	Groups of women	At the beginning of TG classes	At the end of 2 months of TG classes	The difference
Body fat (%)	Obesity of the I degree	40,0±1,2	38,2±1,4*	- 1,8
	Obesity of the II degree	41,9±1,7	40,5±1,6*	- 1,4
	Obesity of the III degree	48,1±4,2	46,8±4,5*	- 1,3
Total body water (%)	Obesity of the I degree	43,0±1,9	45,5±1,1**	+ 2,5
	Obesity of the II degree	40,2±1,7	42,2±1,2*	+ 2
	Obesity of the III degree	38,1±3,5	40,3±2,5*	+ 2,1
Muscle body weight (kg)	Obesity of the I degree	42,6±1,4	43,5±1,9*	+ 0,9
	Obesity of the II degree	48,6±3,5	49,2±2,9	+ 0,6
	Obesity of the III degree	57,3±3,1	56,8±2,9	- 0,5
Bone body weight (kg)	Obesity of the I degree	2,2±0,6	2,1±0,7	- 0,1
	Obesity of the II degree	2,6±0,2	2,5±0,2	- 0,1
	Obesity of the III degree	3,1±0,6	3,0±0,5	- 0,1
Visceral fat level	Obesity of the I degree	5,5±0,5	5,0±0,0	- 0,5
	Obesity of the II degree	6,5±0,7	6,0±0,0	- 0,5
	Obesity of the III degree	11,0±0,0	10,5±0,5	- 0,5
Basic metabolism (kcal)	Obesity of the I degree	1418±46,2	1398±44,4	- 20
	Obesity of the II degree	1648,5±56,8	1614,5±45,5	- 34
	Obesity of the III degree	1972±89,3	1910±82,5**	- 62

* – the reliability of differences between indicators at the level of p≤0,05;
** – the reliability of differences between indicators at the level p≤0,01.

ning of the study was extremely low and dangerous to the health of all women, and throughout the period of TG use, this indicator was normalized only in the group of women with I degree of obesity. In the rest of women, the relative rate of total water also increased, but not enough, compared to the norm for women (45-60%).

The dynamics of the process of body weight correction in obese women with the help of special TG illustrate the changes in the comprehensive indicators of some parts of the body, presented in table III.

The data show that in all women studied, the circumference size of all measured parts of the body decreased within two months, but the largest changes were in the volume of women with III-degree obesity.

The study results of quality-of-life indicators of women with various degrees of obesity are presented in table IV.

The results of the study show that women with the highest BMI rated their quality of life at a low level, and women with lower BMI indicators were satisfied with their lives at the level of low (II degree of obesity) and medium level (I degree of obesity). After 2 months, the indicators for this parameter corresponded to the average and above average levels. The same distribution of levels of indicators was observed in the analysis of the domains of microsocial support, self-perception, physical and psychological well-being.

DISCUSSION

Earlier, we established that a combination of dietary and physiotherapeutic methods is quite effective in the fight against obesity. It is this approach that makes it possible

Table III. Dynamics of comprehensive indicators of the body size of obese women during 2 months of therapeutic gymnastics

Indicators (sm)	Groups of women	At the beginning of TG classes	At the end of 2 months of TG classes	The difference
Abdominal measures	Obesity of the I degree	99,5±3,2	97,2±3,4*	- 1,8
	Obesity of the II degree	111,7±1,5	110,2±1,6*	- 1,5
	Obesity of the III degree	118,8±3,2	115,5±3,5**	- 3,3
Buttocks' measures	Obesity of the I degree	110,7±1,9	108,2±2,6**	- 2,5
	Obesity of the II degree	121,2±2,7	119,2±1,9**	- 2,0
	Obesity of the III degree	137,5±2,5	134,3±3,5**	-3,2
Femur's measures	Obesity of the I degree	69,5±1,5	68,5±1,5	-1,0
	Obesity of the II degree	71,5±2,5	71,0±2,9	-0,5
	Obesity of the III degree	77,5±2,1	75,8±1,9*	- 1,7
Shoulder's measures	Obesity of the I degree	30,8±0,4	30,1±0,3	- 0,7
	Obesity of the II degree	33,4±1,2	33,0±1,2	- 0,4
	Obesity of the III degree	50,5±0,5	49,0±1,1*	- 1,5

* – the reliability of differences between indicators at the level of p≤0,05;
** – the reliability of differences between indicators at the level of p≤0,01·

Table IV. Dynamics of quality of life of obese women during 2 months of therapeutic gymnastics

Indicators (points)	Groups of women	At the beginning of TG classes	At the end of 2 months of TG classes	The difference
Quality of life (max=5)	Obesity of the I degree	3,3±0,3	3,6±0,3	+ 0,3
	Obesity of the II degree	2,8±1,0	3,2±0,6	+ 0,4
	Obesity of the III degree	1,5±0,5	2,5±0,5	+ 0,5
Microsocial support (max=12)	Obesity of the I degree	6,8±1,8	8,2±2,1*	+ 1,4
	Obesity of the II degree	4,2±1,1	6,2±1,2**	+ 2,0
	Obesity of the III degree	2,5±0,5	5,5±1,5**	+ 3,0
Self-perception (max=24)	Obesity of the I degree	14,0±0,0	15,0±0,0	+ 1,0
	Obesity of the II degree	13,8±1,0	15,3±1,5*	+ 1,5
	Obesity of the III degree	6,0±0,0	6,5±0,5	+ 0,5
Social wellbeing (max=32)	Obesity of the I degree	16,3±0,6	19,0±0,0**	+ 2,7
	Obesity of the II degree	15,4±1,1	17,5±1,2**	+ 2,1
	Obesity of the III degree	14,5±0,5	15,0±0,0	+ 0,5
Physical and mental wellbeing (max=28)	Obesity of the I degree	14,5±0,5	15,0±0,0	+ 0,5
	Obesity of the II degree	12,9±0,4	14,4±0,8*	+ 1,5
	Obesity of the III degree	8,5±0,5	10,0±0,0	+ 1,5
Health status (max=5)	Obesity of the I degree	3,6±0,3	4,0±0,0	+ 0,4
	Obesity of the II degree	2,8±1,0	3,9±0,7	+ 1,1
	Obesity of the III degree	2,0±0,0	3,0±0,0	+ 1,0

* – the reliability of differences between indicators at the level of p≤0,05;
** – the reliability of differences between indicators at the level of p≤0,01.

to achieve a stable reduction in body weight over a long period of time [5]. Also, in their previous studies, the decline in the quality of life of women in quarantine conditions and self-isolation, which appeared during the spread of the coronavirus disease, was assessed [18]. We continued the study of this problem and checked the effectiveness of therapeutic gymnastics on the main indicators of vital activity in obese women in conditions of restrictions on motor activity and quality of life during the COVID-19 pandemic.

Dehydration of the studied women can be explained by the high content of fat mass, which is poorly hydrated in comparison to muscle tissue. The muscle component of body weight increased more in women

with I degree of obesity, which also explains the greater increase in the relative amount of total water in women of the same group. Some increase in muscle mass was observed in women with II-degree obesity, and this indicator decreased slightly in women with III-degree obesity. Changes in the mineral component indicator of body weight were insignificant and the same in all women of the three groups, but even such changes in bone mass are dangerous and undesirable in the process of weight correction, so be sure to follow a diet and eat enough calcium-containing foods, to prevent osteoporosis and convulsing. The level of basal metabolism changed most markedly in the group of women with III-degree obesity, apparently due to a decrease in both fat and muscle component of body weight. In women with I and II-degree obesity, the level of basal metabolism also showed a negative tendency to decrease, although not significantly, as there was no desired increase in metabolically active muscle mass and, as a result, basal metabolism did not increase. Such data correlate with the prevalence of overweight in women in Ukraine and the results of the STEPS study, the WHO's stepwise approach to epidemiological surveillance of risk factors for non-communicable diseases [1]. In addition, in other countries where similar studies were conducted, it was established that obesity leads to heart disease and end-stage renal failure [2, 4].

Moreover, the buttocks and abdomen circumferences of women of all three groups decreased to a greater extent, and hip and shoulder girth – to a lesser extent, this indicates a decrease in fat component to a greater extent in the buttocks and abdomen and a possible increase in muscle mass in areas of upper and lower extremities. The data we obtained are consistent with the results of some studies comparing the effects of interval training and diet therapy in obese patients [16].

Indicators of social well-being in all surveyed women were at an average level. At I, II, III degrees of obesity, the state of women's health according to their subjective assessment was above average, average and below average, respectively. The obtained indicators testify to the adequate self-assessment of the studied women regarding the state of their health and their appearance.

CONCLUSIONS

1. The problem of obesity has now reached the scale of a pandemic and requires focused efforts of medical professionals not only at the stage of treatment and correction of human weight, but also at the stage of prevention of overweight, especially among women.
2. Studies of women show a significant impact of overweight on their quality of life, which is reflected in mostly low and below average estimates of almost all quality-of-life criteria.
3. The received indicators in the dynamics of only 2 months indicate the effectiveness of the author's programme of therapeutic gymnastics in the remote mode of training in quarantine restrictions in relation to the pandemic COVID – 19.

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ORIGINAL ARTICLE

CORRELATION BETWEEN DIFFERENT CLINICOPATHOLOGICAL PARAMETERS AND MOLECULAR SUBTYPES OF FEMALE BREAST CARCINOMA IN SOUTH REGION OF IRAQ

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ABSTRACT

The aim: To correlate variable clincopathological parameters with molecular subtypes of the breast carcinoma, which affect the prognosis and management of breast malignancy.

Materials and methods: In this study a total of 511 female patients with breast carcinoma were included, ranging from 32 to 85 years of age, with 35.8% premenopausal and 64.1% being post-menopausal. The sample slides were stained immunohistochemically for estrogen receptors (ER), progesterone receptors (PR), ki67 and HER2, the tumors were graded histologically using the Nottingham criteria system.

Results: Most tumors (72.8%) ranged between 2 and 5 cm in size; the most common histological type of breast carcinoma (49.7%) was invasive ductal carcinoma of no special type, with grade 2 presented in 51.8% cases; most frequent stage at time of presentation was stage 3A, found in 39.9%; the most frequent molecular subtype was ER and/or PR+, Her2- with low proliferation rate ki67<14% subtype in 48.5%, and those group were more likely (statistically significant) to be older, have stage 3 breast cancer, present with tumor size between 2 and 5 cm and tend to be well differentiated histological grade (grade1), mostly with lymph node positive, and most likely have tumor type of invasive ductal carcinoma of no special type.

Conclusions: the most common histological type of breast carcinoma in Iraq south was invasive ductal carcinoma of no special type and most cases showed (ER and/or PR+, HER 2-, low ki67) as the most common molecular subtype.

KEY WORDS: correlation, molecular subtypes, breast carcinoma, ER, PR, HER2

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INTRODUCTION

Breast cancer is an important cause of death in female patients [1]. It is a health problem for both developing and developed worlds and it is the most common malignancy in women [2]. Although there is no accurate statistics about incidence of breast cancer in Iraq, there is obvious increase in such cases in the last few years may be due to radioactive war waste in some regions of the country according to local records. Many risk factors for breast cancer have been recognized like age, age at menarche/menopause, duration of breast-feeding, parity, genetic and nutritional factors, however, in 50% of women the risk factors are not detected [1, 2]. Some studies showed that up to two thirds of the invasive cases being 55 or older of age [3]. Breast cancer is still a major cause of cancer related death worldwide [4]. There are many variables that affect the prognosis and management of breast malignancy, like histological type of the tumor, tumor size, grade, lymph node involvement and hormonal receptor expression status like estrogen receptors (ER) and progesterone receptors (PR) in addition to epidermal growth factor receptors (HER2) protein status. Among these the ER expression is the most important biomarker in breast cancer, because it provides the necessary information for predicting sensitivity to hormonal treatment [4]. Because of this importance, the immunohistochemical assessment of the hormonal receptor status is practically essential and therefore their assessment became almost routine part of the pathologist report as parameter of prognosis [5]. The use of markers for ER, PR, and HER2 are quite useful for managing the expected response to treatment. HER2 over-expression is associated with poor clinical outcome in general and considerable resistance to hormonal therapy, on the contrary, the expression of ER and/or PR, is predicting of response to hormonal and is associated with better prognosis if compared with tumors that are negative for these receptors [6]. Despite the genetic testing is very effective for molecular classification of breast cancer for determining and predicting prognosis, it is not widely used, as it is expensive and may not be available in all centers. Another problem is

that it provide very little information to determine the targeted therapy like tamoxyphin or trastuzumab for tumors expressing hormonal receptors or HER2 receptors, respectively, therefore the immunohistochemistry (IHC) provide better classification regarding both treatment and prognosis [7, 8]. The physiological changes of the breast are mediated to large extent by estrogen. There are two types of the estrogen receptors (ERα and ERβ), the prognostic and predictive value is usually attributed to ERα, and despite that, the majority of ER-positive breast cancers have ERα and ERβ subtypes but ERβ usually decrease its expression during breast carcinogenesis in contrast to ERα [9].

Risk factors of breast carcinoma could be modifiable or non-modifiable risk factors, and the non-modifiable group includes:

Age:

The risk for developing of breast cancer increases with age, so older women are at greater risk [2, 10]. It was found that about two out of three invasive breast cancers occur in women 55 or older [3].

Genetic makeup and family history of breast carcinoma:

Those with one first-degree female relative (e.g. mother, sister, or daughter) having breast cancer, have a chance of a twice risk for getting breast carcinoma, additionally breast cancer patients found to be at risk of 3 to 4 times to get another malignancy in the same or the other breast [3, 10].

Dense breasts:

Dense breasts generally more risky of developing of breast cancer than thin breasts, in addition it may affect for detection of breast tumors by mammogram more than thin breast [10, 11].

Reproductive history:

It was found that early menarche and late menopause may increase the risk of developing of breast carcinoma attributed to longer periods of exposure to hormones [10, 12].

Modifiable risk factors include:

Hormones intake:

It was found that hormone replacement therapy (both estrogen and progesterone) if taken for long period (more than 5 or 7 years) after menopause can increase risk of breast carcinoma [10]

Poor physical activity and overweight:

It was found that breast carcinoma is more likely in physically inactive women or those with overweight or obesity [11-13].

Others:

Factors that may increase risk of breast carcinoma also include non-breastfeeding or if first born was after age of 30 [11, 12]. Breast carcinoma can be in situ or invasive. The in situ category include ductal, lobular, those with characteristic of both or those of unknown origins. Some studies revealed that some cases (20-53%) of DCIS were misdiagnosed as benign lesions, were actually found to be invasive breast carcinoma 10 or more years later [14, 15].

CORRELATION OF THE MOLECULAR SUBTYPES OF BREAST CARCINOMA AND TREATMENT STRATEGY

The hormone receptor status give us information whether such patient may benefit from hormonal treatment, so if the tumor is ER positive status, it will be candidate for hormonal therapy, but in case of ER negative tumor, it is unlikely to get that benefit [16, 17]. There is a significant relationship between major molecular subtypes (luminal A, luminal B, HER2 enriched and basal-like) of breast carcinoma and different modalities of treatment. For example, the luminal A subtype found to respond to well to endocrine (hormonal) therapy, much better than chemotherapy, while although the luminal B subtype also respond to endocrine therapy (tamoxifen and aromatize inhibitors), but it is not as good response as that for luminal A with generally better response to chemotherapy than luminal A, despite such response being variable. The HER2 enriched subtype respond to trastuzumab (Herceptin), however, with patients treated with trastuzumab, it was found there was no significant difference in prognosis regarding the age of patient at time of presentation, this is also noted by other studies [18]. HER2 enriched subtype also respond generally good to anthracyclines based chemotherapy, whereas the basal like type will respond to platinum-based chemotherapy and poly-adenosine diphosphate-ribose polymerase inhibitors well but not to hormonal therapy or trastuzumab (Herceptin) [19-21].

THE AIM

The aim of the study was to correlate variable clinicopathological parameters with molecular subtypes of the breast carcinoma, which affect the prognosis, and management of breast malignancy in patients from south region of Iraq.

MATERIALS AND METHODS

After surgical removal of biological tissues, immuno-histochemical analysis was performed to determine the estrogen (ER), progesterone (PR) receptors and the HER2 protein status by using standard procedures: the

samples were fixed in 10% formaldehyde overnight, embedded in paraffin and sectioned using a microtome at 4-µm. After conventional processing, the slides were placed in epitope retrieval solution. To block endogenous peroxidase bindings, we incubate samples in an oxygen H_2O_2 solution for 5 minutes, then washed with phosphate buffered-saline (PBS). The analysis of data included 511 invasive breast cancer female patients diagnosed in the Al Sader medical city histopathology lab and private labs in Al Najaf city and other prominent pathology centers of south provinces of Iraq, from 2018 through 2021. The histological grade of the carcinoma was assessed based on Nottingham modification of the Bloom-Richardson system. The inclusion criteria of this study include:

1 ready slides stained by H&E that were requested from teaching hospital and some private labs from different south provinces with good quality and submitting their formalin-fixed, paraffin embedded tissue samples for immunohistochemical staining done in this study, except ki67 immunohistochemistry, which was already done, examined again and included in this study;
2 female patients diagnosed with invasive breast carcinoma by mastectomy and core needle biopsies;
3 the histological grade and lymph node status were available for all patients submitted in this study.

IMMUNOHISTOCHEMISTRY

The breast carcinoma classified into four major molecular subtypes [31], which can be determined immunohistochemical. Theses subtypes are: luminal A "ER and/or PR+, HER2- with low proliferation rate –ki67<14%"; luminal B "ER and/or PR+, HER2+ or - (variable) with high proliferation rate –ki67 ≥ 14%"; HER2 enriched "ER-, PR-, HER2+" and basal-like "ER-, PR-, HER2-". We incubate the samples at room temperature for 60 minutes using primary monoclonal antibodies (Clone ER1D5, Immunotech) for ER and (Clone PR10A9, Immunotech) for PR receptors and using a polyclonal antibody (Ref A0484, Dako Heceptest) for HER2, this is followed by incubation with biotin-labeled secondary antibodies (Ultratech HRP Kit PNIM2391, Immunotech Biotinylated Secondary Antibody) for a duration of 10 minutes. Then by using the Chromogenic substrate, Diaminobenzidine (3,3-diaminobenzidine), we can visualize the streptavidin-peroxidase complex. The nuclei were stained by hematoxylin (Harris type). For HER 2 assessment, immunoreaction pattern was divided into four groups of 3+, 2+, 1+ and 0 scores. According to American Society of Clinical Oncology (ASCO)/College of American Pathologists (CAP) guidelines, the HER2 positivity is defined as 3+ on IHC (defined as complete circumferential intense membrane staining within >10% of invasive tumor cells), while tumors scored as 0 or 1+ were considered HER2 negative. If the staining is weak or moderate complete membrane staining within >10% of tumor cells (scored as 2+), it is considered equivocal [31], but fluorescence in situ hybridization (FISH) for equivocal cases was not performed. Regarding ER and PR expression, they considered positive when nuclei of the tumor cells stained were ≥1% [32]. The statistics were done by SPSS version 21.IBM 2012. The categorical variables were presented as frequency and percentage, P-value of < 0.05 was taken as significant value.

RESULTS

In present study a total of 511 female patients ranging in age from 32 to 85 years old (mean = 54.31 years and SD ± 11.447) with 183 (35.8%) were premenopausal and 328 (64.1%) being post-menopausal. Right breast, was most frequently involved (65% of cases). The size of tumors ranged between 1 cm and 10 cm with most cases ranged between 2 and 5 cm found in 372 (72.8%) cases. Most common histological type of breast carcinoma was invasive ductal carcinoma of no special type in 254 (49.7%) patients. Most cases showed ER/PR+, HER2-, ki67<14% as the most common molecular subtype (248 cases, 48.5%). It was found that most common tumor grade in the present study group was grade 2 (265 cases, 51.8%) and most frequent stage at time of presentation was stage 3A found in 204 (39.9%) patients. The frequency and percentage of different features for the cases submitted in this study was illustrated in table I.

The most prevalent histological types are shown in figures 1 and 2.

The histological grade of tumor is better appreciated on higher power magnification as shown in figures 3 and 4.

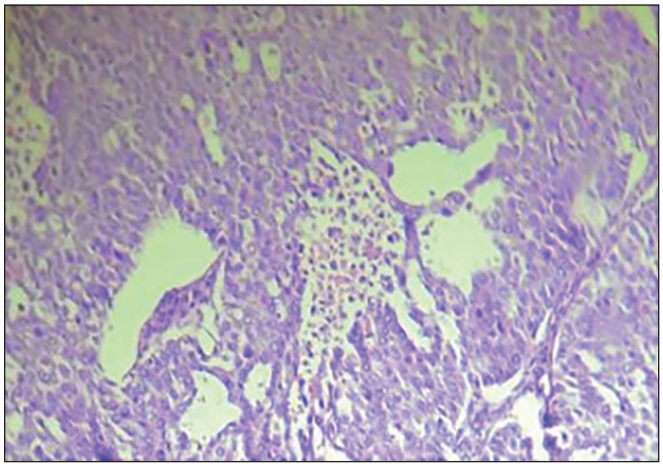

Fig. 1. Microscopic picture of invasive ductal carcinoma of no special type (NST), showing small duct-like formations (H&E stain, x 100 magnification).

Table I. Various clinicopathological parameter of breast carcinoma.

Carcinoma type	Number of patients (frequency)	Grade of the tumor	Number of patients (frequency)	Stage of tumor at time of presentation	Number of patients (frequency)	Size of tumor (cm)	Number of patients (frequency)	Involvement of axillaries lymph nodes by metastasis	Number of Patients (frequency)
Invasive ductal carcinoma of no special type	254 (49%)	Grade 1	166(32.5%)	Stage 2A	131(25.6%)	Less or equal to 2	73(14.3%)	+ ve	371 (72.6%)
Invasive lobular carcinoma	177 (34.6%)	Grade 2	265(51%)	Stage 2B	38(7.4%)	More than 2 to 5	372(72.8%)	-ve	122 (23.9%)
Carcinoma with medullary features	24 (4.7%)	Grade 3	80(15.7%)	Stage 3A	204(39.9%)	More than 5	44(8.6%)	Not examined (true cut biopsy)	18 (3.5%)
Metaplastic	16 (3.1%)			Stage 3B	102(20%)	Cannot be assessed (true cut biopsy)	22(4.3%)		
IDC with neuroendocrine differentiation	14 (2.7%)			Stage 3C	36 (7%)				
Mixed (ductal and lobular)	13 (2.5%)								
Mucinous	7 (1.4%)								
Inflammatory carcinoma	6 (1.2%)								

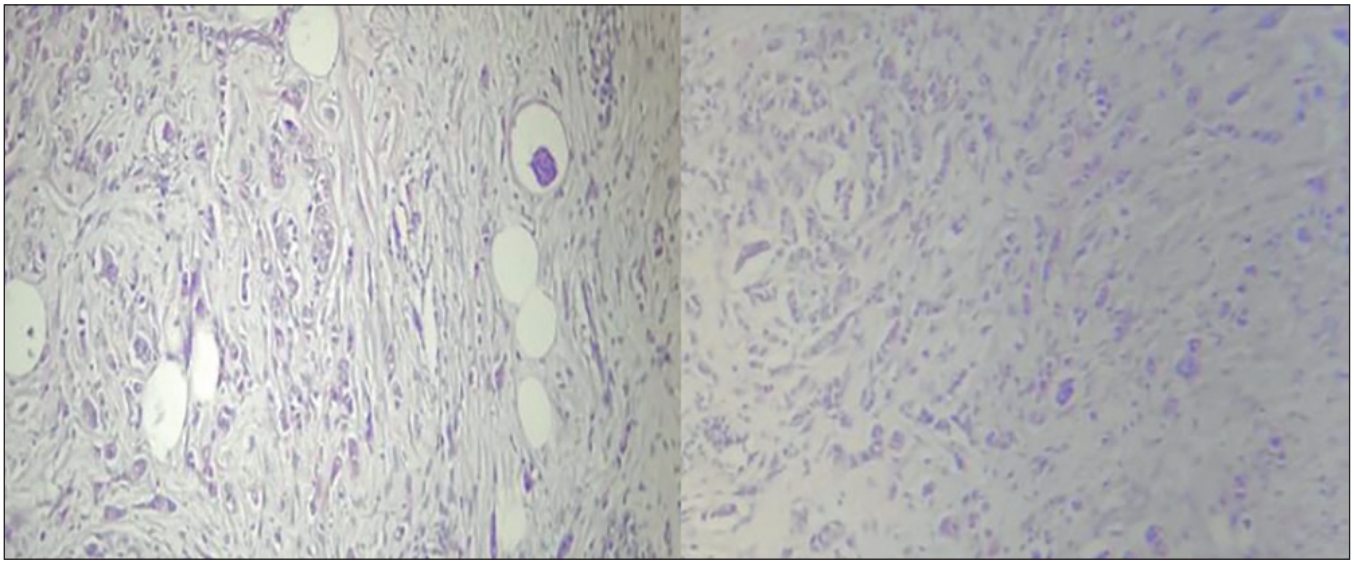

Fig. 2. Microscopic picture of invasive lobular carcinoma, showing Indian file cell pattern (H&E stain x 100 magnification).

From those 511 patients, 248 (48.5%) were ER and/or PR+, Her2-, low proliferation rate; 204 (39.9%) were triple negative; 52 (10.2%) were ER and/or PR+, Her2 variable with higher proliferation rate and 7 (1.4%) were ER-, PR-, Her2+ (Table II).

The immunohistochemical profile for the epidermal growth factor (HER2) and hormonal receptors as obtained during this study was illustrated in figures 5, 6 and 7.

It was found that most frequent hormonal receptor status among patients was ER and/or PR+, HER2- with low proliferation rate subtype – 48.5%, which show statistically significant ($P < 0.05$) correlation with various clinic pathologic parameters as this group were more likely of older age ($P = 0.0005$), have stage 3 breast cancer ($P = 0.004$), present with tumor size between 2 and 5 cm ($P = 0.0005$) and tend to be well differentiated histological grade i.e. grade 1 ($P = 0.0005$). They were mostly lymph node positive ($P = 0.0005$), and have an "invasive ductal carcinoma of no special type" as the most common histologic type ($P = 0.049$). It was

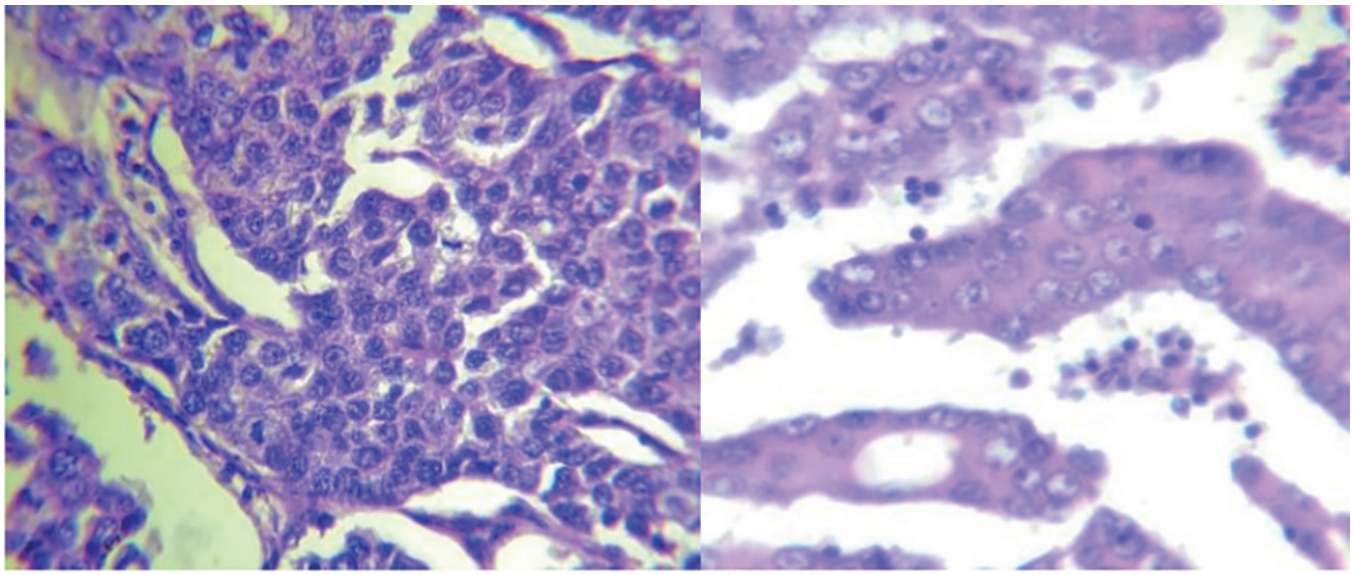

Fig. 3. Microscopic picture of invasive ductal carcinoma NST showing moderate to high nuclear grade (H & E stain, x 400 magnification).

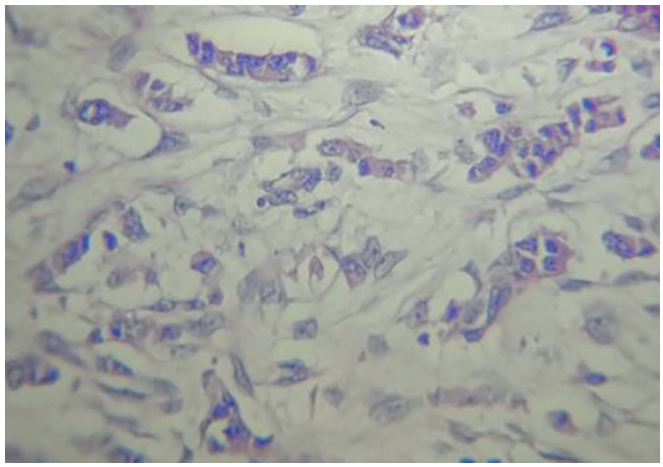

Fig. 4. Microscopic picture of invasive lobular carcinoma showing generally smaller nuclei (H&E stain, x 400 magnification).

found that expression of HER2 receptor is correlated with tumor grade with expression being increased with higher grade (P = 0.001), while there was an inverse relationship regarding hormonal receptor status and tumor grade with ER and/or PR expression being more positive in low grade tumors and decrease with increasing grade (P = 0.0005). The various clinicopathological features as correlated with molecular subtypes of breast carcinoma were illustrated as follows, with statistically significant correlation taken at level <0.05.

The age preference of subtype of breast carcinoma shown in table III.

Table II. Tumor molecular subtypes.

Molecular subtype	Number and frequency of patients
ER and/or PR+, Her2-,ki67<14%	248 (48.5%)
ER and/or PR+, Her2+ or -,ki67 ≥14%	52 (10.2%)
ER-,PR-, Her2+	7 (1.4%)
ER-,PR-, Her2-	204 (39.9%)

Table III. Average age of patients at time of presentation across the molecular subtypes of breast carcinoma.

Molecular subtype	ER and/or PR+, Her2-, ki67<14% (n=248)	ER and/or PR+, Her2+or-, ki67≥14% (n=52)	ER-,PR-, Her2+ (n=7)	ER-,PR-, Her2- (n=204)	P-value
Age (years)	≥ 50	≥ 50	30 - 49	30 - 59	0.0005

Table IV. Distribution of tumor stage across the molecular subtypes of breast carcinoma.

Tumor stage	ER and/or PR+, Her2- ki67<14% (n=248)	ER and/or PR+, Her2+ or -ki67≥14% (n=52)	ER-,PR-, Her2+ (n=7)	ER-,PR-, Her2- (n=204)	P- value
2A	74 (29.8%)	0	0	47(23%)	0.004
2B	25 (10.1%)	23 (44.2%)	0	0	
3A	137 (55.3%)	5(9.6%)	0	52 (25.5%)	
3B	7(2.8%)	0	0	105 (51.5%)	
3C	5 (2%)	24 (46.2%)	7 (100%)	0	

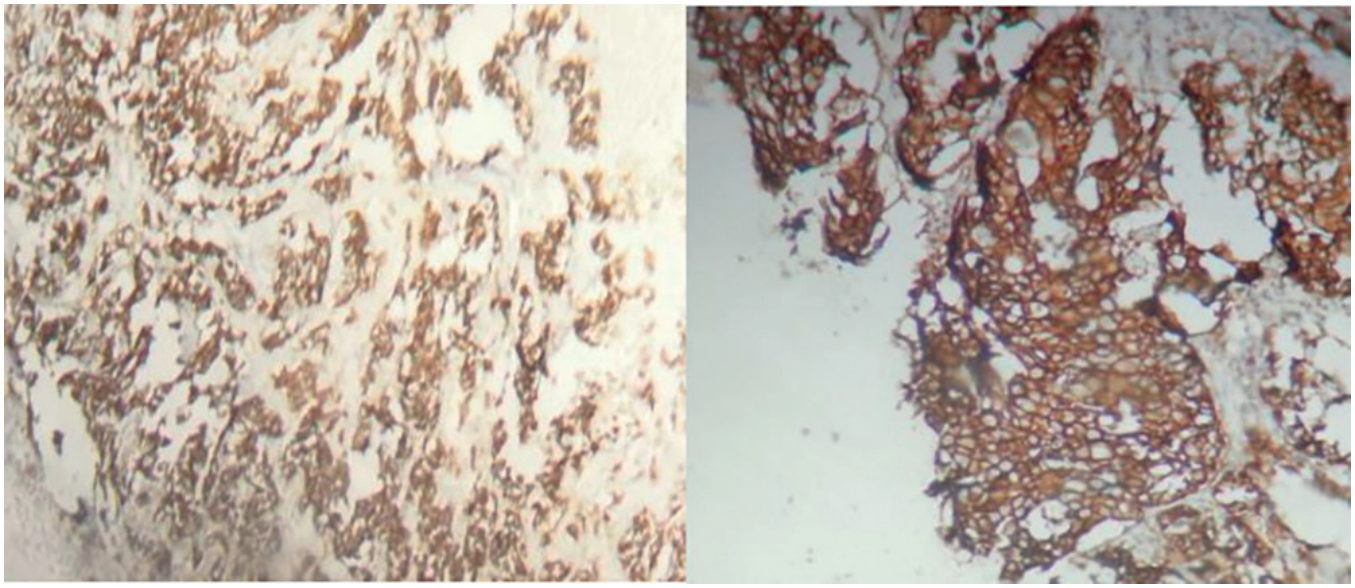

Fig. 5. Immunohistochemical slide stained for HER2, showing complete intense membrane staining in more than 10% of invasive tumor (3+).

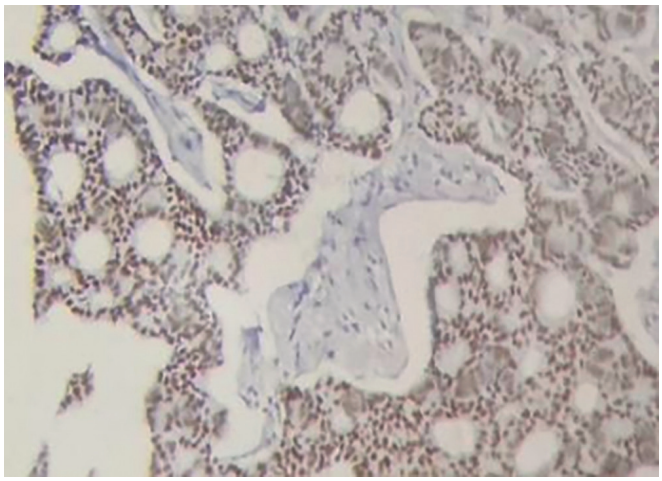

Fig. 6. Immunohistochemical slide stained for ER, showing intense nuclear staining in most of the tumor.

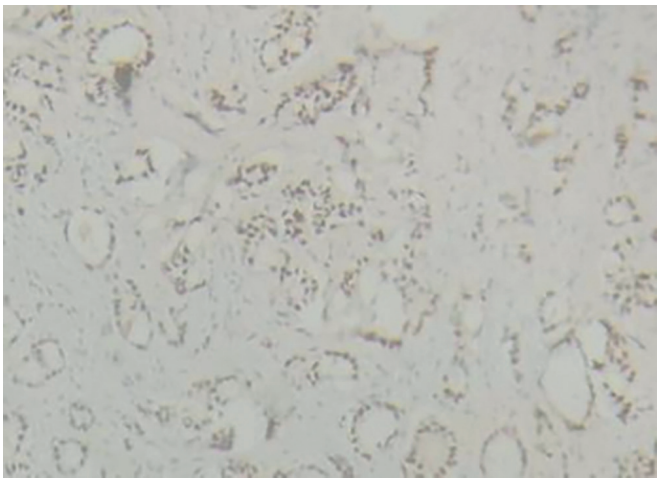

Fig. 7. Immunohistochemical slide stained for PR, showing moderate intensity nuclear staining in most of the tumor.

Table V. Distribution of tumor grade across the molecular subtypes of breast carcinoma.

Tumor grade	ER and/or PR+, Her2- ki67<14% (n=248)	ER and/or PR+, Her2+ or – ki67≥14% (n=52)	ER-,PR-, Her2+ (n=7)	ER-,PR-, Her2- (n=204)	P- value
1	136 (54.8%)	12 (23.1%)	0	17 (8.3%)	
2	109 (44%)	37 (71.1%)	2 (28.6%)	118 (57.9%)	0.0005
3	3(1.2%)	3(5.8%)	5 (71.4%)	69 (33.8%)	

As it noted here, patients with positive ER and PR tend to be older in age at time of presentation than patients with negative hormonal receptor status.

The correlation between stage, grade and molecular subtypes indicated in tables IV and V respectively.

Tumors with positive hormonal receptor status and negative HER2 tend to present at earlier stages than other groups.

Two peaks were noted here, tumors with ER, PR positive hormonal status tend to present at much lower grade than triple negative cases which usually showed higher grade.

The histological type of carcinoma show correlations: the recognized histologic types of breast carcinoma distributed across the molecular subtypes of breast carcinoma (Table VI).

As shown here, the majority of carcinoma histological subtypes in all molecular groups were ductal carcinoma NST and lobular carcinoma.

The correlation of tumor maximal diameter with molecular subtypes was illustrated in table VII.

Table VI. Distribution of histological subtypes of breast carcinoma across molecular profile.

Histopatholog ical type	ER and/or PR+, Her2- ki67<14% (n=248)	ER and/or PR+, Her2+ or – ki67≥14% (n=52)	ER-,PR-, Her2+ (n=7)	ER-,PR-, Her2- (n=204)	P-value
Ductal carcinoma NST	135 (54.5%)	16 (30.8%)	0	103 (50.5%)	0.049
lobular	92 (37.1%)	14 (26.9%)	3(42.9%)	68 (33.3%)	
medullary	4 (1.6%)	1 (1.9%)	0	19 (9.3%)	
metaplastic	1 (0.4%)	7 (13.5%)	0	8 (3.9%)	
Invasive ductal carcinoma with neuroendocrine features	6 (2.4%)	7 (13.5%)	0	1 (0.5%)	
Mixed ductal and lobular	6 (2.4%)	5 (9.6%)	0	2 (1%)	
mucinous	3 (1.2%)	2 (3.8%)	1 (14.2%)	1 (0.5%)	
inflammatory	1 (0.4%)	0	3 (42.9%)	2 (1%)	

Table VII. Distribution of tumor size across the molecular subtypes of breast carcinoma.

Tumor size	ER and/or PR+, Her2- ki67<14% (n=248)	ER and/or PR+, Her2+or -ki67≥14% (n=52)	ER-,PR-, Her2+ (n=7)	ER-,PR-, Her2- (n=204)	P- value
≤2 cm	0	0	0	73(35.8%)	0.0005
2 – 5 cm	248(100%)	0	0	124(60.8%)	
>5 cm	0	37(71.2%)	0	7(3.4%)	
Cannot be assessed	0	15(28.8%)	7(100%)	0	

Table VIII. Distribution of lymph node metastatic status from any histological subtype across the molecular subtypes of breast carcinoma.

Lymph node status	ER and/or PR+, Her2- ki67<14% (n=248)	ER and/or PR+, Her2+or -ki67≥14% (n=52)	ER-,PR-, Her2+ (n=7)	ER-,PR-, Her2- (n=204)	P-value
Positive	248 (100%)	0	0	124 (60.8%)	0.0005
Negative	0	37(71.2%)	0	80(39.2%)	
Not examined	0	15(28.8%)	7(100%)	0	

Most primary tumor sizes were between 2 and 5 cm in greatest dimension, however, few cases cannot be assessed for the actual tumor size as they obtained by core needle biopsy.

The correlation connections of regional lymph node status and the molecular subtypes of breast carcinoma was indicated in the table VIII.

As it shown, most of the tumors that give rise to regional nodal metastasis were positive for hormonal receptor status, followed by those with triple negative profile, and in a few subset of cases not examined for lymph nodes as they are provided as core needle specimens without lymph nodes.

DISCUSSION

The prognosis of each breast carcinoma is well depending on many clinical, pathological and biological factors like primary tumor size, regional lymph node involvement, and tumor grade, the hormone receptor expression like estrogen and progesterone receptor status and growth factor receptors like HER2 status [1]. Breast cancer is considered the most common malignant tumor of the female malignancies. It was found, that it accounts for 22% of all female cancers and that is two folds more prevalent than any other cancer in women at any other site [6]. This study deals with 511 specimens of breast tissue including 489 case of modified radical mastectomy and 22 cases of true cut biopsy specimens of breast carcinoma proven by light microscopy. The study aimed to correlate the histologic type of the cancer and other clinicopathological parameters with the molecular subtypes determined by immunohistochemistry technique to find out the ER, PR and HER2/neu status of the tumor. The age group of all female patients studied range from 32 to 85 years. In routine clinical practice, management of patients with breast cancer is frequently influenced by classic variables as the histological type, grade and stage, ER, PR and HER2 status. Previous studies in unselected breast cancer samples described a correlation between HER2 and hormone receptors [33-36], however, in the present study, HER2 expression revealed a negative correlation with hormonal receptors status, which is in accordance

with a recent study in ductal invasive carcinomas. This negative correlation between hormone receptor and HER2 might be explained by the fact that estrogens suppress HER2 through the ER and that the frequency of expression of ER and PR receptors with that of HER2 may change throughout tumor progression. In addition, the co-expression of hormone receptors with that of HER2 in our study in infrequent (only 8 cases) as previously reported by [37]. Our study reports also a significant positive correlation between ER and PR expression. This correlation may be due to theory of ER-dependent PR synthesis. Grade of tumor in our study, was significantly correlated with the expression of ER, whereas in previous studies in Morocco, both ER and PR expression were significantly correlated to grade tumor. The present cross-sectional study shows that in moderate- or intermediate grade of tumor, HER2 expression is significantly related to ER subtype. This result, suggests that the prognostic value of the ER status in grade II tumors may, therefore, be more important to guides the decision-making process for patient treatment. By contrast, in the current study, there was an association between HER2 expression and vascular space invasion. The prognostic value of vascular space invasion in breast cancer clinical management decisions remains a matter of debate. In a large and well characterized series of patients with operable breast cancers in work of [38], it shown that vascular space invasion provided a strong predictor of outcome in patients with invasive breast cancer and should be incorporated into breast cancer staging systems, however, others studies reported no association. Therefore, further studies are required to clarify these potential relationships. In this study we found, that HER2 status is expressed reversely with hormonal receptors status i.e. ER and/or PR especially in invasive ductal carcinoma types (excluded is triple negative cases in which case both expressions are negative). Such reverse correlation between HER2 and the hormone receptor expression can be explained by knowing that estrogens can suppress HER2 through the estrogen receptor, also the number of times of expression of ER and PR receptors with that of HER2 may change throughout tumor progression, similar findings suggested by other studies [13]. This finding was suggested in this study by finding that the co-expression of hormone receptors ER and/or PR with that of HER2 is actually infrequent and found in only 9 (2.9%) cases. The hormonal receptor status and HER2 expression assessment through IHC or less frequently through other technique, is now routinely done in every case of invasive breast carcinoma for management purposes as patients with ER positive primary breast tumors will benefit from adjuvant hormone therapy, usually tamoxifen, while post-menopausal women may receive aromatase inhibitor and patients with HER 2 overexpression get more benefit from trastuzumab [14]**.** Generally, regarding hormonal receptor status, the ER is expressed in 70-80% of invasive ductal carcinoma, compared to PR that is expressed in 60-70% of invasive breast carcinoma [15]. In this study, however, the ER was found to be expressed in 58.7% of the cases, compared with PR, which was expressed in 45% of cases, and HER2 expressed in only 11.5% of cases. The classification of breast carcinoma based on immunohistochemistry for ER and/or PR or HER2 status to provide both prognostic and therapeutic information cannot be obtained from either alone, therefore adding HER2 expression assessment to that of hormonal receptor status provides better and important therapeutic guidance [22]. In our study, 511 modified radical mastectomy and true cut specimens of breast cancer were involved. It was found that the most common histologic type of breast carcinoma in our study was invasive ductal carcinoma of no special type (IDC NST) that was found in 49.7% of the cases, and followed by invasive lobular carcinoma (ILC) found in 34.6% of the cases. These results were comparable with those obtained by other studies like Rajesh RC, et al. [8]. In this study most patients were postmenopausal (64.1%), probably due to postmenopausal hormonal stimulation; and this is also cleared by others [19], while in other studies like Rajesh RC, et al. [8]. Most hormonal receptor status in this study was positive for ER and/or PR, with most patients of negative hormonal receptor status were younger at time of presentation; and this finding is similar to that obtained by others [2]. The outcomes of breast cancer can vary depending on the type of cancer, extent of disease and the patient age. It was found that the five years survival rates in the developed countries are high (80% to 90%), while in developing countries, the survival generally is poor [23], which may be attributed to the deficient awareness of signs and symptoms of breast lump and poor availability of screening programs in these poor countries, which as a result, leads to advanced disease with larger tumor size and more possibility of nodal involvement at time of presentation [2]. This study done to show the correlation between expression of ER, PR, and HER 2 with one another and too many different clinicopathological features of the breast carcinoma, like histological type of the cancer, its grade, size, lymph node involvement as well as age of patient.

AGE

Our study showed that most cases of ER and/or PR positive patients were in the age group 50 and more with

majority of them being with invasive ductal carcinoma type, and these results is generally similar to those obtained by Chand P, et al. [9], although it showed slightly older age than our study, which may be explained by generally younger age of patient in our country when acquire the disease. Regarding pure HER2 positive status were most at age range of 30 to 49 years, which on average similar to results, obtained by Prem Chand et al. [9], with statistically significant correlation obtained regarding of the age of the patient with ER/PR and HER2 expression (P=0.0005).

TUMOR SIZE

The size of tumors generally ranged between 1-10 cm with 72.8% of cases ranged between 2 and 5 cm. The correlation between tumor size and the four major molecular subtypes of breast carcinoma (meaning ER and/or PR+, HER 2-, low ki67; ER and/or PR+, HER2 +or-, high ki67; ER-, PR-, HER2+and triple negative) was statistically significant (p=0.0005) which was similar to results of other studies [9], and it was found that basal–like and HER2 enriched subtypes were found more often in large tumor sizes, that is also found by others [24].

TUMOR GRADE

We found in our study that 166 patients (32.5%) was with Grade I, 265 (51.8%) cases – with Grade II, and 80 (15.7%) – with Grade III. The majority of ER and/or PR positive tumors (58.7%) were of lower grade (grade I or II) (statistically significant correlation, p=0.0005), but the majority of HER2 positive tumors regardless of ER/PR status were of higher grade (grade II or III) (statistically significant correlation, p = 0.001). A study conducted by Aman NA, et al. [26] and others [25] show similar results.

AUXILIARY LYMPH NODE STATUS

Metastasis in auxiliary lymph nodes was seen in 371 (72.6%) of patients. Out of the positive lymph nodes cases, about 66.7% were ER and/or PR positive primary tumor with significant correlation (p=0.0005), what is in correspondence to found by others [27], and about 95% of lymph nodes with metastasis showed hormonal receptors positive for the metastatic deposits (p=0.0005). The remaining cases were not examined for lymph node status as they obtained by core needle biopsy with no availability of their lymph nodes, and approximate results was obtained by some other studies [28].

RECEPTOR POSITIVITY

In the current study, hormonal receptor (ER and PR) positivity was in 58.7% cases, which is close to the results of the study conducted by [29]. HER 2 positive expression with the primary tumor found in only 16 cases (3.13%), which is actually less than what was obtained by other studies [30], and this may be explained by number of factors including: variation in the populations, technical and biological factors of performance. In this study, regarding ER and PR receptor status, there was intimate correlation with each other (p=0.0005), however this may need to be proved further by other studies.

CONCLUSION

In conclusion, molecular subtypes of breast carcinoma are important in determining prognosis and treatment plans. These subtypes was detected in decreasing order of frequency in this study: luminal A, triple negative, luminal B and HER 2 enriched type. This study showed that the most common histologic type of breast carcinoma was invasive ductal carcinoma of no special type and most of these cases showed (ER and/or PR +, HER 2 -, low ki67) immunohistochemical profile, as the most common molecular subtype. This subtype showed invasive ductal carcinoma of no special type as the most common associated histologic type, and is usually associated with older age patients, having tumors with lower grade (grade 1) and present at stage 3 with positive lymph nodes. In addition, most of primary tumors that give rise to axillary nodal metastasis were ER and/or RP positive, and we found that HER 2 status is expressed reversely with hormonal receptors especially in invasive ductal carcinoma types.

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The Author declare no conflict of interest.

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ORIGINAL ARTICLE

FEATURES OF FORMING SELF-EDUCATIONAL COMPETENCE OF FUTURE DOCTORS

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ABSTRACT

The aim: To analyse the self-educational competence formation for future doctors in higher education institutions. The process has to be analyzed from the presence of motives regarding the educational activity of the individual to personal need for self-improvement.

Materials and methods: The diagnostic stage, which was conducted in 2020-2021, included 300 sixth-year students from three higher educational institutions: I. Horbachevsky Ternopil National Medical University, Danylo Halytsky Lviv National Medical University, Ivano-Frankivsk National University.

Results: Comparative analysis shows that the level of self-educational competence formation for future doctors in higher education institutions depends on the form of educational activityto a large extent. It was established that 196 (65%) future doctors prefer practical training at the patient's bedside, 92 medical students (31%) study in simulation centers, and 12 young people (4%) consider combined classes and generalizing conferences to be important.

Conclusions: Research and experimental verification of the effectiveness of the self-educational competence formation for future doctors took place during the training of sixth-year students at a higher educational institution. Innovative methods of developing critical thinking, information and interactive technologies were used.

KEY WORDS: professional communicative literacy, medical workers, language training, medical terminology, students

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INTRODUCTION

The intensive development of the digital society determines the need to strengthen the role of independent work of medical students and causes the reorientation of the organization of the educational process in institutions of higher medical education to the formation of the ability to learn, readiness for self-development, mastering the methods of adaptation in a changing society. Since the teacher is no longer the primary source of information for the student, the task of organizing training aimed at mastering the main methods of cognitive and research activity becomes important. Traditional forms of classroom-based learning create monotony of the learning process, which leads to student and teacher fatigue. Therefore, the educational activity at the higher educational establishment should be oriented to the individuality of the future doctor, his interests, level of basic knowledge and natural aptitudes [1].

In order to develop the ability to solve life tasksquickly and efficiently, it is necessary to create an appropriate educational environment focused on the formation of the student's cognitive activity, the ability to work with educational material and informationconsciously and independently as well as the readiness to develop.

THE AIM

To analyse the self-educational competence formation for future doctors in higher education institutions. The process has to be analyzed from the presence of motives regarding the educational activity of the individual to personal need for self-improvement.

MATERIALS AND METHODS

The diagnostic stage, which was conducted in 2020-2021, included 300 sixth-year students from three higher educational institutions: I. Horbachevsky Ternopil National Medical University, Danylo Halytsky Lviv National Medical University, Ivano-Frankivsk National University.

RESULTS AND DISCUSSION

At the current stage of the development of society, a cognitive approach with the aim of learning everything does not meet the need for personality development in the age of digital technologies. There is a demand for competency-based education, which involves an approach in which each student masters the educational material at such a level, which gives him the opportunity to successfully study further, to apply the acquired knowledge and skills in practical activities [2].

Currently, there is a reorientation of the traditional educational paradigm to a person-oriented one, which requires a change and the construction of an appropriate language for expressing concepts in their new connections and relationships, establishing the necessary boundaries of concepts. In the works of scientists, there is a discrepancy in the definition of the 2 terms "competence", which are conceptually important in the conceptual series in the implementation of the competence approach – the first "competence" means an ability to work professionally the second one – the authority to perform some actions. The implementation of the activity approach stimulates the development of students' cognitive qualities, the formation of knowledge, abilities and skills, the readiness and ability to apply them in specific conditions [3]. In order to acquire self-educational competence, they need free access to sources of information and a formed readiness to synthesize the received informationindependently. The ability and skills of working with information and computer technologies enable an individual to acquire self-educational competence.Consideration of motivational, procedural and technological aspects of ensuring independent cognitive and research activity of future doctors, which takes into account their individual interests and abilities, requires special attention.

In the interpretation of the concept of «self-education», it is common to consider it as a type of activity aimed at the constant self-development of an individual, the realization of his own needs, socialization, etc. We will consider self-educationas a purposeful and systematic process aimed at the formation of self-educational abilities and skills based on the cognitive and search activity of an individual. In the concept of "self-education", we single out certain components of this term, which we will rely on in the context of our research: the presence of an individual's motives for educational activity, his need for self-improvement; possession of computer, information and search technologies and self-educational skills; the ability to self-organize, implement and self-monitor one's own educational and cognitive activities; formation of self-education reflection.

The problem of improving the educational process requires the development of a set of pedagogical conditions for the effective formation of self-educational competence of future doctors. The primary task is to find ways to increase the effectiveness of tasks of a practical direction, to optimize the methodical support of the educational process. In the educational process, the provision of clear pedagogical conditions is a necessary factor that contributes to the quality assimilation of knowledge and the formation of skills, which are the defining aspects of the main components of the self-educational competence of medical students.

In the process of forming the self-educational competence of future doctors, it is important to ensure that they master the methods of cognition (observation, practical work, experiments), use the personal experience of medical students, and clarify cause and effect of relationships. Involvement of future doctors in research and creative activities is an important means of forming the self-educational competence of an individual [4; 5].

One of the ways to solve this task is to conduct integrated classes, implement educational projects based on the symbiosis of the content of educational subjects, etc. This approach makes it possible to satisfy the interests and needs of students, to apply the knowledge and provisions of other sciences in the educational process-comprehensively. Therefore, we understand that the formation of self-educational competence of an individual is determined by the socio-cultural environment.

In the studies devoted to the problem of the formation of self-educational competence written by specialists of various fields of training (N. Voropai, O. Nozhovnik, E. Spivakovska-Vandenberg), they consider psychological, organizational-methodical, content-targeted, resource conditions for optimizing the educational process, as well as motivational and procedural issues and technological support of subjects of the educational process. The analysis of the scientific works shows that the pedagogical conditions for the formation of self-educational competence of future specialists consist of cognitive and research activitiesin their motivated attitude; reflexive readiness of teachers and students [6]. The main factors that influence the formation of students' positive motivation towards the process of self-development are the awareness of the theoretical and practical significance of the knowledge obtained in the process of studying clinical disciplines, in the form of presentation of educational material, and the selection of tasks of a practical-activity nature.

Therefore, moral principles, value orientations of the personality of the future specialist, motives of self-educational activity produced in the social environment, determine the formation of self-educational competence and personality development.

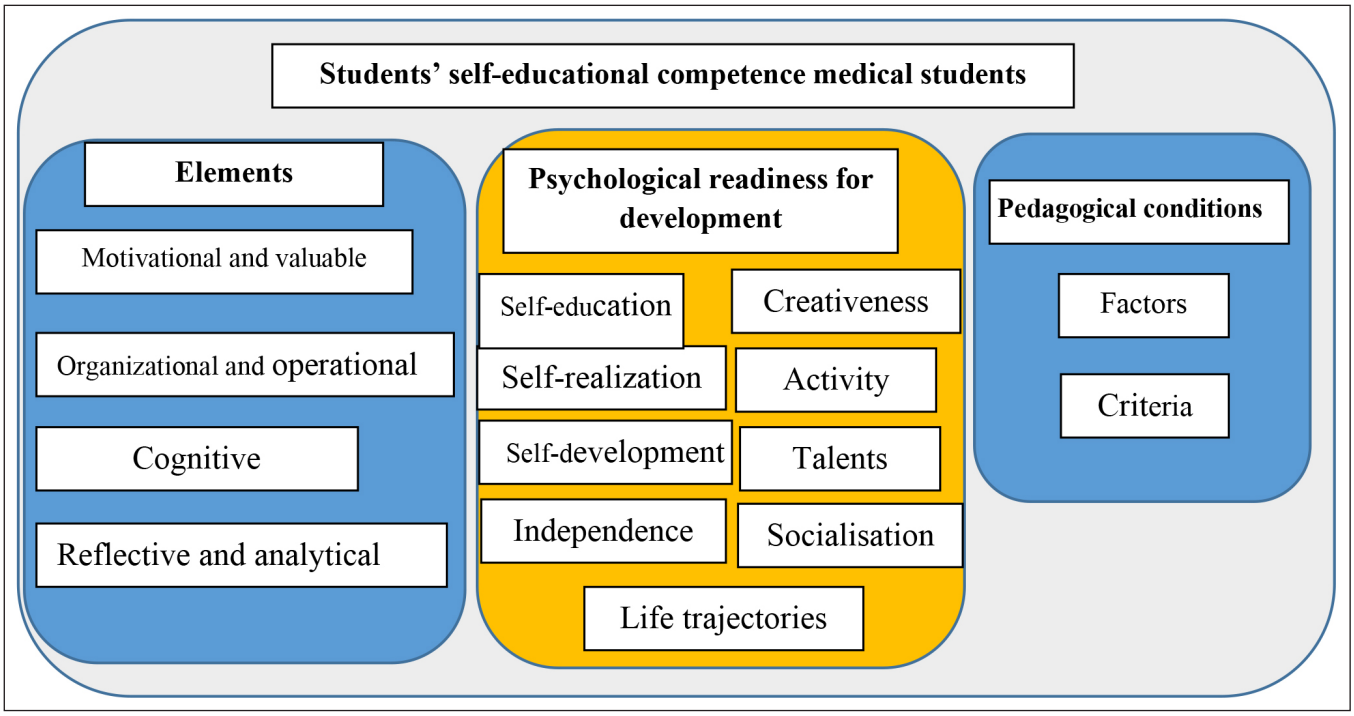

Fig. 1. Self-educational competence of medical students

The content of self-educational competence is supplemented by an orientation towards learning the means of intellectual, spiritual and physical self-development, emotional self-regulation and self-support, psychological literacy, thinking and behavior, care for one's own health, a set of measures related to safety issues of life [7].

