

## ORIGINAL ARTICLE

## EOSINOPHILIC GASTRITIS IN CHILDREN: MORPHOLOGICAL AND IMMUNOHISTOCHEMICAL DIAGNOSTIC CRITERIA

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### ABSTRACT

**The aim:** To study peculiarities of morphological and immunohistochemical changes of stomach's mucosa in eosinophilic gastritis in children

**Materials and methods:** 64.1±6.0% patients with eosinophilic gastritis and 35.9±6.0% patients with lymphocytic gastritis participated in our investigation.

In order to verify the diagnosis morphological and immunohistochemical diagnostics of the stomach's mucosa was performed in all children. To assess morphological changes in tissues the specimens were colored with hematoxylin, eosin and picrofuchsin by van Gieson's. Indirect streptavidin-peroxydase staining method was used for immunohistochemical investigation and the following indexes were assessed: proliferating cell nuclear antigen – PCNA, Bcl – 2, Bax, Collagen Type IV, TGFβ and NF-κβ.

**Results:** Comparative analysis of morphologic investigation has demonstrated that eosinophilic gastritis is characterized by fibrosis and fibroblasts proliferation into basal and superficial parts of mucosa's lamina propria, multiple hemorrhages, thrombosis and erosions on the background of eosinophilic infiltration. Immunohistochemical indexes of cellular restoration in eosinophilic gastritis are characterized by increased proliferative activity and decreased indexes of proapoptotic and antiapoptotic activity. Prevalence of the reaction with the use of monoclonal antibodies to Collagen Type IV in majority of children with eosinophilic gastritis was characterized by separate fragmented foci in basal membranes of superficial epithelium. Remarkable TGFβ immune coloration was detected in majority of children on the background of fibrosis and eosinophilic infiltration of lamina propria. NF-κβ expression in epitheliocytes' cytoplasm and nuclei was uneven. Homogenous remarkable coloration was detected in majority of patients with lymphocytic infiltration of mucosa.

**Conclusions:** Eosinophilic gastritis course in children is characterized by remarkable inflammation, decreased regeneration of the mucosa, impairment of cellular restoration which is prognostic index of fibrous remodeling development.

**KEY WORDS:** eosinophilic infiltration, fibrosis, cellular restoration, children

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### INTRODUCTION

Eosinophilic diseases of the gastro-intestinal tract (GIT) are chronic immunopathological diseases accompanied by predominant eosinophilic inflammation of mucosa of different parts of the gut. Diagnostics of these diseases is quite problematic due to poorness of clinical symptoms and morphological investigation of the gut mucosa's biotates is the own standard of eosinophilic diseases verification.

Taking into account scientific researches data the leading signs of eosinophilic inflammation are aggregation of eosinophils in lamina propria combined with stomach's mucosa fibrosis [1,2]. Among pathogenetic mechanisms of eosinophilic affection of the stomach's mucosa a chief place is occupied by selective chemokine – eotaxin, which regulates migration of eosinocytes with following activation of cytokines. Physiologically eosinocytes are present in mild quantity in stomach's mucosa fulfilling physiological functions of, first of all, regeneration there [3,4]. Being activated eosinocytes produce eosinophilic peroxydase, eosinophilic collagenase and chemokines of 2<sup>nd</sup> generation, which increase vascular permeability, reinforce fibroblasts proliferation and contribute to fibrous remodeling [5]. Today

a leading role of TGFβ in promoting fibrous remodeling of gut's mucosa is proved and it is well studied that expression of this factor is increased in eosinophilic gut diseases in both children and adults. TGFβ belongs to a group of cytokines with remarkable immune suppressive effect regulating the intensity of inflammatory, immune and regenerative processes, and fibrogenesis [6,7]. Scientific researches analysis has demonstrated that activation of transcriptional factor NF-κβ is influenced by infectious and non-infectious work upon epithelial and dendrite cells, macrophages and neutrophils of the gut [8]. Proteins of NF-κβ family are the most important transcriptional factors which estimate nonspecific protection regulating the level of proteins of an acute phase, cytokines, chemokines and antiapoptotic proteins.

