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## RELATIONSHIP BETWEEN INDICES OF CYTOKINE STATUS AND PATHOMORPHOLOGICAL CHANGES OF THE MUCOSA IN CHILDREN WITH CHRONIC GASTRODUODENAL PATHOLOGY

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This article presents diagnostically significant relationships between cytokine status indicators and specific morphological criteria that characterize the condition of the mucosa of the upper gastrointestinal tract in children with gastrointestinal manifestations of food hypersensitivity. Comparative analysis of pathomorphological changes in inflammatory processes of the lining of the stomach and the duodenum demonstrates that children with IgE-dependent gastrointestinal symptoms of food allergy had a more pronounced degree of inflammatory infiltration with high content of eosinophils, as well as dystrophic changes of glandular epithelium and marked stromal edema. Cytokine imbalance in the form of increased interleukin-4 and TARC/CCL-hemokine, along with decreased anti-inflammatory interleukin-10, showed a clear correlation with specific histological changes in the form of eosinophilic infiltration of the gastric mucosa, which allows us to identify specific immunological markers of food allergy.

**Key words:** children, food hypersensitivity, gastroduodenal pathology, interleukin-4, interleukin-10, TARC/CCL-17 chemokine.

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## ВЗАЄМОЗВ'ЯЗОК ПОКАЗНИКІВ ЦИТОКІНОВОГО СТАТУСУ ТА ПАТОМОФЛОГІЧНИХ ЗМІН СЛИЗОВОЇ ОБОЛОНКИ У ДІТЕЙ З ХРОНІЧНОЮ ГАСТРОДУОДЕНАЛЬНОЮ ПАТОЛОГІЄЮ

У даній роботі представлені діагностично-значимі взаємозв'язки показників цитокінового статусу та специфічних морфологічних критеріїв, що характеризують стан слизової оболонки верхніх відділів травного тракту у дітей з гастроінтестинальними проявами харчової гіперчутливості. Порівняльний аналіз патоморфологічних змін при запальних процесах слизової оболонки шлунку та дванадцятипалої кишки свідчать, що діти з IgE-залежними гастроінтестинальними симптомами харчової алергії мали більш виражений ступінь запальної інфільтрації із підвищеним вмістом еозинофілів, а також дистрофічні зміни епітелію залоз та виражений набряк стромі. Дисбаланс цитокінів у вигляді підвищення інтерлейкіну-4 та хемокіну TARC/CCL-17 поряд із зниженням протизапального інтерлейкіну-10 продемонстрував чітку кореляційну залежність із специфічними гістологічними змінами у вигляді еозинофільної інфільтрації слизової оболонки шлунку, що дозволяє виділити специфічні імунологічні маркери харчової алергії.

**Ключові слова:** діти, харчова гіперчутливість, гастроудоденальна патологія, інтерлейкін-4, інтерлейкін-10, хемокін TARC/CCL-17.

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Problematic aspects of allergic pathology have long gone beyond purely medical competence and require serious public attention due to its widespread prevalence, the steady growth of the number of patients, especially in developed countries, and a significant impact on the quality of life of patients. The greatest difficulty in understanding the problem of allergic diseases resides in the simultaneous existence of clear nosologies with lesions of certain organs, as well as pathological changes of organs and systems in response to allergens of various origins. Moreover, the gastrointestinal tract is exposed to constant allergens, food in particular, throughout human life. The development of food tolerance is one of the phenomena of our survival [2]. In the absence of food tolerance formation, which is based on a complex adaptive immune process, the manifestations of food allergies can persistently trouble a person throughout life. Currently, the structure of allergic diseases is represented as follows: 50–70 % of patients have skin manifestations of allergies, 50–60 % have gastrointestinal food allergies, and 20–30 % have respiratory symptoms of allergies [5, 6].

The current stage of research development allows us to distinguish among the gastrointestinal pathologies a group of diseases that cause certain difficulties in diagnosis and treatment, associated with a combination of lesions of both the digestive organs and several other systems. In almost half of the cases, patients with an established localization of eosinophilic diseases of the digestive tract demonstrate a combination of clinical symptoms or signs of other allergic lesions, such as eosinophilic gastroenteritis with eosinophilic esophagitis or colitis [12]. The steady growth of allergic diseases, which are accompanied by impairments of different parts of the gastrointestinal tract, have various manifestations of allergies with the

formation of chronic allergic lesions of the esophagus and stomach [6, 8]. However, it should be noted that the diagnoses of “allergic gastritis” and/or “eosinophilic esophagitis” in children are extremely rare. More often, the symptoms of these nosologies are described as manifestations of acute allergic reactions. The first gastrointestinal symptoms of food allergy are infantile colic, vomiting, diarrhea, flatulence, underweight, irritability, sleep disturbances, refusal to eat, and resistance to the traditional methods of therapy. There is a dependence between the occurrence of these symptoms and the intake of certain types of food [4].