In the research, we took into account that the self-educational competence of future doctors is formed not only in the educational process of the medical institution where the student studies, but also in the family, in the circle of friends, under the influence of culture, politics, religion, etc. There is a need to design the educational process for the purpose of forming competencies [8], including self-education, which involves the involvement of students in the independent implementation of educational projects and preparation for medical practice [9].

In modern trends in education development, the valueof self-education is gaining importance. There is a need in pedagogical practice to find approaches aimed at the development of self-motivation, self-organization, self-control, self-improvement of the student's personality in educational activities.

The diagnostic stage, which was held in 2020-2021, included 300 sixth-year students from three higher educational institutions: I. Horbachevsky Ternopil National Medical University, Danylo Halytsky Lviv National Medical University, Ivano-Frankivsk National University.

Currently, one of the trends in education is the ability of an individual to learn throughout life, which is the basis of continuous learning. Considering society's demands for students' competencies and rapid changes in the organization of the educational process, it is worth analyzing the issue of formation and development of self-educational competence of future doctors, which is one of the key competencies that determine the new quality of education and is gaining special relevance. However, the problems of the structure and ways of developing the self-educational competence of medical students have not been sufficiently researchedyet.

The process of forming the self-educational competence of medical students requires forming their awareness of the need for self-development, mastering the skills of organizing self-educational activities, their readiness to use self-education skills in accordance with the challenges of the information society, the ability to use various sources of information in order to acquire theoretical knowledge and develop practical skills. We understand the importance of forming an individual's need for self-development and their psychological readiness not only to acquire knowledge, but also to improve what they have already learned based on the experience of self-education. In order to determine the rational ways of forming the self-educational competence of medical students and the use of effective means of diagnosing the level of its formation, there is a need to thoroughly study the structure of the term "self-educational competence" (Fig.1).

Competence is an activity characteristic of a person [9; 10], therefore it should structurally contain components of self-educational activity. In the structure of self-educational competence, the scientist singles out four interdependent

components: motivational-value, organizational, practical-active and personal-reflective. The content of the motivational and value component includes the formation of the need for cognitive activity, the value orientation of the individual towards mastering knowledge, and the desire to complete the educational task. The practical-activity component of self-educational competence outlines certain types and methods of independent work, which are aimed at the student's mastery of the "ability to learn".The organizational component in the structure of self-educational competence plays an organizational function and involves rational planning and design of activities, optimal allocation of time for completing educational tasks. The personal-reflexive component affects cognitive independence, volitional and worldview qualities, initiative and responsibility, the scientific composition of thinking [11; 12].

The scientist presented the general model of competence in the relationship of four components (theoretical-informational, value-targeted, practical-activity, and experimental) [13]. Meanwhile, he sees in the competence of a doctor with a higher education "the ability to realize one's own potential for successful creative activity in the professional and social sphere, realizing its social significance and personal responsibility for the results of this activity, the importance and necessity of its constant improvement" [13]. In the structure of competence, the scientist distinguishes five aspects: motivational, value-semantic, behavioral, cognitive, emotional-volitional mechanism, regulation of the process and result of manifestation.

The structure of self-educational competence includes the following components: motivational and value (activity, conscious personal attitude, value orientation on self-improvement); organizational (designing self-educational activity, self-management, purposefulness, self-reflection of cognitive activity); procedural (achieving a cognitive goal by means of self-planned cognitive activity, functionality of abilities and skills, their adjustment); informational (willingness to use information technologies in accordance with the needs of self-education and for the purpose of self-realization) [14].

However, it is singled out three components in the structure of self-educational competence: motivational-value, which refers to needs, motives, value orientations and characterizes the student's attitude to self-education as an important value of personal growth, the presence of moral-willed personality qualities; the cognitive-reflexive component affects the ability to control self-education activities and the level of personal and professional development; the organizational and activity component is characterized by the individual's ability to plan, implement and adjust self-education activities, to apply acquired self-education skills in the process of natural and scientific training [15].

It is worth including in the composition of self-educational competence a motivational component that performs a stimulating function for self-education, determines the need for self-education, and ensures a positive attitude and interest in self-education, awareness of the personal and social significance of self-education. The unity regarding the place of the reflexive component in the composition of the investigated competence, which involves the analysis and self-evaluation of the performed activity, the possession of reflexive technologies, is followed. Most scientists see self-educational competence as an activity component that ensures the mastery of a set of informational, organizational, and communicative skills. Another important component that self-educational competence must contain is cognitive, which determines the level of knowledge about self-educational activities, its content, forms, methods, and forms the future doctor's ability to learn throughout his life. The requirements for mandatory learning outcomes of medical students include the ability to show an emotional and valuable attitude towards a person; to be aware of the social role of the doctor; independently explain the importance of medical science for the sustainable development of society, determine the purpose, tasks and ways of implementing the research, analyze the results of the research according to the provided or independently determined criteria; uses "acquired knowledge and obtained experience to choose a strategy for solving a therapeutic problem, offers own ways of solving them; interacts in a group and is aware of personal responsibility for achieving a common result; independently formulates conclusions based on research results; carries out self-analysis of research activity" [16].

Based on the analysis carried out, we see four elements in the component composition of self-educational competence: motivational-value, cognitive, operational-active and reflective-analytical.

The motivational and value component characterizes the cognitive independence, willpower, initiative and responsibility of the student, determines the schoolchildren's awareness of the goals of learning, a deep understanding of the value of self-education and the cognitive focus of educational activities on intellectual development, the formation of the need for systematic and planned educational activities, manifestations of value orientations of personal development and internal need for self-education.

The formation of this component provides the ability to emotionally experience and understand value orientations for obtaining new knowledge and is manifested in the ability to mobilize forces to overcome difficulties that arise in the process of educational activity, to support cognitive activity and positive emotions, to stimulate activity, to show perseverance and endurance. This

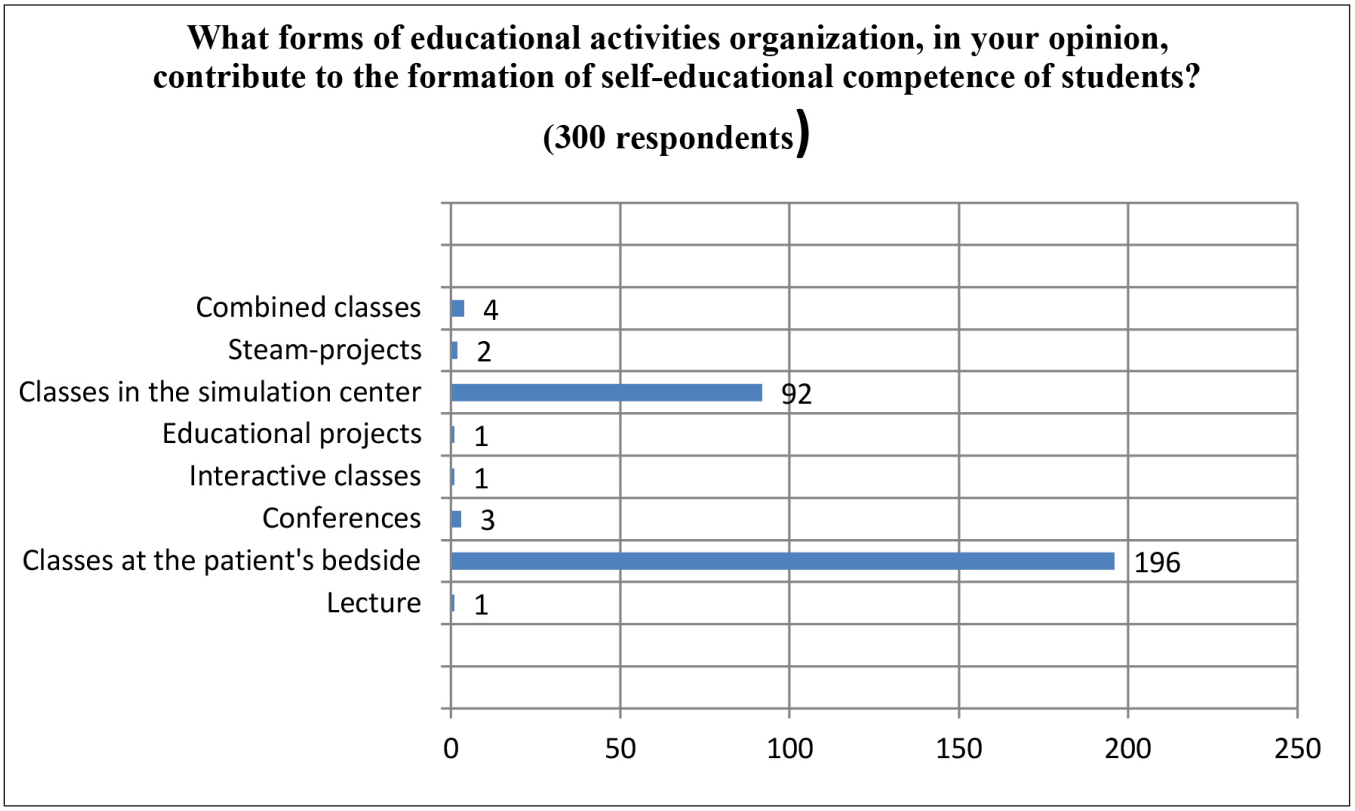

Fig. 2. Forms of educational activities organization.

component of self-educational competence performs the function of stimulating the self-educational activity of a medical student and forming a valuable attitude to the self-education process. The process of forming the self-educational competence of future doctors requires the development of a motivational and value component, which is the determining driver of self-educational activity and is objectively necessary [17].

The cognitive component characterizes the level of knowledge of the future doctor about the methods of self-education, its forms, content, methods, determines the level of mastery of techniques for acquiring general education and subject knowledge, awareness of modern search systems and the possibilities of modern digital technologies. The formation of this component determines the ability to build a student's self-education trajectory taking into account personal needs and abilities, provides the possibility of continuous self-education throughout life.

The organizational and activity component determines the ability of future doctors to plan and manage their own educational and cognitive activities, to choose the types and methods of self-educational activitiesrationally, the necessary training tools, to use services and resources to perform practical and life taskseffectively. Independently determine the content and timing of planned tasks (plan and develop educational research projects, set their duration, determine the sequence of stages of research activity), predict the results of self-education activities, choose effective ways to achieve goals, build your own educational learning trajectory, taking into account personal needs, preferences and abilities, to select forms, methods, means for organizing and implementing self-education activitieseffectively, etc. This component, performing a technological-instrumental function, directs the acquired knowledge, skills and abilities in the direction of activity, which ensures the formation of students' ability to make decisions and act independently.

The reflective and analytical component includes the ability of a medical student to control and reflect on educational activities; carry out self-analysis, form an adequate assessment of one's own achievements when solving difficult tasks; compare the results of activities with the assigned tasks and adjust them, if necessary, develop new tasks. It is connected with cognitive independence, worldview, moral and volitional qualities, the ability to identify achievements in self-education and see negative moments. The reflective activity of the future doctor is improved in the educational process and helps to form self-assessment skills, to adjust the assigned tasks in accordance with the conclusions of self-analysis, thereby performing the orienting function of self-educational activity. The formation of the specified component ensures the readiness of doctors to choose the optimal means of solving practical tasks, effective diagnostics and control over the development of self-education activities, to evaluate the results of activitiesobjectively and critically; to

determine resource opportunities and develop directions for the further process of personal self-development and make qualitative changes in self-educational activities [18].

Comparative analysis shows that the level of self-educational competence formation for future doctors in higher education institutions depends on the form of educational activity to a large extent (Fig.2). It was established that 196 (65%) future doctors prefer practical training at the patient's bedside, 92 medical students (31%) study in simulation centers, and 12 young people (4%) consider combined classes and generalizing conferences to be important.

CONCLUSIONS

Innovative learning technologies are methodological in nature and are implemented through interactive and person-oriented learning, which involves teacher-student dialogue, performance of problematic, searching, creative tasks. Interactive learning includes a set of educational technologies that ensure strong awareness of knowledge, actively form abilities, skills, values, and attitudes. We highlight interactive learning technologies aimed at the expected result of educational activity.

In addition to the mandatory learning outcomes of medical students and own experience, self-educational competence of the future doctor will be considered as an integrated quality determined by clear motivations for activity, the desire for self-improvement, the formation of value orientations, interest in high-quality independent activity, organized and systematized knowledge in a certain way, self-education skills and reflective skills, aimed at obtaining education throughout life.

Thus, we determined the peculiarity of self-educational competence as a pedagogical problem, which includes didactics and teaching methods, and the process of forming self-educational competence of future doctors will be more effective with the controlled implementation of certain organizational and pedagogical conditions.

Systematic stimulation of future doctors to further educational activities develops their qualities of independent research and cognitive activity, which is a prerequisite for the development of self-educational competence.

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ORIGINAL ARTICLE

EXPERIMENTAL GESTATIONAL DIABETES DISRUPTS THE FORMATION OF IMMUNE TOLERANCE IN OFFSPRING

DOI: 10.36740/WLek202301116

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ABSTRACT

The aim: To analyze the mRNA gene expression level of Aire, Deaf1, Foxp3, Ctla4, Il10, Nlrp3 and distribution of NLRP3+-cells in mesenteric lymph nodes (MLNs) of the offspring of rats with GD, both untreated and treated with glibenclamide and in conditions of insulin oral tolerance formation.

Materials and methods: The study involves 160 male rats, one- or six-month-old. The mRNA genes expression was studied by real time quantitative polymerase chain reaction. Structure of Nlrp3+ -cells population was studied by histological sections of MLNs.

Results: We observed AIRE gene repression, reduced mRNA levels of Deaf1 and the transcription factor Foxp3 in offspring of rats with GD. This was accompanied by inhibition of IL-10 gene expression and negative costimulatory molecules Ctla4. The development of the experimental GD was accompanied by transcriptional induction of the Nlrp3 gene in MLNs of descendants. The administration of glibenclamide to pregnant female rats with GD inhibited the transcription of the Nlrp3 gene only in one-month-old offspring (5.3-fold) and did not change it in six-month-old animals. In offspring of rats with GD, the density of the NLRP3+-lymphocyte population in the MLNs increased, more pronounced in one-month-old animals. The administration of glibenclamide to pregnant rats with GD reduced the number of NLRP3+ -lymphocytes only in one-month-old offspring (by 33.0 %), whereas this index in six month-old offspring even increased.

Conclusions: Experimental prenatal hyperglycemia leads to increased proinflammatory signaling and violation of peripheral immunological tolerance formation more pronounced at one month of life.

KEY WORDS: gene expression, insulin, mesenteric lymph nodes, glibenclamide, experimental gestational diabetes, NLRP3 - inflammasome

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INTRODUCTION

Gestational diabetes (GD) – autoimmune disorder, caused by the destruction of β-cells of pancreatic islets by an immune-mediated process, has emerged as a global public health concern [1]. Formation of immunological tolerance to autoantigens is an important mechanism that prevents the development of autoimmune diseases (AIDs). Recently extrathymic expression in number of peripheral tissue -specific antigens (PTSAs), including such pancreatic antigens as insulin and proinsulin was found. Their ectopic transcription is regulated by autoimmune regulator (Aire) [2]. A lot of extrathymic Aire-expressing cells (eTACs) are found in lymphatic nodes and represent one of the critical factors of peripheral immunological tolerance (PIT) [3]. Stromal cells (fibroblast reticular cells, follicular dendritic cells and lymphatic endothelial cells) of mesenteric lymph nodes (MLNs) express PTSAs [4], but their expression is regulated not only by eTACs, but by the regulator of transcription – deformed autoregulatory factor 1 (Deaf1) [5]. Consequently, Aire and Deaf1 are important differentiation regulators of inducible regulatory T-cells (iTreg), which can express transcription factor Foxp3 [9, 10], their action realized through production of suppressor cytokines – IL10, IL13, IL35, TGFβ [6], perforin/granzyme-dependent cytolysis of effector cells and depends on the expression of negative costimulatory molecules such as cytotoxic T-lymphocyte-associated protein 4 (CTLA-4). Yang S. et al. demonstrated ability of Aire to generate in the prenatal period (up to 10 days after birth inclusive) special population of $FoxP3^{+}$Treg-cells, which remains stable in adults and mice [7].

GD can cause the immune disorders in offspring, because during pregnancy all immune mechanisms become activated [8]. Thus, Li Q. et al. demonstrated that interleukin-1β expression could be higher in offspring spleen cells when mother suffering from GD [9]. This phenomenon linked to the activation of NLRP3-inflammasome – multimeric protein belonging to the family

of nod-like receptors, NLRs [10]. Glyburide, parthenolide and glibenclamide are proposed as medications, which have possibillity to change activity of NLRP3-inflammasome e.g. [11]. Glibenclamide is the most prominent, because it not only maintains the adequate glycemic control, but also could dicrease hyperglycemia-associated long-term outcomes in GD [12].

THE AIM

The aim of the current study was to analyze the mRNA gene expression level of *Aire*, *Deaf1, Foxp3, Ctla4*, *Il10, Nlrp3* and distribution of $NLRP3^+$-cells in mesenteric lymph nodes (MLNs) of the offspring of rats with GD, both untreated and treated with glibenclamide and in conditions of insulin oral tolerance formation.

MATERIALS AND METHODS

The experimental animals, white Wistar male rats (n=160) were housed under standard conditions, with proper diet and water ad libitum at the animal facility of Zaporizhzhia State Medical University. Animal treatment and all experimental procedures were performed in compliance with the European Convention for the Protection of Vertebrate Animals used for Experimental and other Scientific Purposes. The study was approved by the Ethical Committee of Zaporizhzhia State Medical University.

Experimental study design comprised eight groups: one-month-old descendants of intact Wistar rats (group 1; n=20); six-month-old descendants of intact Wistar rats (group 2; n=20); onemonth-old descendants of Wistar rats with gestational diabetes (GD) (group 3; n=20); six-monthold descendants of Wistar rats with GD (group 4; n=20); one-month-old descendants of Wistar rats with GD, treated with insulin (group 5; n=20); six-month-old descendants of Wistar rats with GD, treated with insulin (group 6; n=20); one-month-old descendants of Wistar rats with GD treated with glibenclamide during pregnancy (group 7; n=20); six-month-old descendants of Wistar rats with GD treated with glibenclamide during pregnancy (group 8; n=20).

Experimental GD was induced by a single intraperitoneal administration of streptozotocin (STZ) (Sigma Chemical, USA) at a dose of 45 mg/kg body weight on the 15th day of pregnancy. Immediately prior to the administration, STZ was dissolved in 0.1 M citrate buffer (pH 4.5).

Blood glucose concentration was determined on the 3rd day after STZ administration using the glucose oxidase method with BIONIME Rightest TM GM 110 glucometer (Switzerland). Blood samples were taken from the tail vein. Animals with fasting glucose level of > 8.0 mmol/l were selected for study.

Glibenclamide («*Pharmak*», *Ukraine*) was administrated orally at a dose of 5 mg/kg for the 7 days to pregnant female rats after STZ administration.

Short-acting human insulin was administrate d orally using a pipette for the first 14 days of life (*ACTRAPID® HM, NOVO NORDISK, Denmark*) at a dose of 30 IU (1050 µg=1,05 mg, 1 IU corresponds to 35 µg of anhydrous human insulin).

MLNs of experimental animals were studied using real-time reverse transcription polymerase chain reaction (RT-PCR) techniques. Each rat was anaesthetized with ketamine hydrochloride at a dose of 100 mg/kg. An upper midline abdominal incision was made. All the MLNs identifiable along the line of the mesenteric blood vessels were carefully dissected off the mesentery.

Animal euthanasia was carried out by cardiac puncture under deep anaesthesia, in accordance with the requirements of the Animal Care Committee.

MLNs were placed in the Bouin's fluid, dehydrated with ethanol and embedded in paraffin. Molecular genetic studies were performed on archival material hold in biobank up to 2 years. RNA was extracted from 15 µm histological samples. They were dewaxed in xylene and rehydrated with descending concentrations of ethanol (100 %, 96 %, 70 %). Total RNA was obtained using of «Trizol *RNA* Prep 100» (*Isogen Lab LTD, Russia*), that contains *Trizol reagent* (lysis reagent, which includes denaturing agent guanidine thiocyanate and phenol with pH = 4.0) and *ExtraGene* E.

For obtaining cDNA and its reverse transcription RT-1 set «Syntol» (Russia) was used. The reaction mixture was taken in the volume of 25 µl containing 1 µl of random-6 primer, 2 µl total RNA, 8,5 µl deionized water, 12,5 µl 2,5x reaction mixture and 1 µl of reverse transcriptase MMLV-RT. Reverse transcription was conducted at 45°C for 45 min. Inactivation of MMLV-RT was achieved at 92°C for 5 min.

To determine the level of mRNA *Aire* (NM_001106379.1), *Deaf1* (NM_031801.1), *Foxp3* (NM_001108250.1), *IL10* (NM_012854.2), *Ctla4* (NM_031674.1) and *Nlrp3* (NM_001191642.1) we used thermocycler CFX96™Real-Time PCR Detection Systems («Bio-Rad Laboratories, Inc.», USA) with the set of reagents Maxima SYBR Green/ROX qPCR MasterMix (2X) (ThermoScientific, USA). The final reaction mixture for amplification includes coloring SYBR Green, Maxima HotStartTaq DNA Polymerase, 0,2 µl of forward and reverse specific primers, 1 µl cDNA. The reaction mixture brought to total volume 25 µl by adding deionized water. Specific primer pairs (5'-3') for analysis of target and reference genes were selected by the software PrimerBlast (www.ncbi.nlm.nih.gov/tools/primer-blast) and synthesized by Metabion (Germany) (Table I).

Table I. List of primers used for real-time PCR

Gene	Primer	Tm,°C	Product length (bp)	Exon junction
Aire	F = GCCTAAAGCCAGTGATCCGA R = TCTCTACCCTGGGTTCCCTTT	59.82 59.85	43	850/851
Deaf1	F = GCAGAGAGGAAGGAGCAGTC R = GTGCACTCACTCATGGCCT	59.82 60	59	1605/1606
Foxp3	F = CGAGACTTGGAAGTCAGCCAC R = TCTGAGGCAGGCTGGATAACG	60.94 61.91	61	214/215
IL10	F=AGTGGAGCAGGTGAAGAATGA R=GACACCTTTGTCTTGGAGCTTATTA	59.02 59.06	49	445/446
Ctla4	F = TACAGTTTCCTGGTCACCGC R = AGGACTTCTTTTCTTTAGCGTCCT	59.97 59.96	57	567/568
Nlrp3	F = AGCTAAGAAGGACCAGCCAG R = CGTGCATGCATCATTCCACTC	59 60	40	713/714
GAPDH	F = GCCTGGAGAAACCTGCCAAG R =GCCTGCTTCACCACCTTCT	61 60	52	825/826

Table II. Normalized relative quantity of mRNA Aire, Deaf1 and Foxp3 genes in MLN cells

Target	Sample	Expression Fold Change	Fold Regulations	P
AIRE	gd1 vs c1	0,12	-8.1	< 0.05
Deaf1	gd1 vs c1	1,20	1,20	
Foxp3	gd1 vs c1	0,02	-50,0	< 0.05
AIRE	gd6 vs c6	0,44	-2.3	< 0.05
Deaf1	gd6 vs c6	0,11	-9,2	< 0.05
Foxp3	gd6 vs c6	0,39	-2.5	< 0.05
AIRE	gd1+ins vs gd1	13,2	13,2	< 0.05
Deaf1	gd1+ins vs gd1	11,5	11,5	< 0.05
Foxp3	gd1+ins vs gd1	5,2	5,2	< 0.05
AIRE	gd6+ins vs gd6	2,0	2,0	< 0.05
Deaf1	gd6+ins vs gd6	1,2	1,2	
Foxp3	gd6+ins vs gd6	3,3	3,3	< 0.05

*Fold-Change (2^(-Delta Delta CT)) is the normalized gene expression (2^(- Delta CT)) in the test sample (gd1, gd6, gd1+ins, gd6+ins) divided the normalized gene expression (2^(- Delta CT)) in the control sample. Fold-Regulation represents fold-change results in a biologically meaningful way. Normalized to reference gene GAPDH by the method ΔΔCt. c1, c6 – control 1 and 6 months; gd1, gd6 – offspring of the experimental GD rats; gd1+ins, gd6+ins – after insulin administrations.

After initial denaturation at 95°C for 10 min amplification was implemented in 45 cycles including following stages: denaturation – 95°C for 15 sec., annealing at 59–61°C for 30–60 sec., elongation at 72°C for 30 sec. [13].

The reference gene was glyceraldehyde 3-phosphate dehydrogenase *(GAPDH)* gene. Normalized relative quantity of cDNA target genes was determined by the method ΔΔCt. Statistical data analysis of PCR were conducted using available software CFX Manager ™(Bio-Rad, USA). Experiment included negative controls: no template controls (cDNA and mRNA) and no reverse transcriptase control. All amplification reactions were performed on individual samples three times.

Structure of Nlrp3$^+$-cells population was analyzed in the serial histological sections of MLN. Serial sections of 5 µm thick were made on a rotary microtome MICROM HR-360 (Microm, Germany), then they were dewaxed in xylene and rehydrated with descending concentrations of ethanol (100 %, 96 %, 70 %), washed with 0.1 M phosphate buffer (pH=7,4) and colored with Nlrp3 rabbit polyclonal antibodies (Cryopyrin, H-66) (Santa Cruz Biotechnology, USA, sc-66846) for 18 hours in a humid chamber at T=4 °C. After washing with 0.1 M phosphate buffer, samples were incubated for 60 min at T=37 °C with secondary antibody solution to rabbit IgG (Santa Cruz Biotechnology, USA), conjugated with FITC. After incubation, all sections were washed with 0.1 M phosphate buffer and placed in a mixture of glycerol phosphate buffer (1:9) for subsequent fluorescent microscopy. Histological sections were studied with the

software Image J (NIH, USA), than the morphometric and densitometric characteristics of immunopositive cells and were measured. We determined the absolute (number of cells per 1 mm^2) and relative (%) density of different subsets of $Nlrp3^+$-lymphocytes in cortex and medullary cords of MLNs.

STATISTICAL ANALYSIS

The experimental data were processed and analysed using the software STATISTICA 6.0 (StatSoftInc., №AXXR712D833214FAN5, USA). The distribution of data was analyzed by Kolmogorov -Smirnov criterion. The obtained values had a normal distribution, so the difference between the groups was analyzed using the Student's t-criterion. All data were presented as M (mean) ± m (standard error). A probability level (p value) of less than 0.05 was considered to be statistically significant.

RESULTS

Investigation of *Aire* gene expression in MLNs showed that in offspring of rats with GD there is a significant reduction of mRNA of autoimmune regulator by 8.1 times ($p<0.05$) in one month-old (group 3) and by 2.3 times ($p<0.05$) in six-month-old animals (group 4) vs group 1 and 2 (Table II). mRNA content of transcription regulator *Deaf1* in one-month-old animals did not changed significantly, and in six-month-old descendants we observed its reduction by 9.2 times ($p<0.05$) vs group 1 and 2 (Table II). As for mRNA of transcription factor *Foxp3*, there was revealed a significant decrease by 50.0 times ($p<0.05$) in one-month-old rats (group 3), and by 2.5 times ($p<0.05$) in the six-month-old animals (group 4) vs group 1 and 2 (Table II).

Offspring of rats with GD, that were administered orally insulin during 14 first days of life, showed increasing of *Aire* gene transcriptional induction mostly in one-month-old animals (group 5) – the level of mRNA has increased by 13.2 times ($p<0.05$) vs group 3. In six-month-old animals (group 6) this index increased by 2.0 times ($p<0.05$) vs group 4 (Table II). Transcription regulator *Deaf1* in one-month-old animals (group 5) showed a significant increase by 11.5 times ($p<0.05$) vs group 3, and in six-month-old animals (group 6) it was similar to this index in group 4 (Table II).

Studies have shown that expression of the transcription factor *Foxp3* in one-month-old rats was increase of *Foxp3* mRNA by 5.2 times ($p<0.05$) vs group 3, in the six-month-old animals rise was 3.3 fold ($p<0.05$) vs group 4 (Table II).

In experimental groups 5 and 6 (one- and six-month-old offspring of rats with GD that received orally insulin) mRNA expression of costimulatory molecules *Ctla4* and Treg-dependent suppressor cytokine *IL-10* has also been investigated. We have found that relative quantity of *Ctla4* mRNA gene increased by 12.2 times ($p<0.05$) in one-month-old animals vs group 3. In six-monthold rats this index significantly did not changed vs group 4. Contents of mRNA *IL10*, on the contrary, in one-month-old animals was unaltered vs group 3, but in six-month-old rats it increased by 15.0 times ($p<0.05$) vs group 4.

Investigation of *Nlrp3* gene expression in MLNs showed that in the offspring of rats with GD there was a significant (5-fold) increasing of mRNA of this protein in one-month-old rats ($p<0.05$) and 3-fold increasing ($p<0.05$) in six-month-old animals vs group 1 and 2. In rats of group 7 and 8 (one- and six-month-old offspring of animals with GD that received glibenclamide during pregnancy) we have found a significant (by 5.3 times) decrease of *Nlrp* gene expression ($p<0.05$) in one-month-old, and absence of significant changes in six-month-old animals.

Studying the distribution of specific subpopulations of $Nlrp3^+$-cells we have found that total density of immunopositive cells in MLNs cortical plateau of one-month-old offspring of animals with GD increased by 49.0 % ($p<0.05$) vs group 1. In six-month-old animals comparative analysis revealed no significant changes vs group 2. Total number of $Nlrp3^+$-cells in MLNs medullary cords of one-month-old offspring of animals with GD was significantly increased by 44.0 % ($p<0.05$) vs group 1. The study of materials taken from the six-month-old rats showed an increase in the total density by 69.0 % ($p<0.05$) vs group 2. Analysis of MLNs sections in the experimental GD offspring of rats treated with glibenclamide during pregnancy have showed that in cortical plateau of MLNs in one-month-old animals we obtained reducing of the total number of $Nlrp3^+$-cells by 33.0 % ($p<0.05$) vs group 3. In six-month-old rats there were not significant changes in the number of immunopositive cells vs group 4. Total density of $Nlrp3^+$-cells in one-month-old animals did not changed significantly and in six-month-old rats it increased by 29.0 % ($p<0.05$).

DISCUSSION

The modern search for effective targeted therapy [13] for endocrine diseases is based on transcriptome [14], variome [15-16], and proteome data [17-19]. Peyer's patches (PP) and mesenteric lymph nodes (MLNs), which are present in the wall of the intestinal tube are the main components for for immune responses, they play an important role in the mechanisms of preventing the active immune response against usually harmless environmental antigens [20]. PP and MLNs considered

to be the principal site for the induction of oral tolerance (OT) preventing immune response to an orally administered antigen. MLNs have distinctions from PP and peripheral lymph nodes and serve as a crossroads between the peripheral and mucosal recirculation pathways for antigens [21]. Such antigen recirculation occurs from the lamina propria into the MLNs and mediated by $CD103^+$ dendritic cells (DCs) and was found for OT systemic effect [21].

On the other hand, clinical manifestation of T1DM is preceded by the development of autoantibodies to different islet autoantigens, marking the loss of immunological tolerance to β cells. Most trials attempting immune intervention have been conducted in patients with recent onset T1DM (usually within 6 weeks of diagnosis), and have had varying but only limited success. This outcome might partly result from the stage of disease and progressive loss of β cells, in addition to the burden of poor glycaemic control and metabolic β-cell stress over and above the inflammatory insult. Unfortunately, the few attempts to prevent T1DM using immunotherapy in seropositive individuals at risk of the disease were unsuccessful. Bonifacio E. et al. demonstrated that oral administration of 67.5 mg of insulin, compared with placebo, resulted in an immune response without hypoglycemia, allergic and autoimmune reactions [22].

The inflammasomes and the complement system are traditionally viewed as quintessential components of innate immunity required for the detection and elimination of pathogens. But a direct role for NLRP3 in human adaptive immune cells has not been described yet. In recent years, data suggested that NLRP3 could be expressed by mouse and human lymphocytes and has an ability to adjust the differentiation of Th1, Th2 Th17-cells. Recently, Arbore G. et al. have shown that NLRP3 inflammasome assembles in human CD4+ T-cells and initiates caspase-1–dependent interleukin-1β secretion, thereby promoting interferon-γ production and T-helper 1 (TH1) differentiation in an autocrine fashion [23]. Furthermore, Bruchard M. et al. recently showed the ability of NLRP3 to act as a key transcription factor that controls the Th2-differentiation [24]. In Th2 cells NLRP3 binds to promoter IL4 and activates it in conjunction with transcription factor IRF4. In contrast to Th1, where NLRP3 is detected mainly in the cytoplasm by methods of immunofluorescence microscopy, in the Th2-cells it is localized mainly in the nucleus. It is possible that such a nuclear localization function can promote inflammasome transcription. This work showed that NLRP3 should be seen not only as a key inflammasome component, but as a transcription factor in cells $CD4^+$ Th2 [24]. Finally, the mechanisms of IL-1β-induced Th17 differentiation are related to the ability of TGF-β to induce expression RORγt in naive T-cells [25]. Studies in vitro have shown that IL-1β induces the expression of IRF-4, positively regulates IL-21-mediated expression of transcription factors STAT-3 and RORγt [25]. At the same time, NLRP3-inflammasome is one of the sencetive indices of metabolic stress developing diabetes [26-28]. NLRP3--deficient NOD-mice are protected from developing diabetes by reducing migration of diabetogenic lymphocytes in the pancreatic islets.

NLRP3-inflammasome is an important pharmacological target for blocking a number of diabetes complications [29], and the ability of glibenclamide to inhibit the formation of NLRP3 can affect the risk of inflammatory and AIDs in the offspring of mothers with GD. Recent research by Lamprianou S. et al. demonstrated that glibenclamide protects NOD mice from progressing hyperglycemia and loss of insulin-producing β-cells [30]. Although the administration of glibenclamide did not stop the development of insulitis, but induced a shift of the phenotype of immune cells and protects cells of insulinoma MIN6 from apoptosis and loss of connexin Cx36 [30].

CONCLUSIONS

1. The investigation of transcriptional activity of genes-regulators of the peripheral immunological tolerance formation in MLNs of the offspring of rats with GD showed the repression of *Aire* and *Deaf1* mRNA. These changes violate ectopic transcription of pancreatic antigens in MLNs. Reduction of mRNA *Foxp3* level leads to a deficiency of suppressor signaling, which is confirmed by inhibition of suppressor cytokine *IL10* gene expression and negative costimulatory molecules *Ctla4*. Oral administration of insulin during the first 14 days of life stopped these changes, causing transcription activation of *AIRE, Deaf1, Foxp3, Ctla4* and *Il10* genes.
2. The development of the experimental GD is accompanied by transcriptional induction of the *Nlrp3* gene in MLNs of descendants, whose mRNA level increased 5-fold ($p<0.05$) in onemonth-old and 3-fold ($p<0.05$) in six-month-old animals. The administration of glibenclamide to pregnant rats with GD inhibited the transcription of the *Nlrp3* gene only in one-month-old offspring (by 5.3 times, $p<0.05$) and did not change it in the group of six-month-old animals.
3. In the offspring of rats with GD, the density of the NLRP3+-lymphocyte population in the MLNs increased, more pronounced in one-month-old animals. The administration of glibenclamide to pregnant rats with GD reduced the number of $NLRP3^+$-lymphocytes only in one-month-old offspring, whereas this index in six-month-old offspring even increased.

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ORIGINAL ARTICLE

NEPHROPROTECTIVE EFFECT OF GAMMA-SECRETASE INHIBITOR ON SEPSIS- INDUCED RENAL INJURY IN MOUSE MODEL OF CLP

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ABSTRACT

The aim: This study was set out to assess the potential protective impact of MK0752 (a gamma secretase inhibitor) on sepsis-induced renal injury through modulation of inflammatory and oxidative stress pathways.

Materials and methods: Twenty-four Swiss-albino mice aged between eight and twelve week and weighted twenty to thirty-seven grams were randomly allocated into four groups (n=6 in each group). Sham group (laparotomy without cecal ligation and puncture (CLP), sepsis group (laparotomy with CLP), vehicle-treated group (equivalent volume of DMSO before the CLP), MK0752 treated group (5 mg/kg) single daily dose for three days before the CLP. Blood samples were used to assess the serum levels of urea and creatinine. The kidneys were used to assess tissue levels of the TNF-α, IL-10, IL-6, TNFR1, VEGF, notch1, jagged1 and tissue damage by histopathological analysis.

Results: The current study shows that pretreatment with MK0752 ameliorates the renal damage by significantly reducing the proinflammatory cytokines and notch1 signaling.

Conclusions: Taken together, these results suggest that MK0752 could be protective against the renal injury induced by sepsis through its ameliorative impact on renal architecture and modulating cytokines and Notch1 singling pathway. Further studies regarding the role of Notch signaling pathways would be worthwhile.

KEY WORDS: MK0752, endotoxemia, sepsis, a gamma-secretase inhibitor, notch1, jagged1and cecal ligation, puncture

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INTRODUCTION

Sepsis is a common condition, which has considerable impact on life of people and health systems [1]. It is characterized by a massive inflammatory response, which is triggered by infection. Sepsis, often known as septic shock, is a pathophysiological illness marked by severe hypotension, rising metabolic acidosis, systemic inflammatory response syndrome (SIRS), tissue damage and multiple organ dysfunction syndromes, acute respiratory distress syndrome (ARDS), acute lung injury (ALI) and mortality [2]. During sepsis, endothelial vascular permeability increases in numerous organs, resulting in plasma extravasation and subsequent bacterial translocation, which may contribute to the development of severe tissue damage [3]. The discovery of endotoxin, cytokines, and products of arachidonic acid metabolism, as well as demonstration that injecting these compounds into human patients or animal models might result in a clinical state similar to sepsis [4]. Monocytes regulate the innate immune response to pathogens by generating inflammatory cytokines such as interleukin-6 (IL-6, -17, -1), which can lead to SIRS (systemic inflammatory response syndrome), multiple organ failure and death [5]. The illness begins with a localized infection caused by microorganisms such as bacteria, which progresses to tissue invasion via the bloodstream, and as a result of which sepsis and septic shock occur, the major symptoms of which are low blood pressure, ischemia, organ damage, and death [6, 7]. One of the organs deteriorated is the kidney resulting in renal failure accounting for 70–80 percent of the sepsis mortality [8]. Sepsis induced acute kidney injury (AKI) is produced by several molecular processes, including hypoperfusion, renal cell exposure to circulating cytokines and chemokines, and abnormalities in energy metabolism [9]. This leads to a reduction in glomerular filtration rate as indicated by an elevated plasma creatinine level, and oliguria/anuria [10]. Notch receptors are family of transmembrane proteins involved in the determination of cell fate. In the humans, four Notch

receptors have been identified (Notch1–4), with five cognate ligands including Delta-like-1, 3, 4, and Jagged-1, and Jagged-2. Activation of the Notch pathway requires that the receptor binds to the ligand and undergoes protease hydrolysis to produce the Notch intracellular domain (NICD), which is released into the nucleus to activate downstream Hairy/enhancer of split (HES) and HEY through series of reactions [11]. Binding of specific Notch ligands initiates Notch signaling which in turn initiates series of proteolytic cleavages by metalloproteases of the ADAM family (S2 site) and finally the γ-secretase complex (S3 site) [12]. Gamma secretase (GS) is a key mediator of Notch signaling, and inhibitors for γ-secretase are able to prevent Notch receptor activation, MK-0752 is a new potent γ-secretase inhibitor (GSIs), which inhibits γ-secretase to cleave substrates such as Notch receptors [13-15]. Notch pathway genes are expressed in the developing and adult kidney, and Notch activation occurs in the adult kidney following injury [14]. Activation of Notch is responsible for the cellular proliferation, differentiation, and repair. This idea is supported by studies performed by Kobayashi et al. who observed increased expression of Delta1, Notch2 and the downstream target Hes1 using a rat ischemia reperfusion injury model [11, 15]. Jagged1-expressing cells coexist in the subventricular zone (SVZ) with Notch1-expressing cells. It has been demonstrated, in vitro, that it works by Notch1 to keep up proliferating multipotent neural cells. Proliferative stem cells in the SVZ are reduced when Jagged1, Notch-1 signaling is impaired [16]. The gene expression analysis from hair follicles revealed significant suppression of Notch signalling after MK-0752 treatment [17].

THE AIM

This study was set out to assess the potential protective impact of MK0752 (a gamma secretase inhibitor) on sepsis-induced renal injury through modulation of inflammatory and oxidative stress pathways.

MATERIALS AND METHODS

The study was conducted in the Department of Pharmacology and Therapeutics at University of Kufa, College of Medicine and the Middle Euphrates Centre for Cancer Research. The study was carefully reviewed by the Bioethics Committee of the University in Kufa.

DESIGN OF THE STUDY

Twenty-four Swiss albino mice weighting a 25-35 g and ageing an 8-12 week were obtained from the Animal Resources Centre, University of Kufa, Faculty of Science. Animals were kept at 25°C° and 60%-65% of humidity with 12 h light/dark cycle. Animals were divided into four groups, six animals in each group. Sham group was exposed to the anaesthesia and laparotomy with no CLP. Control group was subjected to CLP. Vehicle-treated group were injected with DMSO by intraperitoneal route (IP) before CLP. MK0752-treated group were injected with MK0752 at dose of 5 mg/kg/day, IP, for three days before CLP [18].

EXPERIMENTAL PROCEDURE

Mice were anesthetized by using a 100 mg/kg of ketamine and 10mg of xylazine. Following median abdominal incision, the cecum was identified and ligated under the ileocecal valve. Two punctures were made using a G-20 needle before being returned to its normal position. The abdomen was closed with a 5.0 medical suture [19, 20].

MK0752 TREATMENT

MK0752 powder was prepared in diluted DMSO and given at a dose of 5mg/kg, single dose for three days before CLP [18].

MEASUREMENT OF RENAL FUNCTION

The blood was immediately collected from the heart. Samples were put in appropriate tubes and left at room temperature to clot for 30 min. Samples were then spin down for 10 min at 3000 rpm. Supernatant from each sample was used to measure the serum levels of urea and creatinine [21].

RENAL TISSUE PREPARATION FOR ELISA

The kidney from each animal was gently washed with normal saline to remove any clot. It was weighted and put in a tube containing a phosphate buffer saline (compositions: 1% Triton X-100 and 1% protease inhibitor cocktail) in a ratio of 1:10 w/v. The sample was then homogenized by using of ultrasonic processer [22]. The lysate was then centrifuged for 10 minutes at 4°C at 10,000 rpm, and the supernatant was utilized to measure the TNF-α, TNFR1, VEGF, IL-6, and IL-10 [23] .

HISTOPATHOLOGY EVALUATION

The kidney was putted in 10% formalin solution, the renal tissues were embedded in paraffin blocks, and the block containing tissue was cuted by 5-mm in thickness

stained with Hematoxylin and Eosin (H and E) stain [24]. An independent pathologist assessed renal tissue injury. The renal sections were graded based on the degree of renal injury such as percentage of tubular damage, cellular edema, increased cytoplasmic eosinophil, red blood cell extravasation and inflammation as described previously [25].

The histopathology analysis was carried out under a bench microscope at magnifications of X400. Degree of tissue damage was scored as follows [26]. Score zero indicates no damage. Score one (mild): less than 25% damage. Score two (moderate): 25-50% damage, score three (severe): 50-75% damage, score four (very severe): 75-100% damage.

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Notch1 and Jagged1 level were examined by using of immunohistochemistry. In brief, the sections were deparaffinized in xylazine, and then rehydrated in adjusted ethanol 75-100% and immersed in distilled water. The sections were exposed to a retrieval buffer in a water bath for 20 min at 95°C and washed by washing buffer. Then, the slices were incubated in 3% H_2O_2 for 5 min and followed by washing with washing buffer. The sections were then incubated overnight with anti-(NOTCH1 or JAGGED1)-antibody at concentration 1:100 and 1:200 respectively at 4°C. After 1 h of incubation with specific antibody, the samples were cleaned and treated for 30 minutes with peroxidase. The sections were washed 2 times with buffer and after it 3,3-diaminobenzidine (DAB) was added to the samples to develop the colour. The sections were counter stained with hematoxylin/eosin for 5 min at room temperature and mounted after dehydration in graded alcohol and xylene. Appropriate positive and negative controls were run for each batch. Finally, the protein level of notch1 or jagged1 was measured using H-score technique (ranged 0-300). The immunoreactivity was calculated by multiplying the staining intensity by the percentage of the stained area. The staining strength has been evaluated from 0 to 3. Score 0: no stain; score 1: mild discoloration; score 2: medium discoloration; score 3: severe discoloration. The fraction of tagged cells ranged from 0% to 100% [27].

STATISTICAL ANALYSIS

The data were analysed using SPSS software version 26. Data are represented as mean ± SEM unless otherwise stated. To compare among the study groups, a one-way ANOVA followed by Bonferroni's post-hoc test was used. Histopathological changes and expression levels Notch1 and jagged 1 among the study groups were analysed using Kruskal-Walli's test. Result is considered as statistically significant at level $p \leq 0.05$.

RESULTS

EFFECT OF MK0752 ON RENAL FUNCTIONS

The first set of analyses examined the impact of MK0752 on serum urea and creatinine. Data showed that mice exposed to CLP had decreased levels of serum urea and creatinine compared with sham group. In contrast, pretreatment with MK0752 decreased the serum levels of both readouts (Fig. 1-2).

The figure 1 showing the mean serum levels of urea among the four study groups. Data were expressed as mean ± SEM, n=6. Statistical analysis was performed using one-way ANOVA followed by Bonferroni's test: a – $p \leq 0.05$ versus sham group; abc – $p \leq 0.05$ versus control (sepsis) and vehicle (DMSO) groups.

The figure 2 showing the mean serum levels of creatinine among the four study groups. Data were expressed as mean ± SEM, n=6. Statistical analysis was performed using one-way ANOVA followed by Bonferroni's test: a – $p \leq 0.05$ *vs.* sham group; abc – $p \leq 0.05$ *vs*. control (sepsis) and vehicle (DMSO) groups.

EFFECT OF MK0752 ON TNF-A, IL-6, VEGF, TNFR1, IL-10

To investigate the levels of the TNF-α, IL-6, VEGF, TNFR1 and IL-10 in the renal tissues, ELISA technique was used. The results revealed that levels of TNF-α, IL-6, VEGF and TNFR1in the control and vehicle groups were significantly higher than that of the sham group. In contrast to earlier finding, MK0752-treated group showed a marked decrease in the levels of these proteins. In respect to IL-10, there renal tissue was considerably greater in control and vehicle groups than that of the sham group on the other hand, levels of IL-10 in the renal tissues increased markedly in MK0752-treated group (Fig. 3-7).

The figure 3 reveals the mean tissue levels of IL-6 among the four study groups. Data were expressed as mean ± SEM, n=6. Statistical analysis was performed using one-way ANOVA followed by Bonferroni's test: a – $p \leq 0.05$ *vs*. sham group; abc – $p \leq 0.05$ *vs.* control (sepsis) and vehicle (DMSO) groups.

The figure 4 reveals the mean tissue levels of TNFR1 among the four study groups. Data were expressed as mean ± SEM, n = 6. Statistical analysis was performed using one-way ANOVA followed by Bonferroni's test: a – $p \leq 0.05$ *vs.* sham group; abc – $p \leq 0.05$ *vs.* control (sepsis) and vehicle (DMSO) groups.

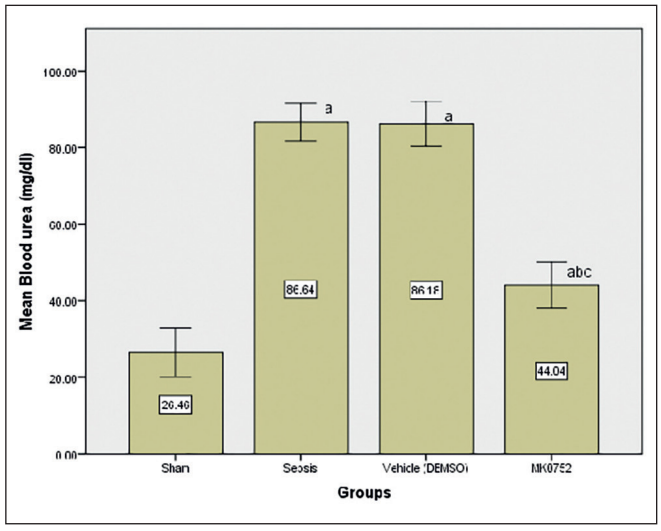

Fig. 1. Mean serum level of urea (mg/dl) of the four experimental groups.

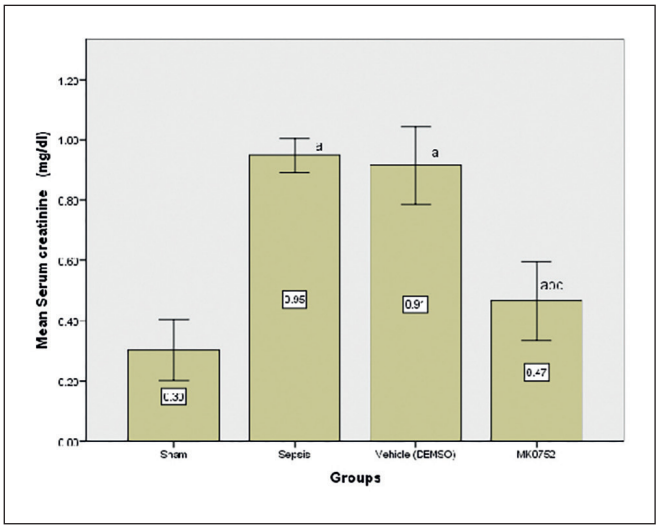

Fig. 2. Mean serum creatinine (mg/dl) of the four experimental groups.

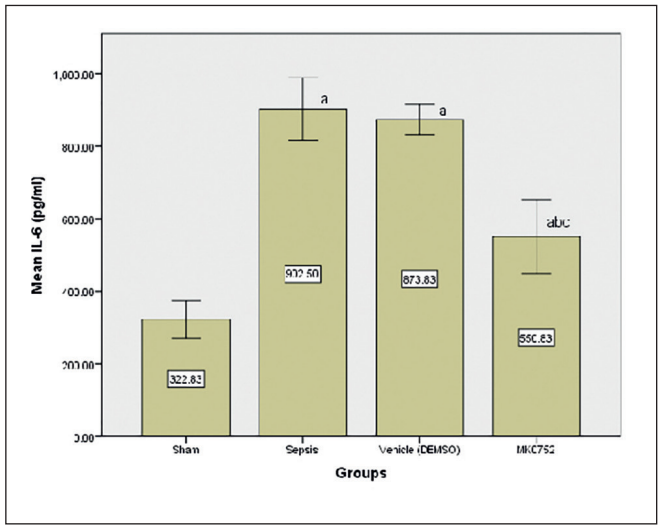

Fig. 3. The mean of tissue IL-6 (pg/ml) of the four experimental groups.

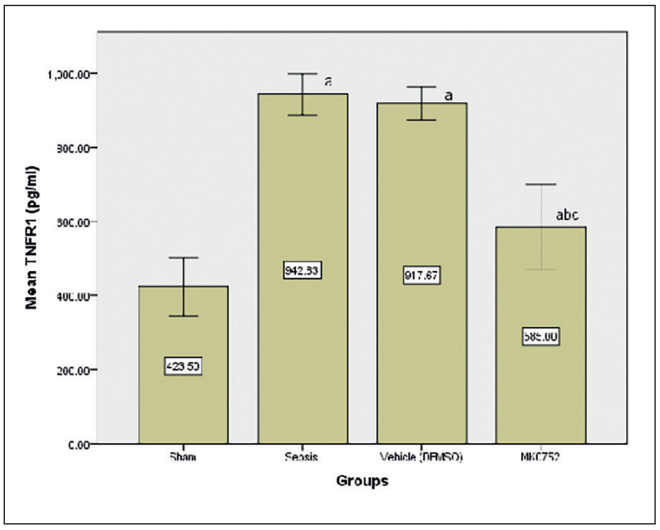

Fig. 4. The mean of tissue TNFR1 (pg/ml) of the four experimental groups.

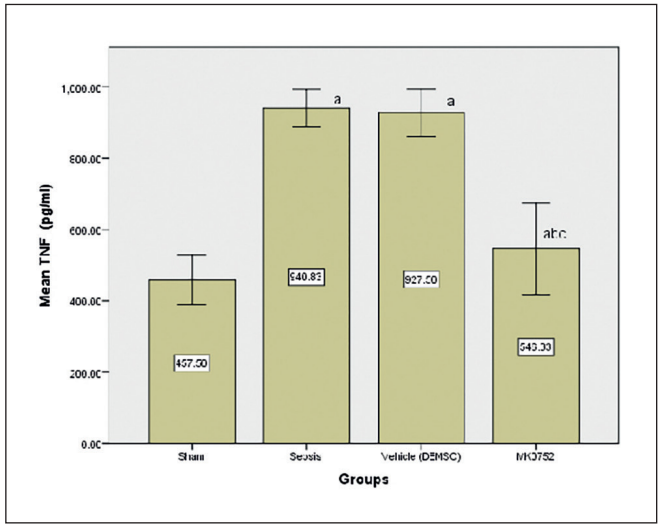

Fig. 5. The mean of tissue TNF-α (pg/ml) of the four experimental groups.

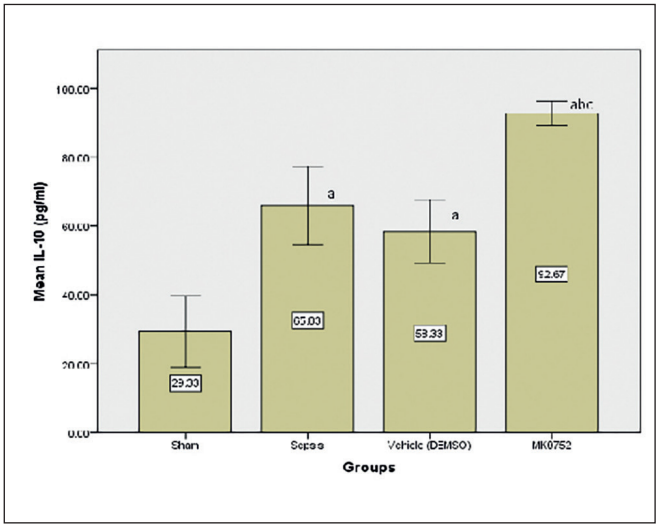

Fig. 6. The mean of tissue IL-10 (pg/ml) of the four experimental groups.

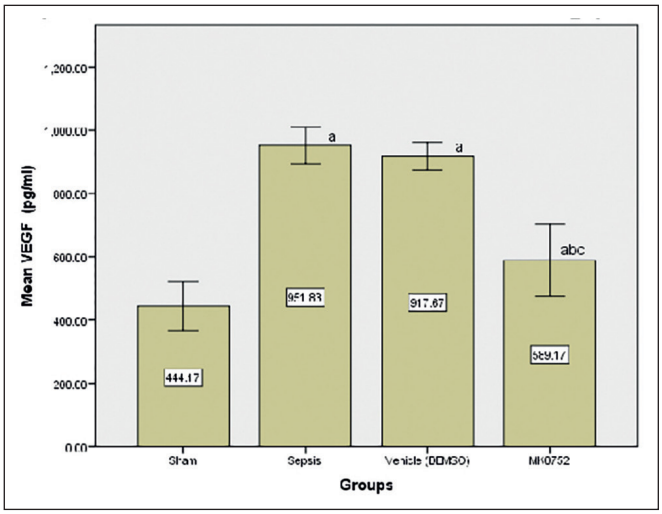

Fig. 7. The mean of tissue IL-10 (pg/ml) of the four experimental groups.

The figure 5 reveals the mean tissue levels of TNF-α among the four study groups. The results were expressed as mean ± SEM, n =6. Statistical analysis was performed using one-way ANOVA followed by Bonferroni's test: a – p≤ 0.05 *vs.* sham group; abc – p ≤ 0.05 *vs.* control (sepsis) and vehicle (DMSO) groups.