In modern classification GIT eosinophilic diseases are differentiated according to main affected organ, so there are eosinophilic esophagitis, eosinophilic gastritis, eosinophilic gastroenteritis and eosinophilic colitis. Eosinophilic gastritis is a chronic immunopathological disease with eosinophilic infiltration of stomach's mucosa. According to the literature data its prevalence in population makes 3 – 8:100000 [9,10].

Modern notions about foundation and development of eosinophilic gastritis are based on generalizing conception of eosinophilic inflammation and have no strict morphological diagnostic criteria. Presented data are a serious argument in favor of necessity of study of morphofunctional and immunohistochemical regularities of stomach's mucosa eosinophilic inflammation development in eosinophilic gastritis.

The aim: to study peculiarities of morphological and immunohistochemical changes of stomach's mucosa in eosinophilic gastritis in children.

## MATERIALS AND METHODS

Investigations have been carried out with a strict adherence to basic statements of the GCP Council of Europe Convention on Human right and biomedicine and basic statements of Ethical principles for medical research involving human subjects of World Medical Association Declaration of Helsinki.

We observed 65 children aged 8-16 years with verified chronic gastritis. Esophagogastroduodenoscopy (upper endoscopy, UE) with a target biopsy of stomach's mucosa has been performed in all children to verify the diagnosis with the help of morphological and immunohistochemical investigation. To assess histological changes of stomach's mucosa a tissue fragments were stained with hematoxylin, eosin and picrofuchsin by van Gieson's. The results of study have been interpreted in accordance with Kyoto Consensus, 2015.

For immunohistochemical examination, sections 4-6  $\mu$ m thick were applied to Super Frost Plus adhesive slides and an indirect streptavidin peroxidase staining method was used. Apoptosis was determined with murine monoclonal antibodies to antiapoptotic Bcl - 2 protein (Clone 124, DAKO, Denmark) and proapoptotic Bax protein (Clone 2D2, DAKO, Denmark). Proliferating cells nuclear antigen (Proliferating Cell Nuclear Antigen - PCNA) (Clone: PC10, DAKO, Denmark) was used to estimate proliferation. Collagen was typed with monoclonal antibodies to Collagen Type IV (Clone COL - 94, DAKO, Denmark). Transforming growth factor TGF $\beta$  and nuclear factor NF- $\kappa$ B were typed with polyclonal antibodies to TGF $\beta$  and NF- $\kappa$ B (DAKO, Denmark). Immunohistochemical staining results interpretation was made according to the type of reaction. When using monoclonal antibodies to antiapoptotic Bcl - 2 protein and proapoptotic Bax protein, color reactions in the cytoplasm of cells were evaluated; when using nuclear antigen of proliferating cells, nuclear staining was taken into account. The count of positive reactions was performed according to the number of edited cells and was expressed as a percentage of the total number of cells in the area of the histological preparation. When interpreting immunostaining using monoclonal antibodies to Collagen Type IV and polyclonal antibodies to TGF $\beta$ , NF- $\kappa$ B, the prevalence and intensity of the reaction was evaluated by a semi-quantitative method in points from 0 to 3 as follows:

a) prevalence:

1) 0 - no coloration;

2) 1 - less than 10% of positively stained cells;

3) 2 - more than 10% and less than 50% of positively stained cells;

4) 3 - homogeneous staining of more than 50% of cells;

b) the intensity of the reaction:

1) 0 - no visible color;

2) 1 - weak color;

3) 2 - moderate color;

4) 3 - expressive color.

Statistical processing of the results was performed according to generally accepted methods of variation statistics.

## RESULTS

Morphological assessment of stomach's mucosa biopates eosinophilic gastritis was detected in  $64,1 \pm 6,0\%$  patients and lymphocytic gastritis - in  $35,9 \pm 6,0\%$  ones.

Biopates of stomach's mucosa in lymphocytic gastritis were characterized by the violation of mucosa's relief in the form of shortening of holes and flattening of rollers and perivascular swelling signs. Superficial epithelium was found to be with areas of desquamation and flattened foci. Mucosa's lamina propria was infiltrated by lymphocytes up to 25 cells in the field of vision, single plasmocytes, eosinocytes and neutrophils which were basically localized in superficial parts of the mucosa and inside the rollers. Foci with atrophic mucosa with decreased quantity of glandules with impaired architectonics were detected as well (Fig.1).

