ORIGINAL ARTICLE

PROGNOSTIC SIGNIFICANCE OF METABOLISM INDICATORS OF CONNECTIVE TISSUE IN PATIENTS WITH UPPER URINARY TRACT OBSTRUCTION

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ABSTRACT
The aim: The aim of the study was a comparative analysis of indicators characterizing the state of connective tissue in patients with hydronephrosis due to upper urinary tract obstruction, with the presence and absence of recurrence after surgery.
Materials and methods: Levels of free and bound hydroxyproline, as well as the key mediator of fibrogenesis transforming growth factor-β1 in serum of patients with congenital and acquired obstructions were determined. Ratio peptide-bound and free hydroxyproline were calculated. Groups were divided according to the presence or absence of recurrence of the stricture for a period of 4.5 years after surgery.
Results: Imbalance of the destructive and synthetic processes in extracellular matrix of connective tissue that is characterized by a higher content of fractions of hydroxyproline and transforming growth factor-β1 in the serum were identified. It is shown that the most pronounced changes are observed in patients with relapsing. In patients with a congenital obstruction and a recurrent course of the disease, the highest activation of the collagen metabolism was observed, which was evidenced by the high levels peptide-bound and protein-bound hydroxyproline, relative to these indicators in patients with acquired obstruction (as with the presence and absence of relapses).
Conclusions: The increase in the ratio of peptide-bound/free hydroxyproline and the level of transforming growth factor-β1 in the blood of patients with stage II-III hydronephrosis on the 21st day after surgery may be a prognostic marker for the development of disease recurrence.

KEY WORDS: connective tissue, hydronephrosis, hydroxyproline, transforming growth factor-β1

INTRODUCTION

The number of patients who experience relapses with urinary tract obstruction, caused by the stricture of the ureteropelvic and ureteral segments remains rather high (15-18%) regardless of the technique of performing the operative intervention [1]. The disease often proceeds asymptomatically, leading to pronounced structural and functional changes in the renal parenchyma when the organ saving surgical operation is already ineffective. A prolonged existing obstruction leads to impairment of the functional state of the kidneys, and ultimately, to the development of chronic renal failure, its morphological equivalent is nephrosclerosis. In connection with this important is not only the surgical removal of stricture with the restoration of normal passage of urine and preservation of kidney function, but also the prevention of its recurrence. Therefore, the problem of assessing the risk of recurrence of strictures continues to be relevant.

In the basis of the cicatricial changes development that leads to recurrence of stricture and hydronephrosis, there is the connective tissue reaction, the disruption of the dynamic equilibrium of its tissue system and the inadequacy of reparative regeneration underlies.

It is common knowledge that the basis of general pathological processes: inflammation, regeneration and sclerosis is the reaction of connective tissue, formative such wise the development of the stereotyped response of the organism to damage and adaptation reserves. All these processes form the basis for the development of recurrences of the disease to determine the current and long-term prognosis [2]. The features of the altered connective tissue lead to more frequent intra- and postoperative complications, including a poor healing of postoperative wounds and the formation of pathological scars. Therefore, it is important to determine changes in the parameters of connective tissue metabolism. One of the indicators used to study of the processes of its metabolism is the level of hydroxyproline in the serum, reflecting the processes of collagen biosynthesis [3, 4].