The figure 6 reveals the mean tissue levels of IL-10 among the four study groups. Data were expressed as mean ± SEM, n = 6. Statistical analysis was performed using one-way ANOVA followed by Bonferroni's test: a – p ≤ 0.05 *vs* sham group; abc – p≤ 0.05 vs. control (sepsis) and vehicle (DMSO) groups.

The figure 7 explaining the mean tissue levels of VEGF among the four study groups. The results were expressed as mean ± SEM, n =6. Statistical analysis was performed using one-way ANOVA followed by Bonferroni's test: a – p≤0.05 *vs.* sham group; abc – p≤0.05 *vs.* control (sepsis) and vehicle (DMSO) groups.

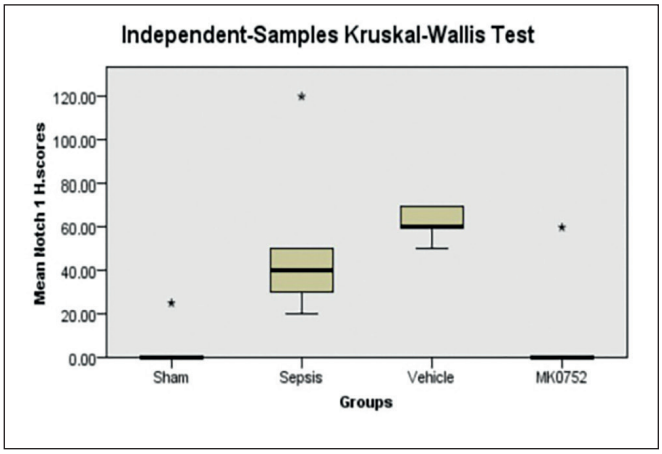

Fig. 8. Mean H-score of notch1 in renal tissue of the four experimental groups at the end of the study (number of animals = 6 in each group). Sham vs. sepsis group and vehicle group, p-value <0.05 (significant). Mk0752 vs. sepsis and vehicle group, p-value <0.05 (significant).

EFFECT OF MK0752 ON NOTCH1 AND JAGGED1 EXPRESSION

In this work, we found that the production of notch1 and jagged1 in renal tissue was considerably (p=0.05) less in a sham group than in the sepsis and vehicle groups. The notch1 and jagged1 values in the kidney tissue of mk0752 mice treated were considerably (p=0.05) less than in sepsis and vehicle groups. Sham *vs.* sepsis group and vehicle group, p-value <0.05 (significant). Mk0752 *vs*. sepsis and vehicle group, p-value <0.05 (significant) (Fig. 8-11).

EFFECT OF MK0752 ON RENAL TISSUE

To examine the histological changes in tissues, the degree of the renal tissue damage among the four study groups were scored from 0 to 4 (Fig. 12). The results revealed a normal renal tissue structure in the sham group whereas increased cytoplasmic eosinophilia and tubule damage were seen in control and vehicle groups (Fig. 13). Pretreatment with MK0752 improved the renal tissue architecture compared with control and vehicle groups.

The results in figure 12 were expressed as mean ± SEM, n=6. Statistical analysis was performed using Kruskal Wallis test. Sham *vs.* control and vehicle groups, p-value <0.05 (significant). MK0752 *vs.* control and vehicle groups, p-value < 0.05 (significant).

DISCUSSION

Sepsis remains a medical problem leading to increased morbidity and mortality in the world. Despite the substantial progress in understanding of the pathophysiological features of this clinical condition, it is still a big challenge. Sepsis-induced AKI is responsible for a 70% of all fatalities. Microbial LPS is recognized to be

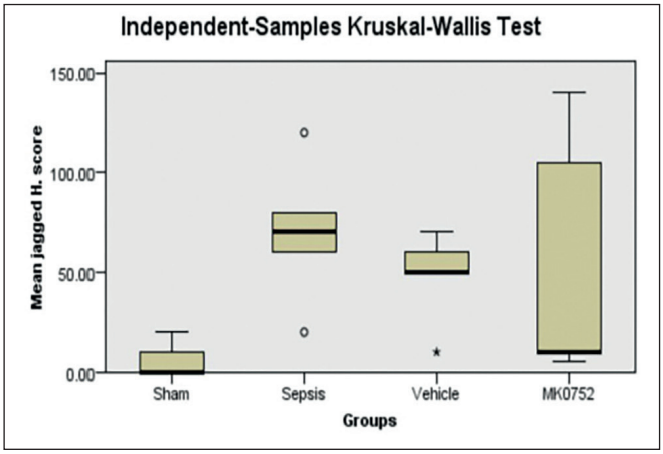

Fig. 9. Mean H-score of jagged1 in renal tissue of the four experimental groups at the end of the study (number of animals = 6 in each group).

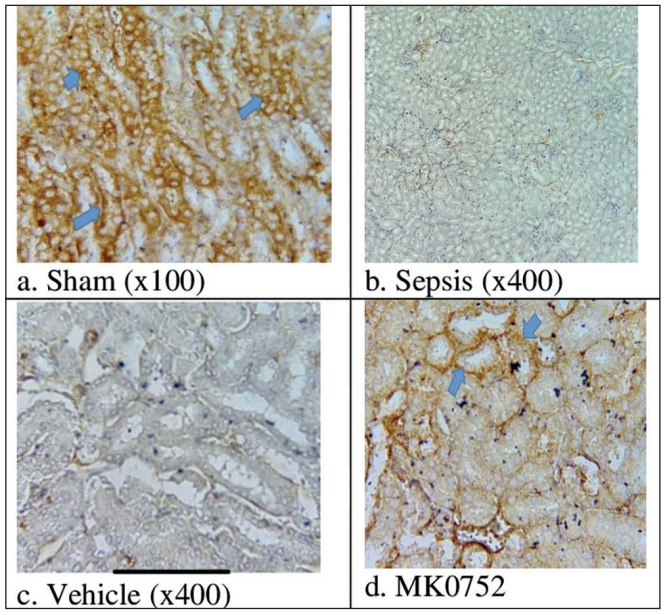

Fig. 10. A cross-section of the kidney showed notch1: (a). Negative sham group; (b). Positive sepsis group/moderate cytoplasmic stain (arrows); (c). Positive DMSO group/ weak cytoplasmic stain (arrows), (d). Negative mk0752 group.

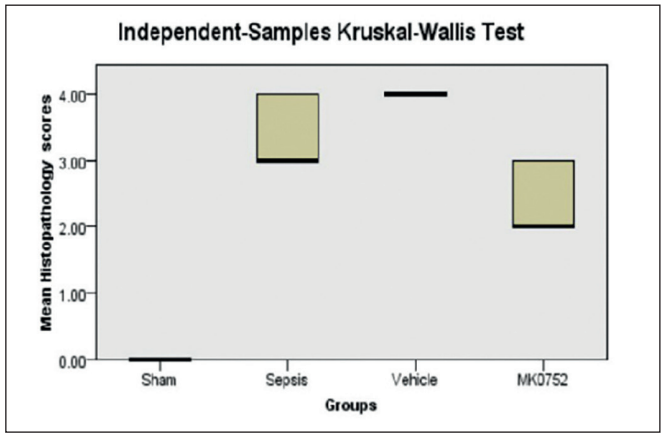

Fig. 12. Mean histopathological scores of kidney tissue of the four experimental groups.

the primary cause of inflammation and clinical manifestations in sepsis, and this is associated with early kidney damage [27]. Glomerulonephritis is a frequent consequence of sepsis, and the prognosis is bleak [28]. The current study found that blood urea and serum creatinine increase in both control and vehicle groups in comparison with sham group. This finding is consistent with that of Harpin et al., and Liu et al., who found high levels of serum creatinine and urea in rats exposed to sepsis by CLP [29]. On the other hand, pretreatment with MK0752 decreased the levels of both urea and creatinine. To the best our knowledge, this is the first study examining the influence of MK0752 on the renal function readouts. A possible explanation for this might be due to that Notch activation occurs during acute

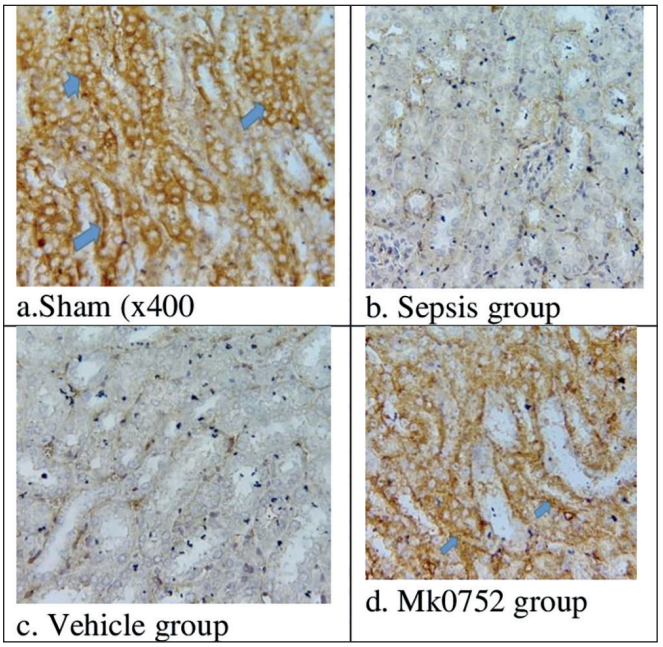

Fig. 11. A cross-section of the kidney showed jagged1: (a). Negative sham group; (b). Positive control group/cytoplasmic stain (arrows); (c). Positive DMSO group/cytoplasmic stain (arrows); (d). Negative mk0752 treatment group.

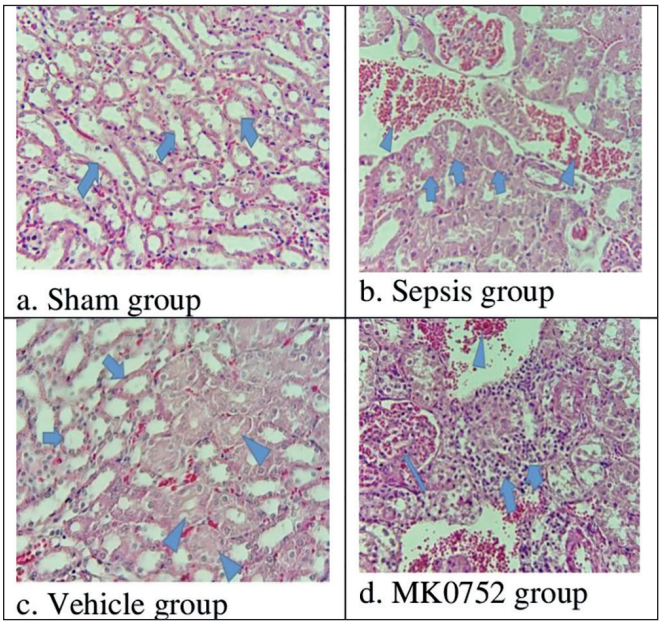

Fig. 13. Histopathological examination of kidney section. The section was stained with hematoxylin and eosin (x400): (a). A cross-section of the kidney showed normal histology, Normal renal tubules (arrows), Sham group. (b). A cross-section of kidney tissue showed a score of 3.4 sepsis changes including control group cytoplasmic swelling, increased cytoplasmic eosinophilia(arrows), and hemorrhage (arrows head). Sepsis group. (c). A cross-section of kidney tissue showed a score of 4 sepsis changes including eosinophilia + swelling (thick arrows), inflammation (thin arrows), hemorrhage (arrow head). vehicle group. (d). A cross-section of kidney tissue showed a score of 2.5 sepsis changes including the area of normal renal tubules with a focal area of damaged tubules Arrows (normal renal tubules).

and chronic renal injury and MK0752 could somehow interfere with Notch signaling pathway activation. The study revealed that levels of TNF-α, IL-6, IL-10, TNFR1 and VEGF in renal tissues were significantly elevated in mice subjected to CLP compared with sham group. These results are in agreement with Riedemann, Guo, and Ward findings which showed that serum TNF-α levels following LPS infusion were 200-fold greater in mice exposed to CLP [30]. IL-6 production is found to be increased in sepsis patients, indicating that IL-6 is a key contributor to the onset of sepsis [31]. IL-10 played a role in suppressing of pro-inflammatory cytokines, reducing the mortality caused by sepsis. Among the septic patients, IL-10 levels are increased highlighting their role in the response to inflammation [32]. TNFR1 seems to have a vital function in pathogens. This receptor not only signals TNF-α, which is immediately increased following bacterial entry, but also recognizes protein A, a crucial staphylococcal surface protein, activating both pro-inflammatory and anti-inflammatory pathways. The findings reveal, that TNFR1 has a massive effect on modulating CD4+ T-cell energy in staphylococcal sepsis, and this receptor activity is essential to microbial clearing throughout the spleen [33]. Serum VEGF concentrations also were found to be increased during a mammalian septic crisis for the first 2 days [34]. MK0752-treated group resulted in a marked reduction in levels of TNF-α, IL-10, TNFR1 and VEGF and elevation in levels of IL-10. These results seem to be consistent with other research which found that treatment of cell cultures with MK0752 provoked a weak angiogenesis suggesting that MK0752 could inhibit angiogenesis-associated with cytokine production. MK0752 has shown to supress many proangiogenic and proinflammatory cytokines and growth factors, especially VEGF when measured in the prepared media [18]. Immunohistochemistry findings showed that expression levels of Notch1 and jagged1 increased in renal tissues of control group in comparison with sham group. These results are in accord with recent studies indicating that levels of Notch1 increased 24 h following injecting mice with LPS [35]. In contrast, pretreatment with MK0752 showed a reduction in immunoreactivity for Notch1 and jagged1 compared with mice subjected to CLP. In accordance with the present results, previous study demonstrated that once-weekly dose of MK-0752 reduced activation of Notch1 signaling pathways for at least 96 h [36]. Another study showed that gamma-secretase inhibitor inhibits NICD expression in dose-dependent manner [37] and a significant inhibition of Notch signaling post-MK-0752 treatment was observed using the gene expression array from hair follicles [17]. Furthermore, the current study showed that severity of renal tissue damage was higher in control group than in sham group. The architecture of renal tissues was featured with a cellular edema, rise in cytoplasmic eosinophils, red blood cell extravasation, an increase in inflammation, and an increase in tubular damage. This finding is in consistent with Lerolle et al. [38], who found that AKI can induce severe acute tubular lesions, strong cytoplasmic eosinophilia, and a high quantity of inflammatory cell infiltration in kidney patients' glomeruli. Maiden et al. showed that in 19 post-mortem renal biopsies, sepsis was shown to induce acute tubular lesions, strong leukocytic infiltration, tubular apoptosis, and sometimes glomerular microthrombi [39]. Pretreatment with MK0752 alleviates the renal tissues as revealed by histopathological evaluation suggesting that MK0752 can reduce the deterioration of renal tissue following CLP.

CONCLUSIONS

Taken together, these results suggest that MK0752 could be protective against the renal injury induced by sepsis through its ameliorative impact on renal architecture and modulating cytokines and Notch1 singling pathway. Further studies regarding the role of Notch signaling pathways would be worthwhile.

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ORIGINAL ARTICLE

A COMPREHENSIVE APPROACH TO MEDICAL-PSYCHOLOGICAL SUPPORT FOR SERVICE WOMEN IN MODERN UKRAINE

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ABSTRACT

The aim: To analyze the current state of the problem and develop a modern methodology for the correction and prevention of stress disorders in female veterans.
Materials and methods: The following methods were used during the research: theoretical and interdisciplinary analysis, complex, clinical and psychopathological examination and methods of mathematical and statistical data processing.
Results: In the course of our work, it was developed an algorithm for medical and psychological support for women who suffered from results of the fighting includes the following components: monitoring of the psychological and mental state of veteran women; increased psychological care; psychological support for veteran women; psychotherapy; psychoeducation; creation of a re-adaptation atmosphere; formation of a health-centred living style and strengthening of psychosocial resources.
Conclusions: The complex system of treatment and prevention of stress-social disorders in women veterans should be aimed at decreasing the level of anxiety-depressive symptoms and the excessive nervous and psychological tension; infective re-evaluation of the traumatic experience; building a positive attitude towards the future and creating a new cognitive model of life.

KEY WORDS: veteran women, combatant, medical suppor, psychological support, complex system of treatment

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INTRODUCTION

Combat operations in Eastern Ukraine has an undoubted negative impact on the psychological and mental state of people who take part in fighting and leads to the development, of disorders of mental adaptation, combat-related psychological trauma, post-traumatic stress disorder and socially-stressful states.

Combat stress is a multilevel reaction of the organism in response to extreme (combat) conditions, which leads to excessive stress of adaptation mechanisms, reactive self-regulation and is an extreme destabilizing state, which depletes the adaptation reserves of the body, increases the risk of desintegration of mental functions and the development of maladaptation states[1, 2].

Stress-related mental disorders that occur during military operations are one of the most important internal barriers to combat readiness and effective performance of professional duties by combatants (even to the point of reducing the ability to conduct active combat operations), and in future - to adaptation in conditions of ordinary life through imposition of new stresses associated with social maladaptation on this substrate [3-5].

Currently, 9,916 women in Ukraine have been given combatant status for participation in the ATO/JFO zone [6]. After military service, some difficulties in reintegrating women veterans into civilian life remain unresolved.

Based on the analysis of data from the study "Invisible Battalion 2.0": returning veterans to civilian life" [7, 8], we can identify the main problems at the stage of returning to civilian life: lack of feedback system for recipients of rehabilitation and reintegration, which makes it impossible to assess the effectiveness of the services provided; lack of needs analysis practice, which makes the reintegration system more gender-neutral than gender-sensitive; a prejudiced attitude of employment centre staff and other employers towards female veterans, lack of understanding of their psychological and communication skills; a prejudiced attitude of medical and social services staff, stigmatisation of the portrait of a female veteran [9-12].

Thus, the development of therapy and rehabilitation system of servicewomen who suffer from combat stress in military conflicts is of great practical importance in solving the many problems that have arisen before the military health care system.

The above mentioned has motivated us develop a modern methodology for the correction and prevention of stress-related disorders in female veterans.

THE AIM

The aim of the work is to analyze the current state of the problem and develop a modern methodology for the correction and prevention of stress disorders in female veterans.

MATERIALS AND METHODS

The following methods were used during the research: theoretical and interdisciplinary analysis, complex, clinical and psychopathological examination and methods of mathematical and statistical data processing.

The theoretical method included theoretical and methodological analysis, generalization of various scientific materials on the topic of the study.

Interdisciplinary analysis of social, psychological, medical literature is aimed at reflecting the current state of the problem.

Clinical and psychopathological examination, which made it possible to draw up a psychological portrait of female veterans.

Statistical data processing was performed using SPSS 26.0 for Windows XP, which allowed provide quantitative and qualitative analysis of the obtained data.

It was provided a comprehensive examination of 96 female veterans of the ATO/JFO zone.

RESULTS

The clinical and psychopathological examination revealed that the clinical structure of stress-social disorders is represented by emotional disorders: depression (68.2% of examined), anxiety (89.2%), feeling of internal tension with inability to relax (88.9%), drudgery (72.8%), anhedonia (49.2%), lack of hope (59.8%), feeling of guilt in the survivor (41.2%); mild cognitive disorders: difficulty concentrating attention (69.8% of those examined), impaired memory (36.8%), difficulty making decisions (56.9%), difficulty planning and organizing (42.8%) and difficulty selecting the necessary words to express thoughts (33.8%); vegetative paroxysms (72.6%); asthenic syndrome (66.9%) and insomnia (66.2%).

A psychodiagnostic study revealed high scores in the Anxiety and Depression Clinical Scales (75.2%) and also in the Neuropsychological Stress Scale (78.2%), severe clinical manifestations of PTSD on the Clinical Administered PTSD Scale-CAPS (68, 7%), the full manifestation of traumatic stress according to the Impact of Event Scale-Revised, IES-R (85.3%), deficient psychological protection mechanisms (62.2%), maladaptive coping (52.6%), and high level of social frustration (72.9%).

In the course of our work, it was developed an algorithm for medical and psychological support for women who suffered from results of the fighting includes the following components: monitoring of the psychological and mental state of veteran women; increased psychological care; psychological support for veteran women; psychotherapy; psychoeducation; creation of a re-adaptation atmosphere; formation of a health-centred living style and strengthening of psychosocial resources.

Psychological and mental state monitoring was focused on identifying individuals with the effects of combat-related psychological trauma; peculiarities of emotional response to stressful situations; presence of psychopathological symptoms (symptoms of combat post-traumatic stress disorder, anxiety, depression, suicidal tendencies, addictive behaviour).

Increased psychological attention was focused on psychoprophylaxis of maladaptive reactions and states in conditions of social stress, raising the level of adaptation to the conditions of civilian life and ensuring psychological well-being, preserving women's psychological and physical health.

Psychological support had a socio-psychological direction and helped optimize the psycho-emotional state of women veterans during their readaptation to a civilian life, reproduction of lost or new socialties, reducing feelings of isolation, stigmatization, harmonization of family relations based on mutual understanding, mutual assistance and mutual support (for married women).

The psychotherapy program included targeted use of cognitive-behavioral therapy, personality-oriented therapy, biosuggestion, non-directive Roger's psychotherapy, arttherapy, biological feedback, trauma-focused therapy, EMDR (EyeMovement Desensitization and Reprocessing). It is advisable to use a self-management program for post-traumatic stress disorder by Pucelik Consulting Group.

Psychotherapeutic intervention was focused on affective re-evaluation of the traumatic experience; correction of behavioural patterns related to combat stress; Developing skills for coping with anxiety and emotional reactions and constructive forms of cognitive and emotional response in the context of a change in living patterns of return to civilian life.

An important component of the program of medical and psychological support is psychoeducation aimed at: forming an adequate system of ideas about the consequences of combat stress and the peculiarities of adaptive reactions to change life stereotypes; understanding the main consequences and psychopathological reactions caused by mental trauma; training in methods of mastering the consequences of combat mental trauma, basic techniques of self-help during intrusive memories, anxious paroxysms, skills of self-regulation and self-management of their condition.

The basis of medical and psychological support for women veterans is the creation of a re-adaptation atmosphere focused on the public recognition of the social significance of participating in combat; recognizing the high social status of women veterans; understanding the specifics of combatants' psychological reactions and behaviour; and creating the conditions for a favourable psychological environment in the family.

It is very important to form a health-centered lifestyle, consolidate healthy habits, correct physical and mental disorders, increase stress resistance, develop constructive coping strategies.

Strengthening the psychosocial resource includes psychosocial reintegration, adaptation to new living conditions, the formation of additional sources of psychosocial support, meetings with volunteers, career guidance.

To prevent the development and decompensation of stressful disorders we have developed a comprehensive system of medical and psychological rehabilitation of combat participants, which consists of four stages:

Stage I - psychophysiological preparation for participation in combat operations, which includes: adaptation to changes in life stereotype; increasing the adaptation capacity; stabilization of the emotional state, decreasing the level of anxiety; increasing the level of stress resistance.

Stage II - psychological support during combat operations: early diagnosis of stress-association disorders; assessment of special features of veterans and prediction of their behavioural reactions.

Stage III - psychological preparation for exiting the war zone and returning to civilian life: Lowering the level of emotional tension; creating and correcting plans for the future; assessing veterans' personality traits and predicting their adaptation reactions when their lifestyle changes; formation of a positive attitude to the future; correction of experiences related to changes in the living stereotype (feelings of hopelessness, difficulties related to social adaptation).

Stage IV of psychophysiological re-adaptation after demobilization: effective reassessment of the traumatic experience; creation of a new cognitive model of living; psychosocial reintegration: adaptation to new living conditions, formation of additional sources of psychosocial support, meetings with volunteers; prophylactic therapy; family psychotherapy, girlfriend psychocorrection; medical-psychological assistance in case of occurrence of stress-association discord.

The approbation of the suggested complex system of correction and prevention of stress-association disorders in women veterans proved its high efficacy.

DISCUSSION

The obtained data on the development of a modern methodology for the correction and prevention of stress-related disorders in female veterans correlate with the data of modern researchers conducted in the field of therapy and rehabilitation of men veterans [13-15] as well as with the experience of medical and psychological support in NATO countries [16, 17].

Developed and tested treatment and rehabilitation modules for combatants with PTSD which include: pharmacotherapy, psychotherapy (trauma-focused therapy, EMDR, rational and group psychotherapy, art therapy, family psychotherapy, autogenic training, training, coping, coping and coping) and socio-psychological support (socio-pedagogical, socio-environmental, career guidance activities). An algorithm for the development of personalized programs for the treatment and rehabilitation of combatants suffering from PTSD has been developed, a key component of which is the personalized identification of target symptoms and critical life circumstances for the restoration of mental health.

Developed and tested treatment and rehabilitation modules for combatants with PTSD which include: pharmacotherapy, psychotherapy (trauma-focused therapy, EMDR, rational and group psychotherapy, art therapy, family psychotherapy, autogenic training, anxiety overcoming training, confident behavior training, inoculation of stress) and socio-psychological support (socio-pedagogical, socio-environmental, career guidance activities).

An algorithm for the development of personalized programs for the treatment and rehabilitation of combatants suffering from PTSD has been developed, a key component of which is the personalized identification of target symptoms and critical life circumstances for the restoration of mental health.

It should be noted that the gender-oriented system of correction and prevention of stress-related disorders in women veterans, which is presented in our study developed in Ukraine for the first time.

CONCLUSIONS

The complex system of treatment and prevention of stress-social disorders in women veterans should include: monitoring of the psychological and mental state of veteran women; increased psychological care; psychological support had a socio-psychological direction; psychotherapy using cognitive-behavioral therapy, personality-oriented therapy, biosuggestion, Roger's non-directive psychotherapy, art therapy, biofeedback, trauma-focused therapy, EMDR (Eye Movement Desensitization and Reprocessing). It is advisable to use the PTSD self-monitoring program from Pucelik Consulting Group; psychoeducation aimed at teaching methods of mastering the consequences of combat mental trauma, basic self-help techniques for obsessive memories, anxiety paroxysms, self-regulation skills and independent management of one's condition.; creation of a re-adaptation atmosphere; formation of a health-centred living style and strengthening of psychosocial resources.

The system of medical and psychological rehabilitation of combatants should consist of four stages: I - psychophysiological preparation for participation in combat operations, II - psychological support during combat operations, III psychological preparation for leaving the combat zone and returning to civilian life, IV - psychophysiological readaptation after demobilization.

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ORIGINAL ARTICLE

SAFETY AND EFFICACY OF THE COMPLEX DEPRILIUM® IN REDUCING SUBCLINICAL SYMPTOMS OF DEPRESSION IN PATIENTS WITH CHRONIC NON-COMMUNICABLE DISEASES: DOUBLE-BLIND RANDOMIZED CONTROLLED STUDY

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ABSTRACT

The aim: To evaluate the effectiveness of the use of the Deprilium® complex for the relief of subclinical symptoms of depression in patients with NCD.

Materials and methods: There were 140 patients involved in the study. To assess the subclinical symptoms, the Hamilton Depression Rating Scale (HAM-D) was used. In order to obtain additional information about the patient's condition, the Somatic Symptom Scale SSS-8 and the Quality of Life Scale (QOLS) were used. Patients were randomized by block randomization to an intervention group, which took Deprilium® complex, and a control group, which took placebo.

Results: After 60 days a statistically significant difference was observed in all clinical indicators between the intervention group and the control group. The median value of the HAM-D scale differed between the groups by 6 points, significantly ($p < 0.000$) lower results were observed in the intervention group, which participants were taking the Deprilium® complex. When comparing the indicators of the intervention group on the 1st and on the 60th day of the study, statistically significant changes ($p < 0.000$) were observed in all three indicators.

Conclusions: The received results confirm the available evidence for the properties of SAMe in depression and complement them with evidence of the effectiveness of the Deprilium® complex that contains SAMe and L-methylfolate with methylcobalamin, which together produce pharmacological and clinical synergy to reduce the severity of subclinical depressive manifestations in patients with NCD. Further studies of the effectiveness of the use of the Deprilium® complex in patients with NCD are required.

KEY WORDS: depression, chronic non-communicable diseases, S-adenosyl-L-methionine

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INTRODUCTION

Non-communicable diseases are the leading cause of death worldwide: four main types of chronic non-communicable diseases (NCD) - cardiovascular disease, cancer, chronic obstructive pulmonary disease, diabetes - cause >2/3 of all deaths, making them a global problem [1]. Currently, in about half of the world's population at least one non-communicable disease is detected, and in 1/4 - more than one [2]. NCD such as cardiovascular disease, diabetes, cancer and chronic respiratory diseases are often accompanied by depressive manifestations, and this connection attracts increasing attention of researchers from around the world [3]. Depression is noted in such patients 2-4 times more often than in people without

NCD, and this combination creates a significant burden on health systems of middle- and low-income countries [4]. Presence of more than one chronic illness, female gender, poor education and lack of permanent relationships are associated with higher risks of developing depression [5]. In this regard, the World Health Organization recommends co-management of the patient with the NCD by an internist and a mental health professional [2-3]. Lack of comprehensive approach to the treatment of such patients can lead to lack of compliance, irrational double diagnostic testing, risks of incorrect interaction of pharmacological drugs, excessive burden on hospitals and even increased mortality [2]. The team approach is important for providing health services for patients with both non-communicable diseases and mental health problems [6].

Thus, there is a need to find approaches to the early therapy for depressive symptoms in patients with NCD, which would allow us to start managing the symptoms at the beginning of their occurrence. Existing approaches to early symptom management, despite their advantages, are still not widely available [6]. Therefore, the search for a therapy that is at the same time effective, safe and doesn't create an economic burden for patients, simple and clear -- this search is still relevant.

A promising candidate for such therapy may be the Deprilium® complex, which contains S-adenosyl-L-methionine (SAMe), L-methylfolate and methylcobalamin. It is a coenzyme that is naturally synthesized in the human body and participates in the construction of neurotransmitters and phospholipids in the brain; adding it to the diet increases the level of serotonin, dopamine and dilseril phosphate [10]. There are reports in the literature that SAMe levels are significantly lower in depressed patients than in people without depressive symptoms [6]. In this case, intravenous or oral administration of SAMe is associated with a significant increase in its levels in the cerebrospinal fluid, which indicates its ability to penetrate the blood-brain barrier. These observations provide a rational basis for the antidepressant effect of SAMe, which has been confirmed in several countries [7]. In a 2020 systematic review, the authors concluded that SAMe had a significantly greater effect on depressive symptoms than placebo, both as monotherapy and in combination with antidepressants, comparable to such widely used antidepressants as escitalopram and imipramine [9]. A large multicenter study found that taking SAMe at a dose of 400 mg per day was indeed associated with a significant reduction in the severity of depressive symptoms after 7 days, and the results kept improving after another 15 days [1]. SAMe has proven to be a quite safe substance, as most side effects are mild, clinically insignificant or transient [1, 7]. Combining SAMe with antidepressants improved their effect in patients with resistant depression and had no increased health risks, among the most common side effects were gastrointestinal disorders and headache [8].

SAMe synthesis is inseparably linked to the presence of folic acids and cobalamin [11] contained in the Deprilium® complex to create pharmacological and clinical synergism of action. This induces the interest to study the effectiveness of the use of the Deprilium® complex in subclinical depressive states in patients with NCD. However, its effect on subclinical manifestations of depression in such population is still insufficiently studied, which makes further research in this area relevant.

THE AIM

To evaluate the effectiveness of the use of the Deprilium® complex to relieve subclinical symptoms of depression in patients with NCD.

MATERIALS AND METHODS

Design of the study: double-blind randomized controlled trial with parallel groups.

On the basis of the Department of Medical Psychology, Psychosomatic Medicine and Psychotherapy of the O.O. Bohomolets National Medical University (monocentric study) in compliance with ethical and deontological norms in accordance with the principles set out in the Declaration of Helsinki, there were 140 patients involved in the study. Before taking part in the study, the participants reviewed the protocol and signed an informed consent.

Criteria for inclusion in the study: men and non-pregnant women who are not breastfeeding, aged 18-65 years; 0–14 points on the Hamilton Depression Rating Scale (HAM-D).

Non-admittance criteria: participation in a study during 1 month before the screening; a mental disorder diagnosis; a traumatic brain injury or a stroke in anamnesis; taking antidepressants ≤1 month before the involvement in the study.

ENDPOINTS

The primary endpoints were the total score on the Hamilton Depression Scale (HAM-D). Secondary endpoints - the total score on the scale of somatic symptoms (Somatic Symptom Scale - SSS-8) and the Quality of Life Scale by O. S. Chaban (Quality of Life Scale - QOLS).

PSYCHODIAGNOSTIC TOOLS

The HAM-D scale was used to assess subclinical symptoms. SSS-8 and QOLS were used to obtain additional information about the patient's condition.

The 17-item Hamilton Depression Rating Scale (HAM-D) consists of 17 items (9 of which are rated from 0 to 4 points, and 8 from 0 to 2 points) filled out by a specialist during a structured clinical interview [12]. Interpretation of the final score in this study was carried out in accordance with the updated in 2019 recommendations of the National Institute for Health and Awareness Institute for Health and Care Excellence (NICE) on therapy and management of depression in adults, where 0–7 points - stand for no depression, 8–13 - subclinical manifestations, 14–18 - moderate manifestations, 19–22 - moderate severity, ≥23 points - severe manifestations of depression [13].

The Somatic Symptom Scale (SSS-8) is a brief self-questionnaire of the somatic manifestations of depression developed by Gierk B. et al. [14], which consists of 8 questions, each rated within 0-4 points, where 0 -"Did not bother at all", 4 - "Bothered a lot". Assessment of somatic symptoms occurs by calculating the total result, which can vary between 0-32 points. The results are interpreted as follows: 0-3 points - minimal, 4-7 - low,

8–11 - medium, 12–15 - high, 16–32 - very high degree of intensity of somatic symptoms [15].

Chaban A.S. Quality of Life Scale (CQLS) is a questionnaire designed to assess the quality of life, containing 10 questions on different aspects of the life of the subject. It is necessary to indicate the number of points that is most suitable, from 0 ("Not at all satisfied" to 10 ("Extremely satisfied"). Assessment of the quality of life occurs by calculating the total score, which can vary from 0 to 100. A score of up to 56 points corresponds to an extremely low level of quality of life, from 57 to 66 – low, 67–75 points correspond to an average level, 76–82 points – high, from 83 points – a very high level of quality of life [16].

PROTOCOL AND DESIGN

The research was conducted on the basis of the Department of Medical Psychology, Psychosomatic Medicine and Psychotherapy of the O.O. Bohomolets National Medical University. Having received information about the study and having given written informed consent, and having undergone a screening procedure for eligibility, participants completed QOLS and SSS-8 questionnaires and were assessed at a structured clinical interview for depression (HAM-D), that met time point T1. Group formation was performed by block randomization to obtain equivalent groups. Patients were assigned to an intervention group that used Deprilium® twice per day (200 mg of S-adenosyl-L-methionine in the form of capsules (total dose of 400 mg / day) in combination with 0.4 mg of L-methylfolate and 0.25 mg of methylcobalamin), or to a control group that received placebo in a similar form and at a similar frequency for 60 days. After 60 days, a re-evaluation was performed on the scales HAM-D, SSS-8, QOLS, which corresponds to the time point T2.

CALCULATION OF THE SAMPLE SIZE

Taking that alpha is 0.05 and the sampling power is 80%, to track the difference between groups of 2 points on HAM-D, assuming a standard deviation is 4 and a dropout risk is equal to 10%, it was necessary to involve 70 people in each group.

STATISTICAL ANALYSIS

Qualitative data are presented through a number of observations and the percentage of the total number of observations. To assess the normalcy of distribution of quantitative indicators the Shapiro-Wilka criterion was used. Quantitative data are presented by mean and standard deviation (M ± SD), or by median and interquartile range [Med (IQR)], as appropriate. To assess the difference between two unrelated samples, the t-test for unrelated samples was used (in case of submission to the law of normal distribution). In the case of a distribution other than normal, to estimate the difference between two unrelated samples the U-test by Mann-Whitney for unrelated samples was used. To estimate the difference between two related samples, in the case of a normal distribution, the t-test for related samples was used, and in the case of a distribution other than normal, the Wilcoxon test for unrelated samples was used. Statistical data processing took place using the programming language R using the environment for statistical calculations EzR v1.54 [17]. Data visualization was performed using the Python programming language with the add-ons matplotlib and seaborn.

RESULTS

The average age of patients was 39.05 ± 9.92 years. 84 (60%) of the subjects were female, 56 (40%) - male. The majority (n = 103; 73.57%) of respondents at the time of participation in the study were married, a minority (n = 37; 26.43%) had no long-term partner.

Almost all the participants (n = 134; 95.71%) were employed and only a few (n = 6; 4.29%) were temporarily unemployed. Prior to randomization, the groups did not differ significantly according to any of the socio-demographic indicators (Table I).

79 (56.43%) participants had hypertension, 33 (23.57%) had bronchial asthma, 27 (19.29%) had diabetes, and 19 (13.57%) had chronic obstructive bronchitis. 122 (87.14%) had one NCD, 18 (12.86%) participants had two. No statistically significant differences in the structure of morbidity were found prior to randomization (Table II).

At the time of involvement in the study all participants (n = 140) noted subclinical depressive manifestations on the HAM-D scale. The mean value in the whole sample was 9.02 ± 0.93. According to the SSS-8 scale, the minimum level of somatic manifestations was observed in 12 (8.57%) participants, low - in 38 (27.14%), medium - in 51 (36.43%), high - in 36 (25.71%), very high - in 3 (2.14%) participants. According to the QOLS scale, the average value for the entire sample was 8.98 ± 3.75. It was found that 46 (32.86%) of the study participants have a very low level of quality of life, 37 – low (26.43%), medium - 34 (24.29%), high - 12 (8.57%), very high - 11 (7.86%). The average in the whole sample was 62.54 ± 14.70. Comparisons of the intervention group and the control group at the pre-randomization stage are presented in table III.

Table I. Social and demographic characteristics of the intervention and control groups

Variables	Intervention group (n=70)	Control group (n=70)	T / χ^2	p
Age	37.72 ± 10.50	40.37 ± 9.18	t = -1.58	0.115
Gender				
female	42 (60%)	42 (60%)	0	1
male	28 (40%)	28 (40%)		
Marital status				
married	52 (74%)	51 (73%)	0	1
single	18 (26%)	19 (27%)		
Employment status				
employed	68 (97%)	66 (94%)	0.174	0.676
unemployed	2 (3%)	4 (6%)		

Table II. Morbidity structure in the study participants

Illness	Intervention group (n=70)	Control group (n=70)	χ^2	p
Arterial hypertension	35 (50%)	44 (62%)	1.85	0.173
bronchial asthma	20 (28%)	13 (18%)	1.42	0.232
chronic obstructive bronchitis	9 (12%)	10 (14%)	0	1
diabetes	15 (21%)	12 (17%)	0.183	0.668

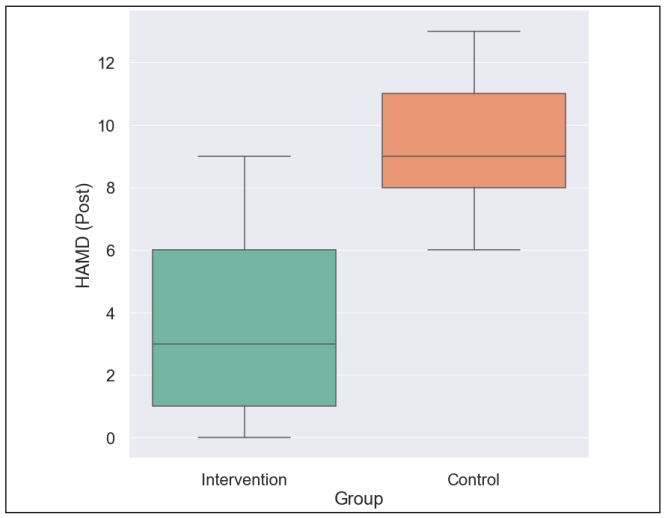

Fig. 1. Median values and interquartile range on a scale HAM-D in the control group and the intervention group

After 60 days (T2), a statistically significant difference was observed for all clinical indicators between the intervention group and the control group. The generalized results are presented in table IV.

The median value of the HAM-D scale differed between groups by 6 points, significantly ($p < 0.000$) lower results were observed in the intervention group which participants had been taking the Deprilium® complex during all that time (Fig. 1), which signaled about the lower degree of depression in this group.

There was also a difference observed in the quality of depressive manifestations. Thus, if at the time of involvement in the study all participants recorded subclinical depressive manifestations, then after 60 days the absence of depressive symptoms was observed in 60 participants of the intervention group, and subclinical depressive manifestations - in 10 participants, while in the control group the spontaneous reduction of depressive manifestations during the time and, accordingly, their absence were observed in 17 participants, and subclinical depressive manifestations - in 53 (Fig. 2). This difference was also statistically significant ($\chi^2 = 50.90$; $p < 0.000$).

The median value of the indicator on the SSS-8 scale also differed between the groups by 8 points, statistically significant ($p < 0.000$) smaller results were also observed in the intervention group (Fig. 3), which reflected a decrease in the degree of somatic manifestations in this group.

Differences were also observed in the mean score on the QOLS. The average values of the two groups differed by 10.04 in favor of the intervention group (Fig. 4), this difference was also statistically significant ($p < 0.000$).

When comparing the indicators of the intervention group on the 1st (T1) and 60th day (T2) of participation in the study, the statistically significant changes ($p < 0.000$) were observed in all three indicators (Table V).

The depressive symptoms dynamics graph (Fig. 5) allows us to visually estimate that for 60 days of using the Deprilium® complex we observed a decrease in the severity of symptoms by ≈66% (decrease in the median value by 6 points).

The decline on the SSS-8 scale was even greater (decrease in the median value by 7 points) - by ≈73% (Fig. 6).

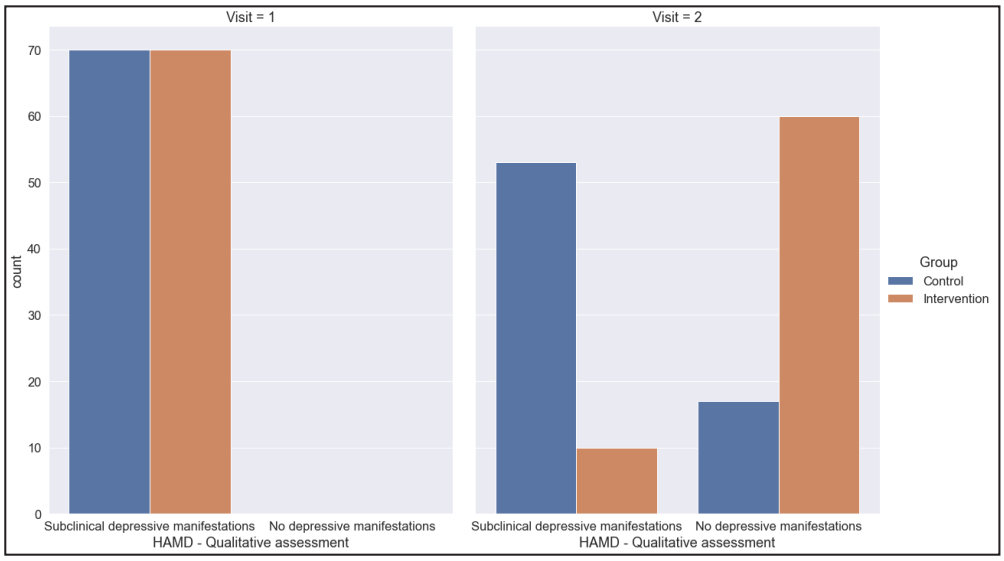

Fig. 2. Qualitative assessment of HAM-D clinical depression scale in the intervention group and the control group on the 1st and 60th day of the study

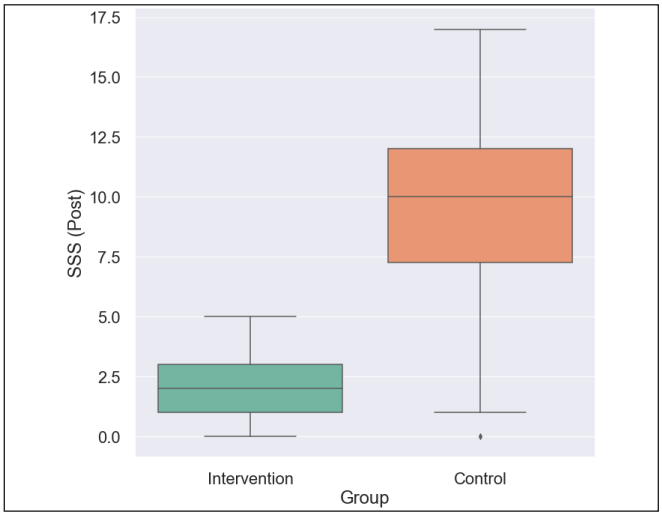

Fig. 3. Median values and interquartile range on the SSS-8 in the control group and the intervention group

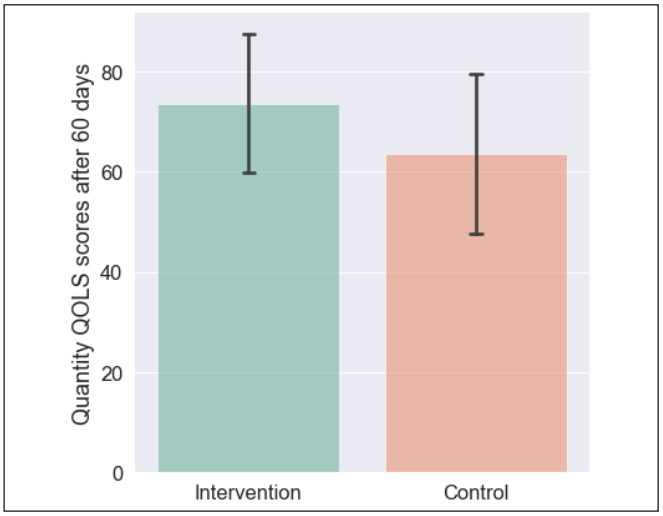

Fig. 4. Mean values and standard deviation of quantity QOLS scores after 60 days in the control group and intervention group

As a result, somatic manifestations in patients from the intervention group decreased significantly ($p < 0.000$). The minimum level of somatic manifestations was recorded in 67 (95%) patients, low - in 3 (5%). In the control group after 60 days there were minimal somatic manifestations in 7 (10%) patients, low – in 11 (15%), medium - in 29 (41%), high - in 18 (25%), very high - in 5 (5%) patients. Visual assessment of the data is presented in Fig. 7.

The increase in indicators was also observed on the QOLS (increase of the average value by 9.9 points) - by ≈13.5% (Fig. 8)

Thus, there was an improvement in the quality of life of patients. After 60 days, 9 (12.9%) participants had a very low level of quality of life, low - 15 (21.4%) participants, medium - 14 (20%), high - 11 (15%), very high - 21 (30%). For comparison, in the control group after 60 days in 19 (27.1%) people a very low level of quality of life was noted, low - in 20 (28.6%), medium - in 18 (25%), high - in 5 (7%), very high - in 8 (11%). This difference between groups was statistically significant ($p < 0.000$). Visual data evaluation is presented in Fig. 9.

Adverse events such as nausea, weakness, decreased blood pressure, diarrhea, itchy skin were more common in the intervention group over the 60 days. Feelings of increased heart rate and anxiety were observed more often in the intervention group. However, statistical processing did not reveal significant differences between the groups (for all cases $p > 0.05$). Results are presented in table VI.

DISCUSSION

The results suggest that the Deprilium® complex is much better than placebo at helping reduce subclinical depressive manifestations in patients with NCD. Although adverse effects were more common

Table III. Indicators of the two groups prior to randomization

Scale	Intervention group (n=70)	Control group (n=70)	t / W	p
HAM-D	9 [8-10]	9 [8.25-9]	W = 2154	0.347
SSS-8	8.52 ± 3.77	9.44 ± 3.69	t = -1.44	0.150
CQOLS	63.72 ± 14.70	61.35 ± 14.72	t = 0.95	0.345

Table IV. Comparison of the two groups on day 60

Scale	Intervention group (n=70)	Control group (n=70)	The difference in the median (Intervention group: control group)	T /W	p
HAM-D	3 [1-6]	9 [8-11]	-6	W = 378	0.000
SSS-8	2 [1-3]	10 [7.25-12]	-8	W = 263	0.000
CQOLS	73.62 ± 13.90	63.58 ± 16.07	+8	t = 3.95	0.000

Table V. Changes in mean values on the 1st and 60th day of the study

Scale	T1 (n=70)	T2 (n=70)	Difference in average/ median	t/W	p
HAM-D	9 [8-10]	3 [1-6]	-6	V = 2275	0.000
SSS-8	9.5 (6.25-9.5)	2 [1-3]	-7	V = 2200.5	0.000
CQOLS	63.72 ± 14.70	73.62 ± 13.90	+9.9	t = 5.56	0.000

Table VI. Frequency of adverse effects in the intervention group and the control group

Variable	Intervention group (n=70)	Control group (n=70)	χ^2	p
Nausea	4 (5%)	2 (2%)	0.174	0.676
Feeling of weakness	5 (7%)	3 (4%)	0.132	0.716
Bounding pulse	1 (1.4%)	2 (2%)	0	1
Decrease of the arterial blood pressure	2 (2%)	1 (1.4%)	0	1
Diarrhea	2 (2%)	0 (0%)	0.507	0.476
Itchy skin	3 (4%)	1 (1.4%)	0.257	0.612
Anxiety	1 (1.4%)	2 (2%)	0	1

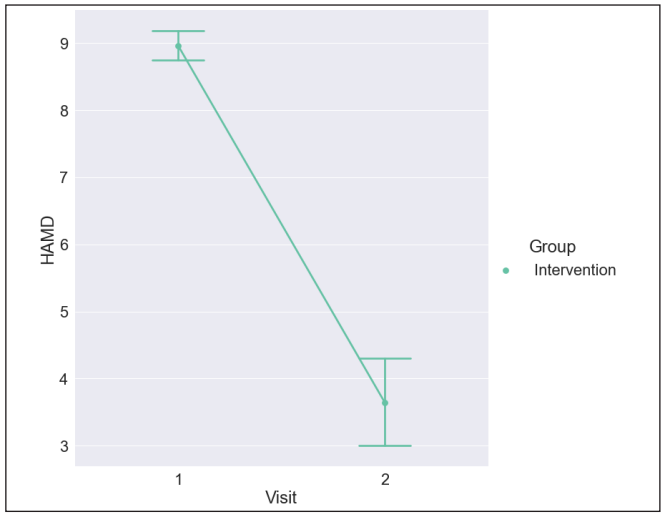

Fig. 5. Dynamics of indicators on the HAM-D scale in the intervention group for 60 days

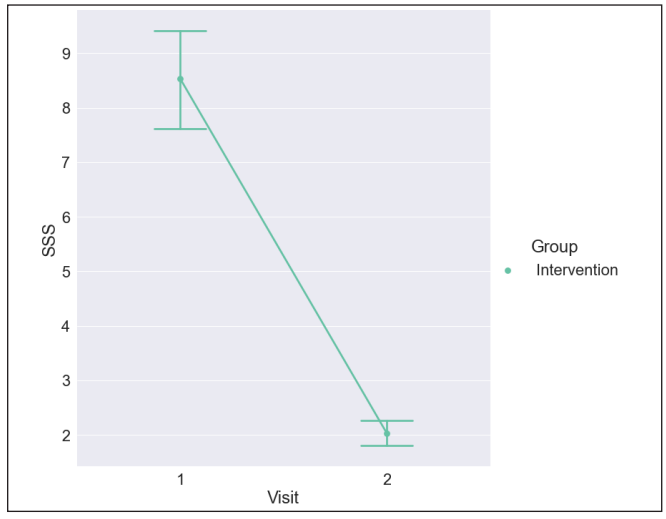

Fig. 6. Dynamics of indicators on the SSS-8 scale in the intervention group for 60 days

in the intervention group, the statistically significant difference with the control group was absent.

There are many approaches to treatment of patients with depression, so each new strategy must be evaluated from

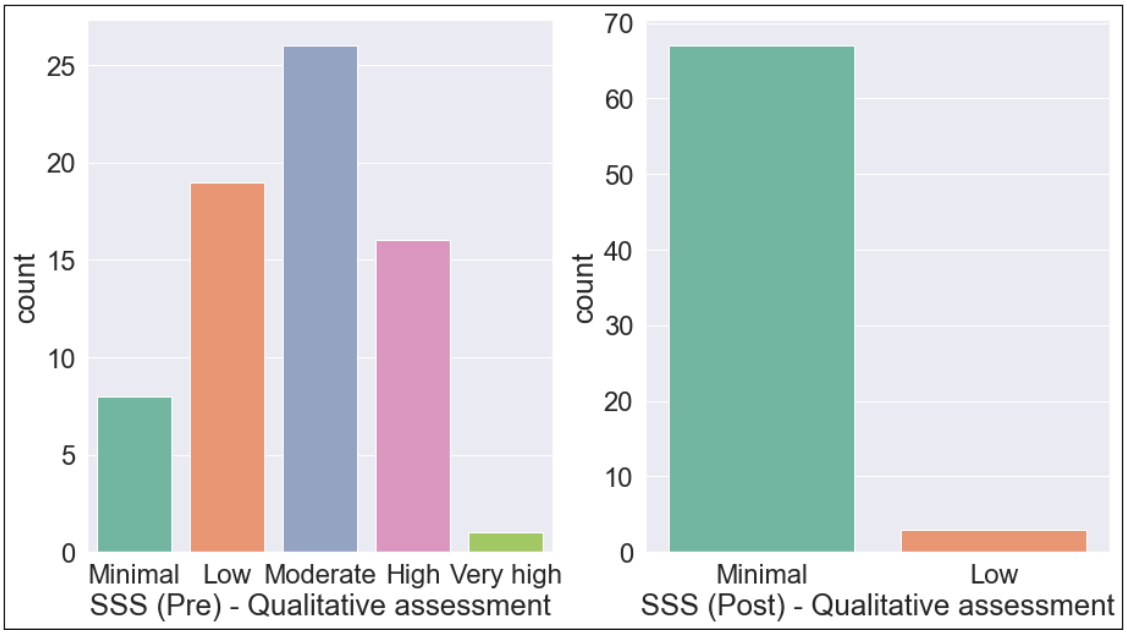

Fig. 7. Qualitative evaluation of somatic manifestations on the SSS-8 scale in the intervention group after 60 days

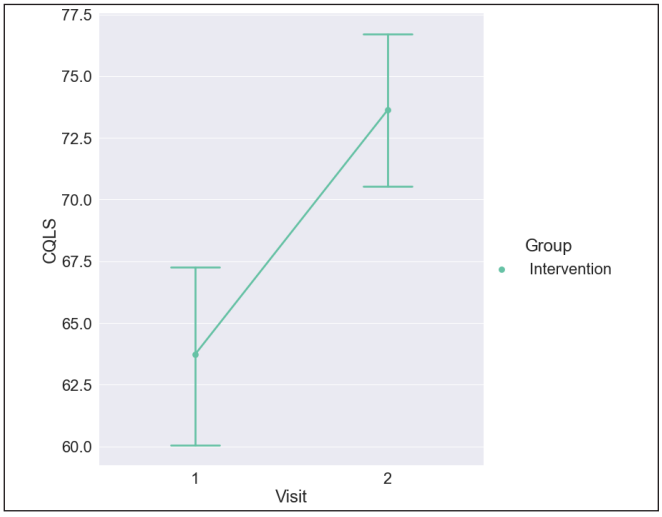

Fig. 8. Dynamics of the indicators on the QOLS scale in the intervention group for 60 days

the standpoint of weighing the risks and potential benefits [23]. Our results are consistent with the results of J. Sarris and co-authors (2014), who obtained similar SAMe efficacy results in major depressive disorder and used a similar design [19], and the results of K.M. Bell and co-authors (1994), who proved that the reduction in depressive symptoms by HAM-D scale is associated with increased concentration of SAMe in blood plasma [20]. Results regarding the side effects are also consistent with the reports of other researchers on the absence of clinically significant side effects reactions [22]. Given that the properties of SAMe can be compared with the effect of standard tricyclic antidepressants and selective serotonin reuptake inhibitors with a small number of side effects [9, 21], this makes it potentially important for the treatment of patients with early subclinical manifestations of depression in patients with NCD.

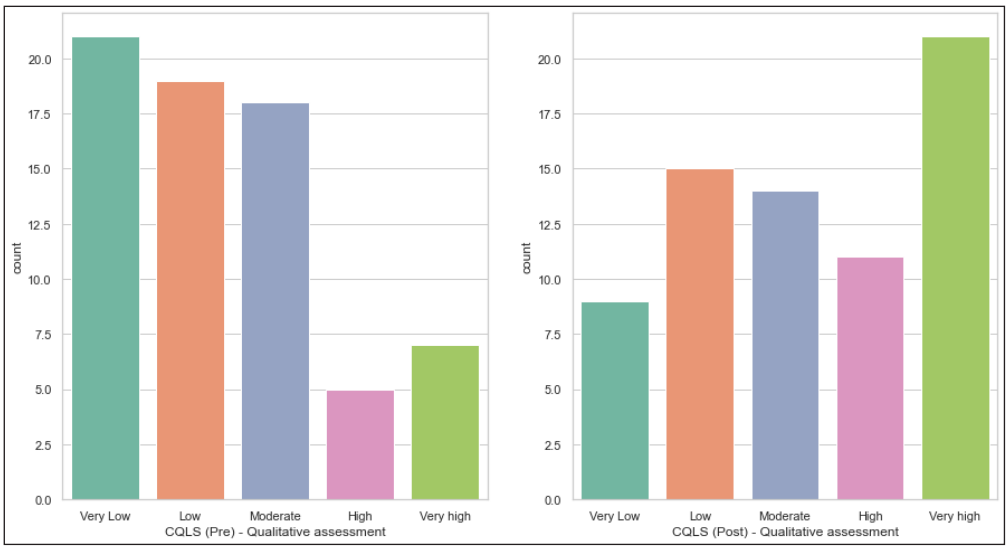

Fig. 9. Qualitative evaluation of quality of life on the QOLS in the intervention group after 60 days.