Stomach's mucosa biopate in eosinophilic gastritis was characterized by impaired mucosa's relief, shortened holes and flattened rollers in all samples. Superficial epithelium was marked with the fields of desquamation and foci of flattened areas. Lamina propria was infiltrated by lymphocytes to 7 cells in field of vision and eosinocytes to 25 - 30 cells in field of vision; areas of perivascular swelling, erosions, hemorrhages and microthrombosis were detected in all biopates. Fibrotic foci sized from 50 - 70  $\mu$ m to 100 - 150  $\mu$ m, areas of proliferating fibroblasts and thin collagen fibers with unclear outlines in basal and superficial parts were detected in lamina propria. Glandules in lamina propria were located unevenly and contained centers of destruction (Fig. 2).

Cellular restoration indexes assessment was performed on the first stage of immunohistochemical assessment (PCNA, Bax i Bcl - 2). Immunohistochemical indexes of cellular homeostasis in eosinophilic gastritis were characterized by the increased proliferative activity due to increased expression of PCNA from 10.6% to 42.1% of positively colored glandular epithelium's nuclei and decreased proapoptotic activity - Bax expression from 10.3 to 29.1% of positively colored cells and Bcl-2 expression - less than 10% of positively colored cells (Fig.3).

A tendency to Bax proapoptotic index increase from 82.4% to 96.1% of positively colored cells in low Bcl-2 expression (less than 10% of positively colored cells) and low PCNA expression from 0.9% to 2% of positively colored cells was detected during analyzing the levels of

indexes of cellular restoration in patients with lymphocytic gastritis (Fig. 4).

Patients' data testifying to fibrin and collagen fibers proliferation in lamina propria as a sign of stromal and epithelial realignment received in eosinophilic gastritis indicated to the necessity of investigation of collagen type IV which characterizes trophic function of lamina propria and regenerative processes quality. Expression of receptors towards collagen type IV was found as separate fragmented foci in basal membranes of superficial epithelium and single glandular structures. In majority of children with eosinophilic gastritis ( $51,2 \pm 7,8\%$ ) prevalence of the reaction with monoclonal antibodies to Collagen type IV was less than 10% of positively colored cells (Fig. 5a) and in lymphocytic gastritis – more than 10% and less than 50% of positively colored cells (Fig. 5b).

The listed above indicates to the actuality of study of transforming growth factor TGF $\beta$  and nuclear factor NF- $\kappa$  $\beta$  which estimate effectiveness of the immune reaction on infectious and noninfectious factors and directly participate in regulation of inflammatory process type and explicitness. Expression of receptors to TGF $\beta$  in cytoplasm and nuclei was uneven. Remarkable immune coloration of TGF $\beta$  with more than 50% of positive cellular elements was detected in  $41.5 \pm 7.7\%$  of patients in eosinophilic gastritis with marked fibrosis and proliferation (Fig. 6a). In lymphocytic gastritis prevalence of reaction with TGF $\beta$  was estimated in only  $13.0 \pm 7.0\%$  of patients and made less than 10% of positively colored cells in mild coloration of the reaction (Fig. 6b).

Expression of NF- $\kappa$  $\beta$  in epitheliocytes' cytoplasm and nuclei was uneven. Homogenous coloration of more than 50% of positive cells was found in  $52.2 \pm 10.4\%$  of patients with lymphocytic infiltration of stomach's mucosa (Fig. 7a). In eosinophilic infiltration of the mucosa prevalence of the reaction with NF- $\kappa$  $\beta$  was estimated just in  $14.6 \pm 5.5\%$  of patients and it was less than 10% of positively colored cells in mild coloration of the reaction (Fig. 7b).

## DISCUSSION

On the basis of the results of the performed research stomach mucosa's morphological changes pathognomonic for eosinophilic gastritis were estimated; these changes were found to include eosinophilic infiltration, fibrosis of stroma of lamina propria, microcirculatory disorders with multiple hemorrhages, thrombosis and erosions. In our point of view microcirculatory disorders of stomach's mucosa are caused by eosinophilic infiltration of lamina propria. As it is known eosinocytes contain high concentrations of peroxydase which increases vascular permeability and leads in vasculitis development.

