**SYSTEMIC ENDOTOXINEMIA AND TENSION OF ANTIENDOTOXIN IMMUNITY ASSESSMENT IN CHILDREN WITH EROSIVE AND ULCERATIVE CHANGES IN THE DUODENAL MUCOSA**

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**Key words:** children, gastroduodenal pathology, Helicobacter pylori, genotyping, endotoxin, antiendotoxin immunity, small intestinal bacterial overgrowth syndrome

**Introduction.** Among the great number of factors, Helicobacter pylori (Hp) is considered to be the main and generally acknowledged cause of chronic gastroduodenal pathology (CGDP) []. Current level of Hp infection in humans is very high. Frequency of diseases associated with Hp infection varies depending on the country and age of patients. In Russia and Ukraine infection of the adult population is about 80%, while the children (depending on their age) make up 40-70% [, , 11]. Frequency of children’s Hp infection doesn’t differ from adults at the age of 12-15. Despite such a significant Hp prevalence, only a small proportion of infected patients suffer from certain clinical form of disease. Majority of the infected are asymptomatic carriers. The problem of what determines the development of the disease form is still being the most complicated and not solved yet. Most researchers make an assumption about the leading role of intraspecific diversity of Hp strains and their impact on the nature of the disease [, , 10]. Genome of this bacterium is currently deciphered, and active investigation of Hp strains, specific for the development of concrete gastroduodenal diseases, is carried out. However, reports on the role of different strains in CGDP development in pediatric patients are scarce and rather contradictory.

Hp genome has vacA, cagA genes associated with increased pathogenicity of the microorganism [, ]. Development of such significant gastric diseases as atrophic gastritis, gastric and duodenal ulcers, gastric cancer is closely connected with their presence. Being the most studied, vacuolating cytotoxin-associated gene (vacA) is coding the formation of vacuolating cytotoxin A, which creates an acid environment inside the vacuoles of gastric epithelial cells, thus ensuring the supply of ammonia and other substances from the intracellular space to the vacuoles. These substances attract water, causing swelling of the vacuoles. Merging with each other, vacuoles cause the rupture of cell membrane and cell death. vacA gene is present in the genome of all the Hp strains, having a mosaic structure and containing variable parts: s-region (coding the signal peptide) and m-region (coding the middle area of the mature protein). Allelic variants of this gene with differing size and nucleotide sequences are described: s1 or s2, and m1 or m2 correspondingly. s1/m1 strains have the highest level of cytotoxic activity, meanwhile s2/m2 strains show insignificant toxic activity [, , ].

Chromosomes of some Hp strains are ascertained to contain common specific sequence comprising more than 40 genes collected in one of its segments called “pathogenicity island”, which is a genetically variable area responsible for the formation of major virulence factors and adhesion of the microorganism to the gastric mucosa. Cytotoxin-associated gene A (cagA) is a pathogenicity island’s marker, which encodes a formation of the cryptic immunodominant protein (CagA). This protein is recognized to be one of the main factors of Hp pathogenicity. It damages the integrity of the epithelium of gastric mucosa, causes induction of uncontrolled proliferation of epithelial and lymphoid cells, secretion of the pro-inflammatory cytokines and inflammatory reaction in the gastric mucosa. None of bacteria isn’t detected to have a homolog of cagA gene, therefore, cagA gene is believed to be a specific one, arising in connection with Hp habitation in the human stomach [, , ].

Examination of microbiocenosis state of various parts of the digestive tube in children with persisting Hp seems to be another promising issue in understanding the development and demonstration of CGDP clinical manifestations []. Long-term existence of Hp infection in the patient’s organism, use of antibacterial drugs upon the eradication of the bacterium can lead to a dysbiosis of various parts of the digestive tract with inhibition of bifidus bacteria and lactobacilli, E. coli and progressive growth of pathogenic flora. Disruption of the normal intestinal microflora correlation in different biotopes of the digestive tube is stipulated by the decreasing local and systemic immune factors and the organism’s natural resistance. Dysbiotic changes in the intestinal microflora can be regarded in their turn as one of the causes of the development of Hp resistance to antibiotics conventionally used in the anti-Hp treatment schemes. These changes contribute to a progressive decrease in the effectiveness of eradication of the microorganism []. Changed biocenosis of one biotope entails translocation of microorganisms to non-typical biotopes with reduced colonization resistance of individual biotopes, as well as the whole microecological system at all []. Small intestinal bacterial overgrowth (SIBO) may serve as the manifestation of such a microflora translocation. It is diagnosed when the number of microorganisms in the small bowel exceeds 104−5/ml. Thus, SIBO can be regarded as the small intestinal dysbiosis [, , 17].

Disruptions of the normal microflora correlation in the different parts of the intestinal tube may involve the accumulation of endotoxins of gram-negative bacteria in the intestinal lumen and their subsequent absorption, entering the systemic blood flow and the development of endogenous intoxication. Endotoxins are the lipopolysaccharides (LPS) of gram-negative bacteria, being a potent toxic factor and playing an important role in the immune regulation and maintenance of chronic inflammation. LPS perform adaptive function upon concentrations of 0,1-1,0 EU/ml in the blood serum, whereas a higher level leads to the development of various inflammatory reactions. Upon massive entering blood flow, LPS are known to be a general pathological factor inducing cascade of reactions and various syndromes []. LPS biological activity is significantly neutralized due to the humoral and cellular antiendotoxin systems. Upon their failure, LPS enter the systemic blood flow, where they form a complex with a specific protein binding LPS – lipolysaccharide-binding protein (LBP), which is a protein of the acute phase of inflammation produced by hepatocytes and enterocytes. LBP firmly binds LPS, puts them on CD14 receptors of mononuclear phagocytes, thus increasing the sensitivity of these cells to this factor of pathogenicity in 100-1000 times. Mediators secreted by them have local effect or cause a cascade of response systemic pathological reactions with the development of cellular hypoxia, impaired metabolic processes and stimulation of inflammatory processes [, ].