Thus, the variety of clinical manifestations of food hypersensitivity, overdiagnosis of food allergy in infants, lack of reliable diagnostic markers, and the difficulty of choosing the treatment tactics determine the relevance of the study of the diagnostic significance of immunological parameters and morphological characteristics in patients with allergic hypersensitivity against the background of food consumption.

**The purpose** of the study was to establish the relationship and diagnostic significance of the cytokine status and the condition of the mucous membrane of the upper digestive tract in children with gastrointestinal symptoms of food hypersensitivity.

**Material and methods.** The study enrolled 34 children aged from 6 to 15 years with clinical signs of lesion of the upper gastrointestinal tract, represented by gastroesophageal reflux disease, chronic gastritis, and duodenitis against the background of food hypersensitivity reactions, with the voluntary consent of parents. The control group consisted of 22 apparently healthy children stratified by age and sex as consistent with the main group. To achieve the aim of the research, all children underwent fibroesophagogastroduodenoscopy with subsequent collection of material from different parts of the esophagus, the stomach, and the duodenum, as well as subsequent morphological evaluation of biopsy samples. Fixation of the obtained material was performed in a 10 % solution of neutral formalin, followed by the preparation of histological specimens according to standard methods. Sections were stained with hematoxylin and eosin. The number of eosinophils was calculated based on the threshold diagnostic criteria depending on the part of the digestive tract as mentioned in the studies by many researchers [7, 9, 11] in five random fields of high magnification, which is a morphological criterion of allergic reaction. Microscopic examination of stained histological specimens was conducted using a light microscope “MICROmedXS-3320” by “NingeroShenghengOpticsandElectronicsCo” with a magnification of  $\times 100$ ,  $\times 200$ , and  $\times 400$ .

In order to clarify the role of immunologically mediated mechanisms in the development of allergic reactions in the consumption of certain food ingredients, we studied the cytokine profile to determine the level of interleukins 4, 10, and thymus and activation-regulated chemokine TARC/CCL-17.

Statistical analysis of the results was performed using the “Microsoft Excel 2010” software. The arithmetic mean of the number of eosinophils and their standard deviation were calculated. The relationship between the eosinophil count and the serum IgE levels was assessed using the nonparametric Spearman and Kendall correlation coefficients ( $r$ ). The relationship between the studied parameters was defined as weak, moderate, and strong if the correlation coefficient was in the range of 0.01–0.29, 0.3–0.69, and 0.70–1.0, respectively. All indices were considered reliable at a value of  $p \leq 0.05$ .

**Results of the study and their discussion.** The study of the diagnostic significance of cytokine profile indicators in children with gastroduodenal pathology revealed the most significant deviations of scientific and practical interest, both in terms of disclosing the processes of tolerance and pathogenesis of allergic diseases of the digestive tract. In order to establish the role of cytokines in the development of food hypersensitivity in children with gastroduodenal pathology, we studied the levels of pro-inflammatory and anti-inflammatory interleukins (table 1).

Table 1

**The level of cytokines in the examined children (M $\pm$ m), pg/ml**

Cytokines	Study groups	
	Children with gastroduodenal pathology (n=34)	Healthy children (control group) (n=22)
IL-4, pg/ml	0.22 $\pm$ 0.05**	0.009 $\pm$ 0.006
IL-10, pg/ml	4.30 $\pm$ 0.60*	8.33 $\pm$ 1.55
TARC/CCL-17, pg/ml	116.56 $\pm$ 16.69**	11.47 $\pm$ 3.67

Note: \* is the significance of difference as compared to the control group ( $p < 0.05$ ); \*\* – ( $p < 0.001$ )

As the results of our research demonstrate, the level of pro-inflammatory TARC/CCL17 chemokine in children with gastrointestinal symptoms of food hypersensitivity was by 10 times ( $p < 0.001$ ) higher than in children of the control group. It should be noted that in almost half of patients (47.1 %) with the pathology of the upper digestive tract against the background of food hypersensitivity reactions, the level of chemokine in the range of  $\geq 100$  pg/ml was observed. In the group of patients, we also noted an increase in the production of anti-inflammatory IL-4, which was by 24 times higher than the norm

( $p < 0.001$ ). Meanwhile, the level of IL-10 was by 2 times lower than in healthy children ( $p < 0.05$ ), which may reflect the tendency to persistent/chronic inflammation.