Decreased indexes of immune coloration to Collagen Type IV as an index of stromal and vascular component of the gut in patients with eosinophilic gastritis, in our point of view, indicate to violation of trophic function of the mucosa's lamina propria, decreasing of regenerating

processes contributing to mucosa's dystrophic changes with further development of fibrosis in eosinophilic gastritis.

Structural peculiarities of dysregeneration characterized by dysbalance of cellular regeneration processes were detected in eosinophilic gastritis in children. Increasing of epitheliocytes proliferation index is associated with increasing of undifferentiated cells which are not able to fulfill their usual function leading in the development of stromal and epithelial rebuilding of the mucosa and its impaired regeneration.

Analysis of peculiarities of receptors expression to TGF $\beta$  in epitheliocytes' cytoplasm and nuclei indicated that in majority of patients with eosinophilic inflammation with fibrosis and fibroblasts proliferation into basal and superficial parts of lamina propria prevalence of positively colored cells and intensity of the reaction was higher. The received results correspond to literature data clarifying the leading role of TGF $\beta$  in potentiating of fibrous remodeling of mucosa in eosinophilic affections of the gut.

Analysis of the results of immunohistochemical investigation of NF- $\kappa$  $\beta$  expression demonstrated that a weak level of prevalence of the reaction with polyclonal antibodies was detected in eosinophilic infiltration of the mucosa which, in our point of view, testifies to the decreased functional activity of local immune system of epitheliocytes and its regulating function in eosinophilic gastritis.

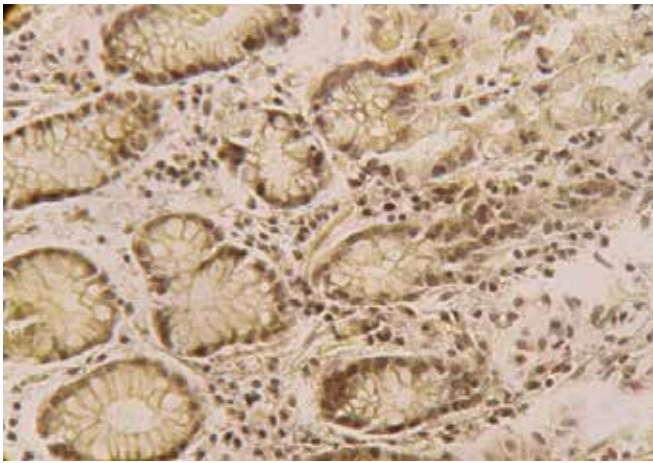
## CONCLUSIONS

Thereby comparative analysis of morphologic and immunohistochemical changes of stomach's mucosa demonstrated that eosinophilic gastritis is characterized by remarkable inflammatory changes and impaired regeneration of the mucosa with further development of fibrosis.

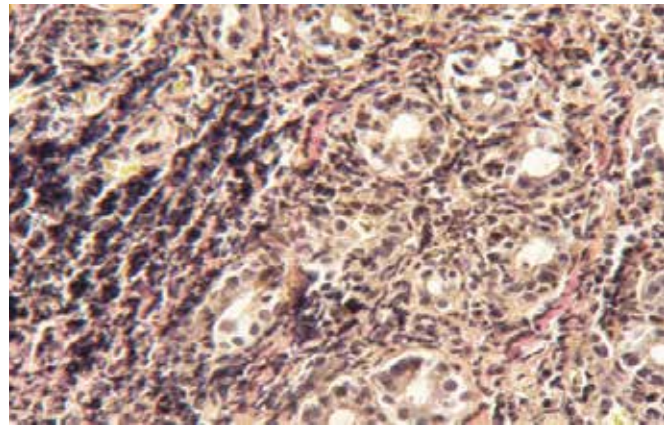
Results of the investigation testify to the important role and multidirectional functional load of TGF $\beta$  and NF- $\kappa$  $\beta$  in formation of eosinophilic gastritis in children. Activation of transcriptional factor NF- $\kappa$  $\beta$  and transforming growth factor TGF $\beta$  estimate inflammatory process activity degree, peculiarities of cellular restoration and mucosa blood supply in eosinophilic gastritis.