The advantages of this study are the design of a randomized controlled trial with double-blindness and a sufficiently long period of use of the Deprelium® complex to identify properties in comparison with placebo. Among the limitations of this study is the lack of follow-up evaluation, which makes it impossible to compare the effectiveness of therapy and durability of effects over time. Another limitation is a certain diversity in the group in terms of NCD, so further studies to provide deeper understanding of the properties and safety of the Deprelium® complex in some homogeneous clinical populations will supplement the obtained results. Also, the data of this study cannot be generalized to populations of adolescents and the elderly due to the age composition of the study participants (adulthood).

CONCLUSIONS

The results we have obtained confirm the available evidence on the effectiveness of SAMe in the treatment of patients with depression and provide the evidence of the properties of the Deprilium® complex containing SAMe and L-methylfolate with methylcobalamin, which together cause pharmacological and clinical synergy aiming to help reduce the severity of subclinical depression manifestations in patients with common NCD. In addition, it was found that the use of Deprilium® for 2 months does not carry increased risk to patient health and does not have significant side effects. Further studies are needed to research the use of the Deprilium® complex for patients with NCD.

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The Authors declare no conflict of interest.

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ORIGINAL ARTICLE

RISK FACTORS FOR DIABETIC NEPHROPATHY IN DIABETES MELLITUS TYPE 1

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ABSTRACT

The aim: To find the risk factors of microalbuminuria and estimated Glomerular Filtration Rate (eGFR) in patients with type 1 diabetes mellitus.

Materials and methods: One hundred ten patients of type 1 diabetes mellitus in this cross-sectional study at diabetic and endocrinology center in Al-Najaf during the period from September 2021 to March 2022. All patients were asked about sociodemographic characteristics (age, gender, smoking, duration of DM type1, family history of DM type1), measured (body mass index BMI, blood pressure) and laboratory investigations done to all patients (G.U.E, s. creatinine, lipid profile, HBA1C, calculated estimated Glomerular Filtration Rate (eGFR) and Spot Urine Albumin-Creatinine Ratio (ACR).

Results: Out of 110 patients, 62 male and 48 female, the mean age was (22±12). The patients with microalbuminuria (ACR ≥ 30 mg/g) show statistically significant with increase HBA1C, duration of DM type 1, total cholesterol (T.C), low density lipoprotein (LDL), triglycerides (TG) and family history of DM type 1, while there were not statistically significant with age, gender, smoking, BMI, eGFR, high density lipoprotein (HDL) and hypertension. Patients with eGFR<90mL/min/1.73m^2 show statistically significant with increase HBA1C, duration of DM type1, LDL, TG, T.C, while significantly decrease in HDL and there were not statistically significant with age, gender, smoking, family history of DM type 1, BMI and hypertension.

Conclusions: The degree of glycemic control, duration of type1 (DM) and dyslipidemia were associated with increased microalbuminuria and reduced eGFR (nephropathy). Family history of DM type1 was risk factor for microalbuminuria.

KEY WORDS: diabetes mellitus type 1, diabetic nephropathy, microalbuminuria, ACR, eGFR

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INTRODUCTION

Diabetic nephropathy is a clinical evidence of kidney injury in response to chronic, long-standing hyperglycemia. Diabetic nephropathy manifests as moderately increased albuminuria (microalbuminuria) [1]. With time, progressive proteinuria is followed by a decline in glomerular filtration rate, with progression to end-stage kidney disease (ESKD) [2]. Kidney disease due to diabetes is combine by extrarenal microvascular complications of diabetes, including retinopathy and peripheral neuropathy as well as macrovascular complications, such as peripheral vascular disease, coronary artery disease, and stroke [3, 4]. Diabetic nephropathy (DN) is one of the most important complications of diabetes mellitus. It is the leading cause of end-stage renal disease (ESRD) in the worldwide. There is evidence that the early diagnosis and treatment of DM has a significant in delaying it is complications [5, 6]. Diabetic nephropathy is important markers of increased cardiovascular (CV) morbidity and mortality in type 1 diabetes mellitus. They need aggressive intervention to improve all CV risk factors (e.g., LDL cholesterol, BP, smoking cessation, exercise, etc.) [7]. The earliest detection of diabetic renal disease is the appearance of microalbuminuria, low amounts of albuminuria (≥30mg/g), DN ('overt' nephropathy) describes a clinical syndrome characterized by: persistent albuminuria (>300 mg/day) on at least two occasions, 3 months apart, and progressive decreased estimated glomerular filtration rate (eGFR) below 90mL/min/1.73m^2 [8] as show in figure 1 [9].

Mortality risk increases at every stage: annual mortality risks were 1.4% (without microalbuminuria); 3.0% (with microalbuminuria); 4.6% (macroalbuminuria); and 19.2% (CKD and ESRD) [10, 11], thus, early monitoring and treatment effect natural history, preventing incipient and progression to overt nephropathy. Annual screening by urine albumin creatinine ratio (ACR) should be performed (preferably an early morning

sample) [12, 13]. The random (spot) urine protein-creatinine ratio and albumin-creatinine ratio correlate with 24-hour urine collections and are sufficiently accurate for screening and monitoring proteinuria [4, 15]. High urine albumin levels indicate glomerular injury, while, absence of albuminuria excludes most glomerular diseases [16, 17]. Creatinine-based formulas are used to estimate GFR by show for factors that affect serum creatinine and creatinine clearance. These formulas take into account the effects of age, race, sex, and muscle mass (estimated by weight) on serum creatinine levels. The Chronic Kidney Disease Epidemiology Collaboration creatinine equation is the most commonly used method for estimating GFR and is the most accurate equation for most persons, particularly when GFR > 60mL/min/1.73m^2. Serum creatinine changes nonlinearly with glomerular filtration rate (GFR), and significant decrement in kidney function at higher GFR may cause only small changes in serum creatinine [18, 19]. In type 1 diabetes, the course of microalbuminuria is unpredictable and timing of glomerular filtration rate (GFR) loss is uncertain. Thus, there is a need to identify the risk factors associated with the development of more advanced stages of kidney disease through systematic analysis [20, 21].

However, two elements to concept have arisen:

- Microalbuminuria has been proven to be a dynamic process that is more likely to remit to normal albumin excretion (termed normoalbuminuric") than to progressive.
- A subset of individuals may experience decline in GFR prior to or during microalbuminuria regardless of the subsequent ends to progression or remission of microalbuminuria [22-23].

The earliest lesions consist of thickened glomerular basement membranes (GBM), mild mesangial expansion and arteriolar accumulation of hyaline. Mesangiolysis and exuberant mesangial repair then develop, ultimately resulting in marked increase in mesangial matrix. Established nephropathy is characterized by mesangial expansion, which may be nodular, so-called Kimmelstiel-Wilson nodules, hyaline in both afferent and efferent arterioles, and markedly thickened GBM by electron microscopy. Glomerulosclerosis is the most common renal complication of DM. Pathophysiology is poorly understood, however: changes to the haemodynamics of the glomerulus is thought to be key, which leads to an increased glomerular capillary pressure.

MECHANISM OF GLOMERULAR DAMAGE

Uncontrolled diabetes removes the negative charge from the filtration slits of the glomerular basement membrane. Normally, negative charges repel the filtration of albumin, which is also negatively charged. Loss of negative charges make albumin to pass through the glomerulus. ACE inhibitors decrease intraglomerular hypertension by dilating the efferent arteriole, this protects the glomerulus from the damage caused by intraglomerular hypertension [27].

THE AIM

To determine the risk factors of microalbuminuria and estimated glomerular filtration rate in patients with type1 diabetes mellitus.

MATERIALS AND METHODS

This is cross sectional study at diabetic and endocrinology center in Al-Najaf during the period from September 2021 to March 2022. The study was done on 110 patients of diabetes mellitus (DM) type 1 with mean age (22±12).

Inclusion criteria:

All patients with diagnosed of diabetes mellitus type 1 who not have exclusive criteria.

Exclusive criteria:

- Dehydration
- Vigorous exercise
- Emotional stress
- Acute illness
- Fever
- Orthostatic proteinuria
- Who are currently taking an ACE inhibitor or an angiotensin receptor blocker
- Urinary tract infections
- Pregnancy
- CKD
- Menstruation
- DKA

DATA COLLECTION

Data collection using three sections:

First section – for socio-demographic characteristics (age, gender, smoking, family history of DM);

Second section – for measurement parameters (weight, height, body mass index (BMI), systolic blood pressure (sys BP), diastolic blood pressure (DIA BP), duration of DM);

Third section – for laboratory parameters (G.U.E, glucose, serum creatinine, serum triglycerides (TG), serum total cholesterol (T.C), LDL, HDL, HBA1C, calculated estimated glomerular filtration rate (eGFR), spot urine albumin-creatinine ratio (ACR).

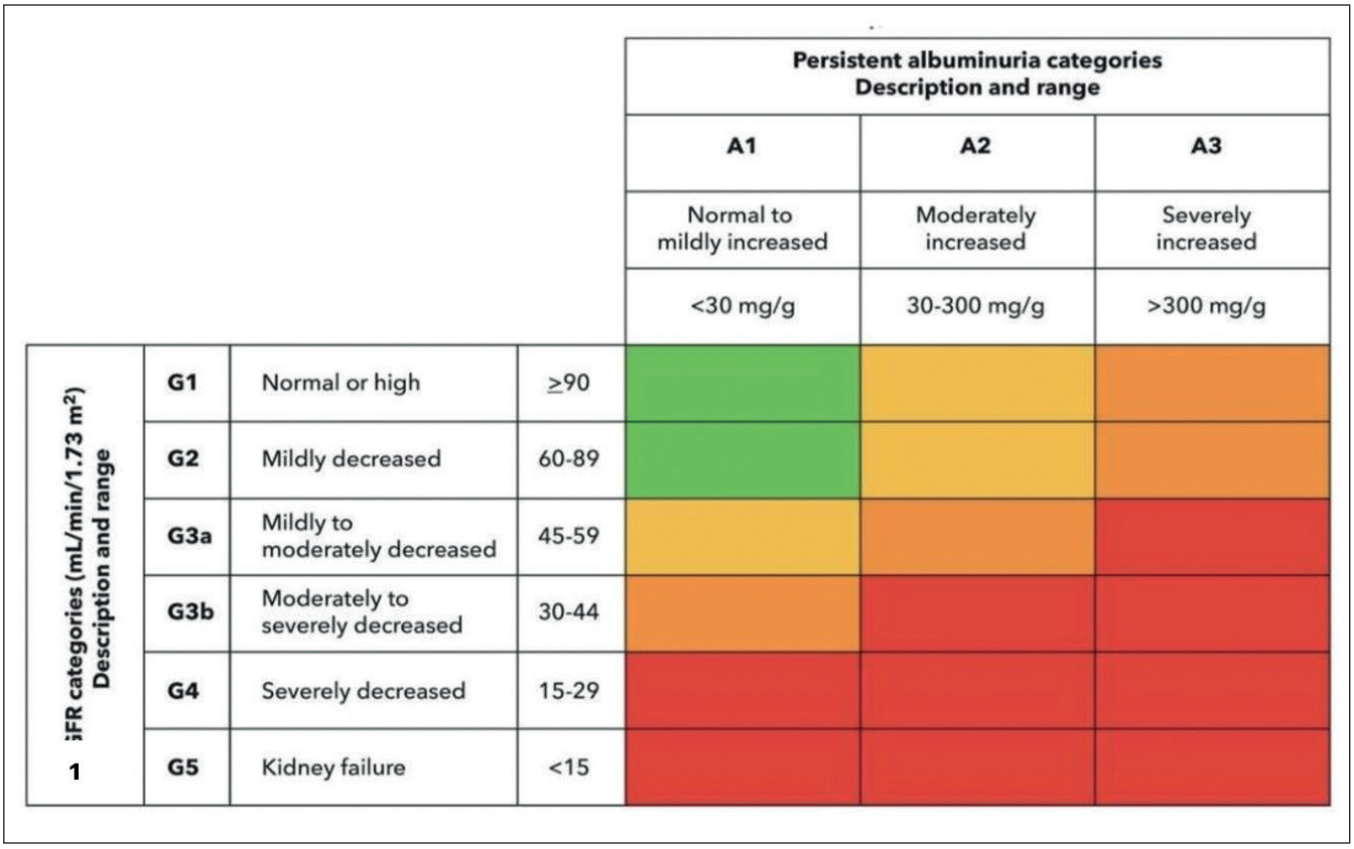

Fig. 1. The kidney disease: Improving Global Outcomes (KDIGO) chronic kidney disease staging system. Prognosis of chronic kidney disease by glomerular filtration rate and albuminuria category, CKD=chronic kidney disease, GFR = glomerular filtration rate. Green: low risk (if no other markers of kidney disease, no CKD); Yellow: moderately increased risk; Orange: high risk; Red: Very high risk.

PROCEDURE

HBA1C was done by autoanalyzer instrument, and the procedure was done by turbidimetric method. Others were done by spectrophotometer instrument, using endpoint enzymatic reaction in blood sugar, lipid, urea and urine albumin and kinetic reaction in creatinine.

SPOT URINE ALBUMIN-CREATININE RATIO (ACR)

Direct quantification using a random (spot) albumin-creatinine ratio is required in all patients with type 1 diabetes [9]:

- ACR: Normal <30 mg/g
- Moderately Increased Albuminuria (Microalbuminuria) 30-300 mg/g
- Severely Increased Albuminuria (Macroalbuminuria) >300 mg/g

ESTIMATED GLOMERULAR FILTRATION RATE (EGFR)

Estimated Glomerular Filtration Rate (eGFR) [28]:

- GI: normal>=90mL/min/1.73m^2
- Abnormal<90mL/min/1.73m^2

Variables include serum creatinine, age, race, and gender, more accurate than MDRD and CGE equations in elderly population and in those with eGFR >60 mU min/1.73 m^2. Preferred formula for calculating creatinine-based eGFR.

eGFR = 141 x min (S.cr/k,1)*
X max (S.cr/k,1)**
X 0.993***
x 1.018 [if female]x 1.159 [if black]
* = -0.329 for females, -0.411 for males; **=-1.209; ***=age
K= 0.7 for females, 0.9 for males;
min = minimum of S.cr/k,1; max = maximum of S.cr/k,1.
Procedures were done for total cholesterol, HDL, serum triglyceride by automated instrument. While LDL calculated by this equation [29]:
LDL= (T.C)- [HDL+(T. G/5)]

ETHICAL APPROVAL

The Scientific Council of the Board of Health Specialization approved the study protocol. Data of the patients in the medical files were kept confidentially and were not disclosed publicly or to unauthorized individual, patient's names replaced with codes to assure keeping the privacy of the patients.

Table I. Demographic and laboratory results according to renal Microalbuminuria ACR ≥30 mg/g.

Parameters			Total Mean (SD)	With microalbuminuria (N=16), ACR≥30 mg/g	Without microalbuminuria (N=94), ACR<30 mg/g	p-value
Sociodemographic	Age		22.4 (±12)	26	21	0.159
	Gender	Male	62	8	54	0.695
		Female	48	8	40	
	Smoking	YES	16	6	10	0.611
		NO	94	10	84	
	Family Hx	YES	34	4	30	0.046
		NO	76	12	64	
Risk factors		eGFR (mL/min/1.7m^2)	128 (±26)	107	131	0.07
DURATION (YEARS)			10.1 (±6)	16	9	0.00005
HBA1C (%)			9.39 (±2)	12.7	8.8	0.000012
BMI (mg/dL)			21.4 (±5)	22.5	21.3	0.173
TG (mg/dL)			212 (±56)	247	206	0.028
HDL (mg/dL)			39.6 (±5)	40	39	0.463
T.C (mg/dL)			232 (±29)	257	228	0.00012
LDL (mg/dL)			162.9	239	150	0.0001
SYS BP			117(±18)	126	116	0.075
DIA BP			70(±13)	73	69	0.17

Table II. Demographic and laboratory results according to Estimated Glomerular Filtration Rate (eGFR).

Parameters			TOTAL Mean (SD)	eGFR < 90 (N=10)	eGFR ≥ 90 (N=100)	p value
Sociodemographic	Age		22.4(±12)	18.2	22.8	0.281
	Gender	Male	62	4	58	0.995
		Female	48	6	42	
	Smoking	YES	16	2	14	0.579
		NO	94	8	86	
	Family hx	YES	34	6	28	0.147
		NO	76	4	72	
Risk factors		DURATION (YEARS)	10.1(±6)	8.2	10.3	0.008
HBA1C (%)		9.39 (±2)	12.36	9	0.0001	
BMI (kg/m^2)		21.4 (±5)	18	21.8	0.058	
TG (mg/dL)		212.7 (±56)	205	213.5	0.015	
HDL (mg/dL)		39.6 (±5)	38.4	39.7	0.014	
T.C (mg/dL)		232 (±29)	230	232.4	0.027	
LDL (mg/dL)		162.9	239.2	155.3	0.0005	
SYS BP		117 (±18)	112	118	0.142	
DIA BP		70 (±13)	71	69	0.081	

STATISTICAL ANALYSIS

Data was entered, managed and analyzed with using of the statistical package for social sciences (SPSS) software version 27. Pearson's correlation test was performed to examine correlations between various parameters. Independent samples t-test (2-tailed) was used to compare means of different parameters. The results were considered statistically significant when $p<0.05$. Variables and parameters presented as mean, standard deviation (SD), and percentages, accordingly.

Table III. Connection between researched parameters (HBA1C, eGFR, ACR) with different age of the patients.

Parameter	Age ≤ 20 (years), N= 58	Age >20 (years), N=52	p-value
HBA1C (%)	9.41	9.56	0.955
eGFR (mL/min/1.73m^2)	129.6	116.6	0.281
ACR (mg/g)	2.44	2.79	0.159

Table IV. Connection between HBA1C with the duration of disease.

Duration (years)	HBA1C ≤ 6.5%	HBA1C > 6.5%	p-value
0-5	4	20	0.119
6-10	6	42	0.012
11-15	0	16	0.277
> 15	0	22	0.934

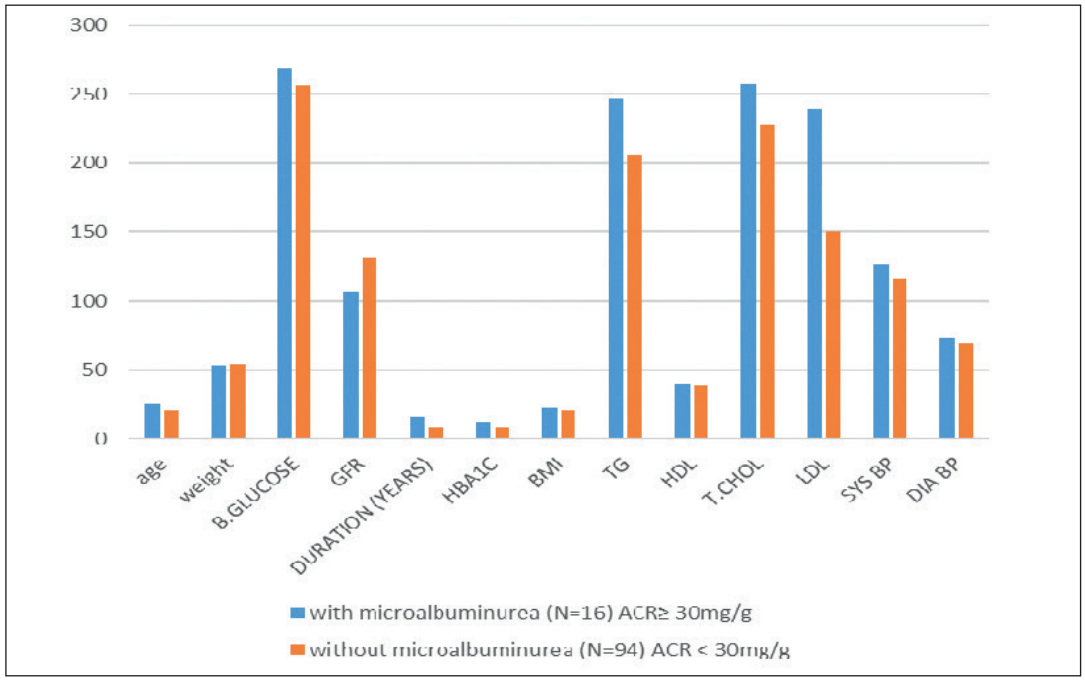

Fig. 2. Relationships between microalbuminuria and investigated parameters (age, BMI, durations, HBA1C, TG, HDL, T.C, LDL, SYS BP, DIA BP).

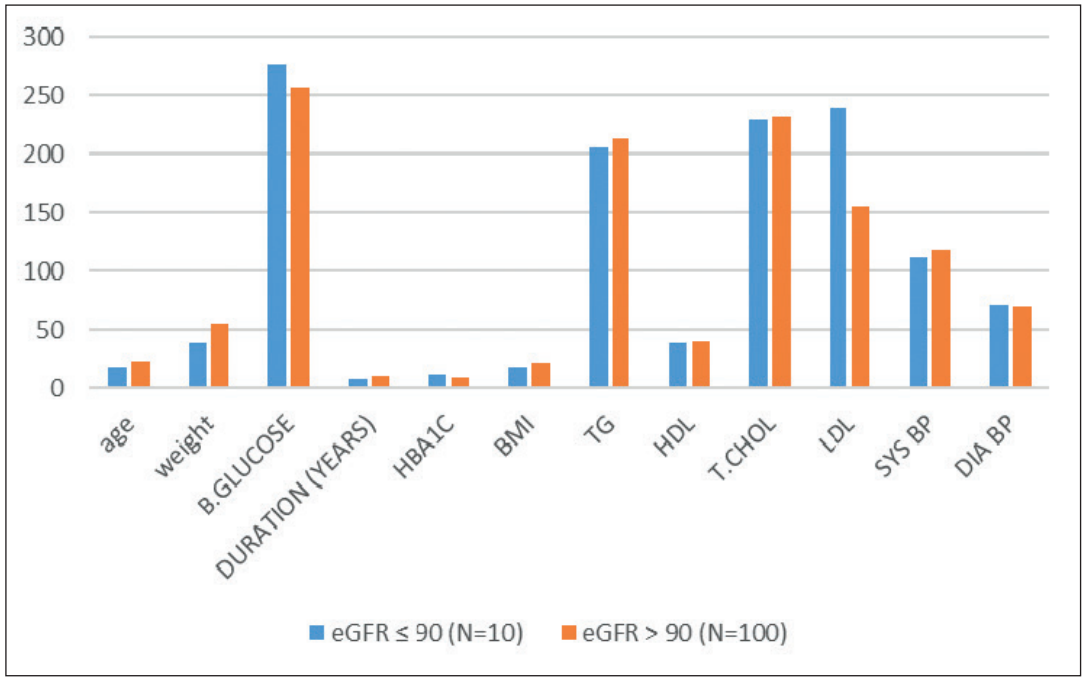

Fig. 3. Relationships between eGFR and investigated parameters (age, BMI, durations, HBA1C, TG, HDL, T.C, LDL, SYS BP, DIA BP).

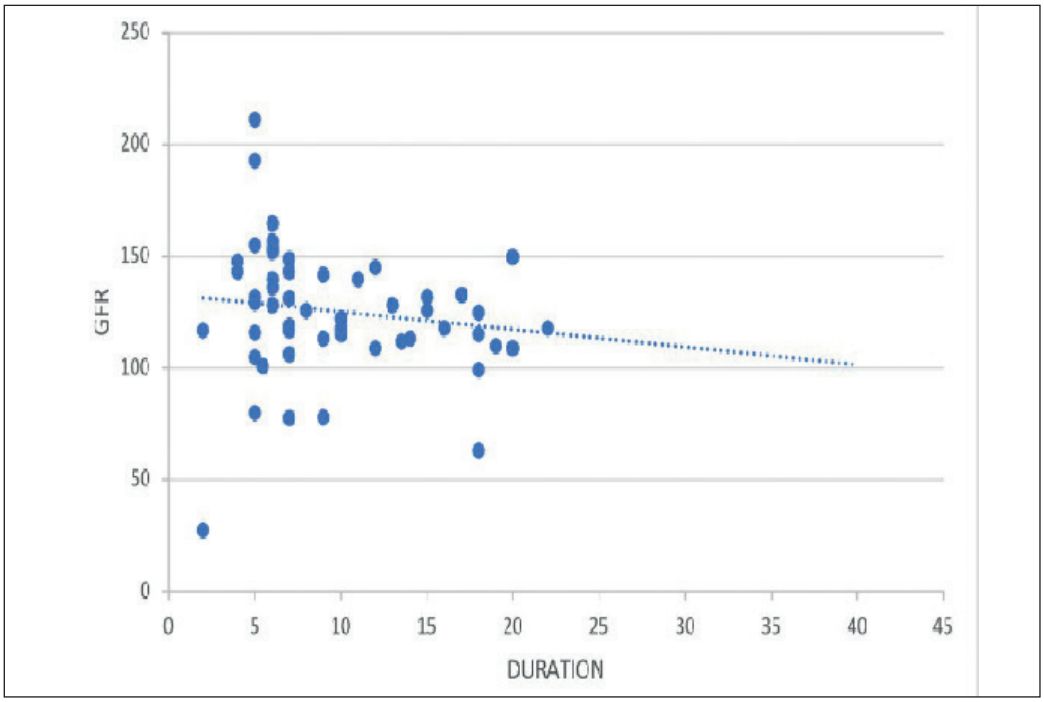

Fig. 4. Scatter-Plot showing the significant direct (positive) correlation between decrease eGFR and duration of DM.

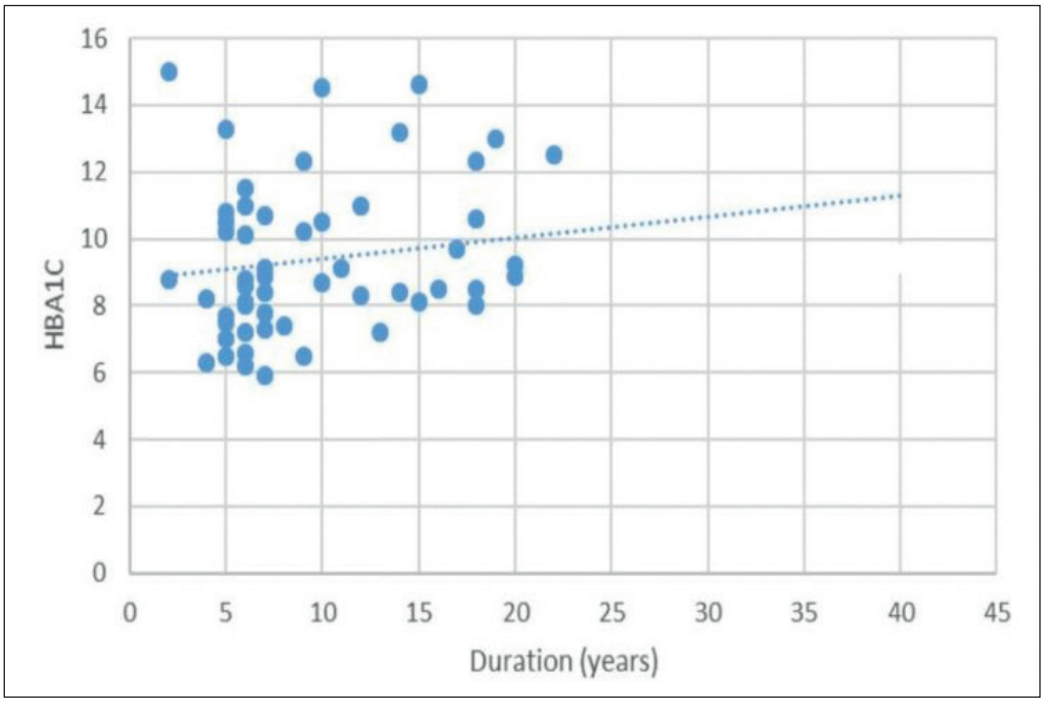

Fig. 5. Scatter-Plot showing the significant direct (positive) correlation between HBA1C and duration of DM.

Cross-tabulation used to assess the relationship between categorical variables with frequencies.

RESULTS AND DSCUSSION

A total of 110 patients with type 1 diabetic, 62 were male and 48 were female, with mean age (22±12) recruited in this study.

The patients with ACR≥30 mg/g show statistically significant with increase HBA1C, duration, total cholesterol (T.C), low density lipoprotein (LDL), triglycerides (TG) and family history, p-value - 0.05 (Table I, Fig. 2).

The patients with eGFR < 90mL/min/1.73m^2 show statistically significant connection with increase HBA1C, duration, LDL, TG, T.C., while significantly decrease in HDL, p-value - 0.05 (Table II, Fig. 3-4).

Results of calculations shows not statistically significant for different parameters (HBA1C, eGFR, ACR) regarding with different age of the patients (Table III).

Glycemic control depending on HBA1C with clear cut is 6.5% related with duration of disease. The patients with HBA1C >6.5% show statistically significant with duration from 6 to10 years, p-value = 0.05 (Table IV, Fig. 5).

Around the third of patients diagnosed with type 1 diabetes mellitus show persistent microalbuminuria within the first 20 years of diabetes, and several potentially modifiable risk factors predict the development of persistent microalbuminuria and persistent macroalbuminuria [30], so that diabetic nephropathy is common complication of diabetes mellitus type 1. DN has been usually found for several years before it is diagnosed due to be asymptomatic in early stage. The prognostic markers that collected here were all verified as risk factors for development and progression of diabetic nephropathy. Current study which show early complications of diabetic nephropathy in DM type 1 by using a method for measuring urinary albumin excretion to determine these risk factors, so that the risk of increased microalbuminuria by using ACR rather than decreased estimated Glomerular Filtration Rate(eGFR), because of early detection of renal impairment, which in agree with the study done by Elnajjar et al. [31] found, that estimated Glomerular Filtration Rate (eGFR) is negative significantly with microalbuminuria, changes in albumin excretion rate, such as the onset of microalbuminuria or its progression to macroalbuminuria, are either caused by or developed in parallel to the early renal decline. In this study, regarding the gender there is no significant relationship with increased microalbuminuria and decrease eGFR. These findings consistent with a study done by Raile et al. [32] and showed that no link between gender and microalbuminuria, while the study was done by Valdivielso et al. [33] showed that the men are at higher risk to develop renal disease in adults and in terms of advanced nephropathy. This suggested the high male sex hormone levels in diabetes worsening of renal decline [34], while the study was done by Mehrdad et al. [35] show that women with diabetes had common risk factors of diabetic nephropathy and increased prevalence of advanced renal impairment compared to men, that because of low level of estradiol in diabetic female compared with non-diabetic female, so that the estrogen may be renoprotective, and estrogen deficiency lead to increase glomerular sclerosis and increase in macroalbuminuria. The present study showed that age of the diabetic patients was not associated with increased microalbuminuria and decrease eGFR, and this was in agreement with the study done by Baek et al. [36], while the study done by Marcovecchio et al. [37] show that early age-onset type 1 DM is an independent risk of diabetic nephropathy. It is unclear why some people developed diabetic nephropathy at an early age than others. It is may be because of strong family history, poor complaining with treatment regimens and ethnically effluence have been noted with incidence of DN. While the early onset of insulin dependent diabetes is said to be associated with the later developed proteinuria, this has been suggested that progression nephropathy in childhood may be slower than in later life. Glycemic control, hypertension, hyperlipidemia and ethnicity may also influence rate of progression. Thus, the early course of diabetes and histological assessment may serve benefit from intensive effort to achieve good glycemic control, in contrast evidence suggest the clinical course individual with heavy proteinuria cannot be altered by good glycemic control, but reduction of hyperlipidemia and control of blood pressure can led to decrease decline in renal function. In present study shown, that renal impairments with microalbuminuria and decease in eGFR have significant relationships with long duration of disease, this was in agreement of study by Elnajjar et al. [31], that show it were significantly higher among the group of patients with diabetes for more than 10 years. In current study, the excess risk of renal impairment (increase in albuminuria and decrease in eGFR) associated with a higher HbA1c level. These findings was suggested that appropriate control of diabetes decrease renal impairment in DM type1 which consistent with finding done by Jenkins et al. [38], that showed the effect of poor glycemic control on the risk for microalbuminuria in youth with type 1 diabetes and highly vulnerable to vascular complications, because the role of oxidative stress in the pathogenesis of vascular complications and effect of longer-term changes in hyperglycemia on free radical production and higher subsequent long-term risk of complication. In this study, increased in dyslipidemia is associated significantly with developing diabetic kidney disease. These findings consistent by study of Raile et al. [32] which showed that both severe hypertriglyceridemia and hypercholesterolemia can accelerate renal injury in the early development of T1DM due to glomerulosclerosis and tubulointerstitial fibrosis and induce renal injury by promoting the intrarenal generation of reactive oxygen species, glomerular infiltration of monocytes and macrophages and podocyte damage. Chaturvedi et al [39] showed that atherogenic lipoprotein profiles are associated with renal dysfunction in type 1 DM. Our study showed that both systolic and diastolic blood pressure were not statistically significant for diabetic nephropathy, because of early detection of DN by microalbuminuria and patients using antihypertensive drugs were excluded from this study. These findings consistent with a study, that is done by Anderson et al. [40] and which showed that systemic blood pressure is not raised prior to the onset of microalbuminuria, while the study of Hovind et al. [41] was consistent with present study, but this fact

might reflect the strict antihypertensive treatment regimens in patients with advanced renal disease were done. But whether or not hypertension is the cause or result of nephropathy, should strictly be treated in pediatric and adolescent patients. While the study done by Raile et al. [32] show that both systolic and diastolic blood pressure to be significantly associated with microalbuminuria. Smoking was statistically not significant with increased level of microalbuminuria and decrease eGFR in this study, which is in agreement with that study was done by Valdivielso et al. [33]. Long-term studies on the effect of smoking on decline in kidney function in DM are lacking, but programs to keep children and adolescents away from starting smoking should be an important factor of continuous diabetes care. While the study is done by Biesenbach et al. [43] show that current smoking is a risk factor for the progression of diabetic nephropathy and the risk increases with the increasing number of cigarettes smoking. Other study that was done by Harjutsalo et al. [44] showed, that smoking accelerates diabetic kidney disease in type 1 diabetes, evidence of increased mortality in smokers as well as increase in cardiovascular mortality in patients with diabetic nephropathy because of toxicity of smoking material on endothelial vessels of glomerular. In this study, the family's history of DM type 1 increased the likelihood of renal impairment by increasing in microalbuminuria, which is statistically significant consistent with finding done by Hunt et al. [45] and show that sibling already diagnosed with DN are markers of an increased susceptibility for DN in siblings of type 1 diabetic patients. Its presence in one sibling increases the risk twofold for the other diabetic siblings, the causes of familial clustering of DN still remain unclear, but genetic factors seem to be strongly involved and diagnosed with diabetes during puberty. This study showed that family history of DM type 1 was not statistically significant for decreased eGFR, and these findings consistent by study of Dasmahapatra et al. [46] which showed that linkage of creatinine clearance to chromosome 10 in families ascertained for cardiovascular disease risk, with relatively normal eGFR due to differences in ethnic populations. In this study, increase BMI were not associated with increase in renal impairment in tpye1 DM, while other study is done by Elnajjar et al. [31] show, that prevalence of nephropathy was clearly significant related to BMI, and the risk of DN was 4.49 times higher in the obese group, than in the non obese one, and also another study done by Dasmahapatra et al. [46] showed that microalbuminuria correlated with BMI. While in current study show no relationships due to selected samples in short time, relatively small number of patients and many patients of type 1 diabetes mellitus are underweight.

CONCLUSIONS

As a result of our research, it was established that the degree of glycemic control, duration of type1 DM and dyslipidemia were associated with increased microalbuminuria and reduced eGFR (nephropathy). Family history of DM type 1 was risk factor for microalbuminuria.

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ORIGINAL ARTICLE

EFFICACY OF ARTHROCENTESIS FOR TREATMENT OF INTERNAL POST-TRAUMATIC TEMPOROMANDIBULAR JOINT DISORDERS

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ABSTRACT

The aim: To study the consequences of temporomandibular joint injury and efficacy of arthrocentesis for treatment of post-traumatic internal temporomandibular disorders.

Materials and methods: 24 patients who experienced trauma history in the head without jaw fractures underwent CT, ultrasound and/or MRI. TMJ arthrocentesis was performed according to a modified method of D. Nitzan (1991) under local anesthesia by means of blockade of the peripheral branch of the auricular-temporal nerve on the background of intravenous sedation.

Results: The ages of the patients varied between 18 and 44 years, and mean was 32,58 years. The causes of trauma were diverse, as traffic accident – 3 (12,5%), assault 12 (50%), hit by materials 3 (12, 5%), and fall-down 6 (25%). According to clinical and radiological signs after traumatic temporomandibular disorders, patients were divided into two groups according to Wilkes classification (1989): 13 patients with stage II (early-middle) and 11 - with stage III (middle).The control ultrasound and MRI carried out 3-6 months after arthrocentesis showed no signs of hemarthrosis in 84.61% of patients with intra-articular disorders of the second degree, and in 72.72% of patients with internal disorders of the third degree, the position and function of the articular disc was restored.

Conclusions: Arthrocentesis with TMJ lavage is a minimally invasive surgical manipulation that has proven itself in temporomandibular disorders of traumatic origin, in particular after fractures of the articular process of the mandible.

KEY WORDS: arthrocentesis, TMJ disorders, traumatic injuries

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INTRODUCTION

Trauma in the area of temporomandibular joint (TMJ) can cause injuries to both soft tissue and the bone, leading to its progressive disorder. For those patients who do not have condylar fractures, soft tissue injury may do more damage to the TMJ than a fracture would have done [1]. The traumatic origin of TMJ disorder, according to foreign authors, was found in 42-62.5% of the examined patients [2-4]. The etiopathogenesis of traumatic lesions of the temporomandibular joint is based on contusions, fractures of the mandible and dislocations. Recent studies based on MRI and arthroscopy have shown that trauma can cause intracapsular damage, including hyperaemia of the capsule, its deformation or rupture, haemarthrosis, synovial ecchymosis, shredding of the disc and articular susfaces, displacement of the disc, violation of the integrity of the articular disc, sprain or rupture of ligaments [6-9].

The incidence of injury to the soft tissues of the TMJ after facial trauma is unknown, there are many reports about results of condylar fracture, but not many about soft tissue injuries [1, 10].

The authors believe that intra-articular changes are more often caused by the internal articular localization of the fracture, but with extra-articular fractures of the mandible can also cause damage to the structural elements of the TMJ [11]. A significant part of internal articular disorders occurs as a result of macro- and microtrauma of the temporomandibular joint.

A common sign of internal TMJ disorders is synovia and is characterized by proliferation of synoviocytes and tissue hypertrophy. Synoviocytes release inflammatory mediators and matrix-degrading enzymes into the joint cavity. At the same time, their activation is secondary in relation to inflammatory mediators and cartilage matrix molecules, after which the mechanism of progressive joint degeneration is triggered in the synovial tissue according to the feedback principle [5, 12].

Being a closed space, inlury to the TMJ can result in bleeding into the superior and/or inferior joint space (hemarthrosis). The development of traumatic disorders is chracterised by the reorganization or ossification of the TMJ hematoma, which causes impaired mobility, adhesion of the articular disc and other components, causing problems with mouth opening, chewing, and the like. Hemarthrosis has been suspected to cause scarring in the join and increases the risk for ankyloses [2]. Studies of the synovial fluid have shown an increase in the concentration of pro-inflammatory cytokines (IL-6, IL-8, IL-11), which results in an increase in adhesive forces and the formation of negative pressure in the upper space of the joint, leadng to the «adhesion» of the disc to the joint fossa [13].

It should be noted that the use of conservative therapy after traumatic injuries of the TMJ (medication, physiotherapy, occlusive splints, selective teeth grinding, electroneurostimulation of the masticatory muscles, diet, psychological support, etc.), is often ineffective, even in combination [14].

Therefore, the next stage in the complex treatment of post-traumatic temporomandibular disorders is the use of minimally invasive surgical interventions, in particular, arthrocentesis.

Arthrocentesis is a puncture and lavage of the temporomandibular joint, which consists in aspiration followed by the introduction of fluid into the joint cavity under pressure, which promotes the removal of metabolic products, hematoma remnants, destruction of adhesions, resulting in increased joint space volume and disc mobilization, with the movement of the lower jaw being restored. In the process of improving the method of arthrocentesis, the classical technique of which describes the introduction of two needles into the upper space of the temporomandibular joint, a method of hydraulic pressure to perform lavage was introduced [15].

THE AIM

The purpose of our work was to study the consequences of the TMJ injury and efficacy of arthrocentesis in the treatment of post-traumatic internal temporomandibular disorders.

MATERIALS AND METHODS

The clinical part of the study included 24 patients (19 men and 5 women), aged 18 to 44 years old, experienced trauma history in the head without jaw fractures. All patients came to the medical dental center of Lviv National Medical University named after Danylo Halytsky in the period of 3-12 months after trauma (traffic accident, assault, hit by materials, fall down). Prior to applying to the Center, they received treatment for TMJ at the place of residence: nonsteroidal anti-inflammatory drugs, analgesics, physiotherapy methods, which did not give the desired positive effect.

Patients underwent radiological examination - orthopantomography, CT, ultrasound and MRI. Pain assessment was performed according to VAS. The examination revealed that a significant number of patients have difficulty in assessing the intensity of pain in points, so we have somewhat simplified the visual analog scale of pain as follows: no pain - 0 points, mild pain - 1-3 points, moderate pain - 4-6 points, severe pain - 7-9 points, excruciating pain - 10 points.

TMJ arthrocentesis was performed according to a modified method of D. Nitzan (1991) under local anesthesia by means of blockade of the peripheral branch of the auricular-temporal nerve on the background of intravenous sedation. First, a tragoorbital line is drawn on the face (Holmund line), then the patient is asked to open his mouth to determine the contour of the articular fossa and articular tubercle and mark these on the skin. The first point for a needle insertion is 10-12 mm in front of the earlobe and 2 mm below the trago-orbital line. The second needle, through which the fluid enters the joint, is inserted 20 mm from the middle of the tragus and 5 mm down from the tragoorbital line [16]. The upper space was insufflated with 200 ml of Ringer's solution. A 20-cm^3 syringe with a 1,8-mm needle was used. The duration of arthrocentesis was 20-30 minutes. Patients were prescribed anti-inflammatory and analgesic therapy, recommended to follow a gentle regimen and diet. Repeated examinations were performed the day after arthrocentesis, for 7 days, after 1, 3, 6 months with assessment of the following parameters: maximal mouth opening (MMO), arthralgia (assessment of pain intensity according to VAS). Difference in treatment outcomes before and after treatment was assessed using the Wilcoxon signed rank test. Changes in MMO and pain scales were compared by Mann-Whitney test. All reported *P*-values were based on two-side tests, and statistical significance was maeked as $P<0.01$, $P<0.05$, $P<0.001$, or not significant.

RESULTS

The ages of the patients varied between 18 and 44 years, and mean was 32,58 years. The causes of trauma were diverse, as traffic accident – 3 (12,5%), assault 12 (50%), hit by materials 3 (12, 5%), and fall-down 6 (25%). On admission, patients complained of TMJ pain, which was rated 1 to 6 according to VAS. Also, all patients noted pain on palpation

Table I. Symptoms of temporomandibular disorders found in patients 3-12 months after facial trauma

Symptoms of temporomandibular disorders	Classification by Wilkes n=24	
	Stage II n=13	Stage III n=11
Clinical symptoms		
Pain associated with mandibular movement: 1-3 points	13	-
Pain associated with mandibular movement: 4-6 points	-	11
Pain not associated with mandibular movement: 1-3 points	-	11
Articulation noises (clicking)	13	11
Limited mouth opening	-	11
Periodic blocking of movements of the articular head	11	-
Deviation of the mandible	7	11
Pain on palpation	13	11
Ultrasound and MRI symptoms		
Deformation of the capsule	5	11
Disk deformation	6	11
Disc adhesion	7	11
Disc protrusion with reduction	13	-
Disc protrusion without reduction	-	11

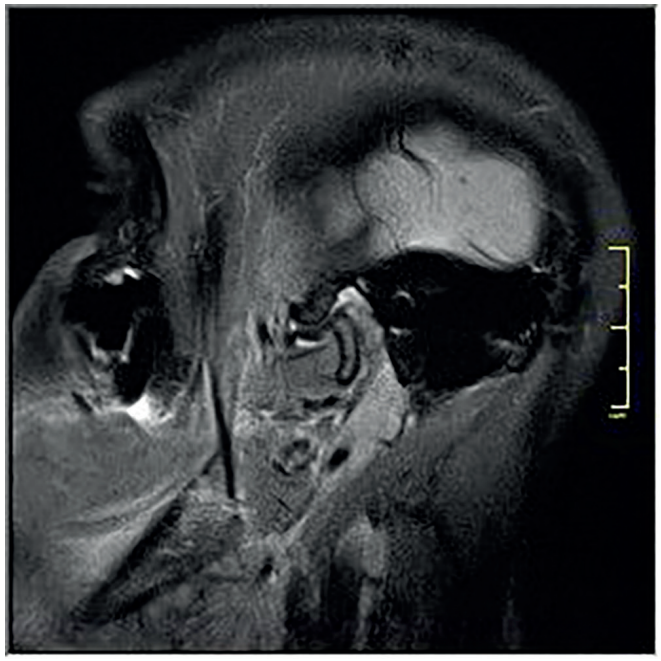

Fig. 1. MRI signs of heamatoma, deformity and penetration of the capsule

of the TMJ. Limited mouth opening ranging from 30 to 38 mm was found in 11 (45,83 %) patients. Lower jaw deviation was observed in 18 (75%) patients. All patients had articulatory noises - clicking, and 11 (45,83 %) had blocked movement of the joint head. Orthopantomograms or CT revealed no signs on mandibular fracteres. Ultrasound and MRI revealed signs of unabsorbed hematoma (consequences of hemarthrosis) (Fig. 1); in 18 patients - deformity

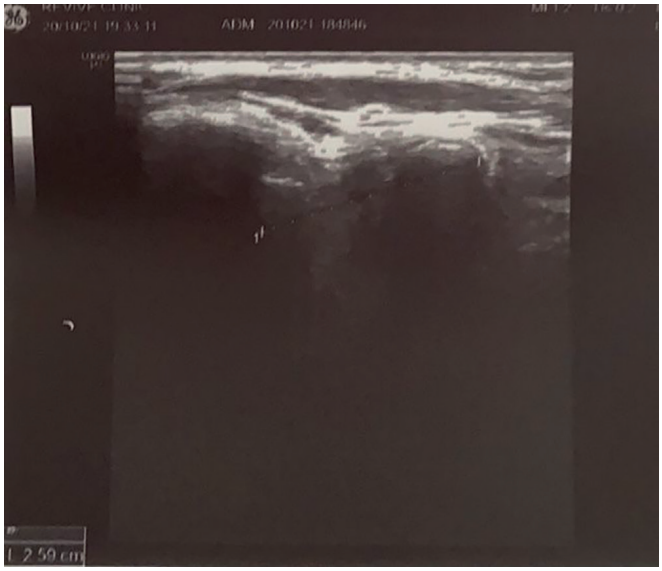

Fig. 2. Ultrasound features of disc adhesion

of the capsule, in 17 - a slight thickening of the posterior edge of the articular disc, in 18 patients - disc adhesion (Fig. 2), in 13 people – forward disc displacement with reduction (Fig. 3), in 11 patients - disc protrusion without reduction (Fig .4) (Table I).

According to clinical and radiological signs after traumatic temporomandibular disorders, patients were divided into two groups according to Wilkes classification (1989): 13 patients with stage II (early-middle) and 11 - with stage III (middle).

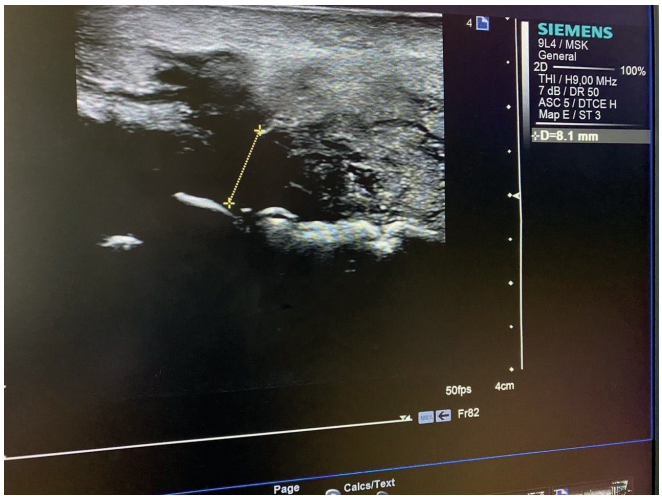

Fig. 3. MRI picture of disc protrusion

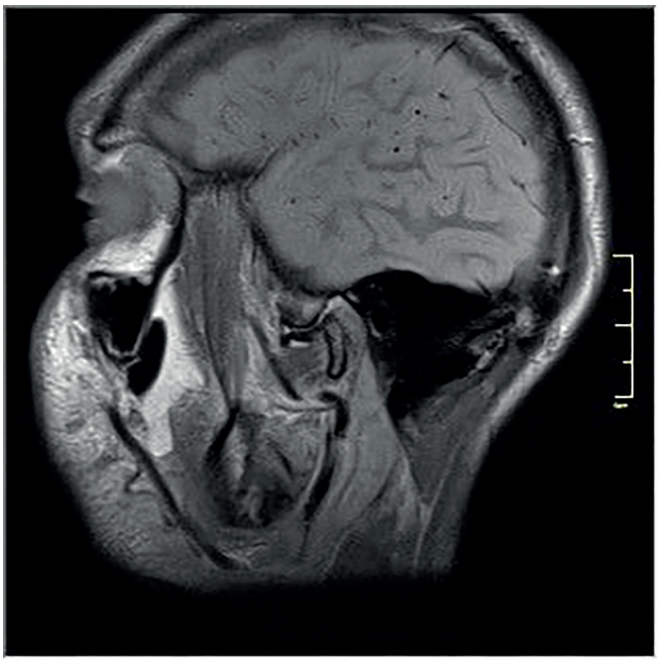

Fig. 4. Ultrasound features of disc adhesion and protrusion without reduction

After arthrocentesis, the condition of the TMJ improved on 2-3 day. In patients with TMJ block, jaw movements resumed immediately after lavage. The opening volume of the mouth on day 3 increased to 4 cm, whereas on day 7 restored fully. Improvement of maximal mouth opening was statistically significant (30-44 mm on average, P<0,001). In the early postoperative period there was dramatic reduction of pain scale - the pain was within 1-2 points, while after 1 month – 0 (6.0-0.0 of 10, P<0,001). The control ultrasound and MRI carried out 3-6 months after arthrocentesis showed no signs of hemarthrosis in 11 (84.61%) patients with intra-articular disorders of the second degree, and in 8 (72.72%) patients with internal disorders of the third degree, the position and function of the articular disc was restored.

DISCUSSION

Because disorders of the TMJ are slow to develop, and are concealed after trauma to the mandible, they are often neglected by doctors. The diagnosis of soft tissue injuries to the TMJ is important for treatment. MRI and/or US are essential for diagnosis because they can show both soft tissue and bony injury at the same time [2]. All our patients had intact surfaces of the condylar bone from CT or panoramic film immideately after injury. If MRI had been taken at the same time of injury, the damage to the soft tissues may well have been detected and so the patients could have been reviewed more closely [2]. All our patients had displaced TMJ disc. Although the position of the discs was not known immediately after injury, and it may have concributed to further damage of the surface. It has been well recognized that the TMJ disc has an important role as a barrier to prevent ankyloses. Howewer, damaged or displased disc may contribute to the development of degenerative disease or ankyloses. Traumatic TMJ injuries often lead to development of internal derangement, osteoarthrosis, and possible fibrose ankyloses, and these possibilities should be considered when these patients are followed up in clinic. Displacement of the disc of the TMJ and damage to the condylar cartilage are the possible causes of these complications. Joint tissue injury can lead to increase of both matrix molecules and complement components released into the synovial fluid. Both csn activate secretion of pro-inflammatory cetokines and chemokines production from synovial membrane cells, which can promote cellular infiltration. This molecular and cellular inflammation can potentiate cartilage erosion via production of enzymatic mediators of matrix degradation. This is a substantional body of evidence from multiply sourses demonstrating a relationship between synovitis and symptoms such as pain, swelling, disfunction in the TMJ [17, 18]. To eliminate inflamed synovial fluin and the remains of haemarthrosis, arthrocentesis and lavage were chosen as the primary treatment option in this study. Arthrocentesis helps to remove blood clots, inflammatory cells, crystalline compounds and tissue breakdown products. Upper joint lavage reduces pain by leaching inflammatory substances (catabolism products, inflammatory mediators - cytokines, arachidonic acid) and tissue detritus products, increases the volume of mandibular movements, reduces the effects of intra-articular adhesions, eliminates negative pressure and triggers the movement of the disc in the correct position by blocking signs [19].

Given the lack of positive dynamics after conservative treatment and the presence of a pathological process in the TMJ, confirmed clinically and radiologically, arthrocentesis with the TMJ lavage confirmed its effectiveness

in traumatic injuries by 81.81-92.31%, which correlates with literature sources [2, 14, 18]. The main advantages of arthrocentesis are the ability to perform manipulations in an outpatient setting, under local potentiated anesthesia, no need for complex expensive equipment (arthroscope), the ease of execution, low invasiveness, and low percentage of postoperative complications [20].

CONCLUSIONS

Arthrocentesis with TMJ lavage is a minimally invasive surgical manipulation that has proven itself in temporomandibular disorders of traumatic origin. Arthrocentesis helps to remove blood clots, inflammatory cells, crystalline compounds and tissue breakdown products. Upper joint lavage reduces pain by leaching inflammatory substances. The opening volume of the mouth on on day 7 restored fully. Arthrocentesis is recommended to be used after ineffective conservative treatment, as well as to prevent post-traumatic intra-articular disorders in the early post-treatment fractures (intermaxillary fixation or osteosynthesis) with the attenuation of acute post-traumatic events, which is our goal of further work.

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ORIGINAL ARTICLE

FEATURES OF THE CLINICAL COURSE OF OSTEOARTHRITIS IN COMBINATION WITH DIABETES MELLITUS

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ABSTRACT

The aim: To examine the features of the clinical course of osteoarthritis in combination with type 2 diabetes on the background of obesity and hypertension.
Materials and methods: 116 patients who were in the inpatient stage of treatment in the rheumatology department of the Chernivtsi Regional Clinical Hospital during 2015-2017 were examined. The epidemiological and clinical features of osteoarthritis in patients with type 2 diabetes mellitus were also analyzed.
Results: It was found that the course of osteoarthritis is extremely severe with limited range of motion in the joints, their deformation and significant deterioration of functional capacity, duration of pain, periodic prolonged exacerbations, the predominance of knee and hip injuries (64.8%) and 14.8 persons - small joints. This showed the progression and generalization of processes in various joints, aggravation of the course and prognosis of osteoarthritis, especially in women. Their prevalence was registered at II radiological stage (59.27% and 74.0%, respectively).
Conclusions: The authors emphasize that such a clinical course indicates the worst prognosis. This multimorbidity of diseases requires treatment, observation and consultation with a traumatologist, rheumatologist and endocrinologist, due to the multisystem approach to the treatment and rehabilitation of such patients with an emphasis on individual clinical features (including gender) and the course of comorbidities or syndromes.

KEY WORDS: metabolic syndrome, osteoarthritis, type 2 diabetes mellitus, multimorbidity, gender features

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INTRODUCTION

Osteoarthritis (OA) is a serious medical and social problem due to the high risk of developing limitations of the musculoskeletal system, which lead to impaired performance, reduced quality of life of patients [1,2]. About half of patients with OA have an additional five or more comorbidities. Some significant factors in the development and progression of OA are diabetes and obesity, which are a cluster of four cardiometabolic risk factors, obesity, along with aging and injury. Chronic low-grade inflammation plays a role in the development and progression of both OA and type 2 diabetes, which makes it possible to consider it a common feature of both diseases [3]. The pathogenesis of OA is associated with a combination of genetic, epigenetic, biomechanical, and metabolic factors that lead to the development of persistent inflammation of all joint structures, involvement in the pathological process of immune system cells, adipose tissue, their mediators, and the formation of various clinical variants [4].

Thus, the study of clinical and pathogenetic features of the combined course of OA and diabetes mellitus 2 is relevant because it contributes to a personalized approach to the tactics of treatment and rehabilitation of such patients.

THE AIM

The aim of the study was to investigate the features of the clinical course of osteoarthritis (OA) in combination with type 2 diabetes mellitus on the background of obesity and hypertension (AH).

MATERIALS AND METHODS

116 patients, who were in the rheumatology department of the Chernivtsi Regional Clinical Hospital during

2015-2017, were examined. In carrying out our work, we were guided by generally accepted world and domestic legal directives: the basic principles of the Helsinki Declaration on Biometric Research (1974), the basic standards of GCP, as well as the "Ethical Principles of Medical Research with the Involvement of People". The Assembly of the World Medical Association (2000), Orders of the Ministry of Health of Ukraine № 281 from 01.11.2000, № 66 from 13.02.2006 and № 690 from 23.09.2009. The design of the study and the informed patient consent form were approved by the Commission on Biomedical Ethics of the Bukovynian State Medical University.