When comparing the features of food hypersensitivity in children with chronic gastroduodenal pathology, depending on the leading marker of atopy – total IgE, the examined patients were divided into two subgroups. The first subgroup consisted of 18 children with IgE-independent allergic reactions to food (Me (Q1-Q3) 35.0 (28.0-77.5)). The second subgroup included 16 patients with IgE-induced allergic manifestations of food hypersensitivity (Me (Q1-Q3) 240.5 (158.0-475.8)).

The study of changes in the concentrations of immunocytokines allowed us to identify the alterations of the cytokine link in different mechanisms of allergic reactions. Analysis of the research findings depending on the IgE status (Table 2) did not reveal statistically significant differences in IL-4 and IL-10 ( $p > 0.05$ ) among patients of both subgroups.

Table 2

**Evaluation of the cytokine profile in patients with gastroduodenal pathology and manifestations of food hypersensitivity depending on the IgE status (M±m), pg/ml**

Cytokines	Children with gastroduodenal pathology and manifestations of food hypersensitivity (n=34)	
	Ig E “+” status (n=16)	Ig E “-” status (n=18)
	M±m	M±m
IL-4, pg/ml	0.32±0.08	0.13±0.04
IL-10, pg/ml	3.58±0.89	4.94±0.82
TARC/CCL-17, pg/ml	153.74±28.57 *	83.52±15.64

Note: \* is the significance of difference as compared to the control group ( $p < 0.05$ )

However, we found an increase in the concentration of anti-inflammatory cytokine IL-4 in more than a half (62.5 %) of patients with IgE-mediated allergic reactions to food (M±m=0.32±0.08 pg/ml) against 38.9 % of children with normal levels of total IgE (M±m=0.13±0.04 pg/ml).

The study found that the TARC/CCL17 chemokine in children with the IgE-mediated reactions to food was statistically significantly higher than in patients with the IgE-negative status. The obtained results provide evidence of the pro-inflammatory orientation of the T-helper (Th) immune response due to increased levels of interleukin – 4 (in 50 % of subjects) and TARC/CCL17 (in 47.1 % of patients) among half of the children with diseases of the upper gastrointestinal tract and manifestations of food allergy against the background of reduced production of anti-inflammatory cytokine – IL-10.

Based on the clinical and anamnestic data, as well as the results of laboratory and instrumental examination, various nosological forms of lesions in the upper digestive tract were diagnosed, among which chronic gastritis prevailed. Inflammatory diseases of the duodenum (DU) occurred in half of the examined children, regardless of the IgE status.

The histological examination of the gastric mucosa revealed the following features: preservation of the structure of the mucous membrane with dystrophic changes of the epithelium, the proper mucous plate was represented by loose connective tissue, which is occasionally compacted with moderate cellular infiltration by lymphocytes, neutrophils, neutrophils, neutrophils (fig. 1).

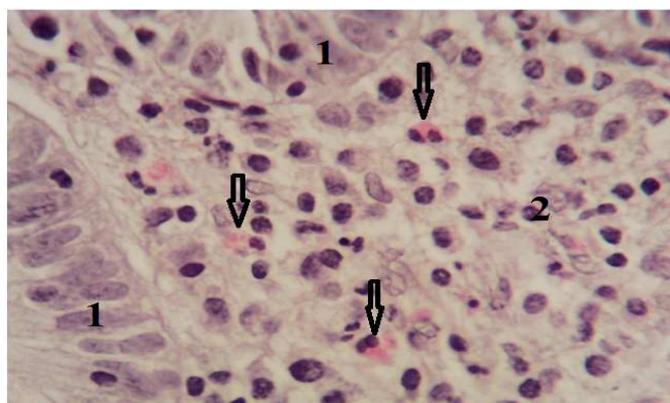


Fig. 1. Microscopic examination of a biopsy sample from the gastric mucosa of patient Ya. with clinical manifestations of gastrointestinal food hypersensitivity and a normal level of total IgE. Staining with hematoxylin and eosin. Objective lens 10<sup>x</sup>, eyepiece 40<sup>x</sup>. 1 – single-layer columnar epithelium; 2 – diffuse inflammatory infiltration of the stroma with a predominance of lymphocytes, plasmacytes, and a small number of eosinophils (indicated by the arrow).