As conclusion we should note that the course of eosinophilic gastritis in children is characterized by dysbalanced processes of cellular restoration which leads in mucosa physiological regeneration violation and is prognostic index of fibrous remodeling. Everything listed allows to make conclusion that the quality of mucosa restoration may be the most important factor in estimation of probability of fibrous remodeling in eosinophilic gastritis. Proper restoration of cytoarchitectonics of the mucosa requires balanced stimulation and cooperation of epithelial elements and components of the connective tissue, activation of growth factors participating in inflammation development, immune answer, cellular apoptosis and tissues reparation.

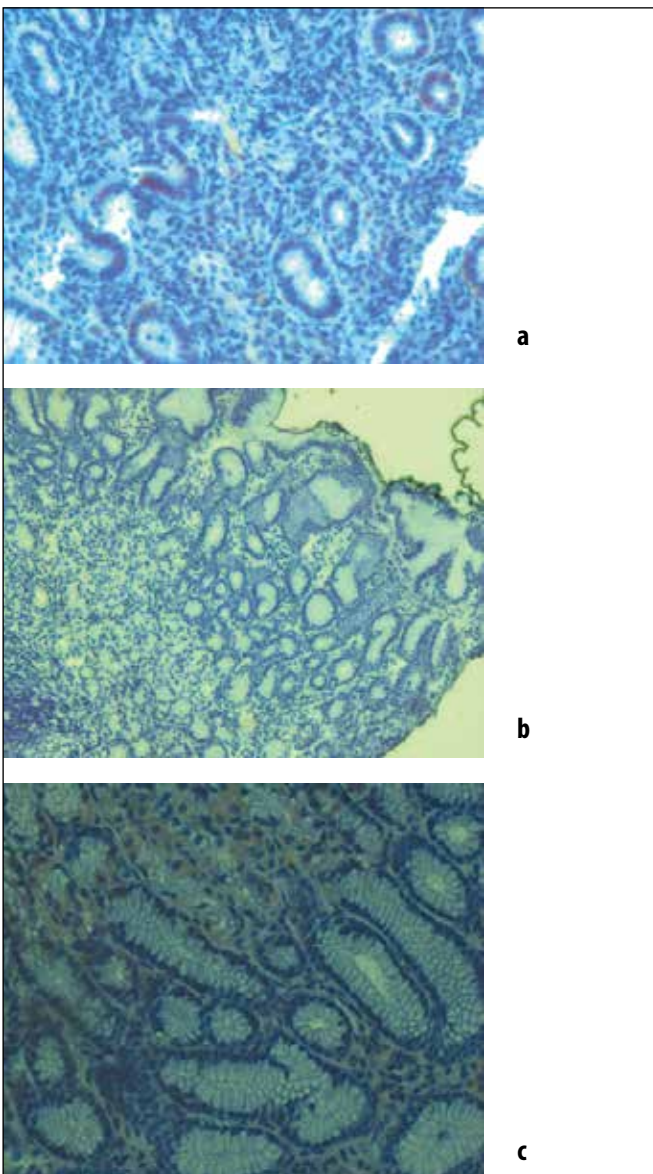




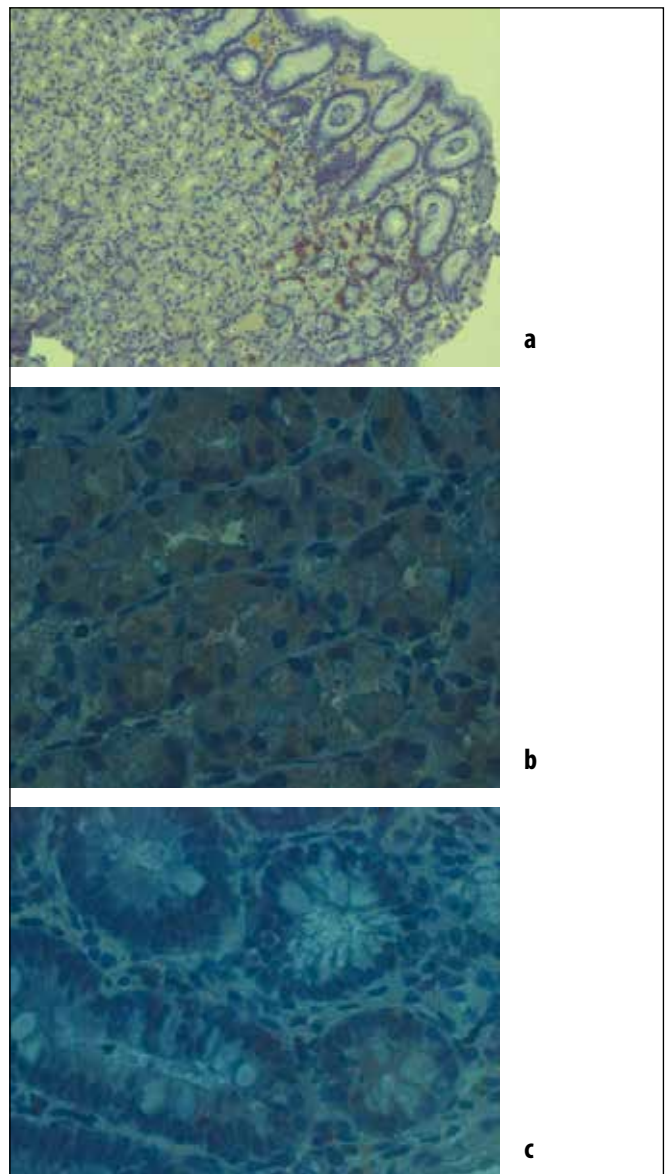
**Fig. 1.** Microphoto of stomach mucosa's biopate. Lymphocytic gastritis x200.