Inadequate response of the connective tissue to the damaging factor is largely realized due to violations of regulatory function and imbalance in the cytokine system [5]. The key cytokine triggering the processes of collagen accumulation is considered to be the transforming growth factor-β (TGF-β), which promotes the synthesis of extracellular matrix of connective tissue, is the dominant regulator
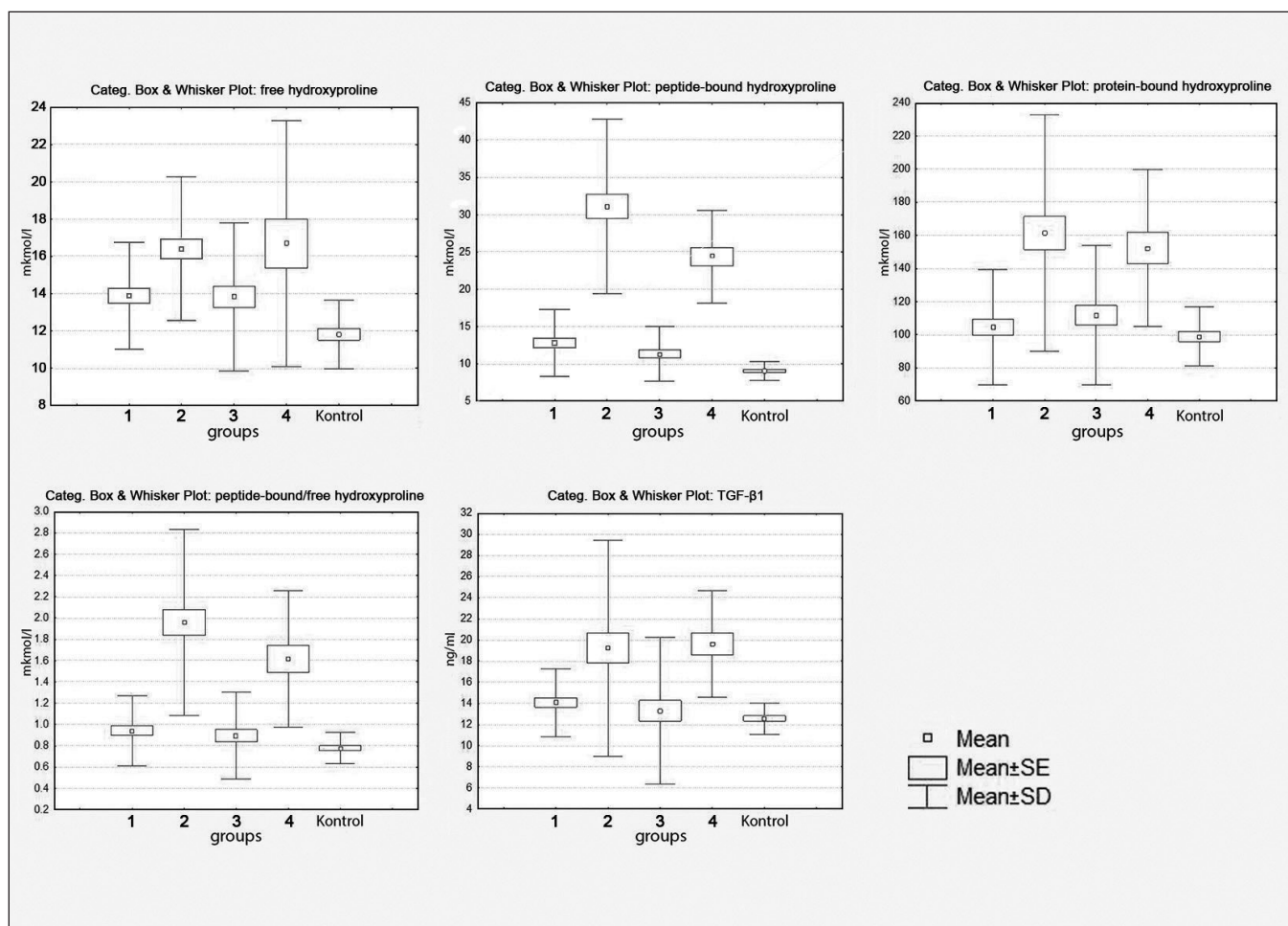
Fig. 1. Content of fractions of hydroxyproline and transforming growth factor-β1 in the blood serum of patients with hydronephrosis of stages II-III depending on the course of the disease

of expression of metalloproteinases [6, 7]. The isolation of informative prognostic risk factors for the development of postoperative complications associated with the disorders in the regulation of the connective tissue system is undoubtedly an urgent task.

The study of the condition and the reaction of the connective tissue of patients with obstructive pathological process of the urinary tract is considered to be relevant, not only from the point of view of assessing the severity of sclerotic changes, but also to assess the reserves of adaptation of the whole organism and the capacity for adequate substitution regeneration.

