ORIGINAL ARTICLE

PATHOMORPHOLOGICAL FEATURES OF GASTROESOPHAGEAL REFLUX DISEASE REALIZATION IN YOUNG PEOPLE WITH AUTOIMMUNE THYROIDITIS

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ABSTRACT

The aim: To evaluate the pathomorphological features of the esophageal mucous membrane in young people with GERD and autoimmune thyroiditis.

Materials and methods: 120 patients with GERD and AIT and 45 people with isolated GERD matched for age, gender and social status were examined. Esophagogastroduodenoscopy, histological study and comparative morphometry of the esophageal mucosa were performed.

Results: The frequency of erosive GERD in the examined groups of patients did not statistically differ. At the same time, integral analysis of the structure of erosive forms of GERD revealed statistically significant redistribution of grades of esophagitis towards its enhancement in patients with comorbid pathology. The histological study showed that in patients with GERD and AIT all the morphometric parameters studied had a significantly more severe course and exceeded similar indicators of the group with isolated GERD: epithelium total thickness, epithelium basal layer thickness, connective tissue papillae height, intercellular space. The analysis of morphological changes frequency showed that epithelium basal layer hyperplasia, dystrophic changes and epithelial edema, elongation of papillae and dilation of intercellular space were significantly more frequent in the group with comorbid pathology.

Conclusions: GERD and euthyroid AIT comorbidity in the student population is accompanied by a statistically significant redistribution of esophagitis grades towards its aggravation. The presence of concomitant euthyroid AIT in patients with non-erosive GERD leads to statistically more pronounced disorganization of esophageal mucosal epithelium.

KEY WORDS: gastroesophageal reflux disease, autoimmune thyroiditis, oesophageal biopsy, pathomorphological study, young population

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INTRODUCTION

The traditional interpretation of gastroesophageal reflux disease (GERD) etiopathogenesis as a chronic disease caused by disorders of gastroesophageal motor-evacuation function with regular reflux of gastric and/or duodenal contents into the esophagus has undergone significant changes [1-3].Not rejecting the importance of chemo-mechanistic aspects («chemical burn» theory) in the development of GERD, modern studies supplement the pathogenesis of the disease with new data on the role of the chronic inflammatory process in the course of nosology; there is an assessment of universal mediators and the search for esophageal-specific mediators of inflammation and sources of their production.

According to the classical «chemical burn» theory, acid-induced death of superficial cells of the esophageal epithelium was thought to provoke an acute granulocytic inflammatory response, which begins in the epithelium and then progresses to the mucosal lamina, with the formation of a defect in the submucosal layer. It has also been suggested that loss of superficial cells of the esophageal mucosa stimulates hyperplasia of progenitor cells in the basal layer of the squamous epithelium, which is a characteristic histological sign of GERD [4-6].

In 2009, the pathogenetic concept of the «chemical burn» was challenged in an animal model of GERD caused by esophagoduodenostomy[7]. Reflux esophagitis in rats did not start from superficial cell death and epithelial infiltration by granulocytes, but rather from T-lymphocytes that first infiltrated the esophageal submucosa and then infiltrated the lamina propria and epithelium. Superficial erosions did not appear until several weeks after esophagoduodenostomy, and basal cell hyperplasia occurred long before the loss of mucosal superficial cells. A culture study of human esophageal epithelial cells found that acids and bile salts caused the release of pro-inflammatory and proliferative cytokines, such as interleukin-8. Based on such data, an alternative hypothesis of GERD pathogenesis was proposed, in which refluxate did not directly destroy esophageal epithelial cells, but rather stimulated them to secrete cytokines [8-10]. The latter caused proliferative changes in the epithelium and mobilized T-lymphocytes and other inflammatory cells, which eventually damaged the mucosa [11-13].

An important factor in pathomorphological realization of GERD may be its combination with other diseases, including autoimmune thyroiditis (AIT), which creates unfavorable immune and humoral background that may contribute to the worsening of GERD course [14,15].