**Fig. 2.** Microphoto of stomach mucosa's biopate. Eosinophilic gastritis x 200.

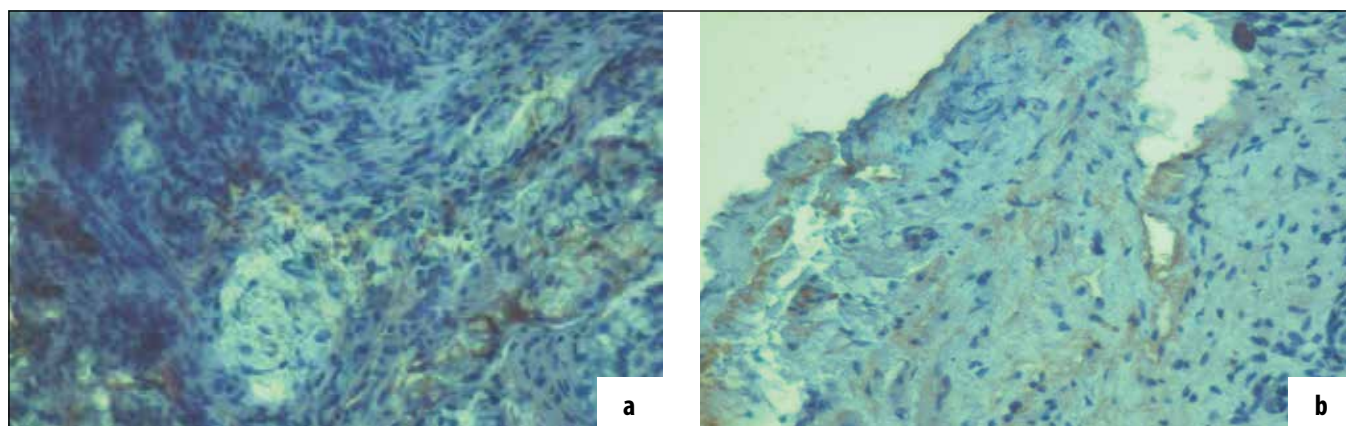


**Fig. 3.** Stomach mucosa's microphoto. Eosinophilic gastritis: a – immunohistochemical reaction with antibodies to PCNA x 200; b – immunohistochemical reaction with antibodies to Bax x 200; c – immunohistochemical reaction with antibodies to Bcl-2 x 200.