Inclusion criteria were the presence of OA, type 2 diabetes, obesity, hypertension (AH), obtaining informed consent to participate in the study.

The exclusion criteria were patients who had suffered an acute myocardial infarction or had recently been hospitalized for heart failure; have treatment-resistant hypertension with CAT ≥200 mm Hg or DBP ≥100 mm Hg); persons with signs of pulmonary heart decompensation, cancer; patients with tuberculosis, bronchiectasis; persons who have undergone surgery during the last 4 weeks; refusal of the patient to participate in the study.

The average age of patients was 58.60 ± 8.25 years, the duration of the disease - from 5 to 23 years (15.52 ± 6.25 years). Among the surveyed sick women there were 91 (78.45%) and 25 (21.55%) men, the ratio of "women: men" was 3.6: 1, which did not differ from the average population. When dividing patients into age groups, qualitative age periods recommended by the World Health Organization (WHO) Committee of Experts were adopted: young age (25-44 years), middle age (44-60 years), elderly age (60-75 years), senile (75-90 years) [5]. Among the surveyed middle-aged women there were 54 females and 18 males. In the group of elderly people there were 5 men and 28 women. The rest of the subjects were elderly (5). The age and sex of the comparison group did not differ significantly.

Taking into account the complaints, anamnesis, objective status, data of general clinical and instrumental methods of examination, the following clinical groups of dynamic observation were identified:

Group I - 37 patients with OA;

Group II - 21 patients with OA in combination with hypertension;

Group III - 41 patients with OA with concomitant hypertension and obesity;

Group IV - 17 patients with OA in combination with abdominal obesity and type 2 diabetes with hypertension and obesity;

Group V - 25 practically healthy persons (PHP). By age and sex patients were comparable with patients of other groups.

The diagnosis of OA was established on the basis of complaints, anamnesis, results of clinical-laboratory and instrumental researches according to the criteria specified in the Order of the Ministry of Health of Ukraine from 12.10.2006 № 676 and the American College of Rheumatology (ACR, 2012).

The intensity of the pain syndrome was expressed by the pain index. The intensity of crunch in the joints was described by the tribal system. Joint deformity was assessed by three types of changes: limitation of range of motion; axial deformation; violation of contact of articular surfaces.

The Lequesne Algo functional Index was used to assess the pain syndrome and the general condition of the patient. According to the questionnaire, 3 indicators were determined: pain or discomfort, the maximum distance when walking without pain and functional activity. The maximum distance when walking without pain was estimated in points. Functional activity was determined in points according to patients' answers to questions.

We also evaluated the WOMAC index (Western Ontario and McMaster University). The assessment was performed on a visual scale in millimeters. The indicator "0" was regarded as the absence of pain, stiffness or difficulty, at 100 mm the intensity of pain, stiffness or difficulty was considered maximum.

These clinical and functional tests were determined on admission to the hospital. All patients underwent X-ray examination of the affected joints to determine the radiological stage of OA.

Body mass index (BMI) was calculated by the ratio of body weight (kg) / height (m2), also determined the waist-to-hip ratio. According to the Kettle index, the diagnosis of "obesity" was established at a BMI> 30 kg / m2. It was believed that abdominal obesity in men showed waist circumference > 94 cm, and in women> 80 cm Insulin resistance was assessed using the method of homeostatic model - HOMA (homeostasismodelassessment). The HOMA index was determined by the formula: IR = concentration of GL (mmol / ml x Ins (μOD / l / 22.5) (GL - fasting glucose index (mmol / ml; Ins - fasting insulin index (μOd / l), 22.5 - coefficient by which the indicators in the numerator are divided). The norm was considered to be the index 2.27-2.77.

The diagnosis of type 2 diabetes was made by an endocrinologist with appropriate research in an endocrinology clinic.

Statistical processing was performed using MS® Excel TM 2010, Primer of Biostatistics® 6.05 and Statistica ™ 7.0 (Statsoft® Inc). A computer register (database) of the obtained indicators was created in the Microsoft Excel system. The normality of the distribution of parameters in the samples was determined by the Kolmogorov-Smirn-

Table I. Frequency of arthralgia symptoms in patients with OA in combination with type 2 diabetes mellitus, hypertension and obesity

Arthralgia symptoms	All patients n = 116		I group OA, n=37		II group, OA+ hypertension, n=21		III group OA+ hypertension+ abdominal obesity, n=41		IV group OA+ hypertension+ abdominal obesity + diabetes, n=17	
	Quantity	%	Quantity	%	Quantity	%	Quantity	%	Quantity	%
Pain without load	19	16,38	4	10,81	4	19,05	8	19,51	3	17,65
Pain during active movements	62	53,45	25	67,57	9	42,86	18	43,90	10	58,82
Pain during passive movements	30	25,86	9	24,32	7	33,33	10	24,39	4	23,53
Pain during palpation	16	13,79	0	0,00	1	4,76	7	17,07	8	47,06
Feeling of tightness	44	37,93	2	5,41	5	23,81	20	48,78	17	100,00
Restriction of movements in joints	116	100,00	37	100,00	21	100,00	41	100,00	17	100,00
Intra-articular crepitation	81	69,83	2	5,41	21	100,00	41	100,00	17	100,00
Swelling	38	32,76	0	0,00	5	23,81	20	48,78	13	76,47
Deformations	116	100,00	37	100,00	21	100,00	41	100,00	17	100,00

Table II. The results of the assessment of the functional state of the joints according to the WOMAC index in patients with OA, combined with type 2 diabetes, obesity and hypertension

Indexes, units of measurement, mm	Groups of examined patients			
	I group OA, n=37	II group, OA+ hypertension, n=21	III group OA+ hypertension+ abdominal obesity, n=41	IV group OA+ hypertension+ abdominal obesity + diabetes, n=17
WOMAC, pain, mm	205,1±18,1	226,5±19,9	249,5±26,3	283,5±11,3p1
WOMAC , stiffness, mm	50,5±5,3	59,8±3,67	87,7±4,2 p1/p2	107,4±5,9 p1/p2
WOMAC, functional insufficiency, mm	754,2±26,8	814,4±25,9	906,9±22,7 p1/p2	1091,6±12,3p1/ p2/p3

Notes: p1 - significant in relation to the indicators of group I ($p < 0,05$); p2 - significant in relation to the indicators of group II ($p < 0,05$); p3 - significant in relation to the indicators of group III ($p < 0,05$).

ov test. Data reliability was calculated using a two-sample (for independent samples) or a pair (for dependent) Student's t-test with a distribution close to normal; Data are given as M ± m. Nominal data are presented in the form of quantitative and percentage values.

Correlation was calculated using Pearson's linear parametric correlation coefficient and Spearman's nonparametric rank correlation coefficient.

RESULTS

Analysis of the causes and social risk factors for OA showed that from the point of view of patients, humidity and cold were most often important (as cleaners, janitors, caretakers, builders). 37 patients indicated a systematically extended working day or the need to work night shifts for several years. Seasonal dependence of exacerbations of the disease (spring and autumn periods) was detected in 79 (68.1%) people. Genetic predisposition to OA (according to anamnestic data) was found in 21 patients. Half of the patients associated OA with menopause. One third of the involved patients indicated an association of disease progression with an increase in body weight. 39 patients indicated that type 2 diabetes and obesity contributed to the worsening of the clinical picture of OA.

Table III. Distribution of patients with osteoarthritis combined with type 2 diabetes, obesity and hypertension depending on the group of affected joints and the degree of functional disorders of the joints

Groups of patients	DFDJ, n=116			Groups of affected joints				
	I degree, n=76 (%)	II degree, n=37 (%)	III degree, n=3 (%)	Gonarthrosis, n=76 (%)	Coxarthrosis, n=3 (%)	Gonarthrosis + coxarthrosis n=18 (%)	Gonarthrosis + small joints, n=12 (%)	Small joints, n=7 (%)
I group OA, n=37	35 (94,6%)	2 (5,4%)	0 (0%)	33 (89%)	0(0%)	0(0%)	0(0%)	4 (11%)
II group, OA+ hypertension, n=21	16 (76,2%)	5 (23,8%)	0 (0%)	16 (76,2%)	0(0%)	0(0%)	4 (19%)	1 (4,8%)
III group OA+ hypertension+ abdominal obesity, n=41	21 (51,2%)	20 (48,8%)	0(0%)	26 (63,4%)	1 (2,4%	7 (17,2)	6 (14,6%)	1 (2,4%)
IV group OA+ hypertension+ abdominal obesity + diabetes, n=17	4 (23,5%)	10 (58,8%)	3 (17,7%)	1(5,9%)	2 (11,7)	11 (64,8%)	2(11,7)	1 (5,9%)

Note: OA - osteoarthritis, DFDJ - the degree of functional disorders of the joints

Table IV. Distribution of patients according to the Leken's index

Leken's index in points	Groups of examined patients			
	I group OA, n=37	II group, OA+ hypertension, n=21	III group OA+ hypertension+ abdominal obesity, n=41	IV group OA+ hypertension+ abdominal obesity + diabetes, n=17
Mild OA (1-4)	-	-	-	-
Moderate OA (5-7)	25 (67,6%)	10 (47,6%)	5 (12,2%)	-
Severe OA (8-10)	9 (24,3%)	7 (33,3%)	18 (43,9%)	4 (23,5%)
Very severe OA (11-13)	4 (7,8%)	4 (19,1%)	10 (24.4%)	3 (17,6%)
Extremely severe OA (14 i >)	-	-	8 (19,5%)	10 (58,9%)

Analyzing the clinical symptoms, it was found that the main complaints in patients of group I were pain in the affected joints, which bothered when descending or ascending the stairs, when moving from a sitting position to vertical, sometimes at rest (by nature they were aching, patients noted their intensity as moderate). The frequency of arthralgia symptoms is showed in Table I.

It was noted that in patients of group III joint pain during active and passive movements occurred in a larger number of patients than in patients of group IU (in 18 against 10 patients and in 10 against 4, respectively). This feature was also observed when comparing the following symptoms: a feeling of tightness (in 20 vs. 17), restricted movement, intra-articular crepitation and joint deformity (in 41 vs. 17).

Analysis of the results of the assessment of the functional state of the joints according to the WOMAC index showed that all examined patients complained of pain associated with exercise (116 people), impaired mobility and daily activities, which significantly increased with increasing OA (61 patients) and accession of comorbid pathology (in 79 patients). In patients of the IV group (17 people) the intensity of pain increased, and this group was also characterized by morning pain, impaired mobility with a significant deterioration in daily activities (Table II).

There was a significant increase in negative feelings in patients of groups III and IV (1.02 times in patients of group III, and in group IV 1.30 times compared with isolated OA). Deterioration of functional capacity was registered in patients of group III 1.2 times, and in group IV - 1.4 times more often compared with the isolated course of OA. Therefore, in patients with comorbidity with type 2 diabetes, these indicators were significantly increased compared with those examined in all groups ($p < 0.05$).

According to X-ray data, some features of the course of comorbid pathology (including gender) were identified. Among them, 37 (67.27%) women with the degree

Table V. Distribution of patients with osteoarthritis in accordance to body weight, type 2 diabetes mellitus, hypertension

Groups of patients	Body weight, n=116				Degrees of blood pressure increase, n=116			Type 2 diabetes, n=17 (%)
	Normal body weight, n=37 (%)	Overweight, n=27 (%)	I degree obesity, n=24 (%)	II and III degrees obesity, n=28 (%)	Normal BP, n=37 (%)	Hypertension of 1 degree, n=34 (%)	Hypertension of 2 degree, n=45 (%)	
I group OA, n=37	37 (100%)	0	0	0	37 (100%)	0	0	0
II group, OA+ hypertension, n=21	0	21 (100%)	0	0	0	18 (85,7%)	3 (14,3%)	
III group OA+ hypertension+ abdominal obesity, n=41	0	6 (14,6%)	21 (51,2%)	14 (34,2%)	0	16 (39%)	25 (61 %)	0
IV group OA+ hypertension+ abdominal obesity + diabetes, n=17	0	0	3 (17,6%)	14 (82,4)	0	0	17 (100%)	17 (100%)

Table VI. Indicators of carbohydrate metabolism in patients with osteoarthritis, M ± m

Indicators	Control	Groups of examined patients			
		I group OA, n=37	II group, OA+ hypertension, n=21	III group OA+ hypertension+ abdominal obesity, n=41	IV group OA+ hypertension+ abdominal obesity + diabetes, n=17
Insulin, μu / ml	8,51±0,74	9,31±0,37	11,23±1,12 p=0,047	13,05±1,44 p=0,007 p_1<0,05	21,25±2,75 p<0,001 p_1<0,001 p_2=0,002 $p_{3=}$0,01
C-peptide, ng / ml	2,05±0,16	2,56±0,53	2,68±0,27 p<0,05	3,08±0,17 p<0,05	3,27±0,34 p=0,002
Glucose, mmol / l	3,94±0,29	4,54±0,21 p=0,099	4,95±0,24 p=0,01	5,23±0,28 p=0,002 p_1=0,05	8,77±0,25 p<0,001 p_1<0,001 p_2<0,001 p_3<0,001
НвA_1C, %	5,36±0,42	5,42±0,26	5,65±0,37	6,21±0,48	8,11±0,23 p<0,001 p_1<0,001 p_2=0,002 p_3<0,001
HOMA-IR	1,48±0,07	1,82±0,14 p=0,03	2,39±0,87	3,12±0,82 p=0,05	9,95±0,34 p<0,001 p_1<0,001 p_2<0,001 p_3<0,001

Notes: p - the probability of differences in indicators with the control group; p_1 – the probability of differences in indicators with group I; p_2 – the probability of differences with the II group; p_3 – the probability of differences with the III group.

of functional disorder I (DFD I) and 15 (32.73%) men were identified, with DFD II two women and one man were found, which amounted to 3.63% of the number patients with I radiological stage. Regarding the second radiological stage, the prevalence of patients with DFD II (34 persons (58.62%) of the total number of persons with radiological stage II) among them were 31 persons (91.17%) and 3 men (8.82%). Thus DFD I was observed at 24 persons from them at 18 women and 6 men).

Since the localization of joint lesions and the severity of osteoarthritis are important for determining the ability to work, the quality of life of patients, the dependence of the degree of functional disorders on the groups of affected joints was studied (Table III).

In 33 (89.0%) persons of the I group of patients (with isolated OA) the lesions of the knee joints with the predominance of the I degree of functional disorders were established. Defeat of knee joints was found in 16 (76.2%) persons and hip - in 4 (19%) patients of group II, with the prevailing degree of functional disorders. In group III (OA + hypertension + abdominal obesity) along with lesions of the knee joints in 26 (63.4%) patients there were lesions of the hip in 7 (17.2%) persons and hip and small joints - in 6 (14.6%) , functional disorders of I and II degree were registered almost equally. Lesions of the knee and hip joints with functional disorders of the II degree prevailed in 58.8% of the examined IV group.

Determination of the severity of OA in patients of group IV according to the Leken's index showed that mild OA did not occur among the examined groups of patients. It was moderate in most patients of group I (25 (67.6%) people). Among the groups of patients with comorbidity, the highest percentage were patients of group III, where OA was combined with abdominal obesity and hypertension, severe course was characteristic of 18 (43.9%) patients. Extremely severe course was observed in patients of groups III and IV (Table IV). This required consultation with a traumatologist rheumatologist to decide on the appropriateness of conservative treatment or surgery.

Whereas body weight is an integrative indicator of carbohydrate and fat metabolism disorders, atherogenic dyslipidemia, hyperuricemia, insulin resistance, microalbuminuria, risk of hypertension, obesity, and type 2 diabetes, increases the risk of coronary heart disease and mortality, its indicators were studied in all patients (Table V). Patients in group I body weight did not exceed the norm. All patients of group II were overweight. In patients of group III obesity of the II degree prevailed (it was established in 21 (51.2%) patients), and in group IV (in the presence of type 2 diabetes) obesity of the II-III degree prevailed in all subjects.

Depending on body weight, pain in patients with OA and severe joint deformity was more common during active and passive movements (70.69%). Deterioration of the clinical course in patients of III and IV groups was established. Pain increased in patients of group III by 1.02 times, in group IV by 1.30 times, and the deterioration of the degree of SFN was registered 1.2 times in patients of group III and 1.4 times in group IV compared with isolated OA. Therefore, in patients with comorbidity with type 2 diabetes, these values were significantly higher than in other subjects ($p < 0.05$).

Since the presence of concomitant insulin resistance in patients with OA contributes to the growth of biomarkers of destruction of cartilage, cartilage matrix and persistence of inflammation of articular cartilage, synovial tissue, we studied the state of carbohydrate metabolism by the content of immunoreactive insulin, C-peptide, glycosylated hemoglobin (HвA1C), and glucose in the blood of patients (Table VI).

It was found that fasting glucose levels were significantly increased in patients of groups III and IV compared with the group of patients with isolated osteoarthritis (group I) ($p1 < 0.05$, $p1 < 0.001$, respectively). As for the indicators of glycated hemoglobin, they showed a similar relationship. The HOMA index was significantly elevated in patients with comorbid course, the highest rate was in group IV patients ($p3 < 0,001$). That is, with type 2 diabetes, insulin resistance increases, which can be reflected in the clinical course of OA. Such conditions can form both symptoms of OA and cardiovascular events in type 2 diabetes, obesity, hypertension, worsening the course of the disease. Confirmation of this can be found in patients of III and II groups increase in blood glucose levels by 1.34 ($p < 0.05$) and 2.23 times ($p < 0.05$) compared with group I ($p < 0.001$) and, even, compared with patients in group III ($p3 < 0.001$).

DISCUSSION

One of the most significant problems of modern rheumatology is OA, which is associated with a steady increase in the incidence of this disease and insufficient effectiveness of treatment, especially in comorbidity with other diseases. The total direct and indirect costs of treating degenerative lesions of the musculoskeletal system account for about 6% of the gross national product, even in the United States, and if the trend of increasing life expectancy continues, they will only increase [6,7].

Our analysis of risk factors for OA showed that the formation of the disease is influenced by working conditions, social factors, seasonality (found in 79 (68.1%) people), genetic predisposition was found in 21 patients. Genetic mutations lead to enzymopathy, and are the cause of chronic recurrent inflammation in the tissues of the joints, especially in cartilage.

Literature data show that patients with OA often have abdominal obesity, which exacerbates the chronic inflammatory process in the joints, aggravates the course of comorbid diseases and worsens the results of treatment [8]. One third of the patients we included in the study also pointed to the association of disease progression with an increase in body weight, contributing to the deterioration of the clinical picture of OA in 39 patients. Pain increased in patients with OA, hypertension and obesity 1.02 times, and in polymorbidity with type 2 diabetes - 1.30 times. Deterioration of the degree of DFD (compared with isolated OA) was registered 1.2 times and 1.4 times, respectively. This can be explained by a violation of microcirculation, which contributes to venous

stasis and hypertension, the occurrence of focal ischemic necrosis of bone. Angiogenesis at the junction of articular hyaline cartilage and adjacent subchondral bone reduces the thickness of subchondral bone, which contributes to the development of abnormal biomechanical stress and enhances degenerative-inflammatory changes in cartilage. The dependence of the intensity of pain during active and passive movements, joint deformities with limited range of motion in the joints, body weight and deterioration of clinical course in patients of these groups can be explained in the atherosclerotic hypothesis. There are reports that high plasma cholesterol and triglycerides are positively associated with joint pain [9-12]. Since the location of joint lesions and the severity of osteoarthritis are important for determining the ability to work and quality of life of patients, the dependence of the degree of functional disorders on the groups of affected joints was studied. Analysis of the results of the assessment of the functional state of the joints according to the WOMAC index showed that patients with polymorbidity OA with type 2 diabetes increased pain intensity, morning pain, mobility impairment with significant deterioration of daily activities, which significantly increased with increasing OA and, especially in polymorbidity with type 2 diabetes ($p < 0.05$). We consider this to be such that comorbidity and polymorbidity aggravate the course of osteoarthritis. Thus, according to our data, the presence of type 2 diabetes and obesity in OA changes the clinical picture, the intensity of the joint syndrome, contributes to greater destruction of cartilage and bone, as evidenced by radiological stages and degrees of joint dysfunction. It can be assumed that this is due to the degree of proliferative sclerotic morphological changes in the joints to a greater extent than inflammatory ones that occur in patients with obesity and hypertension (possibly in type 2 diabetes due to the prevalence of sclerotic and fibrotic processes in the joints clinical symptoms of inflammation, which are typical for obesity without type 2 diabetes, do not dominate). This corresponded to the results obtained in the works of other researchers [13,14].

It is known that OA is often pathogenetically associated with components of MS: insulin resistance, type 2 diabetes, coronary heart disease, hyperlipidemia, hypertension and coronary heart disease [15]. Symptoms such as osteophytosis, osteocystosis, osteosuration, osteoporosis, subchondral sclerosis, meniscus lesions, development of Baker's cysts and enthesopathy due to insulin resistance (IR), as indicated by indicators of adsorption-glycemic activity changes in static surface tension and phase angle of tensiograms.

The combination of OA with MS, obesity is associated by a number of authors with significant disorders of lipid metabolism, which further contributes to the development of atherosclerotic processes and type 2 diabetes [15-17]. In addition, parallels were found between obesity, cardiovascular pathology and the presence of OA, in particular, with lesions of the joints of the hands and lower extremities. According to various sources, the frequency of diagnosing a combination of hypertension and OA in obese patients ranges from 53% to 78%. Our results confirm that polymorbidity of OA with type 2 diabetes, obesity and hypertension in the clinical course of OA increases the degree of obesity, degree and stage of hypertension, which negatively affects the prognosis of such multimorbidity of diseases (with emphasis on the development of negative cardiovascular events) [18]. It is known that the increase in blood pressure in patients with type 2 diabetes can be accompanied by hypoalgesia due to the correlation between high blood pressure values and a decrease in the perception of joint pain due to differences in the level of β-endorphins [19,20]. At the same time, patients have increased anxiety, high levels of psycho-emotional stress, which contributes to the lack of effectiveness of treatment [21].

According to the results obtained, insulin resistance in type 2 diabetes is reflected in the clinical course of OA, worsening the course of the disease, and cardiovascular events. The increase in blood glucose levels in such patients by 1.34 ($p < 0.05$) and 2.23 times, respectively ($p < 0.05$) compared with those in the group with isolated OA ($p < 0.001$) coincide with the results of 19 European cohorts can be caused by oxidative stress, activation of inducible NO synthase in the vascular endothelium, hypoxia, obesity, disorders of carbohydrate metabolism [22,23]. It is known that the energy substrate for chondrocytes is anaerobically metabolized glucose. The presence of hyperglycemia (as the main symptom of MS) leads to the activation of the polyol pathway of glucose metabolism and non-enzymatic glucosylation of proteins, which causes damage to muscles and periatricular tissues. Hyperglycemia and OA interact at both the local and systemic levels. Local effects of oxidative stress and glucosylation of end products exacerbate cartilage damage, and the accumulation of toxic glycolysis products may contribute to the progression of OA (wrist joints in people aged 55-62 years, with a higher incidence in overweight patients, diabetes and hypertension) [24]. The presence of concomitant IR in patients with OA promotes the growth of biomarkers of cartilage tissue destruction: aggrecan - a product of degradation of cartilage matrix, antibodies to collagen II. The cascade of catabolic processes with the participation of aggrecinases, collagenases, metalloproteinases, nitric oxide synthetase, cyclooxygenase-2 and the destruction of cartilage matrix due to the action of IL1β, TNF-α, Progression leads to activation of humoral and persistence of inflammation of articular cartilage and synovial tissue.

Thus, this combination requires careful monitoring of body weight, hyperglycemia, which may be exacerbated by dyslipidemia, disorders of the system "proteolysis-fibrinolysis", which affects the condition of cartilage and synovial membrane of the joint.

CONCLUSIONS

1. The polymorbid course of osteoarthritis with type 2 diabetes, obesity and hypertension is characterized by a higher frequency (by 20.9% - during passive movements, by 13.2% - without load) and intensity (1.4 times) of joint pain, severe damage to hip joints and their combination with damage to knee joints in 64.8% of patients, deterioration of functional capacity (by 1.45 times) with a predominance in a larger proportion (76.5%) of patients of II and III degrees of functional insufficiency of joints in comparison with isolated osteoarthritis.
2. According to the WOMAC index, it was established that patients with osteoarthritis, including in combination with type 2 diabetes, against the background of obesity and hypertension, complain of pain associated with physical exertion, impaired mobility and daily activities, which significantly increased with increasing stage and the addition of comorbid pathology, especially for patients with OA, type 2 diabetes, obesity and hypertension.
3. It was established that the fasting glucose levels are significantly increased in the groups of patients with the combined course of osteoarthritis against the background of arterial hypertension, abdominal obesity, as well as against the background of polymorbidity compared to the group of patients with isolated osteoarthritis ($p1<0.05$, $p1<0.001$, respectively, which confirmed by a similar dependence of glycated hemoglobin indicators

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Conduct research on the clinical effectiveness of rehabilitation measures as a result of courses of physical therapy and physiotherapy.
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ORIGINAL ARTICLE

DESIGN AND *IN VITRO* EVALUATION OF ACRIVASTINE AS ORODISPERSIBLE TABLET USING DIRECT COMPRESSION METHOD

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ABSTRACT

The aim: This study aimed to develop mouth-dissolving tablets of Acrivastine, an antihistamine medication, in order to increase its oral bioavailability.

Materials and methods: Different super disintegrants, such as crospovidone, croscarmellose sodium, and sodium starch glycolate, were used to make Acrivastine oral dispersible tablets (ODTs). These super disintegrants were utilized in various concentrations. The formulation (F3) with 6% w/w crospovidone had a fast disintegration time (less than 30 seconds) and practically total drug release within 10 minutes. All of the formulations were made using the direct compression method and proper diluents, binders, and lubricants. Fourier transform infrared spectroscopy (FTIR) tests were used to investigate the drug-excipient interaction, and all formulations demonstrated improved drug-excipient compatibility.

Results: The average weight of all formulations was between 175 and 180 mg. All formulations' hardness and friability were within acceptable ranges. Direct compression tablets had a hardness of 3.2 to 4 kg/cm2. All formulations were determined to have a friability of less than 1.0%. For oral dissolving tablets, the *in vitro* disintegration time is critical, and this time preferred to be < 60 seconds. The results also showed that crospovidone disintegrated after 24 seconds and sodium starch glycolate disintegrated in 40 seconds *in vitro*.

Conclusions: When compared to croscarmellose sodium and sodium starch glycolate, crospovidone performs better as a super disintegrant. In comparison to other formula, tablets breakdown in the mouth in 30 seconds and have a maximum *in vitro* drug release time in 1-3 minutes.

KEY WORDS: super disintegrants, oral dispersible tablets, crospovidone, disintegration time

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INTRODUCTION

Despite notable developments in drug delivery technology, the oral route for drug administration remains popular due to exact dosage, low therapy costs, self-medication, non-invasive technique, and convenience of administration, all of which contribute to good patient compliance [1]. Elderly persons, children, and some patients, on the other hand, may have difficulty swallowing pills or firm gelatin capsules. Furthermore, such challenges affect not just patients, but also other working persons who do not have access to water. Tablets that disintegrate quickly in the mouth can be used to treat these issues. Oral solid dose forms (ODTs) are oral solid dosage forms that breakdown quickly in the mouth, releasing the medication. They contain super disintegrants, which assist dissolve the ODT in three seconds (s) to three minutes (min) without the use of water [2]. This makes ODTs beneficial to a variety of patient demographics, including geriatrics, and promotes their compliance [3]. Because some medications are absorbed from the mouth, pharynx, and esophagus as saliva flows down into the stomach, some oral dispersible tablets claim to have higher bioavailability than regular tablets. In such circumstances, the drug's bioavailability is much higher than it is in the traditional tablet dose form [4-7]. The taste and disintegration time are the two most important characteristics to consider while creating an ODT. Direct compression ODT formulations often contain high quantities of a super disintegrant to achieve rapid disintegration. The quantities of super disintegrant employed in the formulation might range from 10-20 wt percent of the formulation, depending on the quantity and features of the active pharmaceutical ingredient (API) and the intended release profile. Choosing the best super disintegrant is crucial when constructing an ODT formulation for direct compression. Although the super disintegrant

Table I. Composition of different Acrivastine ODT formulas.

Ingredients	F1	F2	F3	F4	F5	F6	F7	F8	F9	F10	F11	F12
Acrivastine	8	8	8	8	8	8	8	8	8	8	8	8
Crospovidone	2	4	6	8								
Sodium starch glycolate					2	4	6	8				
Croscarmellose									2	4	6	8
Mannitol	90	88	86	84	90	88	86	84	90	88	86	84
Microcrystalline cellulose	63	63	63	63	63	63	63	63	63	63	63	63
Aspartame	15	15	15	15	15	15	15	15	15	15	15	15
Magnesium stearate	2	2	2	2	2	2	2	2	2	2	2	2

primarily impacts the rate of disintegration, it can also affect mouth feel, tablet hardness, and friability when used at high levels [8]. Rapid disintegration of these oral dispersible tablets resulted in active contact of the active material gets with the tastebuds as a result of rapid ODT disintegration, and the necessity for a pleasant flavor becomes a vital aspect of patient palatability. As a result, masking the taste of bitter active constituents is a crucial obstacle to overcome in the creation of ODT formulations. In general, oral administration of bitter active constituents by ODT formulations must improve patient compliance and palatability when compared to ordinary tablets that contain sweeteners and flavors [9]. To accomplish quick tablet disintegration, direct compression method is one of the strategies that needs the use of super disintegrants into the formulation or the use of highly water-soluble excipients. There is no need for water or heat during manufacturing process, making it appropriate for moisture and heat-labile pharmaceuticals [10, 11]. Acrivastine is a triprolidine analog antihistamine indicated for the treatment of allergies and hay fever. It acts through blocking histamine action leading to prevention the symptoms associated with histamine release such as pruritis, vasodilation, hypotension, edema, bronchoconstriction, and tachycardia.

THE AIM

The goal of this study is to make an orodispersible tablet of Acrivastine in order to increase its water solubility, which may improve its oral bioavailability and patient compliance.

MATERIALS AND METHODS

Acrivastine was purchased from Lee chemicals, China. Croscarmellose sodium, sodium starch glycollate and crospovidone obtained from JP & SB Converting Services International S.L. Spain. All other chemicals and reagents were of analytical grade.

PREPARATION OF ORODISPERSIBLE TABLETS

Acrivastine 8 mg, super disintegrants in different ratios and other excipients were blended using mortar and pestle. The drug and super disintegrants were sieved through mesh # 100 before blending.

Angle of repose, bulk density, tapped density, Hausner ratio, and compressibility index were all calculated for the mixture. The mixture, which also included mannitol and microcrystalline cellulose as diluents and aspartame as a sweetener, was combined with 1% magnesium stearate as a lubricant and then compacted using an 8 mm punch on a tablet machine. Table I shows the composition of several these formulations

EVALUATION OF ACRIVASTINE ODT

The general quality tests are carried out in accordance with the USP. For weight variation, the weights of twenty tablets were individually measured, and the mean weight was reported along with its standard deviation [12]. A hardness tester was used to test the hardness of 3 tablets from each batch. Measuring the maximum load that every tablet can resist before breakage was measured in kilograms [13]. Twenty pills were placed in the friability tester for the test. To calculate the friability percentage, the weight before and after accomplishment of 100 revolutions in the fraibilator was documented. Disintegration tester was used to perform disintegration test which was accomplished on six tablets using a disintegration tester with distilled water as the disintegration media at 37°C in accordance with USP [14]. The wetting time was measured by placing 5 rounded filter papers with as diameter of 10 cm in a Petri dish with a diameter of 10 cm. This Petri dish contained 10 mL of distilled water that had been colored by using a water-soluble food coloring solution. After placing the tablet on the wetted paper, the time it took for the colored water to reach the top surface of the tablet was recorded (in seconds). The average of three readings is used in the wetting test [15]. Ten tablets of each formulation were

Table II. Physical properties of the prepared Acrivastine ODT.

Formula	Average weight (mg)	Hardness	Friability	Content uniformity	Disintegration time (sec)	Wetting time (sec)	Drug release at 10 min [%]
F1	178±0.60	3.5±0.24	0.55±0.21	95±0.96	30±0.35	33±1.02	90.2±0.19
F2	179±0.52	3.2±0.20	0.64±0.26	97±0.87	28±0.98	31±1.00	94.3±0.76
F3	180±0.68	3.4±0.23	0.57±0.14	98±0.91	24±0.34	30±0.89	98.2±0.02
F4	180±0.88	3.8±0.21	0.58±0.02	93±0.35	23±0.76	35±0.15	95.3±0.89
F5	175±0.57	3.5±0.03	0.67±0.34	98±0.88	40±1.21	41±0.95	93.3±1.09
F6	180±0.48	3.3±0.31	0.64±0.22	96±0.94	38±0.44	38±0.32	95.3±1.12
F7	179±0.25	3.3±0.36	0.58±0.09	95±1.46	33±0.21	30±0.43	96.4±0.34
F8	178±0.86	4±0.09	0.55±0.45	97±0.92	30±0.75	39±1.12	98.2±0.94
F9	178±0.69	3.5±0.22	0.53±0.54	98±0.83	37±0.11	41±2.56	95.21±0.11
F10	179±1.05	3.4±0.34	0.66±0.23	94±0.22	32±1.34	31±3.06	95.8±0.81
F11	180±0.34	3.9±0.24	0.56±0.16	96±0.55	35±0.87	40±2.02	97.3±0.13
F12	179±0.24	4±0.08	0.57±0.81	94±0.21	29±0.56	39±0.87	96.5±0.17

weighed and pulverized to ensure content consistency. In a 50 ml volumetric flask, a quantity of powder equivalent to 8 mg Acrivastine is placed. Dissolving the powder combination in 10 ml of sufficiently diluted methanol and measuring UV absorbance at 254 nm was used to calculate the amount of drug present in an 8 mg equivalent amount of powder. The concentration of the drug was calculated by using a standard graph. The prepared oral dissolving tablets of Acrivastine were investigated *in vitro* with using of a USP equipment type II with a paddle stirrer at 50 rpm and 900 yml of 0.1N HCl at 37°C as the dissolution medium. At 0.5, 1, 2, 4, 6, 10, and 15 minutes, aliquots of dissolution medium (5 ml) were extracted and replaced with fresh dissolution medium. A 0.45 membrane filter was used to filter the samples. These solutions' absorbance was measured at 254 nm. An equation derived from a standard curve was used to compute the percentage of medication release [16, 17].

RESULTS

The average weight of all formulations was between 175 and 180 mg. All formulations' hardness and friability were within acceptable ranges. Direct compression tablets had a hardness of 3.2 to 4 kg/cm^2.

All formulations were determined to have a friability of less than 1.0%, in order to reduce the risk of tablet breakage during shipping and use. For content uniformity all of the formulations had content homogeneity in the range of 94-98%. The capability of the super disintegrant to swell and engage water determined the wetting time in various formulations. It lasted between 30 and 42 seconds. For oral dissolving tablets, the *in vitro* disintegration time is critical, and this time preferred to be < 60 seconds. The quick disintegration could be owing to the medium's rapid uptake of water, swelling, and burst effect, boosting bioavailability. Table II shows that crospovidone disintegrated after 24 seconds and sodium starch glycolate disintegrated in 40 seconds *in vitro*.

DISCUSSION

Disintegration time is consistent with wetting time results, as sodium starch glycolate swells with greater gelling than croscarmellose sodium and crospovidone, resulting in a longer disintegration time. The disintegration time was found to decrease when the concentration of super disintegrants in the preparations wase increased. When comparing super disintegrants, sodium starch formulations take longer to wet than croscarmellose sodium and Crospovidone. The interior arrangement of the tablets and the hydrophobicity of constituents are linked to wetting. This could be because sodium starch glycolate is dissolved by the swelling mechanism, which results in a longer wetting time. Crospovidone and croscarmellose sodium disintegrate via wicking through capillary action and fibrous structure, with minimal gelling, respectively. According to the data, F3 formulation had a drug release of 98.02%, which was chosen as the best one because of its hardness, friability, DT, wetting time, and percentage cumulative release, which was higher than other formulations. Increasing surface area of the Acrivastine leads to increase in wettability of the drug (i.e., faster disintegration). When comparing different formulas F3, F7, and F11 with the same concentration of super disintegrants but different types, the results showed that F3, which contains crospovidone, provides the best

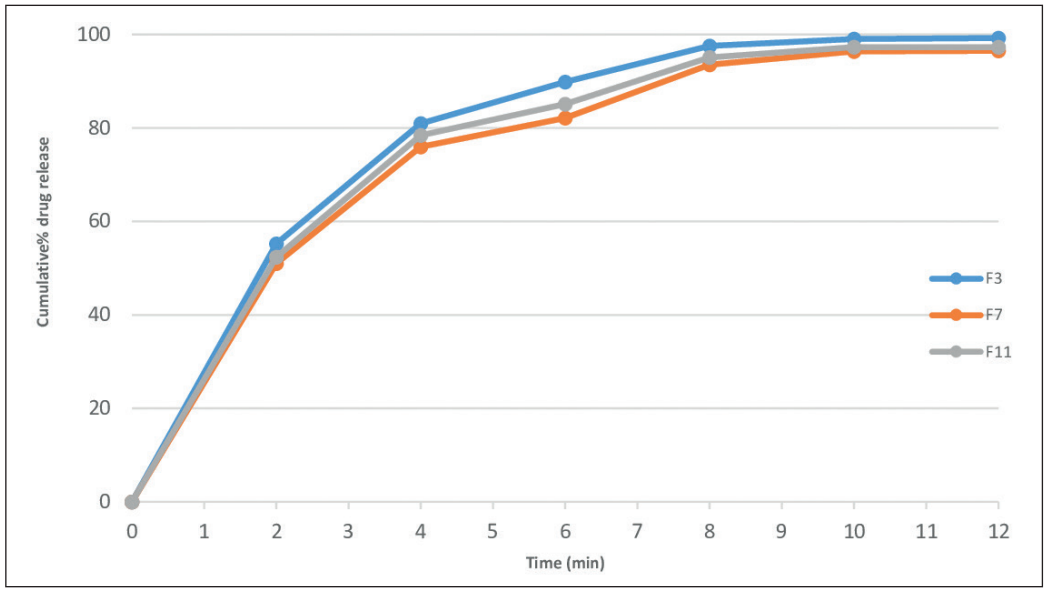

Fig. 1. Cumulative percent of drug release from different Acrivastine ODT formulations that contain different super disintegrants

cumulative amount of drug release when compared to other formulas F7 and F11, which contain sodium starch glycolate and croscarmellose, respectively (Fig 1).

CONCLUSIONS

Acrivastine Orodispersible tablet was manufactured by using the direct compression method employing various classes and concentrations of super disintegrant, as evidenced by numerous characterization and evaluation experiments. When compared to croscarmellose sodium and sodium starch glycolate, crospovidone performs better as a super disintegrant. In comparison to other formulae, tablets breakdown in the mouth in 30 seconds and have a maximum *in vitro* drug release time of 1-3 minutes.

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ORIGINAL ARTICLE

THE ULTRASONOGRAPHY EXAMINATION OF SKELETAL MUSCLES IN TRAUMATIC ISCHEMIA (EXPERIMENTAL STUDY)

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ABSTRACT

The aim: To establish indicators and significance of sonography in the evaluation of muscle necrosis in ischemia of the limb acording to quantitative ultrasonographic indicators and density of collagen by histological method.

Materials and methods: In experiments, rabbits modeled with 6-hour limb ischemia by applying an elastic tourniquet. On days 5, 15, and 30, ultrasound and histological studies of the muscles and correlation analysis were performed between the muscles' entropy and the degree of their damage (atrophy, fibrosis and necrosis).

Results: The relative amount of structurally altered tissue was estimated morphometrically and compared with entropy. A high correlation of muscle damage with vertical δ-entropy indicates that sonography is highly likely to detect areas of necrosis and, to a lesser extent, fibrosis in the development of ischemic limb contracture in the early stages.

Conclusions: Vertical δ-entropy in sonography is a significant indicator of muscle damage after traumatic ischemia and has strong relationship with muscle fibrosis.

KEY WORDS: muscle, ischemic contracture, sonography, necrosis, fibrosis

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INTRODUCTION

One of the most challenging problems that arise in treating patients with ischemic contracture is a clear diagnosis and prognosis of the course in the reactive recovery period (9-18 months from the episode of compartment syndrome) [1,2]. The prognosis is influenced by the immediate consequences of muscle tissue damage at the end of this period, namely the number of muscle fibers and the dynamics of necrotic changes [3]. Therefore, the technique for clear and thorough determination of the nature of the pathological process in the affected muscles is significant. The analysis of the literature on this issue allowed us to identify only a few works [4] devoted to ultrasound examination of skeletal muscles in ischemic contracture, although, in our opinion, it is ultrasonography together with modern methods of digital information processing that can open up new possibilities in predicting the course of the reactive recovery period of ischemic contracture [5,6]. Given a chance to conduct a morphological study, it is feasible to establish the features of fibrosis formation at the site of damaged necrotic muscles. Fibrosis formation is the direct structural basis of contractures that negatively affect the peripheral nerves of the damaged limb and its function [7]. In this experimental work, an attempt made to assess the degree of damage to a limb's skeletal muscles and the development of fibrosis after acute limb ischemia and after local administration of autologous cellular agents.

THE AIM

The aim of our study to establish indicators and significance of sonography in the evaluation of muscle necrosis in ischemia of the limb according to quantitative ultrasonographic indicators and density of collagen by histological method.

MATERIALS AND METHODS

Experiments were conducted on rabbits (Chinchilla lanigera) weighing 4.2-4.5 kg. Rabbits were kept in a clinic for experimental animals on a standard diet with free access to food and water. Premedication and Anesthesia of experimental animals performed by administration of thiopental sodium (i.p., 60 mg/kg). Experimental manipulations performed following the rules «Regulations on the animal use of in biomedical

research», «European Convention for the protection of vertebrate animals used for experimental and other scientific purposes», «Guide for the care and use of Laboratory Animals» (Strasbourg, 1986).

The experimental model consisted of modeling mechanical limb ischemia. To achieve this, a medical elastic tourniquet (5.5 cm wide) has been applied for 6 hours to the left lower limb. Subfascial pressure was measured in the deep posterior compartment of the hindlimb according to the classical invasive Whitesides technique using the serial device «Stryker Intra-comparative Pressure Monitor» (USA) for the determination of indicators at each measurement. Compartment syndrome of the hindlimb fascial compartment deemed to have occurred if the subfascial pressure values exceeded 10 mmHg (according to the recommendations of Harges (1964) at the first hour of the experiment and, later, over 30 mmHg. The animals were injected with autologous cell preparations in the deep posterior compartment of the tibia after tourniquet removal and subfascial pressure control. Animals with ischemia of the limb were divided into 4 experimental groups:

1) main group (n = 5) with ischemia;
2) the group with ischemia and platelet-rich plasma (PRP) concentrate injection (n = 5);
3) the group with ischemia and bone marrow aspirate concentrate (BMAC) injection into the lower limb muscles (n=5);
4) the group with ischemia and stromal-vascular fraction of adipose tissue (SVF) injection (n = 5).

Experimental groups of animals were removed from the experiment at 3 periods – 5, 15, and 30 days. The control group consisted of intact animals (n=5).

Methods for obtaining cellular preparations administered to animals in the experiment provided below.

TECHNIQUE OF OBTAINING PLATELET-RICH PLASMA CONCENTRATE

Blood drawn from the ear vein of a rabbit in the amount of 5 ml. Obtained blood was placed in a special Artrex® tube to obtain platelet mass concentrate. Then centrifugation was performed at 760 g for 8 minutes. The obtained concentrate drawn into a 5 cc syringe. After that, the latter injected into the posterior deep fascial compartment of the lower limb (Fig. 1).

TECHNIQUE OF OBTAINING A SUSPENSION OF BONE MARROW ASPIRATE CONCENTRATE

Autologous bone marrow aspirate concentrate obtained from the proximal thigh of rabbits. A bone trocar (10g diameter) was inserted into the rabbit's proximal thigh, and 5 ml bone marrow aspirate concentrate was performed with a 5 cc syringe. The aspirate separated through a tulip Emulsifier™ filter (Tulip Medical Products, USA). Anticoagulant dextrose citrate ADC-a (Baxter S.A., Belgium) was added to the aspirate. The obtained aspirate was centrifuged at 760 g for 8 minutes, after which 1 ml of the upper plasma layer and the cell layer containing mononuclear leukocytes, mesenchymal and hemopoietic cells was aspirated. Aspiration of 1 ml of the upper plasma layer and 9 ml of the cell layer was performed from a tube.

TECHNIQUE OF OBTAINING STROMAL-VASCULAR FRACTION OF ADIPOSE TISSUE SUSPENSION

A collection of 5 mg of the abdominal omentum, which was crushed mechanically to a state of suspension, was performed through 2 cm access. The specified suspension homogenized by passing it through a system of two connected syringes with a 1 mm hole. Then it was centrifuged at 760 g for 8 minutes.

Ultrasound examination performed just before removing the animals from the experiment. The authors, together with the Institute of Cybernetics of the National Academy of Science named after V.M. Glushkov, developed post-processing software for sonographic data on necrosis area of ischemic muscles and determination of entropy as an integral indicator of the sonographic image texture.

The theoretical basis for the development of this software was to determine the texture of the sonographic image. In our study, we used signs of texture dynamism, which, in our opinion, are more applicable for identifying features in ultrasound images to compare healthy and necrotic areas of ischemic muscles.

The foundations of dynamic information theory are based on the definition of information as a measure of change that accompanies all processes that take place in the world. This measure of information has proved extremely fruitful in the study of information properties of systems and processes and allows to isolate and use of useful (dynamic) information from random stationery and non-stationary signals, images, spatial fields, iterative methods, recursive procedures, etc., significantly reducing its redundancy. δ-entropy of a random process $H\delta$ is defined as the average value of the modulus of the derivative of the process and is a measure of the uncertainty of changes in the random process, i.e. it determines the dynamics of the process.

Considering an image as an implementation of a random process in rows or columns, it is possible to determine the amount of dynamic information in rows and columns that characterize the dynamic properties of the tissue texture in an ultrasound image.

Fig. 1. The technique of obtaining and injecting platelet-rich plasma concentrate into ischemic muscles of the posterior deep fascial compartment of the rabbit lower limb under sonographic control. Note: 1 - blood sampling from the ear vein; 2 – Blood centrifugation to obtain platelet-rich plasma concentrate; 3 - introduction of platelet-rich plasma concentrate into the ischemic muscles of the posterior deep fascial compartment of the lower limb under sonographic control

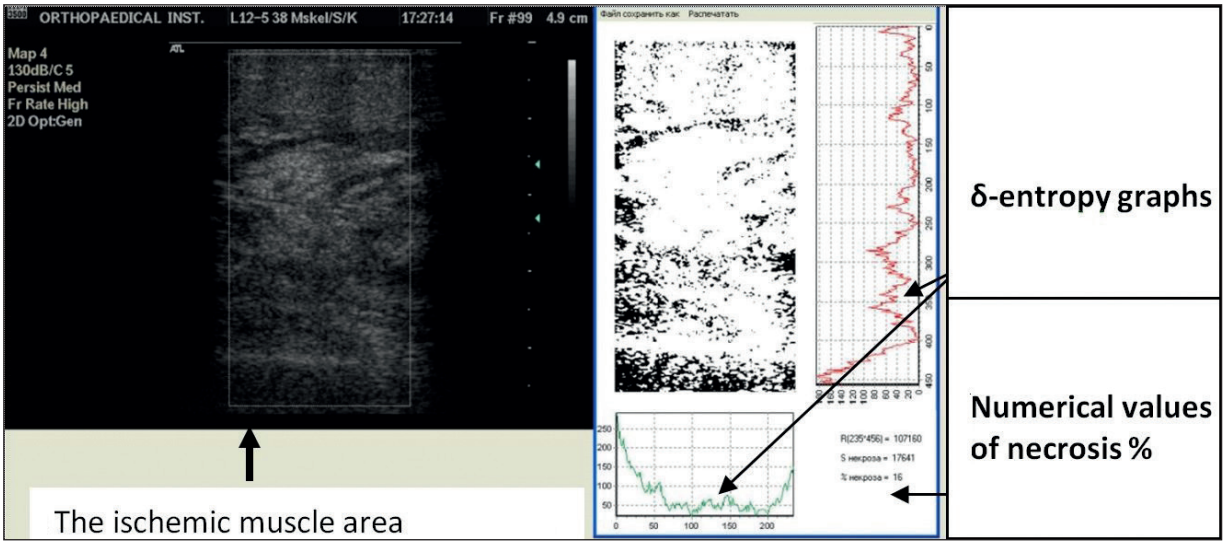

Fig. 2. Example of determining the percentage value of necrosis during computer processing of a sonographic image

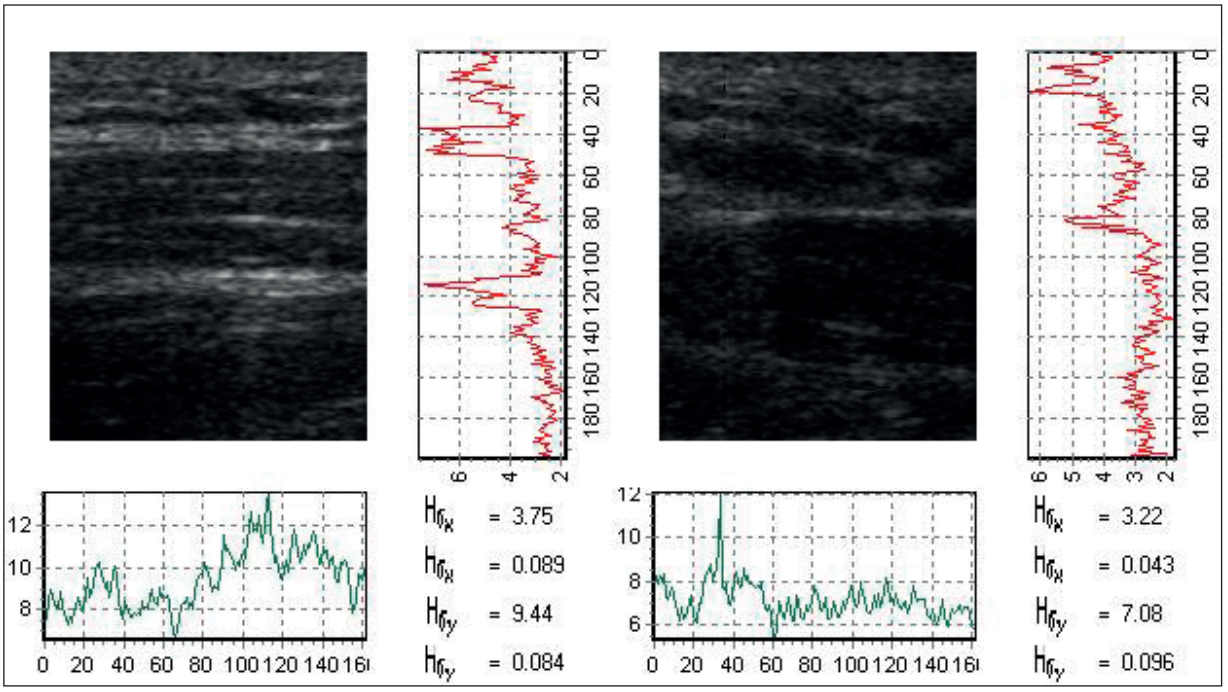

Fig. 3. An example of determining δ-entropy in computer processing of a sonographic image.

№	Group	Day after ischemia					
		Day 5		Day 15		Day 30	
		δ-entropy					
		vertical δ-entropy	*horizontal δ-entropy*	*vertical δ-entropy*	*horizontal δ-entropy*	*vertical δ-entropy*	*horizontal δ-entropy*
2	Ischemia	r=0.90; p=0.04	r=0.70; p=0.19	r=1.00; p<0,001	r=-0.80; p=0.10	r=1.00; p<0,001	r=0.20; p=0.75
3	Ischemia +PRP	r=1.00; p<0,001	r=-0.10; p=0.87	r=1.00; p<0,001	r=-0.50; p=0.39	r=0.30; p=0.54	r=0.10; p=0.87
4	Ischemia + BMAC	r=1.00; p<0,001	r=-0.90; p=0.04	r=1.00; p<0,001	r=0.10; p=0.87	r=0.90; p=0.04	r=0.60; p=0.28
5	Ischemia + SVF	r=0.87; p=0.05	r=-0.30; p=0.62	r=0.90; p=0.04	r=-0.60; p=0.28	r=1.00; p<0,001	r=0.10; p=0.87

Fig. 4. Relationship between muscle necrosis and δ-entropy. Note: intensive blue – strong significant positive correlation; light blue – non-significant positive correlation; rosewood - strong significant negative correlation; pink - non-significant negative correlation

Table I. Indicators of δ-entropy during the sonographic examination of ischemic muscles under the influence of cell therapy

№	Group	Research period of time					
		Day 5		Day 15		Day 30	
		δ-entropy					
		vertical δ-entropy	***horizontal δ-entropy***	***vertical δ-entropy***	***horizontal δ-entropy***	***vertical δ-entropy***	***horizontal δ-entropy***
1	Control			9.25±0.30	5.14±0.49		
2	Ischemia	6.85±0.47*	5.65±0.29	8.03±0.12	5.63±0.25	8.86±0.31	6.12±0.17
3	Ischemia +PRP	8.69±1.00	6.61±0.30	8.46±0.60	6.40±0.42	8.29±0.47	6.22±0.37
4	Ischemia + BMAC	8.46±0.31*	6.18±0.42	8.83±0.37	5.26±0.32	9.19±0.37	5.17±0.42
5	Ischemia + SVF	8.49±0.31	6.05±0.41	8.89±0.37	5.23±0.32	8.37±0.20	5.09±0.42

Note: * P ≤0.05 to control

Table II. Degree of muscle damage during the histological examination of ischemic muscles under the influence of cell therapy

№	Group	Research period of time		
		Day 5	Day 15	Day 30
1	Control		4,74±0.51	
2	Ischemia	53.87±1.61*	52.68±1.47*	69.89±0.85*^@
3	Ischemia +PRP	45.60±6.43*	45.06±2.47*	38.39±2.99*^@**
4	Ischemia + BMAC	50.28±2.90*	52.56±5.32*	39.00±1.03*,**
5	Ischemia + SVF	56.33±1.71*	64.84±2.60*	66.13±4.28*@

Note: * P ≤0.05 to control; ** P ≤0.05 to ischemia; @ P ≤0.05 to 5 days; ^ P ≤0.05 to 15 days

The first step was to determine the necrosis area. A 7.5 MHz sensor was installed along and across the detected necrotic muscle areas. A fixed number of pixels has been taken as 100% for the Philips HD-11 device it was 248 by 587 pixels. We independently selected the area of the sonographic image to be studied. This section was fixed in the form of a rectangle using a specially designed graph builder. Within this defined plane, the program calculated the number of zero pixels (i.e, absolutely black areas – echo-negative) of the sonographic image, which reflected necrotic changes in the muscles (in digital and graphic image) (Fig. 2).

The second step was to determine δ-entropy by comparing the same areas of ischemic and contralateral healthy muscles. Initially, the necrotic muscle area was determined

according to the principles described above, and the identical healthy muscle area was automatically displayed on the sonographic image. After fixing the same planes, the program calculated δ-entropy horizontally and vertically. The corresponding graphs of changes in the dynamics of rows and columns were constructed and the average values of the dynamics were calculated, respectively, for the rows and columns of the selected areas of the image. The average entropy value was determined using statistical methods.

To study structural changes in the hindlimb muscles using sonography, some animals were removed from experiments, and samples of muscles were isolated and fixed in a 10% neutral formalin solution. After fixation, the samples were embedded in paraffin using the isopropanol-paraffin method and 8μm microsections were made on a Thermo Microm HM 360 microtome (Thermo Scientific, USA). Slices were stained with hematoxylin and eosin, and Sirius red for collagen detection and analized as described in the article [8].

STATISTICAL STUDIES

Statistical data processing was performed using StatPlus ver software. 7.3.0. (AnalystSoft Inc. USA). The normality of the data sample distribution was carried out according to the Kolmogorov-Smirnov criterion. The hypothesis of changes in entropy and collagen in damaged muscles after influence of cell suspensions was assessed using a one-parameter analysis of the variance of ANOVA variations with Bonferroni correction. The difference between the groups was considered significant at $P \leq 0.05$. Correlation analysis was performed according to Spearman's criterion. The interpretation of strength and direction of of the relationship between two quantitative variables was carried out according to the Chaddock scale (up to 0,3 practically absent; 0,3-0,5 weak; 0,5-0,7 noticeable; 0,7-0,9 strong). Data are presented as the mean and standard error of the mean (Mean ± SEM).

RESULTS

As a result of processing the δ-entropy data, generalized average values of dynamics were obtained, making it possible to estimate its change between two images, and graphs of changes in dynamics in rows and columns allowed us to use additional signs of changes in tissue density (Fig. 3). Such additional features were:

- maximum and minimum deviations in graphs from generalized averages,
- distances between extremes on graphs that characterize the thickness of fabric layers and their changes,
- low dynamics of the site was a sign of necrosis, which could be segmented and its area and percentage of the studied image could be estimated, etc.