THE AIM

The aim of the study was a comparative analysis of indicators characterizing the state of connective tissue in patients with hydronephrosis due to upper urinary tract obstruction, with the presence and absence of recurrence after surgery.

MATERIALS AND METHODS

Indicators of the functional state of connective tissue in 178 patients with hydronephrosis of stages II-III were

studied, due to the obstruction of the upper urinary tract of various etiologies and course, surgical intervention was required. The work was carried out in accordance with the requirements of the Helsinki Declaration (2013 revision) with the approval of the local Bioethical Committee. Signed informed consent allowing use of their data in this study was obtained from all patients. Among them are 105 women – (59%) and 73 men – (41%), the average age was 38.4 ± 3.8 years. The diagnosis was made based on the results from clinical-anamnestic and laboratory-instrumental studies.

All patients were divided into four groups, representative of age, sex, approaches to surgical intervention and the characteristics of the postoperative period: group 1 – 52 patients who had no relapse after surgery (obstruction against congenital anomalies of the urinary system); group 2 – 51 patients who experienced relapse of the disease (obstruction on the background of congenital anomalies of the urinary system); group 3 – 50 patients who did not have recurrence of the disease (acquired obstruction); group 4 – 25 patients with recurrent disease (acquired obstruction). The observation period after surgery was 4.5 years. Control group – 35 healthy persons, representative by age and sex.

To study the processes of connective tissue exchange, we used the serum hydroxyproline level, which reflects to the
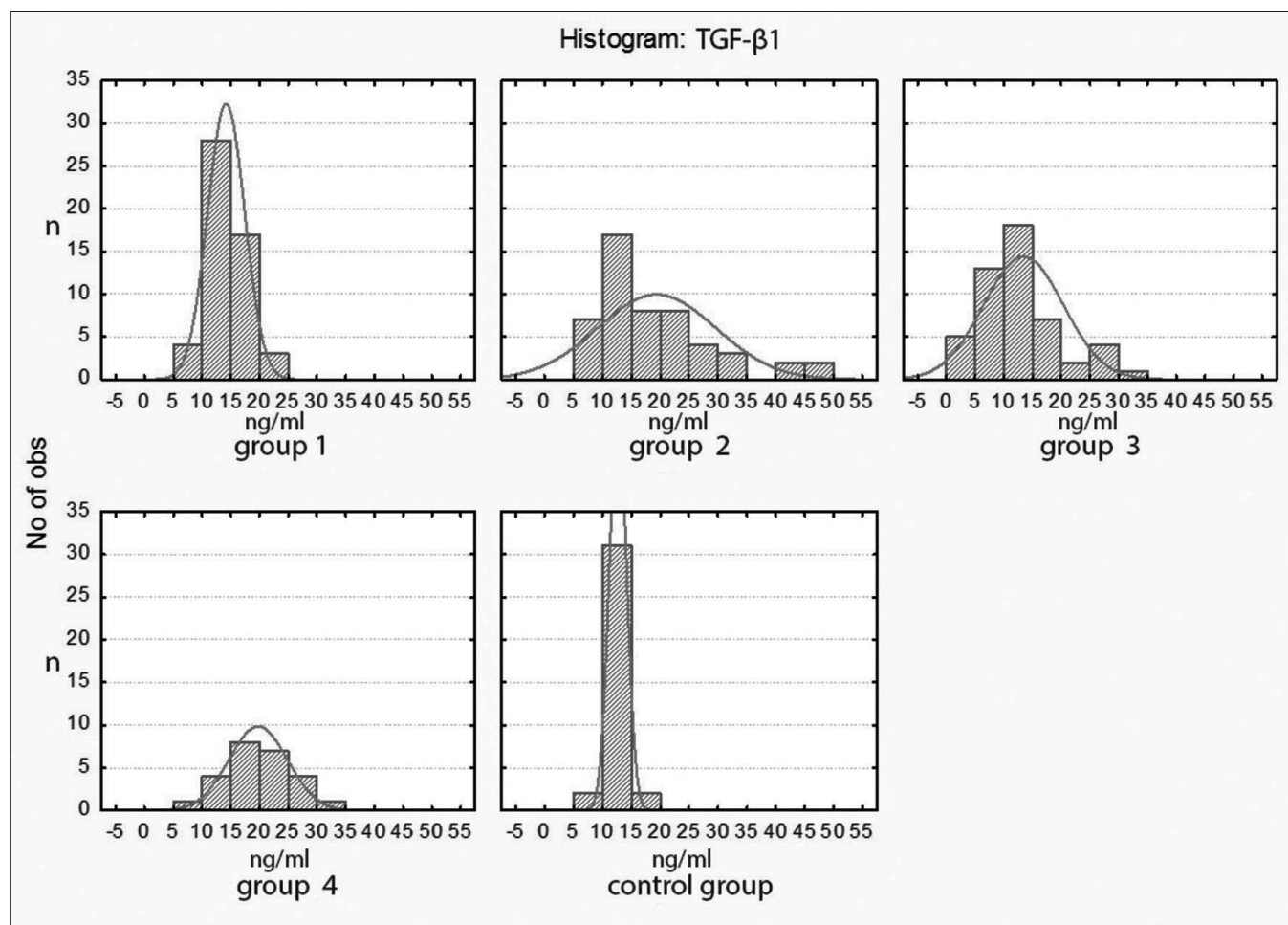
Fig. 2. Deviations of samples from normal distribution when studying the content of TGF-β1 in patients with hydronephrosis II-III stages depending on the course of the disease; axis of abscissae is the concentration of TGF-β1 in the serum (ng/ml), axis of ordinates is the number of patients in the group with the corresponding concentration of TGF-β1