Table I. The incidence structure of different esophagitis grades in the examined patients

Esophagitis grades	GERD and AIT (n=34)	GERD (n=11)	Significance of differences 1
A	6 (17.7%)	7 (63.6%)	df=3 χ2=8.772 p=0.033
B	18 (52.9%)	3 (27.3%)	
C	8 (23.5%)	1 (9.1%)	
D	2 (5.9%)	0 (0%)	

Note: p<0.05 – the difference is statistically significant between groups

Table II. Mucous membrane morphometric parameters of the distal part of the esophagus in the studied patients, M±m

Groups		GERD (n=35)	GERD+AIT (n=50)	Significance of differences 1
Epithelium total thickness, μm		286.1±8.2	319.3±9.1	p<0.01
Epithelium basal layer thickness	μm	49.7±2.1	79.6±3.2	p<0.01
	%	17.3±0.3	25.1±2.9	p<0.01
Connective tissue papillae height	μm	172.7±4.6	224.8±7.3	p<0.01
	%	60.4±3.3	72.3±3.1	p<0.01
Intercellular space, μm		1.12±0.09	1.55±0.11	p<0.01

Note: p<0.05 – the difference is statistically significant between groups

THE AIM

The objective of the study was to evaluate the pathomorphological features of the esophageal mucous membrane in young people with GERD and autoimmune thyroiditis.

MATERIALS AND METHODS

The study was conducted at the Department of General Practice - Family Medicine and Internal Diseases, the Department of Pathological Anatomy, the Department of Internal Medicine no. 1 of Kharkiv National Medical University, Ukraine, between 2017 and 2019. The study was approved by the Ethics and Bioethics Committee of Kharkiv National Medical University. All the procedures and experiments of this study respect the ethical standards in the Helsinki Declaration of 1975, as revised in 2008(5), as well as the national law. Informed consent was obtained from all the patients included in the study.

Criteria for inclusion: students 18 - 25 years old; verified diagnosis of GERD; for the main group - verified diagnosis of AIT.

Criteria for exclusion: hypothyroidism, hyperthyroidism, other endocrine and GIT pathology; diseases of the cardiovascular system, kidneys, lungs; cancer; mental illness; pregnancy and lactation; minority; patient's refusal to participate in the study.

165 patients were examined, including 120 patients with comorbidity of GERD and AIT (main group) and 45 people with isolated GERD (comparison group). The mean age in the groups was 21.9 ± 2.7 and 21.2 ± 2.4 years, respectively (p>0.05). The contingent was represented by students from various universities of Kharkiv (Ukraine); 93 patients (77.5%) of the main group and 34 examined persons (75.56%) of the comparison group were women, 27 (22.5%) and 11 (24.44%) respectively were men. Standard values were obtained while examining 20 almost healthy patients of the same age, gender and social status.

Autoimmune thyroiditis was confirmed by the presence of antibodies to thyroperoxidase and thyroglobulin and thyroid gland ultrasound. The functional state of the thyroid gland was assessed in the previous stages of the study on the content of thyroid-stimulating hormone, free triiodothyronine and thyroxine, all patients were diagnosed with euthyroid status.

The diagnosis of GERD was confirmed by typical complaints, history, clinical and instrumental data. Visual assessment of the esophageal mucous membrane was performed by endoscopic examination of the esophagus (videoendoscopic system "Fuginon", Japan) with biopsy and subsequent histological examination of the material.

Material for the histological study was taken from the mucous membrane of the distal esophagus 3 cm above the conventional circular line connecting the stomach and esophagus. Pieces of the mucosa of the distal esophagus were fixed in formalin, passed through alcohols in increasing concentration, embedded in paraffin and prepared 5 μm thick sections that were stained with hematoxylin-eosin and picro-fuchsin according to Van Gieson method. Microscopic examinations were performed on an Olympus BX-41 microscope. Morphometric parameters were obtained using the Olympus DP-Soft (Version 3: 1). The total thickness of the epithelium, basal layer thickness, the height of connective tissue papillae and intercellular space were determined in 10 random fields of view in high (x40 lens, x10 eyepiece).