In the graph on the right, the image is more "calm", which is reflected by the indicators *Hδx, Hδy* and especially *hδx, hδy,* which are at the maximum value of the brightness difference in the sonographic image and thus allow to evaluate dynamic characteristics in different brightness ranges. *Hδx values* are usually 2-3 times higher than *Hδy values*, which is due to the orientation of tissues in the horizontal direction. The results of the study of δ-entropy in experimental groups are presented in Table I. Statistical analysis showed significantly lower vertical δ-entropy on Day 5 in the group with mechanical ischemia and after bone marrow aspirate concentrate administration. On days 15 and 30, the entropy level returned to control values.

The relative damage to muscle tissue after mechanical ischemia was evaluated morphometrically. Table II shows the percentage of structurally altered tissue (a total of necrosis, atrophy, and fibrotic changes) in histologically examined muscle samples. A significant increase in the density of damage sites was found on day 60 after ischemia and administration of stromal-vascular fraction of adipose tissue aspirate, but after administration of platelet rich plasma and bone marrow aspirate concentrate, the indicator was lower. The injured muscle areas were characterized by muscle fibers atrophy and fibrosis development (the presence of collagen was histochemically confirmed in micro-preparations). On Day 5, muscle fibers with a vividly reduced diameter were found; on day 30, areas of collagenogenesis were already established, which means that necrotic tissue is replaced by connective tissue in irreversibly damaged muscle areas (mainly the external myons of the lower limb muscle group).

Correlation analysis showed a stronge positive relationship between vertical δ-entropy and the degree of muscle damage in mechanical limb ischemia in all four groups ($r>0.8$; $p\leq0.05$) (Fig. 4). Despite the non-significant result on 30 day after injection of PRP, we considered the obtained data as a stable trend. Negative correlation between horizontal δ-entropy and necrosis ($r=-0.9$; $p=0.04$) was found only in the group with bone marrow aspirate concentrate, and these results had opposite directions in different periods of time. Data of horizontal δ-entropy had inconsistencies between periods, did not reach the significant level and therefore we did evaluate it critically. We concluded that vertical δ-entropy is a significant indicator in the study of muscle damage during sonography.

Thus, the proposed assessment of image dynamics was used to determine the correlation between skeletal muscle damage and the sonographic parameters of ischemic muscles. It is concluded that the level of vertical δ-entropy can be used to assess and predict

the severity of compartment syndrome and structural changes in muscles in the reactive recovery period.

DISCUSSION

This study attempted to establish a correlation between fibrotic changes in damaged muscles and sonography results. Experimental animals were simulated mechanical ischemia, which lasted 6 hours, which caused the following disorders: mechanical damage to soft tissues, increased intra-tissue pressure over 30 mm Hg, skeletal muscle necrosis, and the development of fibrosis. We believe that the model used is suitable for studying the pathophysiology of ischemic contracture and can be a model object in assessing the dynamics of muscle atrophy, fibrosis, and the impact of various treatments.

According to the results of ultrasonography studies, ischemic muscle damage had a characteristic sonographic picture, which is the heterogeneity of structural changes, the presence of zones of muscle tissue restructuring (necrosis and fibrosis) and which depended on the duration of the injury, the prevalence of pathological changes in muscle tissue and had characteristic differences from other pathological processes (denervation, traumatic, etc.). Sonography made it possible to non-invasively and objectively assess muscle density in the damaged lower limb and bone-fascial compartment in particular. One can determine the severity and prevalence of the pathological process (the appearance of black, echo-negative areas) by the image intensity changes. A feature of the consequences of muscle ischemia in the first months of the reactive recovery period is destructuring, an increase in thickness due to edema, heterogeneity of changes in muscle tissue with the presence of hypoechoic foci of necrosis, and hyperechoic zones of various sizes and shapes. With an increase in the duration of the pathological process, atrophic and fibrous changes increase, which also affect the heterogeneous increase in echogenicity and mosaic structure of damaged muscles. We consider it appropriate to rely not only on qualitative indicators of muscle structure but also on quantitative parameters – the echo density coefficient, namely vertical δ-entropy, which make it possible to determine the degree of irreversibility of ischemic changes clearly. Computer software made it possible to estimate the percentage of necrosis, which is of great prognostic importance.

Compression ischemia caused extensive muscle necrosis. The 6-hour elastic cuff model proved to be quite successful and causes two pathogenetic factors of ischemic lesion of limb tissues at the same time. This is mechanical damage itself, during which the external (peripheral) muscle mions were necrotized, and mechanical damage to muscle vessels caused secondary ischemic damage to muscle tissue. Given that we cannot differentiate between lymphocytes and blast cells in hematoxylin- and eosin-stained micropreparations, we cannot use techniques that include scales for assessing muscle fiber damage with inflammatory infiltration [9], and there are difficulties in reliably differentiating degrees of damage to muscle fibers (it is not difficult to distinguish intact muscle fiber from atrophied, but there are difficulties in determining them in the early stages of atrophy when cytological signs of damage are weak) [10]. So, we took the other way. Muscle samples were separated along the longitudinal axis. Only longitudinal sections were examined because in severe mechanical damage to the limb muscles of the limb, interest should be on the continuity of muscle fibers [11] and ultrasound also shows a picture of the muscle in longitudinal projection. We found heterogeneity of muscle damage in micropreparations, because as an alternative to estimating a large number of fields, we measured the amount of damaged and structurally altered tissue in the muscle, ie areas that did not contain muscle fibers (areas of total necrosis, complete atrophy and fibrosis of muscle fibers). We presented the degree of damage as the percentage of altered tissue in the total muscle sample as an alternative to the method where the percentage of damaged muscle fibers to the total pure fibers in the muscle sample is determined [12]. A similar technique was used by other authors [13] and additionally converted the percentage of damaged muscle tissue into scores (0 to 5). We decided not to make additional transformations and this presentation of data in our work is convenient for comparison with the level of entropy in the ultrasound examination.

The obtained high positive correlation between the rate of muscle tissue damage (primarily atrophy of muscle fibers and fibrosis) and the results of sonographic data processing, namely vertical δ-entropy is of clinical importance in terms of establishing the severity of necrotic lesions of a particular segment of the extremities and predict fibrous changes, the formation of limb contracture. On day 30 we revealed a delay in structural changes after the introduction of concentrates of platelet plasma and bone marrow aspirate concentrate. However, sonography did not show a difference between 15 and 30 days. This indicates that sonography allows to detect areas of necrosis with a high probability; its sensitivity regarding to fibrosis is less, although we do not rule out an increase in echogenicity in the formation of significant fibrosis. The obtained results expand the diagnostic range of choice of functional and laboratory methods in the treatment of patients in the reactive-recovery period of ischemic contracture.

CONCLUSIONS

In sonography, vertical δ-entropy is a significant indicator of muscle damage after traumatic ischemia and has strong relationship with muscle fibrosis.

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ORIGINAL ARTICLE

INFLUENCE OF CRYOTHERAPY WITH PULSE COMPRESSION ON THE FUNCTIONAL CONDITION OF THE KNEE JOINT AFTER PARTIAL MENISCECTOMY

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ABSTRACT

The aim: To study the effect of cryotherapy with adjustable pulse compression in patients after arthroscopic partial meniscectomy on the functional state of the knee joint in the early period of rehabilitation.

Materials and methods: A total of 63 patients took part in the research: the experimental group included 32 patients (23 men and 9 women), and the control group - 31 patients (21 men and 10 women). In order to determine the effect on the functional state of the knee joint after arthroscopic partial meniscectomy in the experimental group, cryotherapy with adjustable pulse compression was used with the help of «GIOCO CRYO – 2» system; ice bags were used in the control group. In the research process, the following methods were used: visual analogue point scale, sonography, goniometry and myotonometry.

Results: It was found that in the experimental group, under the influence of cryotherapy with adjustable pulse compression, there was a progressive decrease in the intensity of the pain syndrome, the accumulation of reactive synovial fluid, a dynamic increase in the amplitude of movements of the operated joint, and an improvement in the muscle tone of the quadriceps femoris ($p<0,05$-$0,001$).

Conclusions: Thus, cryotherapy with adjustable pulse compression has shown a positive effect on the functional state of the knee joint in the early period of patients' rehabilitation, after partial meniscectomy and can be recommended for use in clinical practice.

KEY WORDS: meniscus, knee joint, cryotherapy with adjustable pulse compression, meniscectomy, pain syndrome, synovitis, range of motion, myotonometry

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INTRODUCTION

Among all injuries of the lower extremity, the frequency of injuries of the knee joint is up to 75% of cases [1]. In the structure of injuries of the knee joint elements menisci constitute up to 85,3% of all cases [2]. The average annual frequency of meniscal injuries is increasing and is becoming the most common surgical intervention on the knee joint [3].

The introduction of arthroscopy in clinical practice has significantly improved the results of the knee joint injuries treatment. However, surgery, even minimally invasive, causes pain syndrome, the development of reactive synovitis, weakness of the quadriceps femoris, impairment of neuromuscular coordination of movements, the elimination of which in the shortest possible time allows speeding up restoration of joint function.

It is believed that the use of cold is one of the most common means used as an effective non-pharmacological intervention for pain treatment in cases of injuries. The following effects of cryotherapy are most often reported - pain relief, reduction of inflammatory oedema and elimination of muscle spasm [4, 5].

THE AIM

To study the effect of cryotherapy with adjustable pulse compression in patients after arthroscopic partial meniscectomy on the functional state of the knee joint in the early period of rehabilitation.

MATERIALS AND METHODS

A total of 63 patients aged 18 to 35 years were under observation. Using the method of forming a random sample, patients were divided into a control group (31 patients, 21 of which were men and 10 - women) and an experimental group (32 patients, 23 of which were men and 9 - women). The average age of patients in

the control group was 27±1,2 years, and in the experimental one - 26±1,6 years. Surgical interventions were performed on both the right and left knee joints. A total of 63 partial meniscectomies were performed: 51 interventions on the medial meniscus and 12 on the lateral one. The surgery was performed under conduction anaesthesia and lasted no more than 30 minutes. All patients underwent a full range of clinical examinations. In addition, radiography of the knee joint in two projections as well as ultrasound investigation and magnetic resonance imaging were necessarily performed. Typically performed surgery, as a treatment measure, provided clear controlled conditions for assessing the role of cryotherapy with adjustable pulse compression in restoring the functional state of the knee joint in the early postoperative period.

In order to determine the effectiveness of cryotherapy with adjustable pulse compression on the functional state of the knee joint of patients in the experimental group after performance of partial meniscectomy during the first hours «GIOCO CRYO - 2» system was used. «GIOCO CRYO - 2» system consists of a pump, cuffs for compressing the area of the joint and a microprocessor that controls the pump (Fig. 1).

In the control group, coating the knee joint area with bags of crushed ice was used for local cryotherapy.

The duration of the procedure was 15 minutes, three times a day, the first ten days after the intervention in the studied groups of patients.

Important indicators of effective recovery of the knee joint function after partial meniscectomy in the early postoperative period are the intensity of pain syndrome, the severity of reactive synovitis, increased range of motion in the joint and the condition of the quadriceps femoris as the main stabilizer of the knee joint.

They formed the basis of evaluating the effectiveness of cryotherapy with adjustable pulse compression on the restoration of knee joint functions.

To do this, the following studies were conducted: assessment of pain syndrome according to the point visual analogue scale; sonography of operated knee joints to determine the severity of synovitis; goniometry to determine the range of motion of the knee joint and myotonometry to assess the tone of the quadriceps femoris.

The indicated research methods were used on the first, third and tenth days after surgery. Examinations of patients of both groups were carried out during a control examination in the clinic.

STATISTICAL ANALYSIS

The results of the research were processed using the software package Statistica 6.0.

Qualitative data are represented as numbers and percentages, while continuous numerical data are represented as mean ± standard deviation. Numerical variables were compared between study groups using Student's t-test. The obtained indicators had a normal distribution (according to the Shapiro-Wilke criterion).

RESULTS

One of the criteria for the effectiveness of knee joint function restoration in the early postoperative period is pain syndrome. For a reliable assessment of pain intensity, we used a point-based visual analogue scale (VAS) [6].

The results of the study of the pain syndrome dynamics according to the point-based visual analogue scale (VAS) of the patients in the postoperative period are presented in Table I. On the first day of using cryotherapy, the pain syndrome index in the control group was 7,49±0,18 points, which is characterized as severe pain, and in the experimental group – 5,81±0,1 points, which corresponds to moderate pain ($p<0,001$).

On the third day of rehabilitation, the pain syndrome in patients of the experimental group decreased to 4,98±0,23 points, while in the control group it decreased to only 6,11±0,25 points, that is, it remained within the range of severe pain ($p<0,001$).

In the subjects of the experimental group, a decrease in the intensity of the pain syndrome in the first days after the surgical intervention made it possible to reduce the use of painkillers. The same tendency was observed up to the tenth day after surgery. The level of pain syndrome in patients of the experimental group on the tenth day decreased to 1,03±0,25 points, and in the control group – 2,70±0,21 points ($p<0,001$).

The exudative component of inflammation is not as sensitive to the patients as the pain syndrome, but is important as an objective indicator of the activity of reactive synovitis. The reactive inflammatory aseptic process in the joint is a necessary, protective reaction of tissues to damage [7].

However, quite often it becomes excessive, predetermining a long and sometimes prolonged course, causing the development of complications [8].

The severity of reactive synovitis was assessed by the presence of clinical signs (pain syndrome, positive symptom of "floating patella") and sonography. The effectiveness of sonography lies in the ability to visualize the structures of the knee joint in the dynamics [9].

To determine the intensity of the reactive synovial fluid accumulation in the upper torsions of the knee joint the following scale was used: 0 - no fluid in the joint cavity, I - maximum fluid layer thickness < 2 mm, II - fluid layer thickness 2 - 4 mm, III - fluid layer thickness > 4 mm [10].

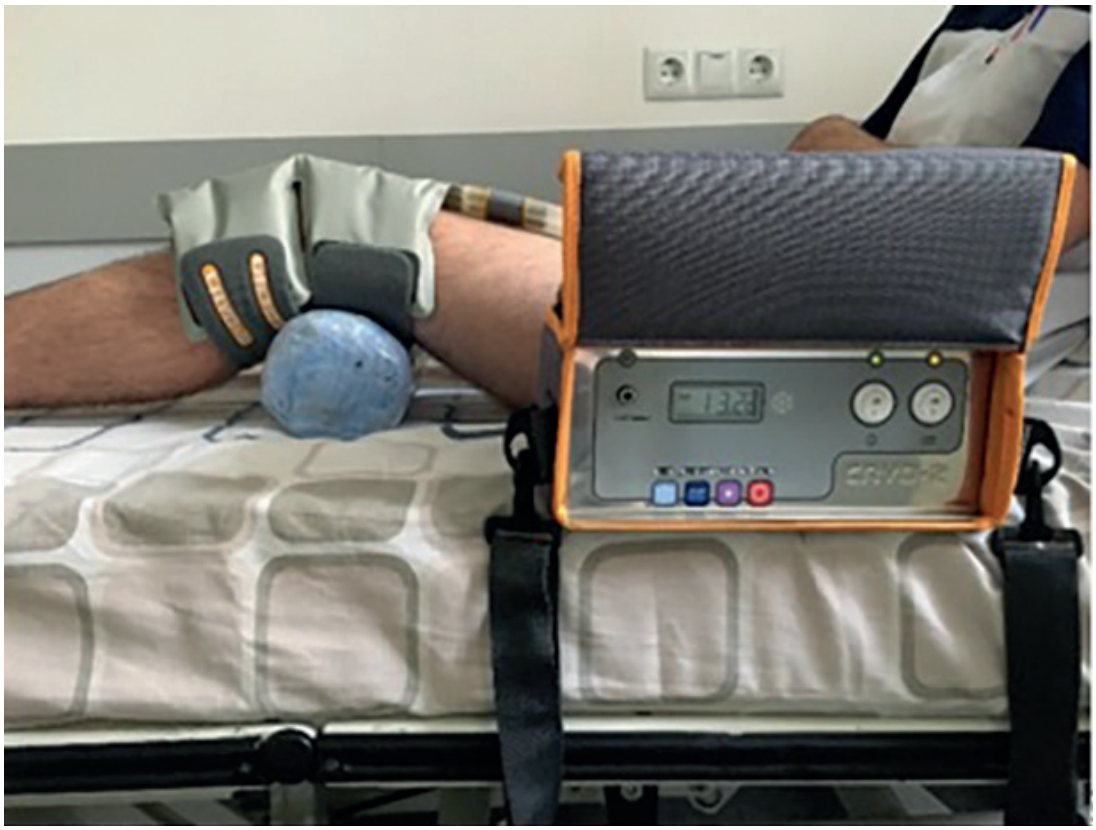

Fig. 1. Appearance of «GIOCO CRYO - 2» system

Table I. Dynamics of pain syndrome and the height of the knee joint reactive exudate layer in the postoperative period

Groups	Pain syndrome (points)		
	Examination day		
	Day 1	Day 3	Day 10
EG (n=32)	5,81±0,12	4,98±0,23	1,03±0,25
CG (n=31)	7,49±0,18	6,11±0,25	2,70±0,21
P	p<0,001	p<0,001	p<0,001
	Height of the reactive exudate layer (mm)		
EG (n=32)	2,14±0,04	1,84±0,04	1,32±0,03
CG (n=31)	2,76±0,04	2,33±0,12	1,64±0,03
P	p<0,001	p<0,001	p<0,01

Table II. Dynamics of the amplitude of knee joint movements and myotonometry of the quadriceps femoris of patients in the postoperative period

Groups	Amplitude of movements (degrees)					
	Day 1		Day 3		Day 10	
	Bending	Extension	Bending	Extension	Bending	Extension
EG (n=32)	30,3±3,0	171,9±2,6	90,0±2,5	175,1±2,0	126,8±2,6	178,2±2,0
CG (n=31)	27,52±2,34	168,30±2,66	67,74±2,38	168,19±2,20	105,42±3,40	170,03±2,43
P	p<0,001		p<0,001		p<0,001	
	Myotonometry (myotones)					
Groups	Day 1		Day 3		Day 10	
	Resting tone	Tension tone	Resting tone	Tension tone	Resting tone	Tension tone
EG (n=32)	62,69±2,36	64,56±1,64	46,16±1,63	54,38±2,08	58,47±2,59	81,97±2,13
CG (n=31)	63,70±1,56	64,90±1,42	43,60±1,89	53,50±1,65	56,70±2,07	69,70±2,82
P	p>0,05		p<0,01	p>0,05	p<0,01	p<0,001

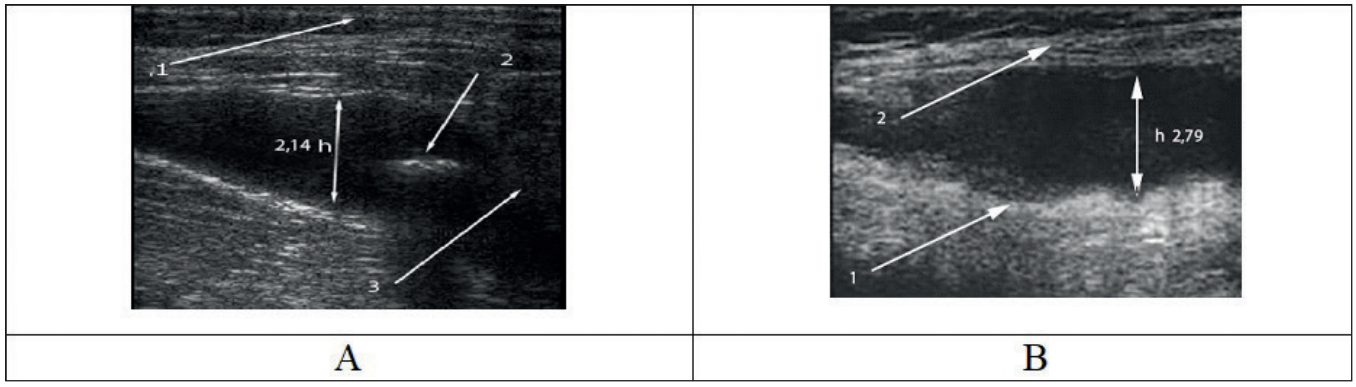

Fig. 2. The height of the layer of reactive exudate on the first day after the intervention in the joint cavity of the patients.
A. Experimental group. h – the height of the layer of reactive exudate in the upper torsion of the knee joint; 1 - the tendon of the quadriceps femoris; 2 - fibrin clot in the upper torsion of the knee joint; 3 - kneecap.
B. Control group. h – the height of the layer of reactive exudate in the upper torsion of the knee joint; 1 – the cortical layer of the femur; 2 - the tendon of the quadriceps femoris.

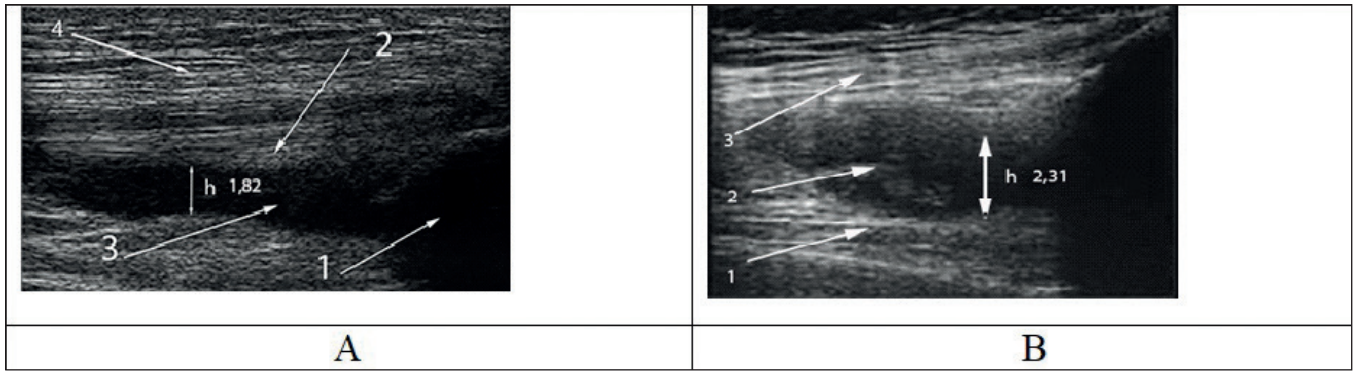

Fig. 3. The height of the layer of reactive exudate on the third day after the intervention in the joint cavity of the patients.
A. Experimental group. h – the height of the layer of reactive exudate in the upper torsion of the knee joint; 1 - kneecap; 2 - synovial membrane of the knee joint; 3 - reactive fluid in the upper torsion of the knee joint; 4 - tendons of the quadriceps femoris.
B. Control group. h – the height of the layer of reactive exudate in the upper torsion of the knee joint; 1 - the cortical layer of the femur; 2 - synovial fluid with fibrine in the upper torsion of the knee joint; 3 - tendons of the quadriceps femoris.

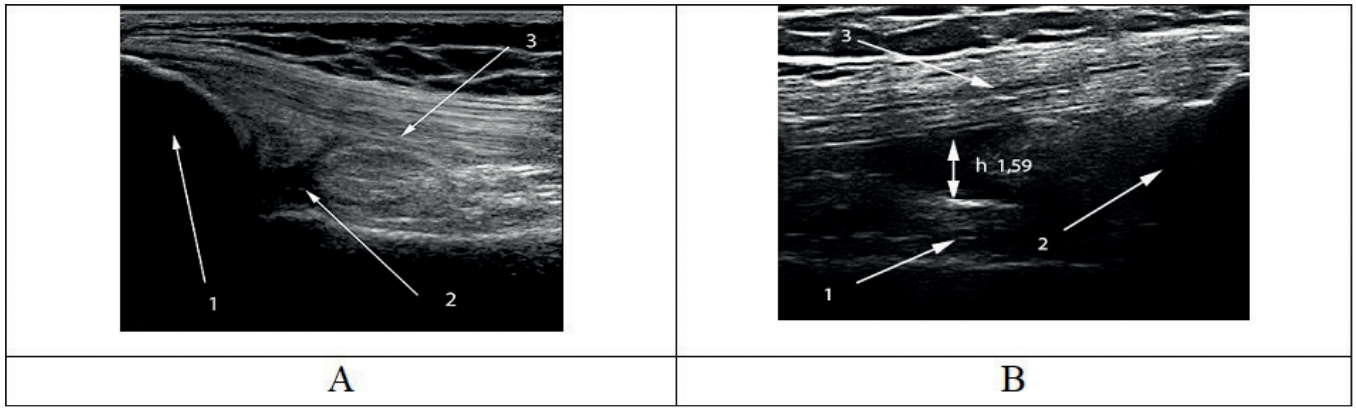

Fig. 4. The height of the layer of reactive exudate on the tenth day after the intervention in the joint cavity of the patients.
A. Experimental group. 1 - kneecap; 2 - upper torsion of the knee joint; 3 - tendons of the quadriceps femoris.
B. Control group. h - the height of the layer of reactive exudate in the upper torsion of the knee joint; 1 - cortical layer of the femur; 2 - kneecap; 3 - tendons of the quadriceps femoris.

On the first day after cryotherapy, the average height of the reactive exudate layer in the knee joint of patients in the experimental group was 2,14±0,031 mm, in the control group it was significantly higher – 2,76±0,040 mm ($p<0,001$) (table I, fig 2).

On the third day of cryotherapy, the amount of exudate gradually decreased in both groups. However, in the experimental group, the height of the exudate layer was 1,84±0,038 mm, and in

the control group, it was higher by 2,33±0,123 mm (p<0,001) (Fig. 3).

Lowering the height of the synovial fluid layer in the knee joint under the influence of cryotherapy with adjustable pulse compression made it possible to reduce the number of punctures. Thus, on the third day, only 15,6% of patients in the experimental group had a joint puncture, while in the control group there were 29% of such patients.

On the tenth day, the height of the synovial fluid layer in patients of the experimental group decreased to 1,32±0,028 mm according to the results of sonography, and in the control group - only to 1,64±0,033 mm (p<0,01) (Fig. 4).

Another important indicator of the effectiveness of the early period of rehabilitation after surgical intervention on the knee joint is the restoration of the range of motion. The results of the assessment of these indicators after cryotherapy are presented in Table II. Thus, on the first day after cryotherapy, the bending in the operated knee joint in the experimental group was 30,3±3,0°, and in the control group – 27,52±2,34° (p<0,001). The extension indicators were as follows: experimental group 171,9±2,6°, control group 168,30±2,66° (p<0,001).

On the third day of observation, the bending in the knee joint in the experimental group increased to 90,0±2,5° (an increase of 99,3%), and in the control group - only to 67,74±2,38° (an increase of 84,50%) (p<0,001). The results of knee joint extension in EG were 175,1±2,0° (an increase of 1,87%) and in CG – 168,0±2,20° (decrease by 0,06%) (p<0,001).

On the tenth day, bending in the knee joint in the experimental group increased to 126,8±2,6° (an increase of 122,90%), and in the control group - only to 105,42±3,4° (an increase of 117,20%) (p <0,001). The joint extension data were, respectively, 178,2±2,0° (an increase of 3,62%) and 170,03±2,43° (an increase of 1,02%) (p<0,001).

The muscle tone is definitely reflex in nature and is an important indicator that reflects the dynamics of functional recovery of patients after trauma and surgery. Deficiency of activity and atrophy contribute to the weakness of the quadriceps femoris as the main stabilizer of the knee joint [11].

One of the methods of objective assessment of the functional state of the muscle is myotonometry. To determine muscle tone, we used «MYOTONOMETER PAT.D. SZIRMAI Gy. sz 64451» (Hungary).

On the first day after surgery in the patients of the studied groups, the resting tone of the quadriceps femoris was increased as a result of pain in the knee joint and, accordingly, was 62,69±2,36 myotones in the experimental group, and in the control group – 63,70±1,56 myotones (p>0,05) (Table II). The tension tone of the quadriceps femoris did not differ significantly in both groups (p>0,05).

Studies of the muscle tone of the quadriceps femoris on the third day showed its decrease due to reduced motor activity of patients in both groups. Thus, the resting tone in the experimental group was 46,16±1,63 myotones (decreased by 30,4%), and the tension tone – 54,38±2,08 myotones (decreased by 17,4%). In the control group, these indicators were as follows: resting tone 43,60±1,89 myotones (decrease by 37,4%), tension tone 53,50±1,65 myotones (decrease by 19,17%).

On the tenth day after surgery, a gradual increase in muscle tone was noted in both groups, but the amount of increase was different. So, in EG, at maximum tension, the tone of the quadriceps femoris was 81,97±2,13 myotones (an increase of 23,8%), at rest, it was 58,47±2,59 myotones (an increase of 7,00%), which indicates an improvement in the condition of the neuromuscular apparatus in patients of this group. In the control group, the indicators were 69,70±2,82 myotones (an increase of 7,09%) and 56,70±2,07 myotones (an increase of 11,63%), respectively. The significance of the difference between the indicators of the groups is at the p<0,01-0,001 level.

DISCUSSION

Cryotherapy is one of the common means of both single and combined treatment of patients after injuries and operations. The use of cold increases vasoconstriction, which reduces tissue metabolism. Slowing down blood flow helps reduce the inflammatory response and swelling [12].

Local cooling slows down the transmission of the pain signal, thus reducing it [13].

The results of our research indicate the effectiveness of both methodologies of cryotherapy in patients after partial meniscectomy. However, the analysis of the dynamics of the indicators recovery such as the intensity of the pain syndrome, the severity of reactive synovitis, the increase in the amplitude of movements in the joint and the tone of the quadriceps femoris shows that in the patients of the experimental group who used cryotherapy with pulse compression, the recovery was more effective and significant (p<0,05-0,001), compared to patients of the control group who used ice bags.

So, already on the first day of rehabilitation after surgery, the patients of the experimental group felt moderate pain, and of the control one - experienced severe pain. The dynamics of decreasing pain intensity indicators in EG was observed until the tenth day of the study in comparison with CG.

Lowering the height of the layer of synovial fluid in the knee joint under the influence of cryotherapy with

adjustable pulse compression made it possible to reduce the number of punctures. So, on the third day, only 15,6% of patients in the experimental group underwent a joint puncture, and in the control group, there were 29% of such patients, which is also a positive indicator of the cryotherapy with pulse compression effectiveness.

Regarding the restoration of the movements amplitude of the operated knee joint, according to our data, a progressive increase in the bending function was established with a growth of 99,3% in patients of the experimental group already on the third day in comparison with the control group (84,5%). There is a statistically significant difference between CG and EG ($p<0,001$). These data are consistent with the results obtained by other authors [14,15].

It is necessary to note studies that demonstrate the average effectiveness of cryotherapy in improving the activation of the knee joint after surgical interventions [16].

However, despite a number of controversial issues and possible complications during cold treatment, cryotherapy devices with pulse compression demonstrate good results after arthroscopic partial meniscectomy in comparison with other procedures due to a significant reduction in pain, swelling, analgesic consumption, increased range of motion and muscle tone.

Both methods have positive dynamics of the functional state of the knee joint restoration after partial meniscectomy. However, patients of the experimental group who used cryotherapy with adjustable pulse compression in the postoperative period had more positive and significant dynamics (on the first, third, and tenth day) than patients of the control group ($p<0,05$-$0,001$) who used bags with ice.

The pain syndrome in patients of the experimental group already on the first day of rehabilitation decreased to moderate (5,81±0,12 points), and in the control group, it was characterized as severe (7,49±0,18 points) ($p<0,001$).

The reduction of clinical manifestations and sonographic indicators of reactive synovitis made it possible to reduce the number of knee joint punctures. It was performed only in 15,6% of EG patients, while in CG there were 29% of such patients, which is also a positive indicator of the cryotherapy with pulse compression effectiveness.

In the experimental group, the restoration of the range of motion in the operated knee joint and the improvement of the muscle tone of the quadriceps femoris occurred faster and more effectively during the entire study period than in the control group.

So, cryotherapy with adjustable pulse compression has shown a positive effect on the functional state of the knee joint in the early period of patients' rehabilitation, after partial meniscectomy and is more effective than cryotherapy using ice bags; that is why it can be recommended for use in clinical practice.

CONCLUSIONS

The analysis of the research results of the various methods of cryotherapy impact made it possible to establish that:

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ORIGINAL ARTICLE

FEATURES OF MORPHOGENESIS OF THE BONES OF THE HUMAN ORBIT

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ABSTRACT

The aim: To find out the sources of origin, the chronology of ossification, the peculiarities of age-related topographical and anatomical changes in the bones of the human orbit.

Materials and methods: The research was carried out on the specimens of 18 human embryos and prefetuses aged from 4th to 12th weeks of intrauterine development and 12 human fetuses aged from 4th to 9th months which were studied by microscopic examination and 3D reconstruction.

Results: The first signs of osteogenesis around the main nervous and visceral contents of the orbit rudiment are observed in 6-week-old embryos in the form of seven cartilaginous bone models. The first signs of ossification in the region of the orbit are found in the maxilla. During the 6th month of intrauterine development, intensive processes of ossification of the frontal, sphenoidal, ethmoidal bones and maxilla are noticeable. From the beginning of the fetal period of human ontogenesis, the ossification of bone rudiments that form the walls of the orbit continues. The processes of ossification of the structures of the sphenoidal bone continue, which leads to morphological transformations of the orbit in 5-month-old fetuses – it is separated from the sphenopalatine and infratemporal fossae by a bone layer, the optic canal is formed, and in 6-month-old fetuses, processes of ossification of the frontal, sphenoidal and ethmoidal bones and maxilla occur, Müller's muscle changes its structure to a fibrous one.

Conclusions: Critical periods of the orbit development are the 6th month of prenatal ontogenesis and the 8th month.

KEY WORDS: morphogenesis, human orbit, prenatal ontogenesis, critical periods

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INTRODUCTION

The study of the features of morphogenesis, the age-related dynamics of topographic-anatomical transformations and the anatomical variability of the bones of the human skull is an important task of modern morphology and an actual direction of anatomical and embryological research, the development of which contributes to the solving of an important medical and social problem – the improvement of methods of prevention, early diagnosis and effective correction of congenital and acquired human diseases, predicting the effectiveness and individualization of operative interventions in maxillofacial surgery, reducing infant mortality. The bones of the brain and facial parts of the skull form the orbit – an important region that includes the organ of vision and its auxiliary apparatus, the external muscles of the eye, blood vessels, nerves, and adipose tissue [1]. All these structures are in close syntopic connections, which affects the morphogenesis and topographic-anatomical changes of the orbit during the prenatal period of human development. Despite numerous scientific studies of the orbit, the organ of vision, and related structures, questions about the time and sequence of the appearance of bone sources that form the orbit, the chronology of their ossification, and critical periods of development are still pending [2, 3]. Elucidation of the sources of the bones of the human head, clarifying the sequence of their ossification will allow to create a morphological basis for the effective interpretation of fetal condition monitoring data [4, 5], will contribute to the early diagnosis of variants of the structure and possible defects in the development of the head, the organ of vision and adjacent structures, to the improvement of algorithms for the interpretation of diagnostic medical imaging data [6, 7, 8].

THE AIM

The aim of the research was to find out the sources of origin, the chronology of ossification, the peculiarities of age-related topographical and anatomical changes in the bones of the human orbit.

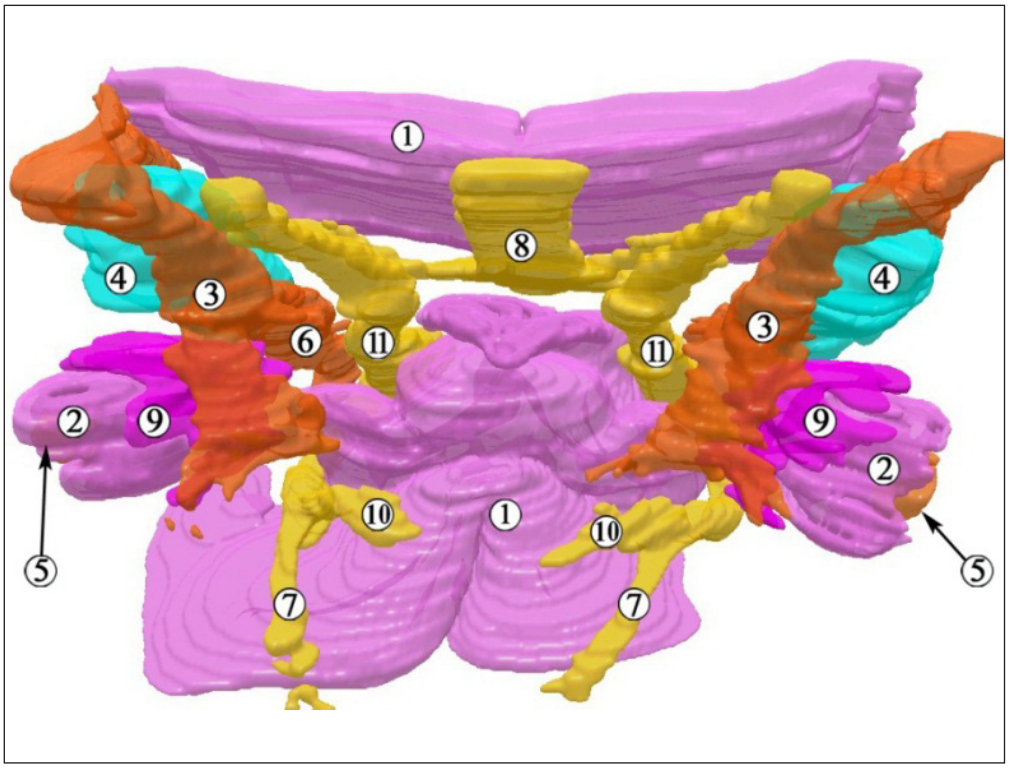

Fig. 1. 3D computer reconstruction of the structures of the head of a human embryo 8.0 mm PCoL (5th week of IUD). Front projection. Magnification: 30x. Signs:
1 – neuroectoderm of the brain; 2 – eye cup; 3 – internal carotid artery; 4 – trigeminal node; 5 – lens; 6 – posterior communicating artery; 7 – mandibular nerve; 8 – optic chiasm; 9 – mesenchymal condensation around the eye cups; 10 – maxillary nerve; 11 – ophthalmic nerve.

MATERIALS AND METHODS

The research was carried out on the specimens of 18 human embryos and prefetuses aged from 4th to 12th weeks of intrauterine development (IUD) (4.0-80.0 mm parietal-coccygeal length (PCoL)) and 12 human fetuses aged from 4th to 9th months of IUD (130.0-450.0 mm parietal-calcaneal length (PCaL)) using a complex of morphological research methods (anthropometry, morphometry, microscopy, macroscopy, 3D reconstruction of a series of histological sections and computer tomograms and statistical analysis).

The investigations were performed keeping to the major regulations of the Resolution of the First National Congress on Bioethics «General Ethic Principles of Experiments on Animals» (2001), ICH GCP (1996), the European Union Convention on Human Rights and Biomedicine (04.04.1997), and the European Convention for the Protection of Vertebrate Animals Used for Experimental and Other Scientific Purposes (18.03.1986), the Declaration of Helsinki on Ethical Principles for Medical Research Involving Human Subjects (1964-2008), EU Directives №609 (24.11.1986), the Orders of the Ministry of Health of Ukraine № 690 dated 23.09.2009, №944 dated 14.12.2009, № 616 dated 03.08.2012.

RESULTS

Our material revealed that the time of appearance of the sources of origin of structures of orbit is 4th weeks of IUD (embryos 4.0-5.0 mm PCoL). In this age period, for the first time, on separate histological sections and 3D-reconstructions of consecutive serial histological sections, mesenchymal condensation around the junction of the eye stalk with the forebrain is observed. The structures of the orbital region are located bilaterally, which is due to the mutual position of the rudiments of the eyeballs.

On the 5th week of IUD (embryos 6.0-8.0 mm PCoL), mesenchymal condensation surrounds the eye cups from all sides, which move from their lateral position (180°) to a more frontal one (Fig. 1), which can be considered the beginning the process of orbit frontalization.

In embryos 9.0-13.5 mm PCoL (6th week of IUD), morphological signs of the beginning of osteogenesis in the mesenchyme of the orbit area were found. 3D-reconstruction makes it possible to distinguish the rudiments of the bones of calvaria, the base of the skull and the face, in particular, the seven bones of the orbit. On the histological sections, the bone rudiments of orbit contain a grid of small zones of osteogenesis in its centers. Each bone sours consists of a thin mesenchymal capsule, which serves as a model for the morphogenesis of particular bones by both membranous and cartilaginous ossification. It should be noted that the maxilla is the first of the bones of the orbit to appear in the form of a single center of ossification above the dental plate at the place of source of the canine. Ossification zones increase in size and thus approach each other, demarcated by sutures.

The beginning of the pre-fetal period (the 7th week of IUD) is marked by intense rates of formation of the facial

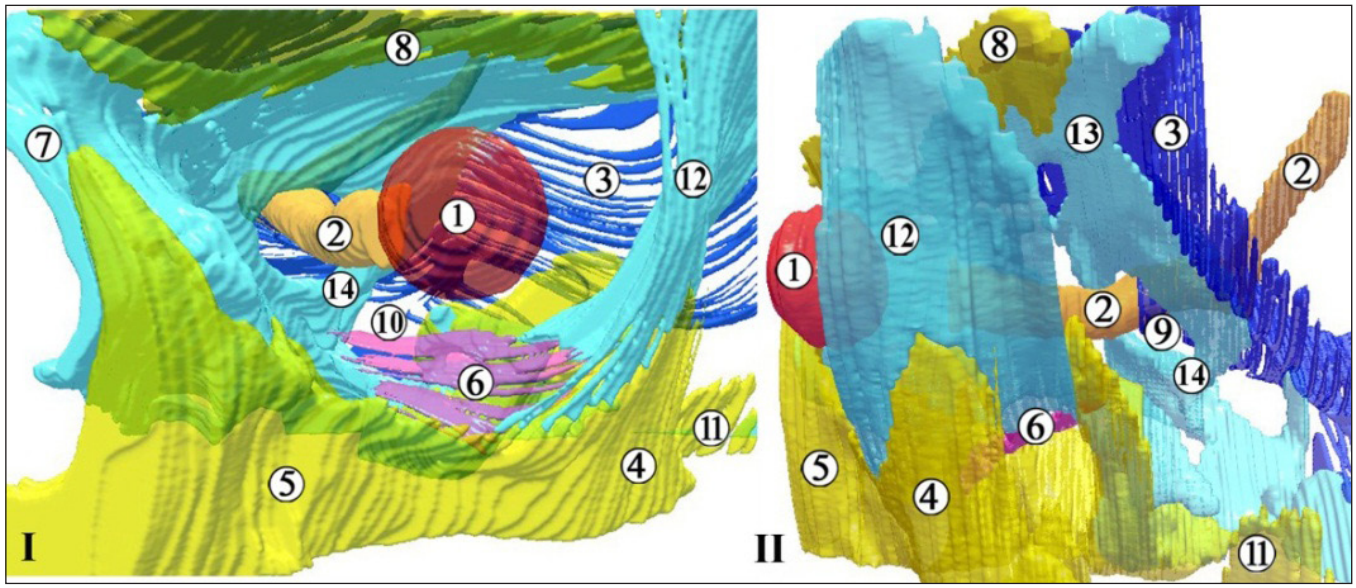

Fig. 2. 3D computer reconstruction of the structures of the left half of the head of the human fetus 22.0 mm PCoL (8th week of IUD). I – front projection, II – lateral projection. Magnification: 25x. Signs:
1 – lens; 2 – optic nerve; 3 – dura mater; 4 – zygomatic process of the maxilla; 5 – maxilla; 6 – Muller's orbital muscle; 7 – cartilaginous nasal capsule; 8 – frontal bone; 9 – optic canal; 10 – inferior orbital fissure; 11 – zygomatic process of the temporal bone; 12 – membranous lateral wall of the orbit; 13 – greater wing of the sphenoidal bone; 14 – lesser wing of the sphenoidal bone.

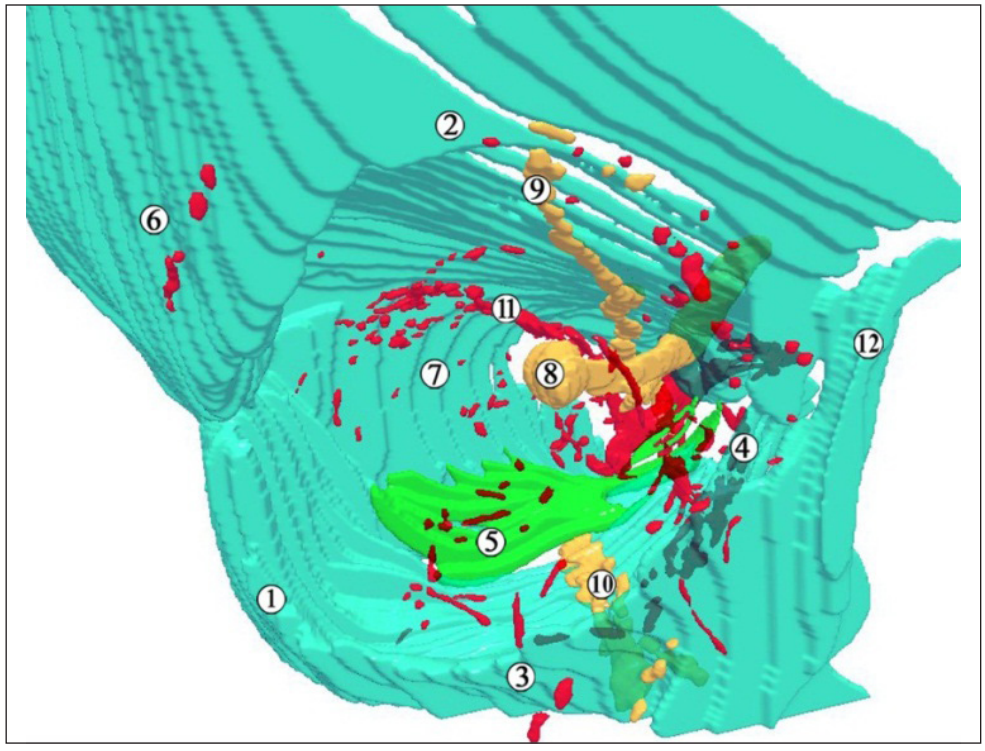

Fig. 3. 3D computer reconstruction of the right part of the head of the prefetus 50.0 mm PCoL (10th week of IUD). Front projection. Magnification: 25x. Signs:
1 – zygomatic bone; 2 – frontal bone; 3 – maxilla; 4 – lacrimal bone; 5 – Muller's orbital muscle; 6 – temporal surface of the greater wing of the sphenoidal bone; 7 – orbital surface of the greater wing of the sphenoidal bone; 8 – optic nerve; 9 – supraorbital nerve; 10 – infraorbital nerve; 11 – supra-orbital artery; 12 – nasal bone.

part of the head and the general growth of body parts. As a result, orbit quickly change their orientation to a more medial one while maintaining a still relatively significant interocular distance. By the end of the 8th week of IUD (prefetuses 24.0-28.0 mm PCoL), the shape of the face gradually acquires anthropomorphic features, but still with signs of hypertelorism. At the end of the 8th week of IUD, the membranous ossification of the frontal bone in the dorsal direction from the supraorbital edge, as well as the rudiments of the lesser wing of the sphenoidal bone in the form of a cartilaginous structure lateral to the optic nerve, are clearly visible (Fig. 2). Between the frontal bone and the lesser wing of the sphenoidal bone, a small cartilaginous structure is visible – sphenoido-ethmoidal cartilage. During the 8th week of IUD, osteogenesis begins through membra-

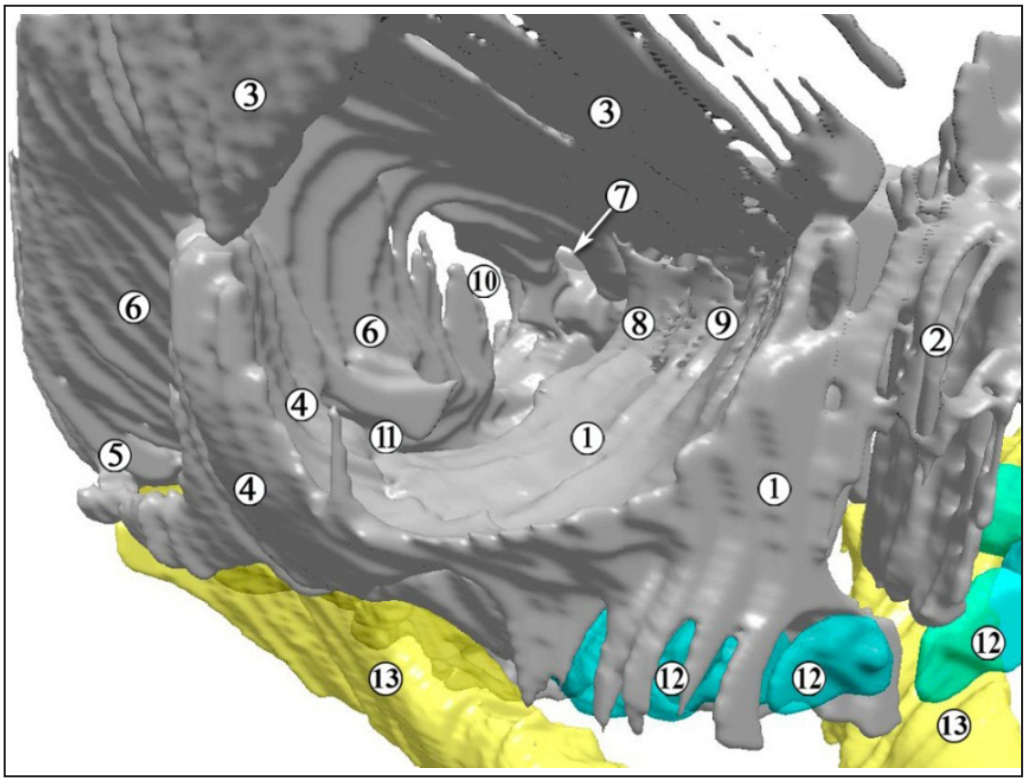

Fig. 4. 3D computer reconstruction of the right orbit of an 11-week-old human prefetus (60.0 mm PCoL). Front projection. Magnification: 25x. Signs: 1 – maxilla; 2 – nasal bone; 3 – frontal bone; 4 – zygomatic bone; 5 – zygomatic process of the temporal bone; 6 – greater wing of the sphenoidal bone; 7 – optic canal; 8 – ethmoidal bone; 9 – lacrimal bone; 10 – superior orbital fissure; 11 – inferior orbital fissure; 12 – teeth rudiments; 13 – mandible.

nous ossification of the zygomatic and palatine bones. An interesting fact is that the lower wall of the orbit is separated from the pterygopalatine fossa by Müller's orbital muscle.

Until the 10th week of IUD, the frontal reorientation of the orbit continues with a gradual slowing of this process. As a result, the interorbital distance decreases compared to the width of the face. Frontalization of the face contributes to the consolidation of the main facial rudiments (Fig. 3), therefore, on 3D-reconstructions, the face of the prefetuses acquires an anthropomorphic appearance.

In human prefetuses of the 10th week of IUD (42.0-52.0 mm PCoL), the ossification of orbital plate of the frontal bone begins already from the medial edge, as well as the bones of the medial wall of the orbit, in particular, the lacrimal bone, and the orbital plate of the greater wing of the sphenoidal bone. A wide spheno-frontal suture occupies most of the superior and lateral walls of the orbit. The peculiarity of this suture is that it is a chondromembranous connection between the frontal bone (membranous ossification) and the greater and lesser wings of the sphenoidal bone (cartilaginous ossification). The spheno-ethmoid cartilage regresses. We believe that this temporary structure provides a supporting framework for the superior wall of the orbit until the spheno-frontal suture is formed, similar to the role of the Müller muscle on the inferior wall of the orbit. Müller's orbital muscle has the appearance of a well-developed muscle plate that occupies more than half of the inferior wall of the orbit (see Fig. 3). The inferior orbital fissure at this stage of the IUD is very wide, since the membranous ossification of the bones that form it is not yet complete.

In the 11th week of IUD (prefetuses 55.0-65.0 mm PCoL), there is a linear increase in such morphometric indicators of orbit as width, height, depth and volume, but the dynamics of these changes are not proportional. Therefore, the shape of the contours of the external bony edges of the orbit (entrance to the orbit) changes from hameconchal (rectangular) at the beginning of the 11th week of IUD to hypsiconchal (rounded) (Fig. 4).

At the beginning of the fetal period (fetuses of the 4th month of IUD) the diameter of the orbit is 6.5±0.5 mm. The ossification of the rudiments of the bones that form the walls of the orbit continues – the unossified cartilaginous precursor of the ethmoidal bone gives rise to three outgrowths – the sources of the nasal concha. Müller's muscle separates the orbit from other regions, but the pterygopalatine fossa remains a continuation of the infratemporal fossa and the region around the sella turcica (Fig. 5).

The greater and lesser wings of the sphenoidal bone have only the lateral and medial centers of ossification, but ossification is more intense on the lateral edge of the lesser wings and on the medial edge of the greater wings of this bone. At the end of the 4th month of IUD, the medial center of ossification of the lesser wings of the sphenoidal bone becomes well defined, their size increases, due to which the connection of the infra-

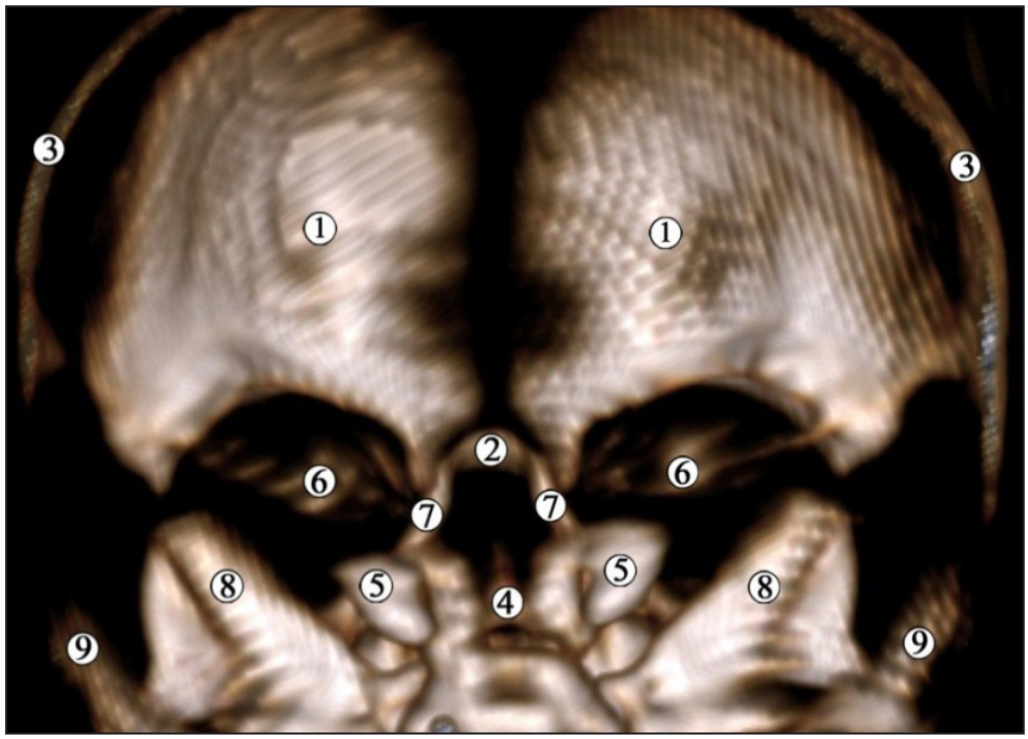

Fig. 5. Computer tomography of the head of a 4-month-old human fetus (180.0 mm PCaL). Posterior projection. Magnification: 3x. Signs:
1 – frontal bone; 2 – ethmoidal bone; 3 – parietal bone; 4 – the body of the sphenoidal bone; 5 – greater wing of the sphenoidal bone; 6 – lesser wing of the sphenoidal bone; 7 – frontal process of the maxilla; 8 – petrous part of the temporal bone; 9 – squamous part of the temporal bone.