intensity of the collagen formation process [3, 8-10] and the level of the key mediator of fibrogenesis – TGF-β1 [11].

The levels of free, peptide-bound and protein-bound hydroxyproline was determined (according to P.N. Sharaev method, 1990) [12], TGF-β1 – by the method of enzyme immunoassay using reagent kits (DRG, Germany). The ratio of peptide-bound and free hydroxyproline was calculated. Studies were performed on the 21st day after the surgical intervention.

STATISTICAL ANALYSIS

The statistical processing was carried out with the help of the statistical software package Statistica 6.0 by using the variation statistics method of the program – the statistical method one-way ANOVA (Fisher LCD post-hoc test). Statistical significance was considered at a p-level <0.05.

RESULTS

The study of the hydroxyproline fractions content in blood serum allowed assessing the functional state of the connective tissue and sclerotic process activity. Features

of collagen metabolism in patients with hydronephrosis transformation were studied (Fig. 1).

Results indicated that free hydroxyproline content significantly increased in patients of all groups compared with control (p=0.015; p=0.001; p=0.019; p=0.001, respectively). When the groups were compared, the results showed that the level of free hydroxyproline was lower in patients without relapse of the disease (groups 1 and 3) than in patients with relapse (groups 2 and 4) (p<0.05 in all cases). There were no statistically significant differences between indicators 1 and 3 in the groups, and between indicators 2 and 4 in the groups.

The content of peptide-bound hydroxyproline was increased in comparison with the control in patients of groups 1, 2 and 4 (p=0.011; p=0.001; p=0.001, respectively), most significantly in patients of groups 2 and 4 (3.5 and 2.7 times, respectively). When the groups were compared, it was observed that the content of peptide-bound hydroxyproline, which simultaneously reflects the degree of decay and pathological synthesis of collagen, was significantly higher in patients with congenital anomalies of the urinary system and recurrent course (group 2) than in patients of other groups (p<0.05 in all cases).

When assessing the level of protein-bound hydroxyproline, its significant increase was found by comparing the control in patients of groups 2 (p=0.001) and 4 (p=0.001) (1.6 and 1.5 times, respectively). The statistically significant differences between the groups were revealed: the level of protein-bound hydroxyproline in patients of groups 2 and 4 was higher in comparison with that of patients in groups 1 and 3 (p<0.05 in all cases).