Statistical processing was performed using Statistica software. The results were analyzed using methods of descriptive statistics: calculation of arithmetic mean, 95% confidence interval, and standard error in the sample. Differences obtained by paired comparisons were considered statistically significant at $p < 0.05$.

Table III. The frequency of morphological changes of the esophageal mucosa in the studied patients

Sign	GERD (n=35)		GERD and AIT (n=50)		Significance of differences (χ2)1
	Abs.	%	Abs.	%	
Epithelium basal layer hyperplasia	22	62.9	44	88.0	df=1 χ2=7.499 p=0.006
Epithelial edema	21	60.0	41	82.0	df=1 χ2=5.049 p=0.025
Connective tissue papillae elongation	20	57.1	41	82.0	df=1 χ2=6.278 p=0.012
Submucosal fibrosis	19	54.3	36	72.0	df=1 χ2=2.829 p=0.093
Intercellular space dilation	19	54.3	39	78.0	df=1 χ2=5.342 p=0.021
Dystrophic changes	23	65.7	45	90.0	df=1 χ2=7.589 p=0.006
Leukocyte infiltration	22	62.9	39	78.0	df=1 χ2=2.33 p=0.127

Note: $p<0.05$ – the difference is statistically significant between groups

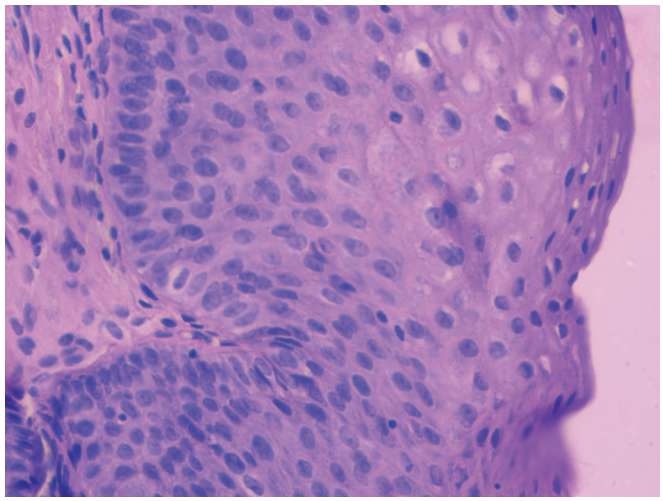

Fig. 1. Esophageal epithelium thickening due to the basal zone hyperplasia in a patient with comorbid pathology (staining with hematoxylin and eosin, x 400)

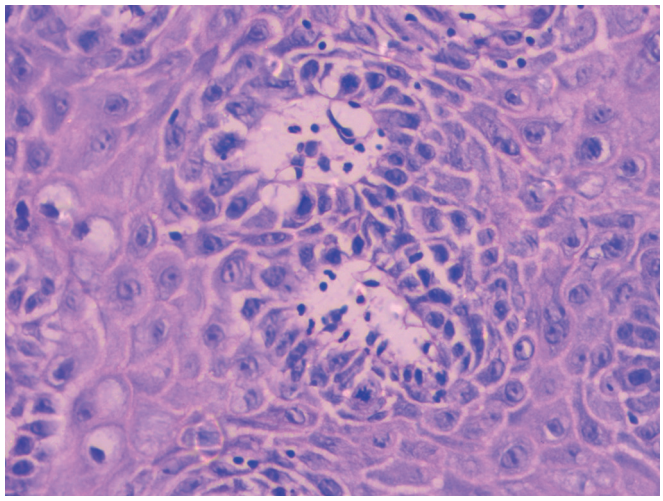

Fig. 2. Leukocyte infiltration of the esophageal mucosa with the presence of rare eosinophils in a patient with comorbid pathology (staining with hematoxylin and eosin, x 400)

RESULTS

The endoscopic examination of patients with comorbid GERD and AIT revealed erosive lesions of the esophagus in 34 (28.3%) and nonerosive – in 86 (71.7%) cases. In the comparison group, erosive GERD was found in 11 (24.4%) and nonerosive – in 34 (75.6%) patients. The grade of esophagitis was determined according to the Los Angeles classification (Tab. I).