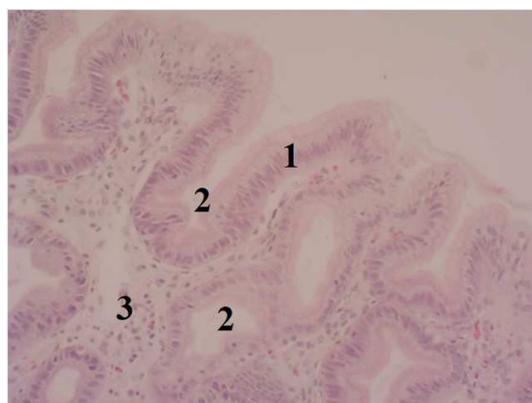


Fig. 2. Photomicrograph of a biopsy sample from the gastric mucosa of child H. with clinical manifestations of food gastrointestinal hypersensitivity and elevated total IgE. Staining with hematoxylin and eosin. Objective lens 10<sup>x</sup>, eyepiece 10<sup>x</sup>. 1 – single-layer columnar epithelium; 2 – gastric pits; 3 – proper mucous plate with an inflammatory infiltration.

Morphological examination of biopsy samples from the gastric mucosa in children with IgE-induced manifestations of food hypersensitivity revealed the preservation of gastric histostructure, along with the signs of chronic inflammation in all examined patients. The analysis of the cellular composition of the infiltrate showed that there was no clear differentiation of cellular poles due to the absence of mucous secretion in the apical part and the presence of dystrophic changes (fig. 2). Desquamation of single epithelial cells or their layers was observed.

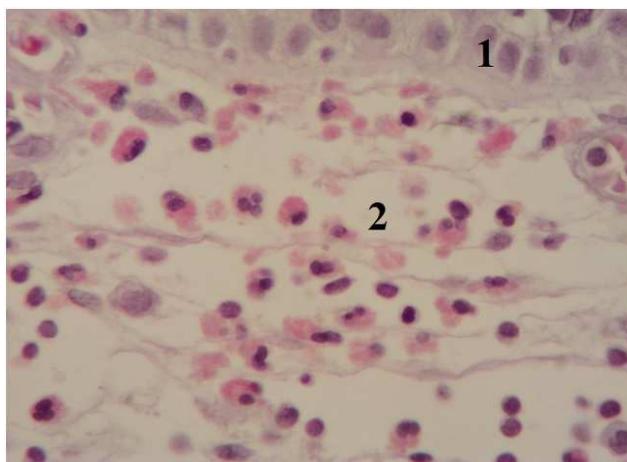


Fig. 3. Photomicrograph of a biopsy sample from the gastric mucosa of patient Sch. with clinical manifestations of gastrointestinal food hypersensitivity and elevated total IgE. Staining with hematoxylin and eosin. Objective lens 10 $\times$ , eyepiece 40 $\times$ . 1 – single-layer columnar epithelium; 2 – marked edema of the stroma with diffuse inflammatory infiltration with a predominance of eosinophils.

Further analysis of morphological studies in a subgroup of children with the IgE-positive status revealed an increase in the indicators that characterize microcirculation disorders: there was an uneven filling of blood vessels with a predominance of venous and capillary plethora with perivascular edema and diapedetic hemorrhage. We found changes with a marked eosinophilic infiltration (fig. 3) and focal accumulation.

Determination of the number of eosinophils in the five high magnification fields of view showed that their average number in patients with IgE-mediated allergic reactions to food was  $39.06 \pm 3.91$  in high magnification fields of view with significant fluctuations in individual patients, which was statistically significantly higher than that in the 1st study subgroup ( $p < 0.01$ ).

The study of correlation dependence in the examined patients revealed the following configuration (fig. 4): with an increase of total IgE, the number of eosinophils in the gastric mucosa ( $r = 0.652$ ;  $p < 0.01$ ) rises.