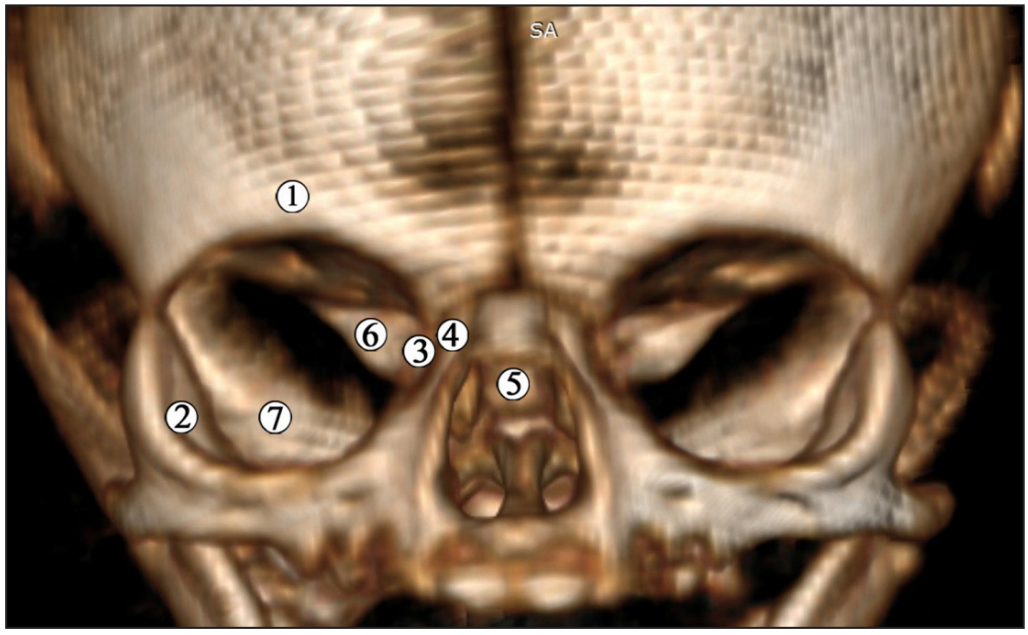

Fig. 6. Computed tomogram of the head of a 6-month-old human fetus 260.0 mm PCaL. Frontal projection. Magnification: 3x. Signs:
1 – frontal bone; 2 – zygomatic bone; 3 – ethmoidal bone; 4 – frontal process of the maxilla; 5 – body of the sphenoidal bone; 6 – lesser wing of the sphenoidal bone; 7 – greater wing of the sphenoidal bone.

temporal fossa with the orbit narrows, but the Müller muscle still delimits the orbit from the sphenopalatine-infratemporal complex.

From the middle of the 5th month of IUD, the diameter of the orbit is 9.7 mm. Due to the union of the medial and lateral centers of ossification, a definitive lesser wing of the sphenoidal bone is formed, which leads to the separation of the sphenopalatine and infratemporal fossae by a bone layer. Due to the development of the lesser wing of the sphenoidal bone, which surrounds the optic nerve, the definitive optic canal begins to form. The space between the greater and lesser wings of the sphenoidal bone is the beginning of the superior orbital fissure. At the end of the 5th month of IUD the orbit changes its shape to a more rounded, mesoconchal one. This is also facilitated by an increase in the height of the fetal orbit due to the development of the facial skeleton and an increase in the side wall of the nasal cavity (due to the development of the paranasal sinuses). Ossification centers appear in the ethmoidal bone and branches of the nasal concha.

On the 6th month of IUD, intensive ossification of the frontal, sphenoidal and ethmoidal bones and maxilla is visible on computer tomograms (Fig. 6). Müller's

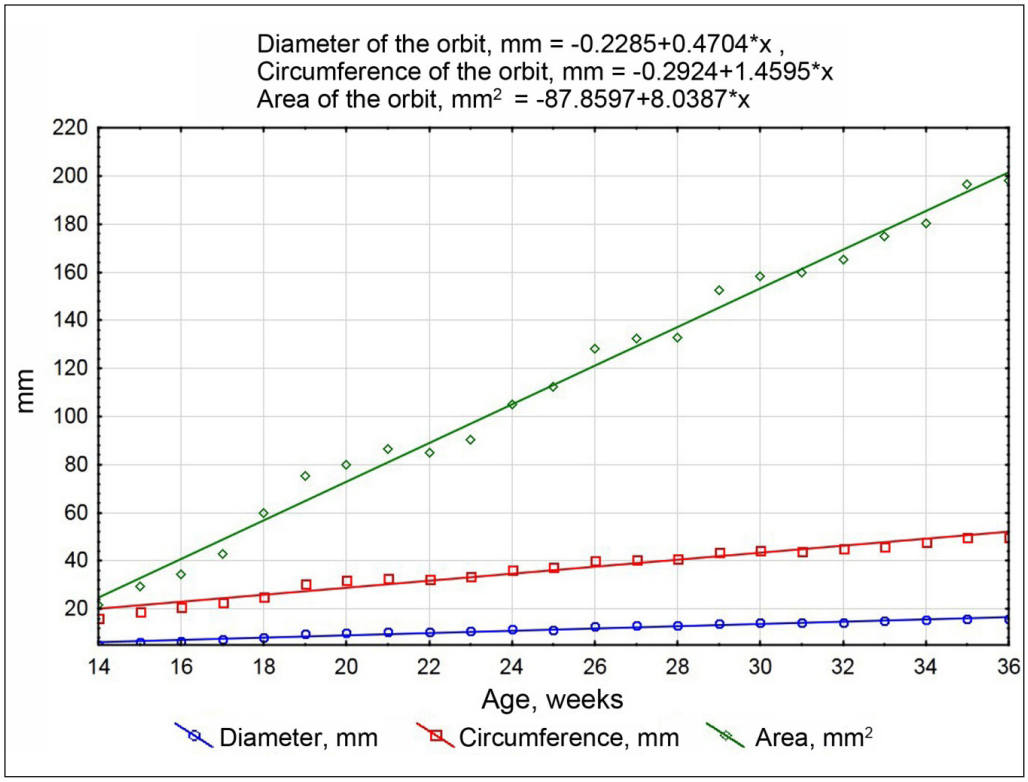

Fig. 7. Morphometric parameters of the orbit (diameter, circumference and area) in the dynamics of the fetal period of human development.

muscle decreases in size, and its smooth muscle fibers are replaced by bundles of collagen fibers.

At the end of the 6th month of IUD, the average diameter of the fetal orbit is 12.8 mm. The cartilaginous tissue of the rudiments of the bones of the orbit apex transforms into bone tissue, and the perichondrium transforms into periosteum with the formation of the tendon ring of Zinn around the optic canal and in the middle part of the superior orbital fissure.

At the end of the 8th month of IUD, the rudiment of the maxillary sinus appears. The structure of the lesser wing of the sphenoidal and frontal bones is approaching the definitive one. These bones come together and form a temporary orbito-spheno-frontal suture. The frontal bone and the greater wing of the sphenoidal bone also converge near the lateral wall of the orbit, forming a permanent lateral spheno-frontal suture. Ossification of the maxilla is progressing, although the ethmoidal bone is still partially ossified.

At the end of the 9th month of IUD, the average diameter of the orbit is 16.5 mm. As a result of the increase in the height of the orbit, it acquires a hypopsychonal form. At the end of the fetal period, the orbit should be considered still rudimentary, since it contains non-ossified connective tissue, primarily in the region of its apex, the ossification of the ethmoidal bone is not yet complete, and almost 50% of the inferior wall of the orbit is represented by Muller's muscle, which decreases in thickness, but not by area.

The analysis of the age dynamics of the morphometric parameters of the orbit during the fetal period of the IUD made it possible to establish the peculiarities of changes in its diameter, circumference and area. In general, these changes are characterized by linear growth (Fig. 7) with periods of slight intensification and deceleration, which are characterized by an uneven course of morphogenetic changes and topographic-anatomical relationships between the bones of the orbit, and therefore they can be critical in view of the appearance of variants of the structure and congenital malformations of the orbit.

Critical periods of the development of the orbit are the 6th month of IUD, during which there is an uneven growth rate of the horizontal size of the orbit relative to the vertical one, and its shape begins to return to the mesoconchal type, which is characteristic of prefetuses. These age-related changes in the shape and size of the orbit are caused by the growth of the eyeball, skull and face, which generally determines the shape of the orbit. Starting from the 8th month of IUD, the structure and topography of the orbit structures begin to acquire signs of a definitive state, the shape of the orbit is finally established, uniform growth rates of all parameters are determined, except for the growth of the circumference of the orbit at the end of the prenatal period of human ontogenesis.

DISCUSSION

Studies of the peculiarities of morphogenesis, structure, constitutional, sex-age anatomical variability of the

bones of the human skull in the pre- and postnatal periods of human ontogenesis do not lose their relevance in connection with the tendency to increase the frequency of congenital malformations that cause severe perinatal pathology, difficulties in diagnosis, treatment and increase of childhood disability of human head structures [5, 6, 7]. Our study of the peculiarities of the morphogenesis of the orbit covers the entire prenatal period – from the origin of the orbit bone sources to the formation of its definitive structure, and the ossification processes continue even after birth.

It is known that the process of ossification of the human cartilaginous skull begins with almost 110 central centers of osteogenesis in the embryonic period of IUD [9]. These centers of ossification form 45 bones of the skull of a newborn, which begin to fuse in the postnatal period of development, and already in an adult their number is 22 bones. After the completion of their ossification, cartilaginous tissue is still preserved in some structures of the skull. Therefore, the formation of the skull continues until a person is 20 years old [1, 2, 6].

On the 8th week of IUD, membranous osteogenesis of the frontal bone occurs, which begins near the supraorbital bulge and spreads from front to back. Also, in this age period, the cartilaginous rudiment of the lesser wing of the sphenoidal bone lateral to the optic nerve is visualized. During the 8th week of IUD, the membranous ossification of the zygomatic and palatine bones is also observed, which generally agrees with the results of other researchers [3, 9]. It should be noted that the inferior wall of the orbit during this period of development is represented by Müller's orbital muscle, which delimits it from the pterygopalatine fossa. Muller's muscle is almost the only example of osteogenesis that begins with muscle tissue [10].

Ossification of the orbital plate of the frontal bone, which begins medially, as well as the lacrimal and orbital plates of the greater wing of the sphenoidal bone occurs in prefetuses of the 10th week of IUD. During this period of development, a lesser wing of the sphenoidal bone is formed, which gradually surrounds the optic nerve, and already at the beginning of the 5th month of IUD, the beginning of the optic canal is formed, and the space between the large and lesser wings turns into the superior orbital fissure. At the end of the 5th month of IUD, ossification centers also appear in the ethmoidal bone. In 6-month-old fetuses, the ossification of the frontal, sphenoidal and ethmoidal bones and maxilla accelerates. The Muller's muscle decreases in size and transforms into bundles of collagen fibers. We agree with Osanai H. et al. [10], who believe that the newly formed periosteum will eventually ossify with the formation of a bone plate along the inferior orbital fissure. At the end of the 8th month of IUD the beginning of the maxillary sinus appears.

The critical periods of the development of the orbit are the 6th month of IUD, during which there is an uneven growth rate of the horizontal size of the orbit relative to the vertical one, and its shape begins to return to the mesoconchal type, which is characteristic of prefetuses. These age-related changes in the shape and size of the orbit are due to the growth of the eyeball, skull and face, which generally determines the shape of the orbit and is consistent with the opinion of other researchers on this issue [11 - 15].

The morphometric regularities of changes in the parameters of the orbit during the fetal period that we have revealed can be useful for early diagnosis of variants of the structure and defects in the development of the orbit and head structures as a whole [16].

Until recently, the issues of typical and sexual variability of the shape and size of the calvaria, base, facial part of the skull and, in particular, the orbit, remained poorly studied. In our opinion, the research of the listed issues is relevant and dictated by the requests of anthropology, neurosurgery, maxillofacial surgery and forensic medicine. The detailing of morphological data on the structure of the bones of the calvaria, base and facial part of the skull is currently also needed to solve the problems of theoretical morphology, anthropology and bioengineering [5, 6, 7, 17].

CONCLUSIONS

1. The first signs of osteogenesis around the main nervous and visceral contents of the orbit rudiment are observed in 6-week-old embryos in the form of seven cartilaginous bone models. The first signs of ossification in the region of the orbit are found in the maxilla. During the 6th month of intrauterine development, intensive processes of ossification of the frontal, sphenoidal, ethmoidal bones and maxilla are noticeable.
2. At the end of the 8th month of intrauterine development due to the processes of ossification of the lesser wing of the sphenoidal and frontal bones, the orbit acquires definitive structural features.
3. From the beginning of the fetal period of human ontogenesis, the ossification of bone rudiments that form the walls of the orbit continues. The processes of ossification of the structures of the sphenoidal bone continue, which leads to morphological transformations of the orbit in 5-month-old fetuses – it is separated from the sphenopalatine and infratemporal fossae by a bone layer, the optic canal is formed, and in 6-month-old fetuses, processes of ossification

of the frontal, sphenoidal and ethmoidal bones and maxilla occur, Müller's muscle changes its structure to a fibrous one.

4. The analysis of the age-related dynamics of the morphometric parameters of the orbit during the fetal period of development made it possible to establish the peculiarities of changes in its diameter, circumference and area, which is expressed by mathematical functions:

Diameter of the orbit, mm = -0.2285+0.4704*x ,
Circumference of the orbit, mm = -0.2924+1.4595*x
Area of the orbit, mm^2 = -87.8597+8.0387*x ,
where x is the age of the human fetuses in weeks.

5. Critical periods of the orbit development are the 6th month of prenatal ontogenesis, during which there is an uneven growth rate of the horizontal size of the orbit relative to the vertical one, and its shape begins to return to the mesoconchal type, which is inherent in prefetuses, and the 8th month, during which the growth of all parameters of the orbit slows down due to intensive processes of organogenesis of its visceral structures.

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ORIGINAL ARTICLE

COMPARISON OF INTRAMUSCULAR VERSUS INTRAVENOUS KETAMINE FOR SEDATION IN CHILDREN UNDERGOING MAGNETIC RESONANCE IMAGING EXAMINATION

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ABSTRACT

The aim: To compare efficacy of intramuscular (IM) versus intravenous (IV) ketamine for sedation in children undergoing brain MRI scanning in children.

Materials and methods: Children who required elective brain MRI were selected for this study. They were randomly divided into two groups; group I received 1.5 mg/kg IV Ketamine and group II received 4 mg/kg IM ketamine. In each group supplementary 0.1 mg/kg midazolam intravenously before positioning on MRI table was given. Patients were monitored for pulse rate, SP02, and respiratory wave.

Results: Children who received IM ketamine had significantly shorter scan time and a greater success rate of sedation with first dose than the IV group. The proportions of scan interruption and scan repeat were significantly higher among the IV group than in the IM group. The scan time was longer among the IV group than in the IM group with significantly more scan interruption and repeat. Satisfaction with sedation as expressed by the technicians was significantly more in the IM group than in IV group (98.1% *vs.* 80.8%, P= 0.004).

Conclusions: Intramuscular ketamine injection was predicted to have a better sedative success rate and takes less time to complete than intravenous administration. This makes IM ketamine more appealing in certain conditions.

KEY WORDS: Intramuscular, Intravenous, Ketamine, MRI, Scan time

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INTRODUCTION

Magnetic Resonance Imaging (MRI) is a non-invasive imaging technique used to achieve good quality image and provides proper diagnosis. It requires patient`s immobility, which is difficult to control in children, especially in dark and noisy environment, therefore they need to be sedated or otherwise anaesthetized in order to attain successful MRI examination without motion artifact [1, 2].

Sedation can be done with different drugs either as a single or in combination. Anaesthetic agents can be administered safely under anaesthesiologist supervision. However, selecting the route of administration is important to control pf sedation with the minimal stress to the child [3]. Intravenous midazolam-ketamine or midazolam-propofol combinations were used among other regimens to sedate children for MRIs examination [4-6]. While a combination of Dexmedetomidine and Ketamine was applied by Kim et al. [4], others, investigated propofol/ketamine versus propofol/fentanyl [5]. Tith et al. [6] compared the safety of propofol, mixed pentobarbital/propofol, and mixed pentobarbital group requiring supplemental sedation in pediatric patients following deep sedation for noncardiac MRI.

This study, therefore, was designed to compare intramuscular (IM) versus intravenous (IV) ketamine to attain satisfactory sedation to maintain patient stability with proper image quality. Ketamine is a phencyclidine analog that causes dissociative anaesthesia. It is a famous drug that known by its rapid-onset of action, rapid recovery and preservation of airway reflexes. This drug is most popular to physicians and can be given safely via different routes. Intravenously administered ketamine has a rapid onset of action but otherwise rapid clearance that necessitates rescue dose when used without supplemented anaesthetic agents [7]. On the other hand, IM ketamine injection can provide slow release of the drug from the muscle and maintain a plasma level for a reasonable time.

Unlike adults, MRI examination in children should be completed and interpreted at the same session. Scanning time depends on the type of MRI machine,

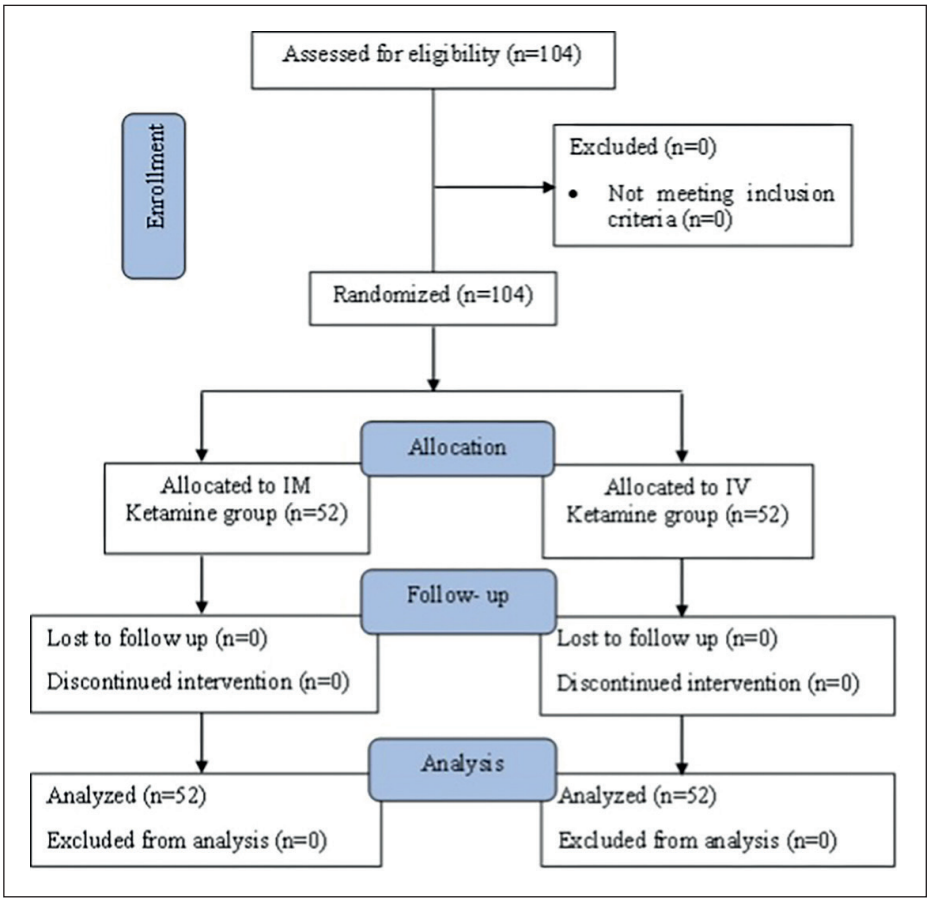

Fig. 1. Design of the research (CONSORT Diagram).

requested sequences, examining aria and imaging techniques [8]. Any interruption of procedure leads to delay in scanning time that mandates additional dose of anaesthesia. In certain cases, when image artifact is present or body movement was noticed the technician decides to repeat the scan. As a rule, MRI scanning is either interrupted when patient movement is obvious or there is any obstacle, that needs intervention and scan pause.

THE AIM

The aim of study is to compare efficacy of intramuscular versus intravenous administration of ketamine for sedation in children undergoing brain MRI scanning.

MATERIALS AND METHODS

This prospective randomized interventional study ware done from August 2021 to July 2022 at the Basrah University Medical Centre, and include children aged 2-7 years who were referred to the center for brain MRI examination during that period. This study was conducted in line with the Consolidated Standards of Reporting Trials (CONSORT) checklist (Fig.1). The Ethical Committee of College of Medicine, University of Basrah approved the study [Project ID: 030409-051-2022]. A written information consent was obtained from all participants before enrolment in the study.

The sample size was determined by using of the sample size formula and for comparison of the means values of scan time (the primary outcome) of the two groups selected significance level of 0.05 with test power of 90% [9]. Due to the lack of a previous study with a similar design, the results of the pilot study on 42 patients (21 patients in each group) were considered. The sample size was determined to be 90 patients (45 patients in each group). Given the possible attrition rate of 15%, the ultimate sample size was anticipated to be 104 patients. The participants were randomly assigned into two equal groups (52 patients in each group) using a computer-generated random number list in 1:1 ratio from the website of https://www.randomizer.org. The first group was assigned to receive ketamine (2 mg/kg) IV; the second group was given ketamine (4 mg/kg) IM, and the lateral side of the thigh of the child was used for injection. The protocol of study was explained to the parents and written informed consents were obtained from the parents or accompanying guardian before enrollment of the children in the study. These children comply with the American Society of Anaesthesiologists (ASA) physical classes 1 and 2. Children were

excluded if they were on antiepileptic drugs, allergic to the proposed medications used in the study, children who were recently fed, those with recent history of upper respiratory tract infections or when the MRI was not applicable.

The MRI SIEMENS MAGNETOM Avanto (1.5T) model No. 07391167 (Germany) was used. It is fully equipped with a broad range of dedicated Syngo MR applications for every clinical field with high-quality morphological and functional techniques and high-resolution imaging. For the imaging the Syngo MR B19 imaging software was used.

Prior to imaging, proper assessments of the child were made, and the parents were asked to ensure that the child was not fed solid food for about 4 hours or clear fluid for 2 hours before the examination. All children had their baseline heart rates, mean arterial pressure, and peripheral oxygen saturations (SpO2) recorded using MRI compatible monitor.

Ketamine used in the study was available as hydrochloride 50 mg/ml. To ensure painless injection, a topical local anaesthetic EMLA cream (contains lidocaine 2.5% and prilocaine 2.5%) was applied on the thigh at the site of IM injection or at the site of IV cannula insertion.

When the machine became ready, the child was admitted to the MRI unit. In Group 1 after inserting of a suitable IV line, the child received 1.5 mg/kg IV Ketamine followed by 0.1 mg/kg midazolam IV. While in Group 2, the child was given Ketamine 4 mg/kg IM in the lateral side of the thigh and was observed for loss of consciousness. Soon after that, an IV line was fixed and 0.1 mg/kg midazolam was given IV.

After proper positioning was achieved on the MRI table and the coil placed properly, the child passed to the MRI tunnel. The technician initiates settings and scan started thereafter. Breathing pattern was continuously observed on the examination screen by placing breathing paddle on the patient chest.

While the anaesthesiologist thoroughly monitors SpO2, pulse rate, blood pressure and respiration wave using MRI compatible monitor and looks for any obvious movement throughout the examination. During scanning, if there was breathing obstruction, SpO2 below 95%, vomiting, hiccup, and visible movement of the patient or any doubtful changes in the vital signs, the anaesthesiologist alerted the technician to stop examination until the event was controlled and an additional dose of ketamine (1 mg/kg) was given intravenously and the scan resumed. The sequence was repeated when there was motion artifact.

The primary outcome was scan time, which was calculated from starting of first sequence until ensuring successful last sequence of MRI examination. It was calculated using mobile stopwatch. Then, the child was transferred to a recovery bed and maintained under close observation until full recovery and ready to be discharged.

The secondary outcomes were: successful sedation as reflected by successful rate of MRI examination, and presence of adverse effects such as scan interruption, scan repeat, and vomiting. Sedation was considered successful if MRI was completed without image distortion that needs scanning repeat (Fig.2). Each sequence was assessed individually and considered, as successful when there was uniform navigation.

Patients were discharged when they respond to commands (Ramsay sedation scale score is 3).

Otherwise, hazy image, artifact or overlapping images on navigation reflected unsuccessful sequence (Fig. 3).

STATISTICAL ANALYSIS

Statistical analysis was performed by using of SPSS (Statistical Package for Social Sciences) version 23, (IBM, Chicago, Illinois, USA). Numbers and percentages were used to describe categorical variables, whereas continuous variables were expressed as means ± standard deviations. Continuous data were compared with independent sample t-test while categorical data were analyzed with Chi-square and Fisher tests. A P value < 0.05 indicates statistical significance.

RESULTS

A total of 104 children subjected to brain MRI examination and required sedation were included in this study. The first group received ketamine (IV) and the second received ketamine (IM). Of the total number, 68 (65.4%) patients were males. The mean age was 3.9±1.4 years. No significant difference was revealed between the two groups regarding demographic characteristics. Similarly, no significant difference were found between the two groups regarding hemodynamic variables. At base line, there was no significant difference in pulse rate between the IV and IM groups (94.8 ± 6.1 vs. 93.9 ± 7.3, $p = 0.492$). The mean maximal pulse rate reached during sedation in the IV group was 96.0 ± 15.1 compared to 92.6 ± 7.1 in the IM group, with no significant difference ($p = 0.145$). The pulse rate was increased in the group treated with IV ketamine compared to the baseline value, while the pulse rate was decreased in the group, which received IM ketamine.

After 5 minutes of sedation, the mean arterial pressure (MAP) in both groups declined and rose to baseline after 10 minutes. There was no difference between the two

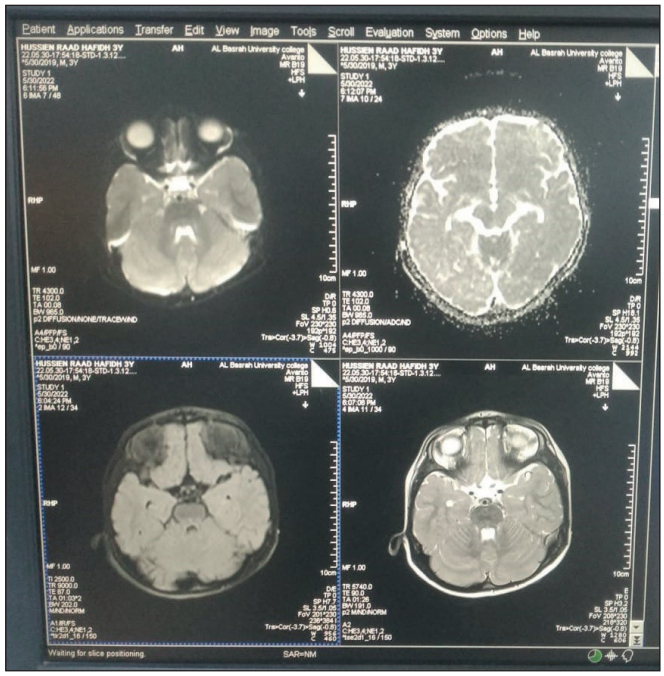

Fig. 2. MRI completed without image distortion.

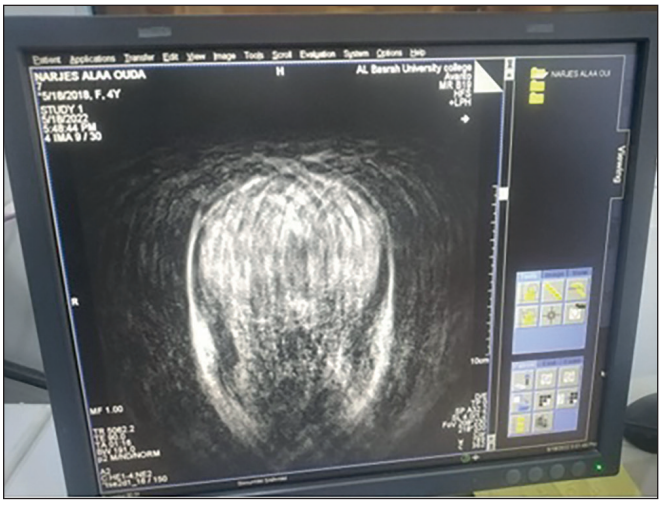

Fig. 3. Unsuccessful sequence.

groups. At baseline, there was no significant difference in SpO2 saturation between the two groups. However, both groups showed a drop in SpO2 levels during sedation, with the IV group experiencing a greater decline than the IM group. The difference in SpO2 between the two groups during sedation was significant ($p = 0.031$) (Table 1).

The success rate of sedation, as defined by neither interruption of MRI examination nor scan repeat, was significantly higher among IM group than the IV group ($P \leq 0.001$). The scan time was significantly longer in the IV group than in the IM group. The proportion of vomiting was lower in IM group than in the IV group, but the difference between the two groups did not achieve significant difference (6.0% vs. 7.7%, $P = 0.999$). The proportions of scan interruption and scan repeat were significantly higher among the IV group than in the IM group. Repeated doses were instantly required in the IV group and the difference is statistically significant. The satisfaction with sedation as expressed by the MRI technician was significantly more in the IM group than in IV group (98% *vs.* 80.8%, P= 0.008) (Table II).

DISCUSSION

Successful MRI scanning in children should be completed in one session and this mandates persistent steady child until completing the scan as any mobility can affect image quality [10]. Child movement is difficult to control in dark and noisy environment, therefore they need to be sedated or otherwise anaesthetized to prevent motion artifact [11].

Ketamine is a famous and widely used drug in anaesthetic practice outside the operation room [12, 13]. It is a popular drug that was used safely by doctors preferably in children since many decades [14, 15]. Furthermore, it can be given via many routes. Nevertheless, the drug is associated with unpleasant hallucination which can be controlled by a benzodiazepine like midazolam [5, 16].

The present study was designed to compare IV and IM ketamine for successful scanning on children presented for MRI. To the best of our knowledge, there were no published data on the use of IM ketamine injection in MRI examination, however there were studies using this route in other fields [3, 7, 12, 14, 17, 18]. The combination of midazolam and ketamine is good and safe choice to offset adverse events of other sedative agent [19-21]. Promising results were obtained in this study with IM route of ketamine. One of these advantages was easy and comfortable IV cannulation, which was seen in this study, as the child who got IM injection appeared calmer than when an IV line insertion in awake patient [22]. Successful sedation is reflected by successful procedure when no interruption happens. Although all scans were completed, there was a difference in time required to finish the requested task.

In this study scanning interruption was remarkably lower in the IM than that of IV. This can be explained by prolonged sedation time achieved by IM injection of ketamine, which assists in slow absorption from the injection site. Many studies agreed with this explanation. On the other hand, rapid metabolism of the IV administered drug resulted in early patient movement.

There was good hemodynamic stability in both groups, despite different rout of administration. Similar results were found in a study used midazolam and ketamine intravenously [23].

Upper airway obstruction occurs in sedated children and is manifested by abnormal breathing patterns and a de-

Table I. Demographic, hemodynamic and respiratory changes during sedation

Character	IV group (n=52)	IM Group (n=52)	P value
Male, No. (%)	36 (69.2)	32 (61.5)	0.150
Age (years), Mean ± SD	3.8 ± 1.3	3.9 ± 1.4	0.766
Weight (Kg), Mean ± SD	16.3 ± 4.0	16.5 ± 3.4	0.794
Pulse rate, (Beats/min.), Mean ± SD			
Baseline	94.8 ± 6.1	93.9 ± 7.3	0.492
Maximum	96.0 ± 15.1	92.6 ± 7.1	0.145
MAP (mmHg), Mean ± SD			
Baseline	80.3 ± 3.5	79.9 ± 2.9	0.588
After 5 minutes	72.8 ± 3.2	73.7 ± 3.7	0.185
After 10 minutes	80.3 ± 4.9	79.8 ± 3.9	0.550
SPO2 (%), Mean ± SD			
Baseline	98.6 ± 0.7	98.7 ± 0.8	0.519
Minimum	95.6 ± 1.5	96.6 ± .9	0.030

Table II. Outcome of the procedures and adverse effects

Character	IV group (n=52)	IM Group (n=52)	P value
Scan time (min.), Mean ± SD	22.3 ± 4.5	18.7 ± 1.6	<0.001
Success rate, No. (%)	38(73.1)	49 (94.2)	0.004
Scan interruption, No. (%)	14 (26.9)	2 (3.8)	0.001
Scan repeat, No. (%)	12 (23.1)	3 (5.8)	0.012
Vomiting, No. (%)	4 (7.7)	3 (5.8)	0.696
Second dose required, No. (%)	26 (50.0)	5 (9.6)	<0.001
Satisfaction, No. (%)	42 (80.8)	51 (98.1)	0.004

crease in SpO2 that was considerably higher in the IV group and consequently leading to more interruption. The higher decrease in SpO2, noticed in the IV group, was caused by uncontrolled head movement, which resulted in airway misalignment. Some researchers, that utilized intravenous ketamine in their studies noticed a decrease in SpO_2 [18, 24].

There have been studies that examined the combination of intravenous midazolam with intravenous ketamine or propofol in MRI with varying rates of sedation success [16, 23].

MRI scan sequences were repeated in (23.1% of the IV group) as a single sequence due to obvious artifact discovered after completing of scanning. Interruption or repeating resulted in prolonged scan time which was seen mainly in IV group. Compared to IV, the IM ketamine required less repetitive doses of ketamine with subsequent smooth recovery. Similar finding was reported by Gharavifard et al. [7]. Some authors who used IV ketamine with propofol infusion also mentioned additional sedation requirement [25].

No significant difference was observed in both groups regarding vomiting which was recorded in the recovery in the group of children who received IV and IM ketamine. On the contrary, few studies reported higher incidence of vomiting after IM injection. Overall, the two routes of administration of ketamine are safe.

Recovery time for the two groups was reasonably accepted. The findings of the present study were in agreement with that reported by Ramaswamy et al. [18]. Though the majority of children in the IM group only received a single IM ketamine injection, just five children required a second dose; hence this group spent less time in the MRI tunnel. While in those who received the drug intravenously, a considerable number of them required repeated doses which subsequently affect the scan time but ultimately the two groups finished the session at the same time.

The proportion of vomiting was not significantly different between the children who received the IM and those who received the IV ketamine (5.8% vs. 7.7%, P=0.696 respectively); the low incidence of vomiting observed in the two groups could be probably related to the antiemetic effect of timely administered midazolam. This is in agreement with the findings reported by others [26-28]. Some authors showed higher rate of vomiting in patients sedated with ketamine/propofol [5].

The convenience with sedation, as expressed by the MRI technicians, was significantly more in the IM group than in IV group (98.1% *vs.* 80.8%, P= 0.004). All patients were discharged within convenient time when they matched the criteria for discharge.

CONCLUSION

To keep a motionless child undergoing a brain MRI examination, intramuscular ketamine injection was predicted to have a better sedative success rate and takes less time to complete than intravenous administration. This makes IM ketamine more appealing in certain conditions.

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ORIGINAL ARTICLE

THE LEVEL OF REACTIVE OXYGEN SPECIES AS A MARKER OF ASTHMA SEVERITY IN CHILDREN

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ABSTRACT

The aim of the research was to assess the reactive oxygen species (ROS) levels in granulocytes of patients with asthma.

Materials and methods: The study involved 35 children aged 5 to 17 years. 26 children with persistent asthma, partially controlled course in the period of exacerbation were divided into groups: 1 group - mild asthma (n = 12), group 2 - moderate asthma (n = 7) group 3 - severe asthma (n = 7) and control group included almost healthy children (n = 9).

ROS levels in granulocytes were evaluated using BD FACSDiva™. The spirographic complex was used to assess the function of external respiration

Rresults: The level of ROS in granulocytes of patients with severe asthma was significantly reduced compared with children in the control group and patients with mild and moderate asthma ($p_{1-3} = 0.0003$, $p_{2-3} = 0.0017$, $p\ c-_3 = 0.0150$).

The concentration of ROS in granulocytes ≤ 285 a.u. was prognostically significant with high specificity and sensitivity with severe asthma.

Conclusions: The concentration of ROS levels in neutrophils in patients with severe asthma probably reflected the suppression of their products, which suggests the depletion of the reserve capacity of neutrophils.

Decreased concentrations of reactive oxygen species in children with asthma can be considered as a possible marker of asthma severity.

KEY WORDS: asthma; flow cytometry; granulocytes; reactive oxygen species; children

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INTRODUCTION

Asthma is a chronic inflammatory disease of the respiratory tract. It is characterized by bronchial hyperreactivity and reversible airflow limitation [1-2]. At present, asthma is one of the most common chronic pathologies among children and adolescents [3].

According to WHO estimates about 300 million people suffer from asthma. Asthma incidence rates are 1-18%. In children, this figure varies 5 to 10% [4 - 5].

There are many diagnostic possibilities for asthma in the world, but achieving control remains an open and relevant topic [6-8].

Identification of biomarkers of pediatric asthma is an active area of research. It can potentially bring great clinical benefits and represent a step forward to individual treatment: the so-called precision medicine [9-12].

It is well known that inflammation of the respiratory tract occupies a major position in the pathogenesis of asthma. Studies suggest that eosinophils and neutrophils are the major cells that contribute to inflammation. Increased neutrophils in sputum, bronchoalveolar lavage fluid (BALF) or biopsy is more common in severe asthma [13 - 16].

Recent studies have found that the bronchial biopsy of children with controlled and uncontrolled severe asthma contains varying amounts of intraepithelial neutrophils. In the group with increased neutrophilia, the number of intraepithelial airway neutrophils correlated with better lung function, better symptom control, and a lower maintenance dose of inhaled glucocorticosteroids [11, 17].

Neutrophils are cells that have a unique function of phagocytosis and form the first line of non-specific protection. An important feature of neutrophils is the "respiratory explosion", which results in the production of reactive oxygen species (ROS). The ability to generate

ROS also characterizes the functional activity of neutrophils, namely the possibility of complete phagocytosis [18 - 20]. ROS plays several important roles in an organism. First, the ROS formation is a natural physiological process that constantly takes place in an organism. Secondly, ROS, formed in increased quantities, act as damage factors. Third, ROS is seen as a signaling system involved in key regulatory mechanisms of the living cell. The resulting ROS are involved in two divergent but ongoing biochemical processes - the catabolism of old and the synthesis of new molecules [21-23].

Various methods are used for in-depth study of asthma biomarkers, but in pediatric practice the technique of conducting some of them is difficult. For example, biopsy and bronchoalveolar lavage are invasive, and the collection of induced sputum has age restrictions [11-12].

Therefore, it is advisable to study the concentration of biomarkers in peripheral blood, as in the most accessible environment in routine practice.

That is why peripheral blood is a promising source of biomarkers.

In this study, we conducted a clinical and prognostic assessment of the levels of reactive oxygen species in granulocytes of children with persistent asthma, considering ROS as a signaling system of functional activity of neutrophils capable of phagocytosis.

THE AIM

The aim of the study: to assess the level of reactive oxygen species (ROS) in granulocytes (neutrophils) in children with asthma.

Tasks of the research: 1) to study the clinical and anamnestic features of asthma in children;

2) assess the ROS levels with varying degrees of persistent asthma;

3) Investigate the relationship between the ROS levels and indicators of the function of external respiration, which reflect the disease severity in childhood asthma at different stages of the disease. 4) Based on the results obtained to create a model for predicting the asthma severity in children.

MATERIALS AND METHODS

DESCRIPTION OF PATIENTS

This was a prospective cohort examination of children from 5 to 17 years with asthma: allergic (Ig E-dependent or Ig E-independent), persistent mild, moderate, severe (2 - 4 degrees of severity), partially controlled, on exacerbation. Patients' diagnoses were in line with accepted GINA 2020 recommendations [4].

Exacerbations were mild to moderate in severity. The study was conducted from September to December 2020 at a children's hospital. The study included all children who were admitted to the pulmonology department and met the criteria for inclusion and exclusion.

Inclusion criteria: patient's age from 5 to 17 years with asthma, patient's with symptoms of asthma exacerbation; in 1-2 days from the beginning of asthma exacerbation; signing informed consent by the both patient's parents and patients older than 14.

Exclusion criteria: children under 5 years of age; patients without written consent to conduct the study; patients with acute bronchitis simple, acute obstructive bronchitis, intermittent asthma, pneumonia; patients diagnosed with remission and controlled asthma; with congenital and chronic cardiopulmonary or neurological diseases; hereditary diseases that lead to changes in the functioning of the respiratory tract, including cystic fibrosis; proven immune deficiency; patients with severe somatic condition and decompensation of vital functions; suspected or confirmed gastroesophageal diseases; patients with neoplasms of any localization; pregnant girls.

Assessment of asthma exacerbation was performed according to the recommendations of GINA 2020 using the criteria of severity of asthma exacerbation [4].

To assess the control used diagnostic tests - c-AST, taking into account the age of children. Test for children aged 4-11 years and 12 years and adolescents [4,21,22]. According to the obtained results and GINA criteria, there was a partially controlled asthma (2 criteria were positive).

Patients received basic treatment for asthma according to the GINA 2020 recommendations [4].

Children with persistent asthma (grade 2 severity), partially controlled during exacerbation, mild exacerbation, received therapy according to step 2 GINA (low-dose ICS and short-acting β2-agonists (if necessary)) [4].

Children with moderate asthma (grade 3), partially controlled during exacerbation, mild exacerbation, received treatment according to GINA step 3 (combination drugs (low-dose ICS / β2-long-acting) and short-acting β2-agonists). for needs)) [4].

Children with persistent severe asthma (grade 4), partially controlled during exacerbation, mild exacerbation, received treatment according to step 4 GINA (combination drugs (medium doses of ICS / β2-long-acting) and short-acting β2-agonists). for needs)) [4].

All patients underwent physical and laboratory examination. The viability and level of reactive oxygen species (ROS) in granulocytes were also studied in children. The study of these indicators was performed in the first two days of hospitalization in the presence of clinical manifestations and wheezing.

The control group consisted of 9 healthy children (of similar age / sex) without any signs of chronic or acute illness during the previous three months who were referred for age control or vaccination. Parents of all patients were informed of the objectives of the study and received written informed consent before enrollment in the study.

Patients were divided into groups depending on the degree of asthma: 1 group - asthma mild persistent (n = 12), 2 group - asthma moderate persistence (n = 7), 3 group - asthma severe persistent (n = 7), the fourth is the control group (n = 9).

Collection of blood samples and preparation of leukocyte suspensions

Blood samples from patients and control subjects who participated in the study were collected in the morning on an empty stomach. Sterile K2EDTA Vacutainers were used for blood collection (IMPROVACUTER Evacuated EDTA K2 Spray Dried PET Tubes, Guangzhou, China). Within two hours after the collection of samples, they were used to obtain leukocyte suspensions. Briefly, 50 µl of blood were lysed using a working solution of BD Pharm LyseTM Lysing Buffer (Becton, Dickinson and Company, BD Bioscience, San Jose, CA, USA).

ROS levels were evaluated in granulocytes using H2DCFDA staining

Leukocyte suspensions were stained with a ROS-sensitive dye 2',7'-dichlorodihydrofluorescein diacetate (H2DCFDA, InvitrogenTM, USA). Its 10 mM stock solution in dimethyl sulfoxide (DMSO, Sigma-Aldrich, USA) was used to prepare a working solution in PBS. Working solution was added to leukocyte suspensions (10 µM). Incubation with H2DCFDA lasted for half an hour in the dark. Simultaneously, 10 µl of 7-aminoactinomycin D (7AAD) was added to the samples. This dye is used to distinguish viable from non-viable cells, since it can penetrate inside the cells only when the cell membrane integrity is compromised. 7AAD becomes fluorescent upon binding to DNA. Thus, non-viable cells are 7AAD-positive (Fig. 1).

DATA ACQUISITION AND POST-ACQUISITION ANALYSIS

The population of granulocytes was allocated using BD FACSDiva™ software (Becton Dickinson, USA) based on forward and side scatters. Thereafter, the region of viable 7AAD-negative granulocytes was gated based on the 7AAD fluorescence acquired in the FL3 channel.

H2DCFDA is known to be converted to a fluorescent dichlorofluorescein (DCF) after the cleavage by esterases inside the cells and subsequent interaction with ROS. DCF fluorescence in viable 7-AAD-negative granulocytes was detected in the FL1 channel by BD FACSCanto™ II Cell Analyzer (BD Biosciences, USA). DCF fluorescence is known to be proportional to intracellular ROS levels. To assess intracellular ROS levels, the mean fluorescence intensity (MFI) of DCF fluorescence in 7AAD-negative granulocytes was compared between groups [26,27].

The assessment of the children's external respiration function was performed using the spirographic complex "SpiroCom AINC.941311.005 I". It was manufactured by the National Aerospace University "HAI" STC of electronic medical devices and technologies "HAI-Medica", Kharkiv, Ukraine (TU U- 33.1-02076 005-2002). The study was performed according to the standard method of spirography.

All statistical calculations were performed using batch program StatSoft STATISTICA version 8 (Tulsa, OK) and MedCalc statistical software versions 17.2.

BIOETHICS

The planned clinical study was carried out after receiving approval by the Ethics and Bioethics Commission of Kharkiv National Medical University on October 2, 2019, protocol № 6 and was conducted in accordance with the principles of the Helsinki Declaration, amended in October 2013.

RESULTS

GENERAL INFORMATION

Of the 28 children surveyed, 42.8% (12) had persistent mild asthma, 25% (7) had persistent moderate asthma, and 25% (7) had persistent severe asthma. No significant statistical difference was found between the groups in the collection of life history and disease. This concerned children's sex and age, the presence of atopy in the patient and close relatives (the presence of atopic dermatitis, allergic rhinitis, allergic diseases in the family, asthma in the family). The laboratory examination revealed no difference in the levels of eosinophils and neutrophils. The increase in IgE level in patients with moderate and severe asthma relative to mild asthma was statistically significant (Table I).

Examination of lung function revealed a statistically significant decrease relative to the severity of asthma: FEV1(Forced Expiratory Volume in one second), FEV1 / FVC (Tiffno test), PEF (Peak Expiratory Flow) were significantly lower in patients with severe asthma relative to mild (Table I).

Correlation between ROS levels in 7AAD-negative granulocytes and FEV1, FEV1 / FVC, PEF.

There were also a direct and positive correlations between ROS levels in 7AAD-negative granulocytes and FEV1 $r = 0.6394$ $p < 0.05$; FEV1 / FVC $r = 0,7322$ $p < 0.05$; PEF $r = 0.6387$ $p < 0.05$.

Table I. Demographic and clinical characteristics of the subjects

Sign	units	Mild asthma (Group 1)	Moderate asthma (Group 2)	Severe asthma (Group 3)	p
	n	n = 12	n = 7	n = 7	
Gender, M/F	n	7/5	1/6	3/4	P1 > 0,05 P2 <0,05. P3 > 0,05
Age, years	Me (Lq; Uq)	9,5 (5,0;17,0)	11,4 (8,0;16,0)	12,7 (7,0;17,0)	p1-2 – 0, 125 p1-3 – 0, 132 p 2-3 – 0, 612
Presence of atopic dermatitis	%, n	58,3% (7/12)	85,7% (6/7)	85,7% (6/7)	p1-2 – 0,1114 p1-3 – 0,5000 p 2-3 – 0,1114
Present of allergic rhinitis	%, n	41,6% (5/12)	57,1% (4/7)	28,5% (2/7)	p1-2 -0,2549 p1-3 -0,2896 p 2-3 -0,1476
Present of allergic disease in relatives	%, n	41,6% (5/12)	71,4% (5/7)	71,4% (5/7)	p1-2 – 0,1193 p1-3 – 0,1193 p 2-3 – 0,5000
Presence of asthma in relatives	%, n	25% (3/12)	42,8% (3/7)	42,8% (3/7)	p1-2 – 0,2135 p1-3 – 0,2135 p 2-3 -0,5000
High eosinophil blood	%, n	8,3% (1/12)	14,2% (1/7)	28,5% (2/7)	p1-2 – 0,6821 p1-3 – 0,2419 p 2-3 – 0,5075
IgE increase, IU/ml	%, n	66,6% (8/12)	100% (7/7)	100% (7/7)	p1-2 – 0,0279 p1-3 – 0,0279 p 2-3 – 1,0000
High neutrophil blood	%, n	0% (0/12)	0% (0/7)	0% (0/7)	p1-2 – 0,5000 p1-3 – 0,5000 p 2-3 – 0,5000
FEV1 %	Me (Lq; Uq)	104,0 (101,0; 122,5) %	100,0 (89,0; 104,0)	78,0 (73,0; 78,0)	KW: H= 16,286; p= 0,0003 MW: p1-2 – 0,0692; p1-3 – 0,0003; p2-3 – 0,0026.
FEV1/FVC %	Me (Lq; Uq)	107,5 (106,5; 110,5) %	102,0 (95,0; 104,0)	92,0,0 (89,0; 97,0)	KW: H= 16,975; p= 0,000 MW: p 1-2 –0,0060; p1-3 – 0,0004; p 2-3 –0,0350.
PEF %	Me (Lq; Uq)	106,0 (104,5; 118,5) %	104,0 (80,0; 109,0)	61,0 (42,0; 69,0)	KW: H= 14,674; p= 0,0007 MW: p1-3 – 0,2907; p1-3 – 0,0004; p 2-3 –0,0040.

KW — Kruskal-Wallis test; Me (Lq; Uq) — median (lower quartile; upper quartile); MW— Mann-Whitney test; p significant with the Bonferroni correction.

Concentration of ROS in neutrophils

In the current study, ROS production by granulocytes was analyzed in patients with different stages of asthma: Me (Lq; Uq) (Group 1) - 393,0 (353,0; 457,0), a.u.; (Group 2) - 355,0 (290,0; 411,0), a.u.; (Group 3) - 274,0 (204,0; 283,0), a.u. H2DCFDA staining indicated that mild and moderate asthma was not associated with reduced ROS levels in 7AAD-negative granulocytes, i.e. viable

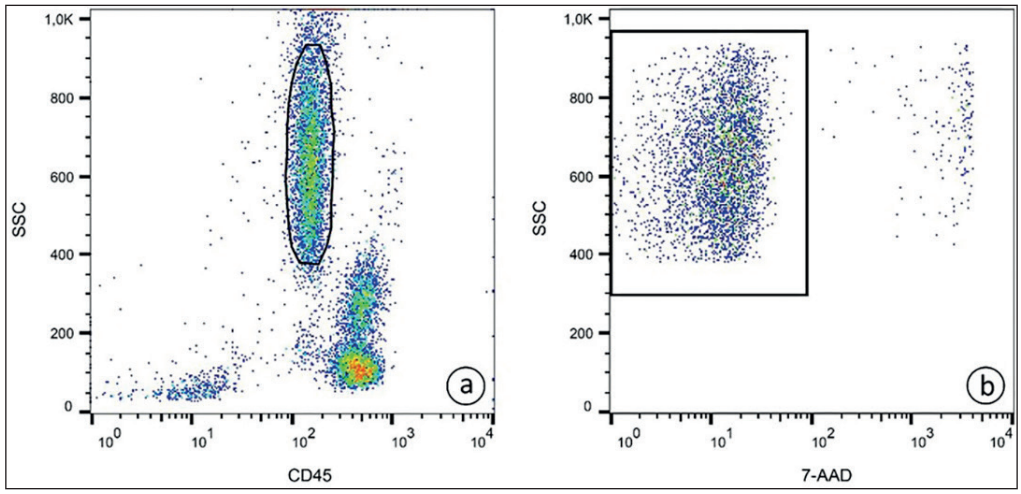

Fig. 1. Representative images that reveal the gating strategy used for isolating the subpopulation of granulocytes in the population of CD45-positive cells (SSC/FL6 dotplot, panel a) and viable cells stained negatively with the DNA intercalator 7-aminoactinomycin D (panel b).

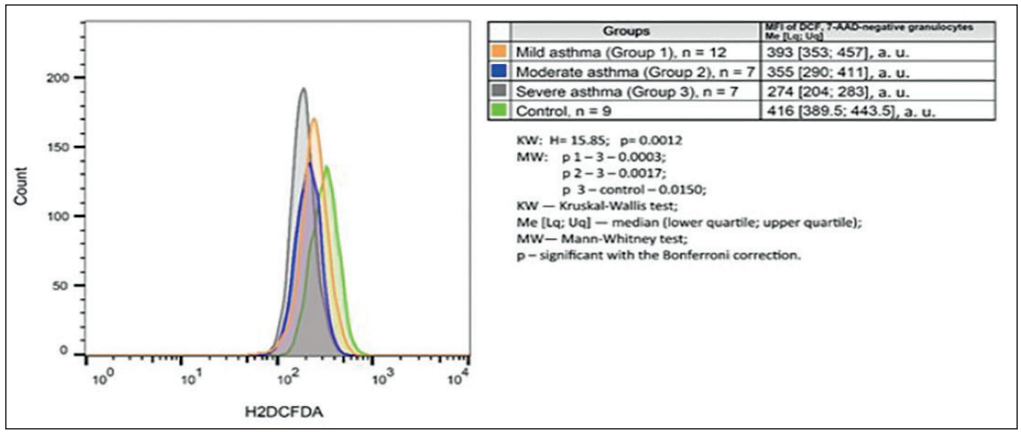

Fig. 2. Representative histograms that demonstrate dichlorofluorescein (DCF) fluorescence in the gated population of viable granulocytes obtained from patients with mild, moderate and severe asthma, as well as healthy individuals. Asthma is associated with a decrease in DCF fluorescence in granulocytes suggesting the reduction of intracellular reactive oxygen species (ROS) levels.

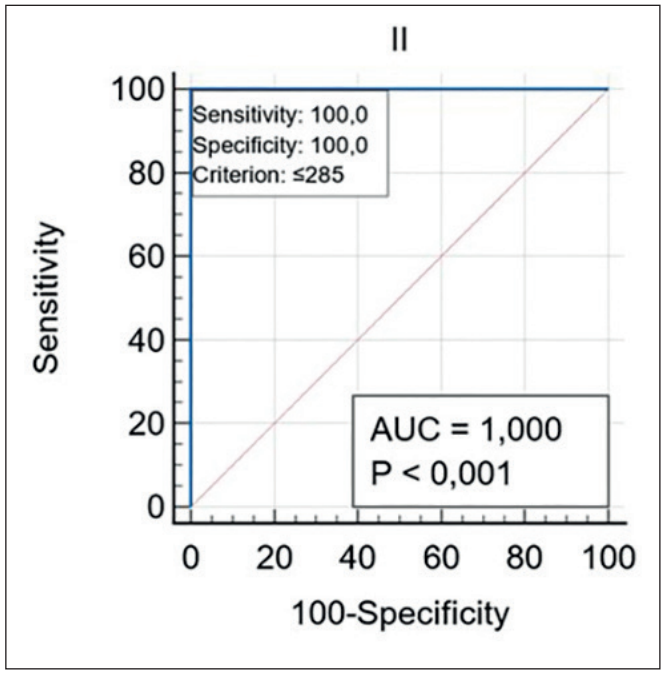

Fig. 3. ROC curves for ROS levels in granulocytes as a biomarker of the severity asthma in children.

cells. No statistically significant differences were found in DCF MFI values. MW: p 1 - 2 = 0.1083; p 1 - c = 0.6996; p 2 - c = 0.0933. However, the severe asthma was accompanied by a statistically significant decrease (p=0,0150) in MFI values of DCF in 7AAD-negative granulocytes. MW p 1 - 3 = 0,0003; p 2 - 3 = 0,0017; p 3 - C = 0,0150. Such changes in MFI values indicate that ROS levels in granulocytes are reduced in patients with severe asthma (Fig. 2).

Prognostic criteria for ROS levels in granulocytes.

ROC analysis was performed to determine the prognostic value of ROS levels in peripheral blood granulocytes. The relationship between the level of ROS in granulocytes and the asthma severity was determined. The limit value for the level of ROS in granulocytes is below 285 a.u. led to a specificity of 100% and a sensitivity of 100% for a predetermined endpoint, namely the formation of severe asthma (Fig. 3).

DISCUSSION

It is known that neutrophils occupy one of the key positions in inflammation of the respiratory tract and are the first line of nonspecific immune defense [13-17]. The neutrophils ability to generate ROS characterizes their functional activity and the possibility of complete phagocytosis [18-20].

Neutrophils play a crucial role in the transmission of redox potential signals due to their early recruitment and the wide variety of ROS released [23].

Researchers have identified the involvement of ROS in many important processes in the body - from damage to recovery. Elevated levels of ROS act as a factor in damage, including asthma [28]. But at the same time, ROS are involved in key regulatory mechanisms of the living cell (catabolism of old and synthesis of new molecules), playing the role of signaling system [21 - 23]. It is noteworthy that ROS generated during the inflammatory process play an important role in the healing and activation of neuroprotective pathways [23]. Scientists associate this with the recovery process.

This study found that patients with varying degrees of asthma had different levels of ROS production in 7AAD-negative granulocytes (neutrophils), ie viable cells in peripheral blood.

In our study, patients with mild to moderate asthma had higher levels of ROS in neutrophils compared with patients with severe asthma, where there was a statistically significant reduction. The results we obtained are no exception. Similar results have occurred in other scientific studies, where the object of study was a severe degree of asthma [11, 17]. In the context of this study, higher neutrophil levels were found to be associated with better asthma control, higher external respiration function, and lower doses of inhaled corticosteroids.

The relationship between ROS and FEV1, FEV1 / FVC, PEF was evaluated in our study. A direct, positive correlation between these indicators was established. This has shown that higher levels of ROS in neutrophils are associated with better lung function and milder asthma.

In a scientific study of cerebral ischemia, early ischemia was associated with an increase in ROS in brain tissue. The researchers concluded that elevated levels of ROS are the result of cerebral ischemia and exacerbation of the disease by inducing cell death, apoptosis and aging due to oxidative stress. In the stage of recovery of brain tissue, ROS play the role of a signaling molecule and may be useful for regulating angiogenesis and preventing tissue damage [22].

Therefore, the higher concentration of ROS in neutrophils in children with mild to moderate asthma can be considered as an assistant in the repair of damaged lung tissue by removing dead cells and cell debris.

The ROC analysis determined the predictive concentrations of ROS in granulocytes (neutrophils) in children with severe asthma. The concentration of ROS in granulocytes ≤ 285 a.u. was prognostically significant with high specificity and sensitivity in children with severe asthma. We did not find similar data in scientific works.

Decreased levels of ROS in granulocytes (neutrophils) in children with varying degrees of asthma can be considered as a possible predictor of its severity.

However, there are limitations. First, there are a small number of patients included in this study. The sample size will be expanded in the future. Second, the study was performed without specifying the heterogeneity of asthma (eosinophilic, neutrophilic or paucigranulocyte phenotype) [29-30].