The mean level of TGF-β1 for all the examined patients was significantly higher than the control and was 16.1 ± 0.57 ng/ml (at a norm of 12.6 ± 0.25 ng/ml) (p<0.05). However, in patients within the groups 1 and 3 (without relapses), there were no significant differences with the control group. When comparing the revealed statistically significant differences between the groups 1 and 2 (p=0.001); 1 and 4 (p=0.001); 2 and 3 (p=0.001); 3 and 4 (p=0.001); the levels of TGF-β1 in patients within group 1 and 3 were significantly lower. To further evaluate the results of the study, depending on the course of the disease, we estimated the distribution of TGF-β1 content in the samples (Fig.2).

When analyzing the concentration distribution graphs, it was noticed that in the 1st and 4th groups the distributions differ slightly from the normal ones. In group 2, there were very high values which were absent in other groups, as a result of which the distribution of the concentration values of TGF-β1 differs significantly from normal and shifted towards high values. In group 3 there were both high and very low values, absent in other groups. In general, the researchers observed that the distributions for patients of all groups were characterized by a wide range of values in comparison with the control most pronounced in patients of groups 2 and 3.

DISCUSSION

Since the level of free hydroxyproline in the blood serum reflects the degradation of collagen [13], an increase in its content in patients of all groups indicates a predominance of degradation of collagen fibers, which is characteristic of an active inflammatory process At the same time changes in the physicochemical properties of not only collagen, but also proteoglycans, structuring fibrils and collagen fibers were observed. These changes are accompanied by the activation and proliferation of the cellular component of the connective tissue (macrophages, mast cells, leukocytes, neutrophils), which in its turn leads to the activation of proteolytic enzymes, a violation of the normal structural and spatial organization of the collagen fibers.

Peptide-bound hydroxyproline is characterized to a greater degree by the products of incomplete collagen degradation and its inability to be involved in secondary synthesis [14]. The increase in its content in patients with a recurrent course, and especially with congenital malformations of the urinary system: may indicate the disruption of homeostatic mechanisms at different levels (including intercellular interactions). This led to a distortion of the stereotyped dynamics of the process of reparative regeneration that loses its adaptive character, and reflect changes in the ultrastructure of collagen.

An increase in protein-bound hydroxyproline indicates a predominance of collagen biosynthesis [15]. Excess collagen synthesis and accumulation of collagen fibers occurs as a result of disturbances in the collagen synthesis / degradation system. Such imbalance leads to fibrosis of the organ, to proliferation of fibrous connective tissue, and in chronic inflammation of the mucous membranes and to replacing of the loose fibrous connective tissue with a dense one. Replacement of damaged own differentiated tissues (as a result of inflammation and surgical intervention) leads to the formation of coarse connective tissue (scar tissue), and, consequently, to the development of relapse. Defects in the biosynthesis of collagen and fibrillogenesis lead to the development of structural defects in the connective tissue, and, consequently, to the deficiency of its function. The discrepancy between the structure of the fibers and the load on them leads to a gradual disorganization of collagen fibers and fibrils, which in turn increases functional insufficiency. In this case, in patients with a recurrent course, there is a combination of several damaging factors: inflammation, mechanical trauma, and in patients with congenital pathology, probably hereditary deterministic disorders at the level of the physiological system of connective tissue.

It is noteworthy that the ratio of peptide-bound and free hydroxyproline levels underwent characteristic changes in patients with recurrent disease (groups 2 and 4), and exceeded the control values by 2.5 and 2.1 times, respectively. The revealed changes may indicate the pathological course of reparative regeneration, the altered reactivity of the extracellular matrix, which is reflected in the rate of synthesis and degradation of biopolymers directly involved in the formation of scar tissue. In this case, the values of this ratio in patients of groups 1 and 3 did not have statistically significant differences from the control and were significantly lower than those of groups 2 and 4.