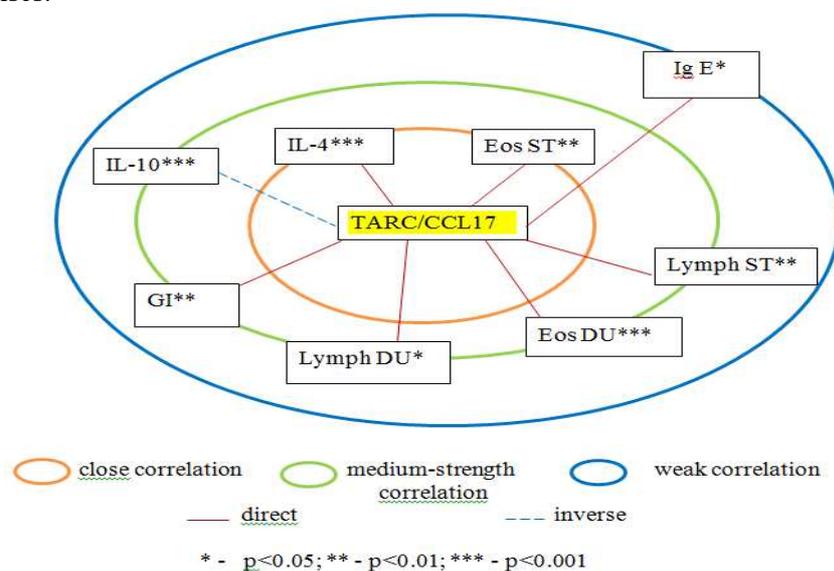


Fig. 4. Correlation between the TARC/CCL17 chemokine values and immunological and histological changes in children with gastrointestinal symptoms of food hypersensitivity

The identified changes indicate that in children with IgE-mediated allergic reactions to food, along with pronounced morphological signs of chronic gastritis, there was a greater number of eosinophils, which were part of the inflammatory infiltrate in both the lining of the stomach and the duodenum, as compared to patients with IgE-mediated allergic reactions. There is a different relationship between the severity of eosinophilic infiltration of the gastric mucosa ( $r = 0.770$ ,  $p < 0.001$ ) and the duodenum ( $r = 0.419$ ,  $p < 0.05$ ) and the level of total IgE.

The obtained data suggest no statistically significant correlation between total IgE levels and eosinophil counts in the duodenal mucosa of patients with IgE-independent gastrointestinal symptoms of food hypersensitivity ( $r = 0.291$ ,  $p > 0.05$ ).

When comparing the correlation structures, we found a relationship between the level of TARC/CCL17 and IL-4 ( $r = 0.809$ ,  $p < 0.001$ ), the severity of eosinophilic infiltration in the lining of the stomach ( $r = 0.718$ ,  $p < 0.01$ ) and the duodenum ( $r = 0.470$ ,  $p < 0.001$ ). In contrast, the concentration of IL-10 was inversely related to lymphocytic infiltration of the gastric mucosa ( $r = 0.756$ ,  $p < 0.001$ ), lymphocytic infiltration of the duodenal mucosa ( $r = 0.643$ ,  $p < 0.001$ ) and the level of TARC/CCL17 ( $r = 0.588$ ,  $p < 0.001$ ).

Improving the diagnosis and prediction of food hypersensitivity in children with the pathology of the digestive system is possible on the basis of identifying certain immunological markers and their comprehensive assessment.

The data obtained during the study indicate that the morphological significance of eosinophilic infiltration is a reflection of increased permeability of the mucous membrane for potential allergens under conditions of the disrupted structure of the extracellular matrix and, therefore, is decisive in the development of antigenic properties of protein structures in the tissues of the gastrointestinal tract [3, 6, 12]. Inflammatory processes in the upper gastrointestinal tract in children with food hypersensitivity have a higher risk of early chronicity with the formation of atrophic changes in the mucous membrane. In most cases, immune inflammation with a predominantly eosinophilic tissue infiltration in the absence of other causes for tissue eosinophilia is the morphological basis of the clinical presentation. Clinically, eosinophilic diseases of the gastrointestinal tract are characterized by the delayed development of symptoms and the absence of manifestations of the skin and respiratory system, which are typical for the reagin mechanism [1, 8, 10].

The comparative analysis of pathomorphological changes in inflammatory processes of the lining of the stomach and the duodenum demonstrates that children with IgE-dependent gastrointestinal symptoms of food hypersensitivity had a greater degree of inflammatory infiltration with a significant content of eosinophils, dystrophic changes of glandular epithelium, and marked edema of the stroma. The detected changes lead to the disruption of the barrier function of the gastrointestinal tract, which in turn increases the sensitization by allergens [6, 7, 12]. The developing complex of hypersensitivity reactions, in addition to the systemic effect, influences the structural and functional state of the cells of the lining of the stomach and the duodenum, which may be one of the causes for chronic inflammation recurrence and insufficient effectiveness of therapeutic measures. The mutual correlation of biological markers, which are detected in dysregulatory changes in a child's body in response to antigenic stimulation, demonstrates the involvement of the mucous membrane of the digestive tract in the pathological process with maximum damage to the provoked organs, more often the esophagus and the stomach, rarely the duodenum.

### Conclusion

The study of the cytokine status revealed a dependence on histological changes in the examined children and demonstrated a significant increase in interleukin-4 and TARC/CCL-17 chemokine, which correlated with eosinophilic infiltration of the gastric mucosa and inverse relationship with a decrease in anti-inflammatory interleukin-10, which allows us to identify specific immunological markers of food allergy.

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