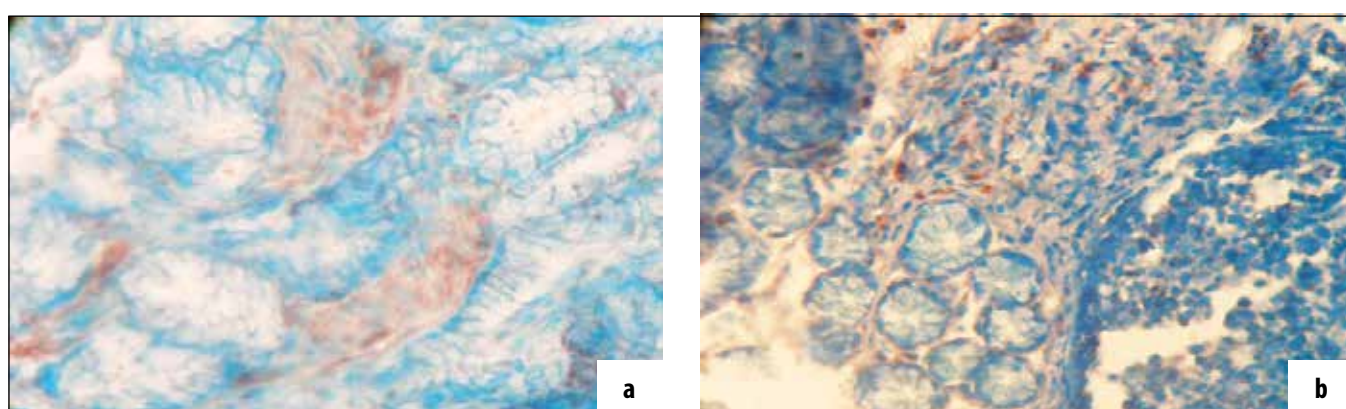


**Fig. 4.** Stomach mucosa's biopate. Lymphocytic gastritis: a – immunohistochemical reaction with antibodies to PCNA x 100; b – immunohistochemical reaction with antibodies to Bax x 200; c – immunohistochemical reaction with antibodies to Bcl-2 x 200.

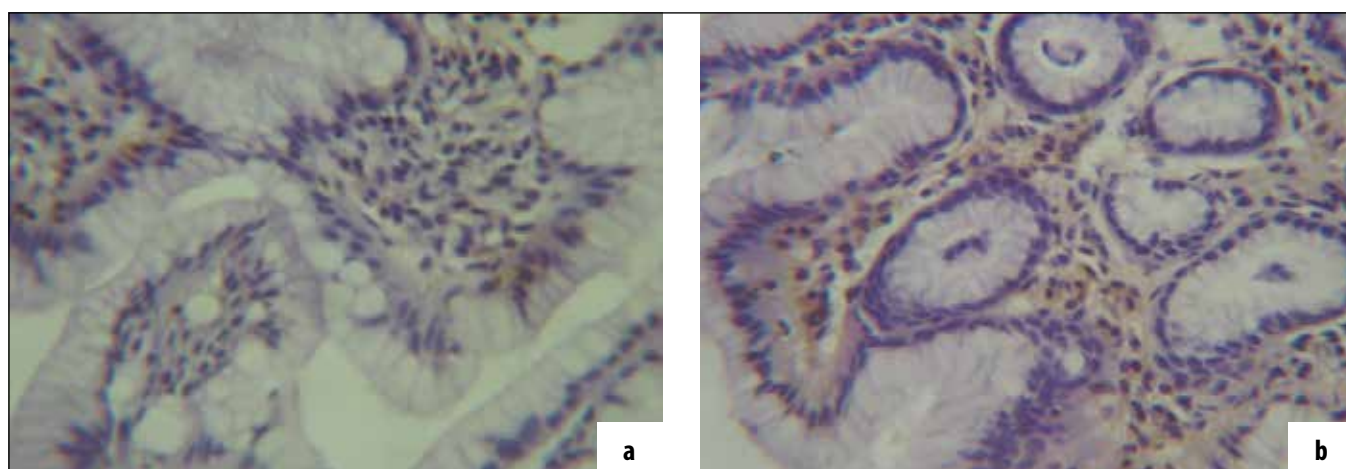




**Fig. 5.** Microphoto of stomach mucosa's biopate. Expression with monoclonal antibodies to Collagen Type IV x 200: a – eosinophilic gastritis; b – lymphocytic gastritis.



**Fig. 6.** Microphoto of stomach mucosa's biopate. Expression with polyclonal antibodies to TGFβ x 200: a – eosinophilic gastritis; b – lymphocytic gastritis.



**Fig. 7.** Microphoto of stomach mucosa's biopate. Expression with polyclonal antibodies to NF-κβ x 200: a – lymphocytic gastritis; b – eosinophilic gastritis.

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**Conflict of interest:**

*The Authors declare no conflict of interest.*

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