The frequency of erosive GERD in the examined groups of patients did not statistically differ (df=1, χ^2=0.250, p=0.618). At the same time, integral analysis of the structure of erosive forms of GERD revealed statistically significant redistribution of grades of esophagitis towards its enhancement in patients with comorbid pathology (df=3, χ^2=8.772, p=0.033).

Taking into account the fact that erosive esophageal lesions were observed in a small number of patients, who also had different grades of esophagitis, which made statistical processing impossible. Thus, samples of patients

with a non-erosive form were chosen for histological study: 35 cases of isolated GERD and 50 – of GERD and AIT comorbidity.

The following histological peculiarities of esophageal mucosal epithelium were noticeable during the examination of biopsy material in both groups of patients. First of all, significant changes in multilayer squamous epithelium were noted: quite often stratification of its layers was disturbed, basal zone hyperplasia and elongation of stromal papillae were noted.

As a rule, the thickness of the basal layer is formed by a few cells and is less than 15% of the total thickness of the epithelium, and the length of the papillae does not exceed 50%[16-18]. In the studied groups, the thickness of the basal epithelium zone was greater both in absolute value and in relation to the total thickness of the epithelium. (Tab. II)

It should be noted that in GERD and AIT group, the epithelium thickening was mainly due to the basal layer, which was 25.1%, whereas in GERD isolated group this index was close to the physiological norm at the level of 17.3% (Fig.1).

During the histological examination, it was noticed an increase in the size of the nuclei, their hyperchromicity, the presence of physiological mitoses, which also indicated the activation of regenerative processes. Characterizing histological changes in epithelial cells in patients with isolated GERD, we should note significant dystrophic and focal necrotic changes on the background of pronounced edema of the spinous and basal layers.

At the same time, in patients with GERD and AIT comorbidity, epithelial cells were polymorphic with cytoplasm vacuolization, some of them were sharply increased in size with signs of parenchymatous protein (hydropic) degeneration and nuclear dislocation to the cell periphery. The above changes in the epithelium were observed in combination with significant intercellular edema with loss of normal orientation of superficial epithelial cells, in some places with the presence of intercellular bridges.

Morphologically, a significant increase in the length of papillae was detected both in isolated GERD and in combined pathology. However, an intergroup comparison revealed that in patients with GERD and AIT the thickness of the basal epithelial layer and the length of connective tissue papillae was significantly greater (Tab.II).

It should be noted that the increased thickness of the basal layer may reflect the increased proliferative activity (intensification of regenerative processes) of its cells. The length of connective tissue papillae reached 75% of the epithelial layer, and its increase is most likely explained by secretion of inflammatory mediators, stimulating proliferation of fibroblasts, endothelium and smooth muscle cells. The submucosal layer showed morphological signs of fibrosis with sclerotic changes in the lamina propria with thickening of collagen fibers, which also can cause changes in the shape and length of villi.

The leukocytic infiltration in submucosa was less pronounced in the group with isolated GERD, where lymphocytes and macrophages predominated in the infiltrate. In the group of combined pathology, infiltration was more intensive and polymorphic, the presence of neutrophilic leukocytes, especially in foci of dystrophic and necrotic changes was determined, accumulation of eosinophils was observed in some preparations (Fig. 2).

Thus, the main morphological signs of reflux esophagitis in both groups were: basal layer hyperplasia; elongation of connective tissue papillae; intercellular edema with the intercellular space dilation; dystrophic changes with cytoplasm vacuolization, and in some places focal necrotic changes of epithelial cells; the presence of marked inflammatory infiltration in submucosa layer. In spite of the fact that above mentioned signs were noticed both in patients with a combination of GERD and AIT and with isolated GERD, the intergroup comparison revealed some significant differences in these values (Tab. III).