Thus, in patients with hydronephrosis, there was a disturbance in the balance of destructive and synthetic processes in the extracellular matrix of connective tissue, which is characterized by an increase in the content of fractions of free and peptide-bound hydroxyproline in the blood, and in patients with a recurrent course of protein-bound and the ratio of peptide-bound and free hydroxyproline with an increase in the proportion of peptide-bound. Similar changes in the indices of collagen metabolism indicate fibrillogenesis, the intensity of which does not correlate with the degree of collagen degradation; and the preservation of destructive processes, combined with the processes of fibrogenesis.

The most pronounced changes in connective tissue metabolism were observed in patients of groups 2 and 4, especially in patients of group 2 with congenital pathology. The most pronounced changes in the indices of connective tissue exchange were observed in patients of groups 2 and 4, especially in patients within the 2 groups with congenital pathology. Probably, in the patients of these groups, disturbances of reparative regeneration in time (slowing down,

acceleration) and in the quality of the resulting connective tissue (change in the ratio of the main components) determine the course of the disease and the development of relapses. In patients with non-recurrent course (in groups 1 and 3) these processes were more balanced.

An increase in the level of TGF-β1 in patients with in the groups 2 and 3 was observed due to a sharp increase in the values of some patients, while a significant number of patients retained values close to normal.

In the pathogenesis of the formation of a pathological condition, an inadequate response of the connective tissue to the damaging factor plays a significant role. This happened largely due to violations of the regulatory function and imbalance in the cytokine system. TGF-β1 is a multipotent cytokine, an important modulator of cell growth, inflammation, proliferation and differentiation, extracellular matrix deposition and apoptosis [16]. TGF-β1 is a profibrotic cytokine that stimulates the production of extracellular matrix proteins, its excessive expression leads to the development of fibrosis. TGF-β1 has a pronounced activating effect for myofibroblasts, which plays a key role in wound healing. There is experimental evidence that the inhibition of TGF-β1 synthesis or signaling pathways realized with this factor significantly reduces fibrotic changes [17]. Therefore, the normalization of the expression of this cytokine can be considered as a favorable prognostic sign.

Thus, our studies have revealed differences in the content of markers of connective tissue exchange in patients with relapsed compared with those in patients without relapsed. These changes may indicate a violation of the general scheme of regulation of the remodeling processes of connective tissue and a decrease in the reserves of adaptation of the organism.

It is known that the regulation of collagen metabolism is provided by both local and systemic mechanisms. The study of the concentration of hydroxyproline fractions and the production of TGF-β1 made it possible to reveal some facts that broaden the notion of the peculiarities of connective tissue metabolism and inflammatory response in patients with hydronephrosis. The study of these indicators allows us to assess the functional state of the connective tissue and the activity of the sclerotic process. The results of the study can be used in the prediction of the disease course and the development of new directions for pathogenetically substantiated therapy based on assessing the state of the physiological system of connective tissue. The study of the functional state of connective tissue in patients with hydronephrosis, who require surgical intervention, will allow to develop a pathogenetically justified correction of previously predicted complications. The algorithm of the differentiated approach to the choice of the method of surgical intervention and evaluation of the risk of postoperative complications can be based, including on laboratory methods for assessing the functional state of connective tissue.

CONCLUSIONS

1. In patients with obstruction of the urinary tract due to stricture of the ureteropelvic and ureteral segments, changes in the functional state of the connective tissue, most pronounced in patients with a recurrent course of the disease:
   – changes in the direction of metabolism and the rate of synthesis of hydroxyproline, the main biochemical marker of connective tissue, which is manifested by an increase in the content of its fractions in the blood serum;
   – increase in the content of the transforming growth factor-β1 in the blood serum.
2. Disturbances in regulation at the level of the physiological system of connective tissue can be considered as a factor contributing to the development of relapses, and methods for studying the functional state of connective tissue can be proposed as additional criteria for assessing the risk of recurrence of strictures.
3. The most informative prognostic criteria were the peptide-bound/free hydroxyproline ratio and the level of the transforming growth factor-β1 in serum.

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Conflict of interest:

The Authors declare no conflict of interest.

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A - Work concept and design, **B** – Data collection and analysis, **C** – Responsibility for statistical analysis,
D – Writing the article, **E** – Critical review, **F** – Final approval of the article