In addition, one of the limitations of this study was the use of only one biological material for examination - blood. Researchers searching for the role of neutrophils in childhood asthma asthma used bronchoalveolar lavage (BAL), primary bronchial epithelial cells obtained during fibrobronchoscopy, endobronchial cleansing, and biopsy as material [11,17]. But for us, determining the severity markers of childhood asthma in the least invasive way was crucial.

The priority of the current study was to combine clinical characteristics and available biomarkers of bronchial asthma. Further studies of asthma biomarkers in children are needed to improve the prediction of key clinical outcomes and, as a consequence, the development of individualized treatments.

CONCLUSIONS

1. Patients with mild to moderate asthma have higher levels of ROS in neutrophils compared to patients with severe asthma.
2. The ROS concentration in granulocytes of peripheral blood in children with asthma below 285 a.u can be considered as an additional marker of its severity.
3. Higher levels of ROS generated by granulocytes had a positive correlation of external respiration function, which indicated better lung function.

The results of our study will probably be able to influence the further tactics of examination and treatment of patients with asthma.

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ORIGINAL ARTICLE

MORPHOLOGICAL PECULIARITIES OF THE SKIN GRANULATION TISSUE IN PATIENTS WITH MALIGNANT NEOPLASMS OF THE ABDOMINAL ORGANS

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ABSTRACT

The aim: To examine morphological peculiarities of the skin granulation tissue from the laparotomy wound in patients with malignant neoplasms of the abdominal organs.

Materials and methods: 36 bodies of deceased people were examined after midline laparotomy performed for surgical treatment of diseases of the abdominal organs. The main group included 22 bodies of deceased people suffering from malignant neoplasms of the abdominal organs, mostly in III-IV stages of diseases. The group of comparison included 14 bodies of deceased individuals suffering from acute surgical diseases of the abdominal organs. An average length of the laparotomy wound was 24,5 ± 0,28 cm. An average distance from the reticular elements to the external border of the granulation tissue was measured by means of computed histometry (mcm), the optical density (OD) of staining of the collagen fibers was determined by means of computed microdencitometry (expressed in OD absorbance coefficient – the absorbance of the solution per unit length per mole of solute), the specific volume of the blood vessels in the granulation tissue – by means of computed histostereometry (%), the granulation tissue cells were calculated by means of the score test (within eyeshot 10000 mcm^2). The specimens were stained with hematoxylin, eosin and methylene blue/Chromotrop 2B.

Results: The obtained results of the investigation conducted are indicative of more prominent chromotropic properties in the samples of the main group, confirming certain biochemical changes and features of the collagen fibers respectively. Moreover, slide mounts of the main group possess reliably lower optic density of staining of the collagen fibers which is indicative of their slow formation. It may suggest a reduced solidity of the postoperative scar on the laparotomy wound skin promoting easier wound disruption, that is, occurrence of subcutaneous eventration in patients with malignant neoplasms of the abdominal organs.

Conclusions: Oncological process in the body results in the aggravation of swelling and chromotropophilia in the deep layers of derma during more remote terms after surgery and reduced optic density of the collagen fibers staining, which promotes easier laparotomy wound disruption and occurrence of true postoperative eventration.

KEY WORDS: postoperative eventration, abdominal hypertension, skin granulation tissue

Wiad Lek. 2023;76(1):213-217

INTRODUCTION

Nowadays postoperative eventration is one of the most dangerous complications occurring after surgery performed on the abdominal cavity of weakened patients. In spite of a considerable advance of abdominal surgery the rate of postoperative eventration does not decrease for many years, and lethal outcome is 24%, though certain authors admit 65% [1].

The choice of the method of treatment of postoperative eventration is known to depend on its kind and availability or absence of complications. Thus, in case complete or true eventration occurs the majority of authors prefer urgent surgery after a short preoperative preparation, but in case of subcutaneous eventration a conservative tactics is more preferable. Surgery is indicated only in case of such complications as strangulation of the intestinal loop or development of secondary peritonitis with underlying subcutaneous eventration [2, 3].

Subcutaneous eventration allows avoiding repeated operation or gaining time for preoperative preparation, which is very important for the patients suffering from malignant neoplasms of the abdominal organs. Tumor intoxication causes various changes, secondary immune deficiency, cachexia, anemia etc. [4 -6].

Examination of morphological peculiarities of the granulation tissue in this group of patients, and the laparotomy wound skin in particular, will enable to better understand the role of malignant neoplasm in occurrence of subcutaneous eventration and help in further investigations in order to predict its development.

THE AIM

The aim was to examine morphological peculiarities of the skin granulation tissue from the laparotomy wound in patients with malignant neoplasms of the abdominal organs.

MATERIALS AND METHODS

To achieve the purpose of the study 36 bodies of deceased people were examined after midline laparotomy performed for surgical treatment of diseases of the abdominal organs, including 19 (52,8%) females and 17 (47,2%) males. An average age of the deceased individuals was 66,3 ± 0,73 years. An average length of the laparotomy wound was 24,5 ± 0,28 cm.

The main group included 22 bodies of deceased people suffering from malignant neoplasms of the abdominal organs, mostly in III-IV stages of diseases. The group of comparison included 14 bodies of deceased individuals suffering from acute surgical diseases of the abdominal organs.

Table I presents the distribution of patients depending on the surgery performed on the abdominal organs.

All the patients during their hospital stay were provided with standard postoperative treatment according to the protocols of medical aid given to patients with urgent surgical pathology of the abdominal organs.

For morphometric purposes (computer program ImageJ 1.48 v) an average distance from the net elements to the external border of the granulation tissue was measured by means of computed histometry (mcm), the optical density (OD) of staining of the collagen fibers was determined by means of computed microdencitometry (expressed in OD absorbance coefficient – the absorbance of the solution per unit length per mole of solute), the specific volume of the blood vessels in the granulation tissue – by means of computed histostereometry (%), the granulation tissue cells were calculated by means of the score test (within eyeshot 10000 mcm^2). The specimens were stained with hematoxylin, eosin and methylene blue/Chromotrop 2B [7].

The obtained results were statistically processed by means of electronic tables Microsoft Excel and the package of statistical processing program PAST. The Shapiro-Wilk test was to detect all departures from normality when the p-value is less than or equal to 0.05. The Mann-Whitney test was used to compare differences between the groups. The results was considered to be reliable when the p-value was ≤ 0,05, which is commonly accepted in medical-biological studies.

RESULTS

On the 1st day after surgery in the majority of samples from the main group many epidermal cells were found to be in the state of apoptosis or mitotic activity. Keratosis and disturbed stratification of the epidermal layers were found in the samples from the group of comparison.

The dermal papillary layer was without specific features, but contrary to the main group a prominent swelling of the deep dermal layers was found practically in all the samples from the group of comparison.

A weak staining of the collagen fibers with methylene blue was found in the samples of the main group in the papillary and reticular dermal layers, which is indicative

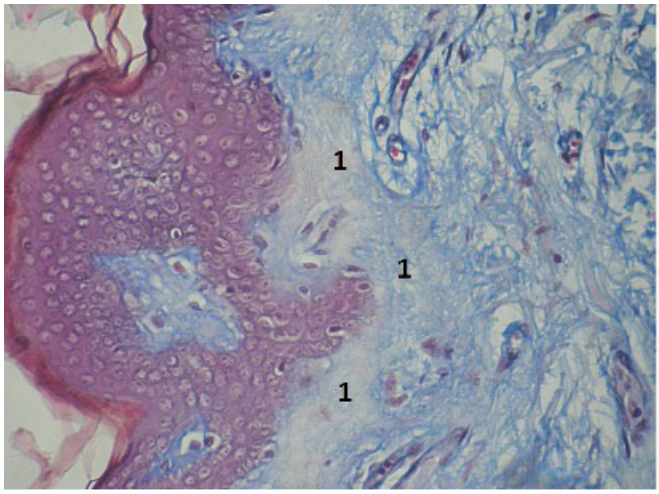

Fig. 1. Micrograph of the skin (the 1st day after the surgery performed). Patient M., 68 years old, № 7 from the main group. Diagnosis: sigmoid adenocarcinoma. $T_3N_1M_0$, III B stage. A weak staining of the collagen fibers in the papillary and reticular dermal layers (1). Methylene blue / Chromotrop 2 B stain. Ob. 10×. Oc. 10×.

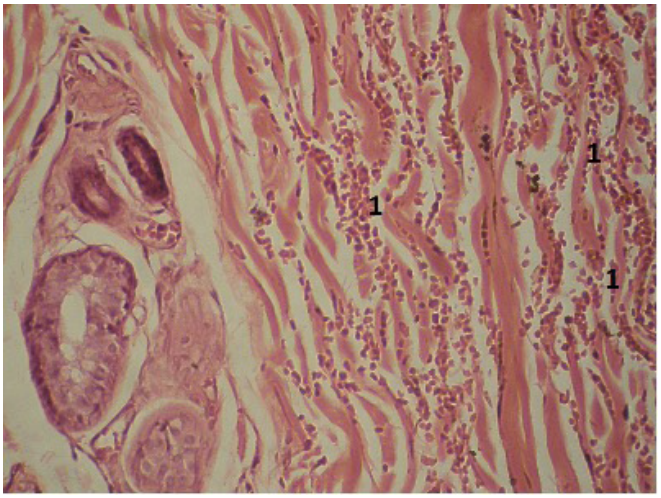

Fig. 2. Micrograph of the skin (the 1st day after the surgery performed). Patients Ch., 64 years, № 12 from the main group. Diagnosis: highly differentiated clear cell adenocarcinoma of the head of the pancreas. $T_3N_1M_0$, III stage. Mechanical jaundice. Many hemorrhages in the derma (1). H&E stain. Ob. 20×. Oc. 10×.

Table I. Distribution of patients from both groups of examination depending on the surgery performed, abs., %

Surgery performed	Group of patients		Abs.	%
	Comparison	Main		
Stomach resection	4	6	10	27,8
Gastroenteroanastomosis	2	3	5	13,9
Cholecystojejunoanastomosis	1	3	4	11,1
Enterectomy	2	1	3	8,3
Left hemicolectomy (colonic resection)	2	3	5	13,9
Sigmoid resection	2	2	4	11,1
Hartmann's operation (rectosigmoid colon resection)	1	4	5	13,9
Total:	14	22	36	100

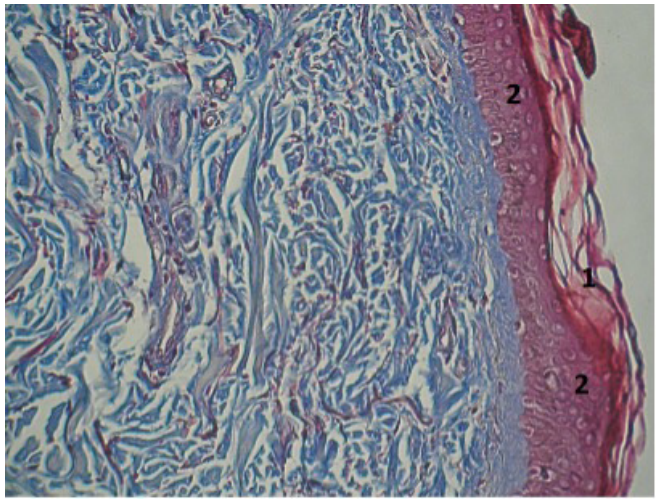

Fig. 3. Micrograph of the skin (the 1st day after the surgery performed). Patient C., 72 years, № 16 from the main group. Diagnosis: adenocarcinoma of the pyloric part of the stomach. $T_3N_2M_1$, IV stage. Stenosis of the stomach outlet. Many epidermal cells with the signs of apoptosis (1). The papillary layer not changed (2). Methylene blue / Chromotrop 2 B stain. Ob. 10×. Oc. 10×.

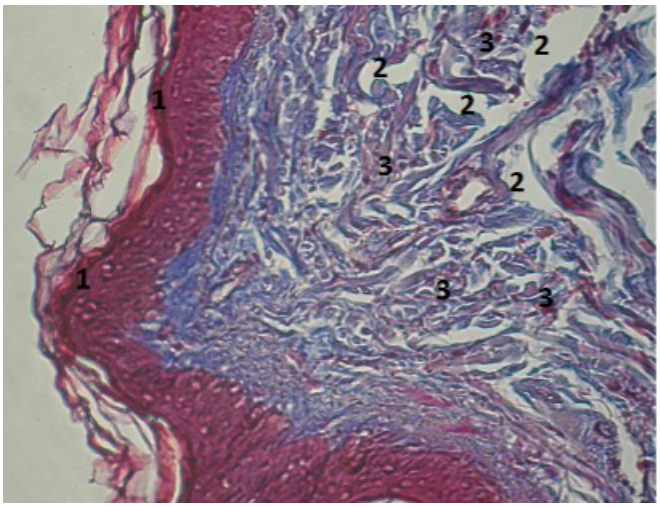

Fig. 4. Micrograph of the skin (the 2nd day after the surgery performed). Patient K., 67 years, № 14 from the main group. Diagnosis: adenocarcinoma of the colon splenic angle. $T_2N_1M_0$, II B stage. Many epidermal cells with the signs of apoptosis (1). Deep dermal layers look like edema (3). Separate collagen fibers are susceptible to chromatropophilia (red color) (3). Methylene blue / Chromotrop 2 B stain. Ob. 10×. Oc. 10×.

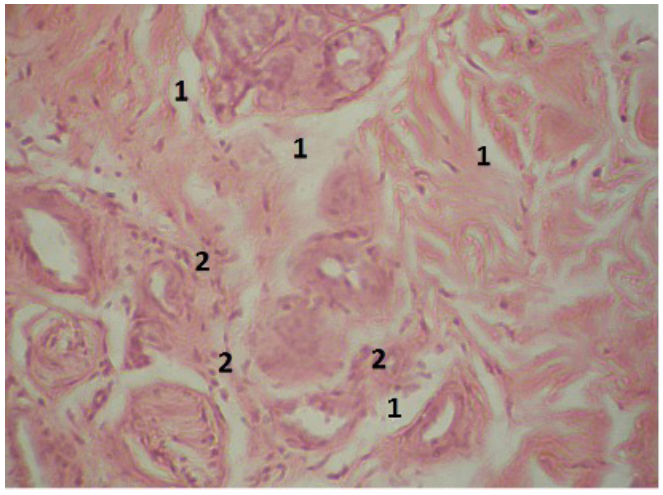

Fig. 5. Micrograph of the skin (the 5th day after the surgery performed). Patient Z., 74 years, № 4 from the group of comparison. Diagnosis: peritoneal commissures. Acute intestinal obstruction. Swelling, serous inflamation (1) Leukocytes (2). H&E stain. Ob. 20×. Oc. 10×.

of fresh regeneration. The specific volume of the collagen fibers in the above mentioned layers is considerably lowered. Numerous hemorrhages are detected.

Chromotropophilia (red color) was detected in the samples of both groups in the study. In the group of comparison it was 2,64 ± 0,308%, which is higher than that in the main group – 2,14 ± 0,211% (p>0,05). Inflammatory changes were found in the layers of the skin closer to fascia in both groups of the study. These changes were more prominent in the slide mounts from the group of comparison.

On the 2nd day the signs of apoptosis were found in many samples of epidermal cells from the main group. The granular layer was absent, and the keratinized one contained nuclei (parakeratosis). The papillary layer was comparatively less compact.

In the samples from both groups of the study swelling of the connective tissue fibers was found, where

chromotropophilia involved about 0,5% of fibers. Optic density and specific volume of the collagen fibers are lower.

On the 3rd day of the early postoperative period increased mitosis was found in the slide mounts of the main group. Swelling of the papillary and deep dermal layers was detected in the samples from both groups of the study. Chromotropophilia in the samples from the main group was 6%, and in the group of comparison – 8-10%.

On the 4th and 5th days after surgery swelling and chromotropophilia in the samples of the reticular layer and deep dermal layers were found in approximately 3% of fibers in the main group. In the samples from the group of comparison, in addition to swelling of the deep dermal layers there was serous inflammation detected, and chromotropophilia of fibers was about 6%.

During more remote periods swelling of the deep dermal layers in the samples from both groups of the study remained, but chromotropophilia in the main group increased and became about 10%. Collagen fibers in the slide mounts of the main group are disharmonious. Thick fibers are darker and thin fibers are lighter in color.

Optic density of staining of the collagen fibers in the samples from the group of comparison was 0,254 ± 0,001, which was reliably higher than that of the main group – 0,203 ± 0,003 ($p<0,001$).

DISCUSSION

Summarizing the results of the study, it should be noted that the presence of a malignant neoplasm in the human body significantly suppresses and slows down the processes of maturation of laparotomy granulation tissue.

This is explained by the fact that in the samples of patients of the main group, a more pronounced chromotropophilia is noted, which indicates certain biochemical changes in the granulation tissue and, accordingly, has an effect on the properties of collagen fibers. The negative impact of a malignant neoplasm is explained by a less pronounced, weak inflammatory reaction of the granulation tissue of the skin of the laparotomy wound in all periods of observation. Also, in the micropreparations of the main group, the collagen fibers are disharmonious and their optical density of staining is probably lower, which indicates their delayed maturation.

The worse maturation of the granulation tissue of the laparotomy skin wound is explained by the surgical trauma caused against the background of already existing pathological changes in the body, due to the presence of an oncological process, which collectively leads to their significant deepening [1, 3].

This allows us to assume a decrease in the strength of the postoperative scar of the skin of the laparotomy wound, which will contribute to an easier separation of its edges, that is, the occurrence of complete eventration in patients with malignant neoplasms of the abdominal cavity, which is much more dangerous and requires immediate surgical intervention [2, 5].

This feature must be taken into account in order to timely prevent this postoperative complication by strengthening not only the muscle-aponeurotic layer of the laparotomy wound, but also the skin in patients with malignant tumors of the abdominal cavity.

CONCLUSIONS

Oncological process in the body results in the aggravation of swelling and chromotropophilia in the deep layers of derma during more remote terms after surgery and reduced optic density of the collagen fibers staining, which promotes comparatively easier laparotomy wound disruption and thus occurrence of true postoperative eventration.

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REVIEW ARTICLE

PUBLIC AUTHORITIES AND LOCAL SELF-GOVERNMENT BODIES COOPERATION IN THE FIELD OF HEALTH CARE DURING THE COVID-19 PANDEMIC: UKRAINIAN EXPERIENCE

DOI: 10.36740/WLek202301130

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ABSTRACT

The aim: To characterize cooperation of public authorities and local governments in the field of health care to identify problems that arise when providing free medical care to citizens of Ukraine through state and municipal health care facilities under conditions of COVID-19.

Materials and methods: The methodological basis of the research is the general methods of scientific cognitivism as well as concerning those used in legal science: methods of analysis and synthesis, formal logic, comparative law etc. The norms of the adopted new legislation of Ukraine, as well as the practice of its application are analyzed.

Conclusions: The following proposals for amendments and supplements to the legislation of Ukraine are substantiated: lack of clear definition of the role of hospital councils within the legislation of Ukraine; providing health care facilities that have separate buildings and isolation of COVID-19 patients; provision of medical aid to COVID-19 patients by a family doctor; establishment and functional activity of ambulance crews in the newly formed united territorial communities; ect.

KEY WORDS: patient, free medical aid, state-guaranteed medical care package, state health care institution, municipal health care institution

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INTRODUCTION

Since 2014, Ukraine has been reforming local self-government and territorial organizations of power, and since 2015, the Government of Ukraine has initiated transformational reform of the health care system, also, since 2018 Ukraine has been reforming the health care financing system [1-3]. The acute respiratory illness COVID-19 caused by the coronavirus SARS-CoV-2 has become an indicator of the effectiveness of these reforms and it has demonstrated once again the necessity for consolidation of efforts from public authorities and local governments to prevent pandemic, ensure accessibility and quality of health care in local communities.

Thus, the study of Ukraine's experience within interaction of public authorities and local self-government bodies in the field of health care is relevant up to date, being as well theoretically and practically mature.

THE AIM

To characterize cooperation of public authorities and local governments in the field of health care to identify problems that arise when providing free medical care to citizens of Ukraine through state and municipal health care facilities under conditions of COVID-19.

MATERIALS AND METHODS

The methodological basis of the conducted research is the general methods of scientific cognitivism as well as concerning those used in legal science: methods of analysis and synthesis, formal logic, comparative law etc. The norms of the adopted new legislation of Ukraine, as well as the practice of its application are analyzed. It is also emphasized upon own and delegated powers in the field of health care, referred to the executive bodies of village, township, city councils.

REVIEW AND DISCUSSION

Ukraine is the unitary state. Local self-government is the right of a territorial community — residents of a village or a voluntary association of residents of several villages into one village community, residents of a settlement, and of a city — to independently resolve issues of local character within the limits of the Constitution and the laws of Ukraine [4].

According to the Constitution of Ukraine the material and financial basis for local self-government is movable and immovable property, revenues of local budgets, other funds, land, natural resources owned by territorial communities of villages, settlements, cities, city districts, and also objects of their common property that are managed by district and oblast councils. On the basis of agreement, territorial communities of villages, settlements and cities may join objects of communal property as well as budget funds, to implement joint projects or to jointly finance (maintain) communal enterprises, organizations and establishments, and create appropriate bodies and services for this purpose. The State participates in the formation of revenues of the budget of local self-government and financially supports local self-government. Expenditures of bodies of local self-government, that arise from the decisions of bodies of state power, are compensated by the state (Article 142) [4].

In the Constitution of Ukraine it is stated: «Certain powers of bodies of executive power may be assigned by law to bodies of local self-government. The State finances the exercise of these powers from the State Budget of Ukraine in full or through the allocation of certain national taxes to the local budget, by the procedure established by law, transfers the relevant objects of state property to bodies of local self-government. Bodies of local self-government, on issues of their exercise of powers of bodies of executive power, are under the control of the respective bodies of executive power» (Article 143) [4].

According to the Law of Ukraine "On Local Self-Government", the executive bodies of village, settlement, and city councils are responsible for: a) their own (self-governing) powers; b) delegated powers (Article 32) [5].

The National Health Service of Ukraine procures medical services and medicines under the program of medical guarantees and reimbursement [6]. Health care facilities are being financed today with "the money following a patient" basis [7]. During the COVID-19 pandemic this principle proved its effectiveness. First, the health care facility receives funds exclusively for the services it provides, according to the tariff for them. As a result, financial funding is transferred to health care facilities that provide major aid to a greater number of patients and accordingly they work more intensive. Since the population density and the number of cases of COVID-19 population from region to region are different in Ukraine, this principle promotes rational use of funds, and also makes it possible to increase the amount of money quicker, under agreements with the National Health Service of Ukraine, to direct greater funds to those health facilities that accept greater number of patients. Second, it has been identified what public authorities and what health care facilities owners are responsible for.

Thus, the administration of the health care institution which is in communal (public) ownership and the owner of it (the district health care institution is owned by local governments, which, in particular, can allocate funds to make their hospital more and more technologically equipped, being able to provide better medical services) bear responsibility for material and technical equipment supply of the hospital, and also for consumables availability.

International documents: changing the paradigm of interaction of public authorities and local self-government.

The right for life is an absolute right, that the state is obliged to ensure. The right to health is one of the fundamental human rights. It is reflected in international documents of universal and regional status: Universal Declaration of Human Rights 1948 [8], International Covenant on Economic, Social and Cultural Rights 1966 [9], International Covenant on Civil and Political Rights 1966 [10], Declaration on the Rights of Persons with Disabilities 1975 [11], Declaration on the Rights of Persons with Mental Disabilities 1971 [12], etc.) and of regional nature (Convention for the Protection of Human Rights and Fundamental Freedoms 1950 [13], European Social Charter (revised) 1996 [14], etc.).

On December 14, 2020, the UNGA adopted Resolution 75/130 "Health of the World Population and Foreign Policy: strengthening the resilience of the health system through affordable medical aid for all" [15]. The Resolution impose legal obligations onto the member-states: they are to take all necessary steps, including ones of legislative and administrative nature, regardless of the level of economic development. First of all, this lawfully normative act reaffirmed the importance of national ownership. The Resolution is such a signal: the objective necessity for in-depth cooperation in the field of healthcare is explained by importance of this type of activity for each person, people – citizens of the state of all nationalities, society. The Resolution also reaffirmed the central role and responsibility of governments at all levels – public authorities and local self-government – in defining their own path to universal health coverage, taking into account national circumstances and priorities.

The UNGA urged Member States to strengthen national health systems by ensuring affordable medical aid for all, with a focus:

1) on first medical aid;
2) the availability of physical and financial accessibility of quality health services as well as safe, effective, affordable and essential medicines, vaccines, diagnostic devices and medical technologies.

Likewise, the UNGA recommends that Member States:
1) implement highly effective strategies to protect people's health and take full account of the determinants of health through inter-sectoral work using approaches that involve all government agencies and mainstream health in all policies;
2) to pursue effective health financing policies, including through close collaboration between relevant authorities, including financial and health authorities;
3) to pursue a policy of more efficient allocation and usage of resources with adequate financing on for the first medical aid, innovative financing.

So, the UN established new international legal requirements for national health systems, which changed the paradigm of interaction between public authorities and local governments in foreign countries during the period Covid-19 pandemic, but they did not change the paradigm – ensuring the human right to quality medical care is the main responsibility of the state. The state performs these responsibilities via public authorities and local governments. If the public authorities and local governments do not do this effectively, it will mean that the state does not fulfill the duty to defend the right to health protection, medical care.

The involvement of community residents into the local decision-making processes in health care sphere.

O. Shevchenko and V. Romanova rightly emphasize, that cohesion presupposes the involvement of community residents into the local decision-making processes in health care sphere, as well as increasing the state's ability to meet the needs of citizens [16]. Thus, because of the application of the social cohesion in France, developed medical system was created, in which, for example, the number of places in intensive aid wards is twice as high as in Italy.

So, due to the implementation of the social cohesion, the role of the territorial organization of government and local self-government within the formation of health policy is growing.

The Ministry of Health of Ukraine is in charge of the establishment and functioning of hospital districts. One hospital district is to be placed on the territory of one region. "There was a kind of "centralization" of hospital districts. This has been done in order to ensure the development of health care facilities on the regional level, a network of facilities in accordance with the needs of the population, as well as to create effective, efficient patient routes, in particular in connection with the agreements on medical care for the population institutions have to contract. For example, we understand that the facility has a contract for a heart attack insult, and local authorities must ensure that patients can get right into these facilities, which have equipment and specially taught medical staff, as the Chairman of the NSZU Andriy Vilensky points out [17]. Matviy Khrenov, a health care expert, rightly emphasizes that the hospital district and the enlarged district are fundamentally different entities: "the enlarged district is an administrative entity with prescribed regulations, and its clearly assigned powers [17]. Instead, the hospital council is only an advisory body. «Volodymyr Krasyokha, Chief physician of Voznesensk Multidisciplinary Hospital (Mykolaiv Region), underlines that "decisions of the hospital council are submitted to the governor for signing, so they cannot be advisory. This is going to be an order to execute» [17].

According to Eva van de Rakt and Florian Christl «… uncoordinated measures have exacerbated social inequality ..., hampered democratic decision-making processes and struck the principle of social cohesion» [18].

FUNDING FOR COMMUNITY HEALTH CARE FACILITIES

The National Health Service of Ukraine has the right to directly finance a health care institution through the contract [19; 20]. Also, as it has been mentioned above, the decision to increase or decrease funding except financing transferred directly from the National Health Service is made by the owner. Therefore, in order to solve the problem of the lacking of doctors in the context of the COVID-19 pandemic, the Nizhyn City Council, for example, allocates a plot of land to an anesthesiologist who is coming from another city and builds a house for him to live in. In Mena town (Chernihiv region), local authorities built a 6-apartment house and invited doctors from Chernihiv.

However, in Ukraine there are medical institutions of communal (public) ownership which are not be switched into a new form of work, like payment for medical care in accordance with the contract concluded to the National Health Service of Ukraine at the tariff of the relevant package of medical aid provided due to inconsistency of health care facilities, understaffing, etc. The owners of such communally owned health care facilities do not have funds within the local budget to allocate them for the renewal of material and technical base provoking interest of medical personnel to work in particular institution. The consequence of this fact becomes a social tension, as well as the fact that the residents of the territorial community are deprived of the opportunity to receive affordable and quality medical aid in accordance with the state-guaranteed packages of free medical care[1].

[1] Payment on for health care institution for medical services provided is implemented by the National Health Service of Ukraine in accordance with the agreement concluded with this institution.

Similar situation was considered by the Court in the case of the Center for Legal Resources on behalf of Morita Malak and others vs. Romania: improper care and treatment, as well as inadequate, poor hospital living conditions directly contributed to the untimely deaths of five patients [21]. In the Resolution of the European Court of Human Rights in the case of the Center for Legal Resources on behalf of Valentin Campeanu vs. Romania dated 17 July, 2014, it is stated: "... the Court established, that the Article 2 (right fir life) of the Convention was violated from material and procedural points of view. In particular, it was consolidated that Valentin Campeanu stayed at medical institutions that were not supplied with necessary equipment to ensure proper care for his condition; the fact is about his being transferred from one department to another without an established diagnosis; and that the authorities did not provide him with adequate treatment in regards of antiretroviral therapy. Authorities, being aware of this difficult situation as for lack of staff, malnutrition and lack of central heating around the psychiatric hospital premises where he was transferred, unjustifiably endangered his life" [22; 23].

In Austria a single network of inpatient and out-patient institutions was established, regardless of their subordination and ownership [6]. The developed tariffs for medical services eliminate price competition between hospitals, which stimulates them to increase the quality of work. In Estonia, a system of contractual relations is used in which the Health Insurance Fund concludes contracts only with selected medical organizations - the most effective service providers are selected. So, the main tool for quality control of the services provided is the conduct of audits, a comprehensive assessment of the performance of medical institutions.

According to the decision of the Constitutional Court of Ukraine in the case of free medical care free health care in Ukraine and communal health care institutions does not exclude such opportunities through the financing of this industry through the development of extra-budgetary mechanisms to raise additional funds, including through the establishment of hospital mutual help cash desks (unions, funds), which activities should be regulated by law [24].

PROVIDING MEDICAL AID TO COVID-19 CONTAMINATED AND OTHER PATIENTS

As in Austria, Estonia, such uniform tariffs for medical services under state guarantees are implemented in Ukraine to eliminate price competition between hospitals for medical services included in the "package". Since June 1, 2020, the following state-guaranteed medical care packages have been applied: primary health aid; emergency medical aid; acute myocardial infarction; acute stroke, provided in stationary hospital conditions; four packages for COVID-19 treatment. At the initiative of the National Health Service of Ukraine, health care facilities were divided into two groups: 1) health care facilities that provide medical aid to COVID-19 patients [25]; 2) health care facilities that provide medical care to patients who are not ill for COVID-19 [26; 27]. Health care facilities of the first group must have the appropriate number of staff and equipment to provide care to patients with any manifestations of the disease of any severity, as well as to enter into an agreement with the National Health Service of Ukraine. Such facilities receive funds from the National Health Service of Ukraine according to the tariff [28; 29]. Under these agreements, the institution is to be provided with medicines, consumables, as well as additional payments of up to 300% to medical staff who work directly with COVID - 19 patients.

In case that a health care facility has not entered into a contract for a COVID-19 treatment package with the National Health Service of Ukraine, this establishment may provide medical aid to patients, who are not ill for COVID-19 only. Today, the National Health Service of Ukraine has an agreement on inpatient package for the treatment of COVID-19 with 437 health care facilities [28]. The total network of inpatient health care facilities in Ukraine is more than one thousand institutions. Instead, it will will stimulate these hospitals to increase the quality of work performance [3].

SURCHARGES FOR MEDICAL STAFF

According to the agreement concluded by the health care institution for the inpatient COVID-19 package with the National Health Service of Ukraine, a 300% surcharge is provided for the team of physicians working with COVID-19 patients. To provide inpatient care to COVID-19 patients, the health care facility must have minimum of four teams of 12 people each, consisting of three doctors and three health employees per doctor (48 people). In practice, many health care facilities do not increase the number of teams (for example, to 5, 6, 7 and more), but include at least 4 teams of more doctors and health professionals [28]. For example, there may be 4 teams, but medical employees are not 48, but more (56, 96, 120, etc.) [28]. This increase in doctors and health employees in the teams, rather than the teams themselves, means that there is a lack of funds for surcharges for doctors and medical employees. In this case, the issue of deficit surcharges comes to the attention of the owner and head of the health care in-

stitution, but to the National Health Service of Ukraine. The National Health Service of Ukraine has simplified the requirements for teams: if a health care institution has four teams with an anesthesiologist, the institution can create more teams, where there can be any set of medical doctors.

Visit of the family doctor to the patient's home, establishing and functioning of ambulance crews within the newly formed united territorial communities

The decision to visit the patient's place or not is to be made by the family doctor himself [29]. The doctor's work should be optimized [3; 30]. If a patient needs urgent medical aid, an ambulance is to arrive to the patient's and provide all necessary medical aid or hospitalize the patient·he State guarantees the package of urgent medical aid as well as the package of primary medical aid [31; 32]. The family physician uses remote communication methods and thus provides medical aid to COVID-19 patients. If a local community or health care facility sees the need to create an ambulance crew, Ukrainian law does not prohibit this. Some primary aid centers create regular teams that work on weekends, as well as round-the-clock teams (contact center, teams that can arrive on call). However, the National Health Service of Ukraine reimburses the costs of medical care only in accordance with the concluded agreements with the health care institutions and within the framework of the tariffs of the state-guaranteed package of free medical care.

PROVISION OF HEALTH CARE FACILITY WITH OXYGEN

According to Eva van de Rakt and Florian Christl Germany's general resource readiness for such crises and the application of the social cohesion principle played perhaps the most important role in confronting the epidemic. The high level of development of the healthcare system, the availability of appropriate material and financial resources allowed Germany to prepare its system and society for resistance to the coronavirus epidemic through relatively short time [18]. Moreover, Germany itself has become an international aid donor and an active player on the front in the fight against the epidemic [33].

The responsibility for providing oxygen to the health care facility laid upon its administration. Taking into account that the demand for oxygen has increased tens and hundreds of times, and the Ukrainian oxygen market is not able to meet this demand, the heads of health care facilities and local governments cannot solve this problem just by themselves. This issue needs to be addressed at the State level. Licenses to supply oxygen to businesses have been intensified, leading to an increase in the number of such suppliers.

CONCLUSIONS

1. The UN established new international legal requirements for national health systems, which changed the paradigm of interaction between public authorities and local governments in foreign countries during the period Covid-19 pandemic, but they did not change the paradigm – ensuring the human right to quality medical care is the main responsibility of the state.

According to the practice of ECHR the Article 2 (right for life) of the Convention was violated from material and procedural points of view if public authorities and local governments, being aware of a difficult situation as for lack of staff and the availability of appropriate material and financial resources , unjustifiably endangered people life: there were improper care to COVID-19 and treatment, inadequate, poor hospital living conditions, person stayed at medical institutions that were not supplied with necessary equipment to ensure proper care for his condition; person transferred from one department to another without an established diagnosis.

2. The high level of development of the healthcare system, the availability of appropriate material and financial resources allowed Germany to prepare its system and society for resistance to the coronavirus epidemic through relatively short time. In Austria and Estonia the main tool for quality control of the services provided is the conduct of audits, a comprehensive assessment of the performance of medical institutions.
3. In Austria, Estonia, Ukraine some common problems remain unsolved: the formation of an effective model of health care financing; promoting greater access to medical aid to COVID-19 contaminated and other patients.

The following problems that arise when providing free medical aid to citizens of Ukraine in state and municipal health care facilities in the conditions of COVID-19, the solution of which requires consolidation of efforts of public authorities and local governments are as follows: the functioning of hospital districts; financing of health care facilities; lack of clear definition of the role of hospital councils within the legislation of Ukraine; providing isolation of COVID-19 patients patients; providing health care facilities that have separate buildings and isolation of COVID-19 patients patients, planned medical care when establishing quarantine and the introduction of enhanced anti-epidemic measures in areas with significant spread of acute respiratory dis-

eases and COVID-19 caused by coronavirus-SARS-CoV 2; increase the number of doctors and health employees in the teams that provide inpatient care to COVID-19 patients instead of the practical necessity and expediency of increasing the number of these teams; provision of medical aid to COVID-19 patients by a family doctor, establishment and functional activity of ambulance crews in the newly formed united territorial communities; providing oxygen to health care facilities.

The following ways of solving these problems in Ukraine are proposed: 1) to establish the legal responsibility of officials of public authorities and local governments for violating the Law of Ukraine "On State Financial Guarantees of Medical Care of the Population" when developing regional budgets, district budgets, budgets of territorial communities, district budgets in cities; 2) clearly specify in the legislation of Ukraine the mechanism for controlling the distribution of funds by hospitals and the legal liability of hospitals and chief doctors for violating them; 3) specify in the legislation of Ukraine the mechanism for monitoring the formation of the list of necessary medical equipment by state and municipal healthcare institutions and specify in the legislation of Ukraine the mechanism of the involvement of community residents into this processes; 4) specify in the legislation of Ukraine the mechanism for monitoring the purchase of necessary medical equipment by state and municipal healthcare institutions and specify in the legislation of Ukraine the role and the mechanism of the involvement of community residents into this mechanism for monitoring the involvement of community residents into the local decision-making processes in health care sphere; 5) to increase the additional revenues for health care financing in general (specify in the legislation of Ukraine the official direct payments of the population for medical services of secondary importance, to develop the territorial community savings programs, to specify in the legislation of Ukraine the mechanism for government medical loans and local medical loans).

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REVIEW ARTICLE

MEDICAL CONTRACTS WITH CONDITIONS CONTRARY TO PUBLIC POLICY

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ABSTRACT

The aim: To reveal some features of medical contracts with conditions contrary to public policy.

Materials and methods: The study is based on the statutory acts of countries of European Union. The author also uses acts of international law in the field of medical services, the law and cases court practice of EU.

Conclusions: The sphere of medical services objectively requires increased control by the state. There are various legal mechanisms for ensuring the rights of the patient and the proper level of medicine. It is important to invalidate the unfair terms of medical contracts, compensation for losses and moral damage. These remedies are obtained through judicial protection and, in some cases, through other jurisdictional means. It is important to implement European standards in national legislation.

KEY WORDS: contracts in the field of medical services, invalidity of contract, restitution, Draft Common Frame of Reference

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INTRODUCTION

In the area of health care delivery, there may be cases of low levels due to the quality of medicines or inappropriate methods of treatment. This can lead to a serious threat to the life and health of the patient.

Health care is one of the most dynamic and rapidly changing sectors of our economy. In order to compete, health care companies (including hospitals, medical groups, health insurers, pharmacy benefit managers and managed care organizations) must adopt and rely on information technology and technology-enabled products to deliver care and services to their patients and customers. The acquisition and implementation of these technology-based solutions is critical to the success of health care companies [1].

Civil law is required to have adequate legal regulation of this issue and the development of mechanisms to provide of poor-quality medical services. This process is regulated by medical services contract, which can be defined as a contract whereby the medical institution is authorized or duty-bound to perform medical treatment, epidemic prevention, health care or medical inspection for patients.

Most of the reformers agree that the current law and practice relating to medical malpractice are unsatisfactory for many reasons. In particular, standards of health care are set too high, and with too little regard to cost; damages, especially for pain and suffering are too high and are socially wasteful; the collateral benefits rule duplicates compensation for a lucky handful and adds to costs; too few people are compensated in any case, given the need to prove fault; and the legal and administrative costs of the whole system are excessive. Therefore, they conclude, contract would be a better instrument for regulating the physician/patient relationship [2].

THE AIM

The paper is aimed at researching of invalidity of contracts in medical sphere, kinds of their invalidity and several consequences. The author also plans to the law doctrine of this sphere as well as legislation and judicial practice in this issue, and also proposes ways to solve the identified problems.

MATERIALS AND METHODS

The grounds of this paper are the statutory acts of countries of European Union, USA and some others. In scientific researching the author also deals with:

1) the acts of legislation Draft Common Frame of Reference (DCFR); "The Rules Governing Medicinal Products in the European Union"; Directive 2001/83/ EEC for

available translations of the preceding and in Regulation (EC) No 726/2004; Council Directive 93/39/EEC of 14 June 1993 amending Directives 65/65/EEC, 75/318/EEC and 75/319/EEC in respect of medicinal products Regulation (EC) No 726/2004 of the European Parliament and of the Council of 31 March 2004; Directive 93/42/EEC of 14 June 1993; Regulations No. 726/200411, which provided for the establishment of the European Agency for the Evaluation of Medicinal Products (EMEA); Lex of Germany "On Medicinal Products"; The Order of the Ministry of Health of Ukraine of November 22, 2011 No. 809 "On Approval of the Procedure for Prohibiting (Stoppage) and Renewing the Market Circulation of Medicinal Products on the Territory of Ukraine"; The Order of the Ministry of Health of Ukraine of June 06, 2012, No. 422 "On Certain Issues of the Prohibition of the Advertising of Medicinal Products";

2) typical or standard forms of medical contracts (Standard Personal Medical Services Agreement 2018/19 (developed by National Health Service), Standard General Medical Services Contract;

3) case law (Judgment of the Court of 11 December 2003. Deutscher Apothekerverband eV v 0800 DocMorris NV and Jacques Waterval. Case C-322/01//European Court reports. 2003.P.I-14887).

The author also deals with the acts of international law. Moreover, judicial practice, law doctrine and views on this issue have been used. Also the paper is based complex of methods. Dialectical method bases on two principles: the principle of universal connection and the principle of development medical law. The author makes extensive use of comparative method - comparing the law of Ukraine to the law of other European countries, which makes the basis for comparison to ensure integration process of Ukraine. Functionalism typically applies at the level of micro-comparison. From a broader perspective a more structural analysis of (parts of) legal systems may be used. The analytical method is a process that goes beyond the merely mechanical. The analytical method abstracts the essence of knowledge in an organized and premeditated form in legal sphere.

REVIEW AND DISCUSSION

The requirements and procedures for quality of medical products, are primarily established by Directive 2001/83/ EEC for available translations of the preceding and in Regulation (EC) No 726/2004 for available translations of the preceding. They also include harmonised provisions for the manufacture, wholesale or advertising of medicinal products for human use.

There are a wide number of medical contracts: Physician Employment Contract, Physician Recruitment Contract, Managed Services Contract, Medical Director Contract, Care Transfer Agreements, Contracts Related to the Use of Medical Technology, Compensation and Benefits Contracts, Joint Venture Contracts, Contracts for Equipment [3].

Additionally, EU legislation provides for common rules for the conduct of clinical trials for available translations of the preceding to test the safety and efficacy of medicines under controlled conditions) in the EU. Various rules have also been adopted to address the particularities of certain types of medicinal products and promote research in specific areas.

The momentous Code of rules in this sphere is "The Rules Governing Medicinal Products in the European Union".

The most important acts of European legislation in this area are Council Directive 93/39/EEC of 14 June 1993 amending Directives 65/65/EEC, 75/318/EEC and 75/319/EEC in respect of medicinal products Regulation (EC) No 726/2004 of the European Parliament and of the Council of 31 March 2004 layingdown Community procedures for the authorization and supervision of medicinal products for human andveterinary use and establishing a European Medicines.

The EU legal framework for human medicines sets standards to ensure a high level of public health protection and the quality, safety and efficacy of authorised medicines. In addition, it promotes the functioning of the internal market, with measures to encourage innovation. It is based on the principle that a medicinal product requires a marketing authorisation by the competent authorities before being placed on the market.

In the past several years, many independent physicians have become affiliated with or acquired by entities such as hospitals, health systems or large group practices. Such unwinding may mean the departure of one physician, or the departure of a whole physician practice (we refer to "physicians" in this document to cover both situations). Physicians may depart from a Health Organization for a variety of reasons, including the desire to pursue alternative practice models and to practice medicine with greater clinical autonomy [4].

The aim of European legislation is to remove obstacles free trade within the EU, imposed by national regulators by establishing national requirements for product labeling and packaging. The EU authority thus limited states in their ability to introduce different standards, even if they set a higher level of consumer protection or public health. Thus, member countries do not may prohibit or prevent the placing on the market of their country of medicinal products on the basis of reasons related to labeling or instructions for use, if they comply with the requirements of the EU`s directives.

The important step in the reform of admission was the adoption of Directive 93/42/EEC of 14 June 1993 concerning medical devices, which established a new procedure, which come in force since 1995 until the present. The introduction of a single admission procedure, the so-called centralized procedure, was fundamentally new. However, the states retained, in certain cases, their own national procedures. The procedure for implementing a single admission procedure is regulated by the Regulations No. 726/200411, which provided for the establishment of the European Agency for the Evaluation of Medicinal Products (EMEA). The latter draws his conclusion regarding of the new medicine under consideration, on the basis of which the EU Commission takes a decision. The main criteria for research and evaluation are established standards for quality, safety and efficacy.

Tamara Hervey, Bart Vanhercke notes that european integration creates a problem-solving gap where 'member governments have lost more control over national welfare policies, in the face of the pressures of integrated markets, than the EU has gained de facto in transferred authority'. A provision of goods or services forms a part of a national health care system is not sufficient in itself to remove it from the application of EU law [5; p. 94-95].

General debate of last few years comes down to an attempt to resolve hesitation between legal attempts for regulation of 3-D biobrinting and concept of complete prohibition of such activities. Off course, the "ban" is the easiest way out, but such approach will inevitably stop or at least limit the progress of science and technology efforts. "Regulation" approach poses a complex challenge in developing of key principles for 3-D bioprinting because regulatory level of existing synthetic biology is not as comprehensive to give us the answer; it is surely not ready for spreading of unique scientific products to the market, making them available almost for everyone. Therefore, an adequate response to the mentioned challenge is a reasonable position between some aspects of prohibition and self-regulation, resulting in a moderate number of regulations and standards for developing and marketing. Such regulations may concern an intellectual property (IP) rights, regulation of distribution, premarket restrictions, control mechanism etc [6].

Given the insufficient level of regulation of the health insurance institute in Ukrainian legislation, the article attempts to investigate global experience in the context of this issue. In particular, a number of international documents were investigated, their content was characterized and the main provisions were highlighted. Based on collected and analyzed data, the authors outlined the areas of adaptation of Ukrainian legislation to the highest standards of the EU, made a proposal for the ratification of the conventions of the International Labor Organization. Supported a proposal for the development and adoption of a separate special law on general-compulsory state health insurance, which would regulate issues related to health insurance [7].

The absence in the national legislation of clearly defined concepts of medical law limits the potential of legal protection of the rights of patients and necessitates the development and adoption of a single legal act to efficiently regulate the rights and obligations of the physician and the patient, provide classification of healthcare services, procedures and conditions of the services provision, quality criteria, etc. The prospective Medical Code of Ukraine shall become such a normative legal act, since the need for its development provides prospects for further research in this area [8].

As an example of national regulation of this issue, we can refer to the experience of Germany.

In Germany, its implementation is regulated the Lex "On Medicinal Products", which also reflects the requirements of EU directives in the pharmaceutical field. In accordance with this act, the manufacturer (or importer) must confirm the quality, effectiveness and safety (Unbedenklichkeit) of the medicine. At the same time, the quality it is "such a property of a drug that is determined through its structural composition, purity (presence of impurities), identity, through other chemical, physical and biological properties, or through the process of its direct manufacture." The concept of impact efficiency is not defined in the law, but is revealed in judicial practice and scientific literature. Dangerous is such a drug, in respect of which, based on scientific findings, there is a reasonable suspicion that it, when used, has a harmful effect on health (the German Lex "On Medicinal Products").

The other direction of the improvement of legal regulation is formation of a distance selling or mail order market. For example, in Germany one pharmaceutical firm posted on its website offers for the sale of medicines. It was possible to get the ordered medicines either at a pharmacy in Holland, not far from the border with Germany, or by mail. Only offered for sale medicines approved for the German market. Soon against this company a lawsuit was filed because in Germany there is a ban on the sale of medicines by mail. The company referred to the fact that this rule is contrary to freedom of movement of goods. The case was referred to the EU Court of Justice.

At that time Standard Personal Medical Services Agreement 2019 is used in Great Britain. Its current form was prepared by Hill Dickinson LLP on behalf of NHS England and NHS Improvement Classification. Its

aims are: given due regard to the need to eliminate discrimination, harassment and victimisation, to advance equality of opportunity, and to foster good relations between people who share a relevant protected characteristic (as cited under the Equality Act 2010) and those who do not share it; given regard to the need to reduce inequalities between patients in access to, and outcomes from, healthcare services and in securing that services are provided in an integrated way where this might reduce health inequalities." [9]

The European Court of Justice, referring to the Dassonville case [10], found that the prohibition of distance selling in Germany leads to a restriction on the freedom of movement of goods. For German pharmacies, mail order is an additional or alternative way of sales, at the same time, for foreign pharmacies, the established ban excludes access to the German market. Accordingly, such the ban affects national and foreign pharmacies differently and falls under scope of Dassonville case. Checking if the given restriction to be justified in the general interest pursuant, the Court noted that in the presence of a less severe restrictive measure, which, if this is equally effective in achieving the goal (protection of public health), the original restriction is not subject of ban.

Prepares the decision (order) of the State Service for prohibition (stopping) the production, sale (trade), storage and use of medicines that do not correspond the requirements specified by regulations and regulatory documents, including those for which notifications of unforeseen side effects have been received. reactions and / or death of a person as a result of the use of a set or series of medicines for investigation their causes.

In the legislation of Ukraine such acts plays the main role: Order of the Ministry of Health of Ukraine of November 22, 2011 No. 809 "On Approval of the Procedure for Prohibiting (Stoppage) and Renewing the Market Circulation of Medicinal Products on the Territory of Ukraine", registered by the Ministry of Justice of Ukraine of January 30, 2912 No. 126/20439 and The Order of the Ministry of Health of Ukraine of June 06, 2012, No. 422 "On Certain Issues of the Prohibition of the Advertising of Medicinal Products", registered by the Ministry of Justice of Ukraine on July 16, 2012, No. 1189/21501.

Recently in Ukraine there has been a liberalization of the terms of trade in pharmaceutical products. Licensing of pharmacy kiosks in Ukraine has been prohibited since 2013. The application for a licence to open a pharmacy is dealt with by Ukraine's State Administration for Medicines and Control of Narcotics. According to anew regulation, the owner of the licence should not have connections (or be influenced in their activities) by representatives of countries that offer military aggression to Ukraine.

In addition, in the scientific literature, it is quite rightly noted that formation of a state policy on ensuring the rights of citizens to health and life, taking into account the various consequences of such a policy, cannot be narrowed down only to the proclamation of such rights, but also requires planning and development of relevant state programs [11].

Such contracts in law enforcement practice are considered to violate public order. Generally, a contract or an act is thought to be contrary to public policy if it results in a breach of law, harms citizens, or causes injury to the state. Public policy means that courts will occasionally find a contract invalid because it is against the public good.

An example of a legislative definition of a violation of public policy is the article 817 of German Civil Code, which provides: If the purpose of performance was determined in such a way that that the recipient, in accepting it, was violating a statutory prohibition or public policy, then the recipient is obliged to make restitution. A claim for return is excluded if the person who rendered performance was likewise guilty of such a breach, unless the performance consisted in entering into an obligation; restitution may not be demanded of any performance rendered in fulfilment of such an obligation.

Of great interest is the case Kohll, which was considered by The Court of Justice [12]. In 1994, Mr Kohll, a Luxembourg national, wanted his daughter (a minor) to receive treatment from an orthodontist established in Germany and he requested the relevant authorisation from the Luxembourg health insurance fund. That fund refused authorisation on the ground that the treatment was not urgent and could be provided in Luxembourg. Relying on the freedom to provide services (and not Regulation No 1408/71), Mr Kohll took the view that he had the right to seek treatment for his daughter in Germany without prior authorisation and to claim reimbursement of his costs from his health insurance fund, not in accordance with the tariffs of the country of treatment (Germany) but in accordance with the tariffs applied for that type of treatment in his country of insurance (Luxembourg). The Court of Justice held that treatment performed by a healthcare professional should be regarded as a service. In those circumstances, rendering payment for scheduled outpatient treatment, at the tariffs applied in the patient's country, subject to prior authorisation constitutes a barrier to freedom to provide services, since such authorisation deters insured persons from approaching providers of medical services established in another Member State. The Court furthermore observed that such rules are not justified either by a risk of seriously undermining the

financial balance of the social security system or on grounds of public health.

The other issue of contracts with defects may be lack of capacity. Furthermore, to discuss the criterion of legal capacity for medical treatment, it is important to examine the age of majority and competence first, because both of them closely related to the legal capacity. The age of majority is the age that children become adults by law. This means that they are legally in control over their actions and decisions, and their parents are no longer responsible for them. The opposite is a minority, which means being a minor or a child. The age of majority is a legally fixed age and the idea of adulthood which is different in different places [13].

No less important and fundamental for judicial practice is the case Decker [14]. A patient may be prescribed medicines or medical devices by a doctor established in a Member State and decide to purchase the products in a pharmacy located in another Member State (whether by going there in person or by mail order). That was true in the case of Mr Decker, who, in 1992, purchased a pair of glasses for himself in Belgium prescribed by an ophthalmologist established in Luxembourg. The Luxembourg health insurance fund refused reimbursement of those glasses on the ground that the purchase had taken place abroad without prior authorisation. The Court of Justice held that the refusal to reimburse medical products purchased without prior authorisation in another Member State constitutes an unjustified barrier to the free movement of goods, inasmuch as such a requirement is not justified on grounds of public health in order to ensure the quality of medical products supplied in other Member States. Since then, patients have been able to buy their medical devices or medical products in another Member State without prior authorisation and to claim reimbursement from their health insurance fund in accordance with the tariffs applied in their own country (28 April 1998, Decker, C-120/95).

The 1971 Regulation, replaced by Regulation No 883/2004, provides that an employed or self-employed person whose state of health immediately requires healthcare during a stay in another Member State (emergency medical care) is entitled to have that care covered by his health insurance fund, without obtaining its prior authorisation, in accordance with the tariffs applied in the country of treatment. Where a pensioner travels to another Member State and must receive emergency hospital treatment there, his health insurance fund cannot render its provision of cover for the medical costs dependant on the grant of prior authorisation or on the requirement that the illness from which that person suffers manifested itself suddenly, even if that requirement is applicable to employed and self-employed persons. The difference in treatment between pensioners and workers can be explained by the legislature's desire to promote effective mobility of pensioners having regard to their greater vulnerability and dependence in health terms [15].

Among the acts of European law, the 2009 Draft Common Frame of Reference (DCFR) should be singled out [16].

Particular attention is paid to cases of non-compliance with the contract on the grounds of violation of fundamental principles or mandatory rules (Section 3). The DCFR provides for the nullity of a treaty that violates the fundamental principles of EU law and this kind of invalidity is required to have the effect of the relevant principle (Article 7: 301).

If the contracts do not comply with the mandatory rules, these violations affect the validity of the contract in accordance with the provisions of these rules. If the mandatory rules do not explicitly establish the consequences of such a breach, the court may: declare the contract valid, invalidate the contract in whole or in part, change the contract or its consequences. It should be noted that only in this case it is a question of judicial procedure for declaring the contract invalid. In deciding the case, the court must take into account a number of circumstances: the purpose of the violation, the category of persons protected by this rule, the sanction provided for violation, the severity of the violation, whether the violation was intentional, the strength of the relationship between the violation and the contract (Article 7: 302). The consequences of the nullity or invalidation of the contract on these grounds are similar to the consequences provided for out-of-court challenge of the contract (Article 7: 303).

An actually new method of protection, which is not known to Ukrainian law, is the possibility of the court to invalidate certain conditions of the contract, replacing them with the conditions provided by law. Such discretionary power of the court is effective in the context of combating unfair contract terms. Thus, on September 2, 2021, the European Court of Justice heard the case of OTP Jelzálogbank and Others (C-932/19), the essence of which was that in 2007 a Hungarian consumer concluded credit agreements denominated in foreign currency with Hungarian banks. The consumer went to court, claiming that the agreement was invalid, citing the unfairness of the condition that the exchange rate at the time of the loan, which corresponded to the exchange rate of the foreign currency against the Hungarian forint, differed from the exchange rate applicable at the time of repayment funds, which corresponded to the selling rate of this currency to HUF. Solving the dispute,

the Hungarian court stated that under Hungarian law it could not invalidate credit agreements in full, but could invalidate only the conditions specified by the consumer, replacing them with the conditions established by law. The European Court of Justice agreed with the arguments of the national court and noted that the law of an EU Member State may provide that if a consumer agreement on exchange rate differences is recognized when issuing a foreign currency loan and its return is unfair, the loan agreement is invalid only. and not completely. In this case, an unfair condition is replaced by a condition provided by law. This approach is in line with EU law if it allows the court hearing the dispute to restore the balance of rights and interests of the parties of the contract [17]. This approach is fully in line with the provisions of the EU Directive on unfair terms in consumer contracts [18].

CONCLUSIONS

Thus, it should be noted that the problem of good quality of medicine in the field of medical services is one of the most important topics. It should be pointed out that the definition of invalidity, grounds of invalidity and consequences of invalidity are parts of effective ways of protection of rights of a patient. The harmonization of the legislation of the EU member states requires further improvement of the standards for the provision of medical care. This should also include the processes of recodification of the civil legislation of Ukraine. The main consequence of the provision of poor-quality medical services, the use of prohibited methods of treatment and untested preparations is an immediate prohibition. The law orders the medical institution to pay compensation the plaintiff for harm caused or loss suffered.

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