Thus, epithelium basal layer hyperplasia, dystrophic changes and epithelial edema, elongation of papillae and dilation of intercellular space were significantly more frequent in the group with comorbid pathology. Besides, patients with GERD and AIT had a higher frequency of inflammatory leukocytic infiltration (78%) combined with signs of submucosal fibrosis (72%) in comparison with the group of isolated GERD – 62.9% та 54.3% respectively, however, these differences were not significant.

DISCUSSION

Research data of recent years indicate significant progress in the study of pathogenetic links, pathways and mechanisms of GERD progression, naturally accompanied by optimization of diagnostic approaches and therapeutic strategy. Therefore, in recent years, the increasing attention of scientists is attracted by the comorbid course of GERD with other diseases. This problem is of particular importance when combining with autoimmune pathology, which is usually associated with the development of systemic inflammation that can act as an additional factor of GERD progression. The development of GERD in young people is of great importance, since the formation of chronic pathology at an early age, especially in the presence of such " insidious" companion as AIT, may be accompanied by rapid progression of nosology and early development of complications.

However, at the current stage of medical science development, there are practically no studies of pathomorphological features of the esophagus in young patients with GERD and AIT comorbidity.

The presented study identified the significant dilation of the intercellular space in patients of both groups, which is considered by the overwhelming majority of scientists to be a classic marker of GERD. At the same time, some authors believe that dilation of the intercellular space in the esophageal epithelium is not a pathognomonic sign of GERD and can be observed in patients with psychological stress [19], which is relevant to the student population and may have been an additional trigger in this category of patients. In addition, according to Lori A Orlando et al., dilation of the intercellular space may be a sign of epithelial barrier disorder due to increased intercellular permeability [20].

The study of Jeremy R Parfiftt et al. compared the histological picture of esophageal mucosa in GERD and in eosinophilic esophagitis and showed the commonality of certain changes for both nosologies – elongation of villi, hyperplasia of the mucosal basal layer and intercellular edema [21]. Meanwhile, the key differential diagnostic criteria for eosinophilic esophagitis are the presence of 15 or more eosinophils in the field of view, eosinophilic micro abscesses, superficial eosinophilic infiltrates and eosinophil degranulation. Eosinophilic esophagitis is known to be an immune-mediated inflammatory disease of the esophagus, so the presence of eosinophil accumulation in selected preparations of patients with comorbid pathology may have been a consequence of the influence of an additional autoimmune inflammatory component brought on by AIT.

It should be noted that eosinophils are physiologically present in the GI tract, but their presence in the esophagus is pathological. Eosinophilic infiltration leads to thickening of the esophageal mucosa, basal layer hyperplasia, and villous deformity [22]. In addition, eosinophils are able to secrete cytotoxic granules, directly act on neurons and damage axons of the esophageal sphincter muscle fibers. The latter leads to decreased tone and progression of GERD [23,24]. The presence of chronic inflammation in the mucosa and submucosa layer of the esophagus also leads to the activation of fibroblasts and causes the formation of fibrosis and esophageal stenosis [25].

CONCLUSIONS

GERD and euthyroid AIT comorbidity in the student population is not associated with the prevalence of an erosive form of GERD, but it is accompanied by a statistically significant redistribution of esophagitis grades towards its aggravation.

The presence of concomitant euthyroid AIT in patients with non-erosive GERD leads to statistically more pronounced disorganization of esophageal mucosal epithelium due to basal layer hyperplasia, edema of spinous and basal layers, dilation of intercellular space, elongation and deformity of connective tissue papillae.

Signs of submucosal fibrosis, marked dystrophic and in some places, necrotic changes in epithelial cells and inflammatory infiltration in the submucosal layer should also be considered as characteristic features of GERD in the examined patients, meanwhile, the presence of concomitant euthyroid AIT non significantly, but increases the expressiveness of these deviations.

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The Authors declare no conflict of interest.

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