Small amount of research on the influence of systemic endotoxinemia and state of antiendotoxin immunity on the CGDP course in children infected with the different Hp strains determines the relevance of the present study.

**Aim of research** is to study the indices of systemic endotoxinemia and state of humoral antiendotoxin immunity in children with erosive and ulcerative changes in duodenal mucosa infected with different Hp strains.

**Materials and methods.** On the basis of the Donetsk city children’s clinical hospital № 1, 60 children aged from 14 to 17 with erosive and ulcerative changes in the duodenal mucosa associated with Hp were examined: 20 patients with duodenal peptic ulcer disease (UD) and 40 – with erosive bulbitis (EB). In order to confirm the diagnosis, all the children underwent gastric and duodenal endoscopy with a biopsy of the mucosa. Hp diagnostics was carried out by two methods: invasive – rapid urease test with biopsy material, and non-invasive – urea breath test using the test system «Helic» with detector tubes («AMA», Russia). Hp infection was determined in case of positive results of both diagnostic methods.

Study included children with Hp genotyping that was performed with the use of kits «Helicopol» («Lytech», Russia). All the children were divided into two groups according to the clinical virulence of Hp strain. The first comparison group (n=32) consisted of children with virulent Hp genotype – cagA+vacAs1/m1 or cagA+vacAs1s2/m1m2. Second group included patients with avirulent Hp genotype (n=28) – virulent combination of genes wasn’t found in this group of patients upon Hp genotyping. Control group consisted of 20 conditionally healthy children without CGDP.

SIBO diagnostic assessment was performed for all the children by the hydrogen breath test with lactulose load, using a digital analyzer of the exhaled hydrogen «LactofaH2» («AMA», Russia).

Investigation of systemic bacterial endotoxinemia and antiendotoxin protection factors was conducted in studying the concentrations of a number of indices. LPS concentration in the blood serum was determined by LAL-test «E-toxate» («SIGMA», USA), adapted to the clinical conditions and based on the endotoxin ability to cause coagulation of lysate protein fractions of crab hemolymph Limulus polyphemus, EU/ml. Quantitative determination of LBP in the blood serum of patients was conducted by ELISA method («HyСult biotechnology», Netherlands), mcg/ml. Determining the level of IgG (anti-LPS-IgG), IgM (anti-LPS-IgM), IgA (anti-LPS-IgA) antibodies separately from endotoxin core-region was conducted by a quantitative method in the blood serum with the use of kit «EndoCab» ELISA («HyСult biotechnology», Netherlands) by ELISA method, MU/ml. LPS received from such four types of gram-negative bacteria as Escherichia coli, Salmonella typhimurium, Klebsiella aerogenes, Pseudomonas aeruginosa were used as antigen. Each of LPS contained entirely inner core-part, but external one or part of O-specific polysaccharide chain was absent.

**Results and discussion.** Upon the hydrogen breath test with lactulose load, it is stated that translocation of the colon bacterial flora into the small intestine was typical for children with destructive changes in the duodenal mucosa. Thus, SIBO was diagnosed in 19 (95,0±4,9%) patients with duodenal UD and 30 (75,0±6,8%) patients with EB. SIBO was detected in 2 (10,0±6,7%) patients of the control group.

It is stated that the level of systemic endotoxinemia in examined patients, regardless of the depth of the lesions of gastroduodenal zone mucosa significantly exceeded (p<0,001) indices of the control group (0,52±0,1 EU/ml), and was equal to 1,9±0,1 EU/ml upon EB, and 2,1±0,1 EU/ml upon duodenal UD. The highest LPS concentration in the blood serum was observed in children with duodenal UD (Table 1). Endotoxinemia, being within the physiological norm and not exceeding 1.0 EU/ml, was diagnosed in all children of the control group. Physiological level of endotoxinemia was detected only in 2 (10,0±6,7%) patients with duodenal UD and 5 (12,5±5,2%) patients with EB. Patients infected with virulent Hp strains had a higher level of endotoxinemia. LPS concentration mean value in the children’s blood serum in the first group was 2,3±0,1 EU/ml, which was significantly higher in statistical respect (p<0,001) in comparison with children of the second group (1,6±0,1 EU/ml).

Table 1

**The mean value of systemic endotoxinemia and antiendotoxin humoral immunity in the patients examined depending on the severity of destructive process in the duodenal mucosa**

|  |  |  |  |
| --- | --- | --- | --- |
| **Index** | **Children with duodenal UD (Hp+)****n=20** | **Children with EB (Hp+)****n=40** | **Healthy children****n=20** |
| LPS, EU/ml | 2,1±0,11 | 1,9±0,11 | 0,52±0,1 |
| LBP, ng/ml | 32,7±2,41,.2 | 22,3±1,61 | 6,7±0,7 |
| anti-LPS-IgG EndoCаb, МU/ml | 74,9±7,01 | 84,2±3,41 | 107,2±5,3 |
| anti-LPS-IgM EndoCаb, МU/ml | 77,7±5,31 | 84,6±2,81 | 110,2±4,3 |
| anti-LPS-IgA EndoCаb, МU/ml | 79,0±7,31 | 78,9±4,21 | 104,9±6,7 |

Note: 1 ― difference from the group of healthy children is statistically significant (р<0,05); 2 ― difference from the group of children with EB is statistically significant (р<0,05).

Increased LBP concentration is detected in the blood serum of children examined on the background of the progressing inflammatory process in the duodenal mucosa. Thus, LBP level in most of the children in the control group didn’t exceed the permissible concentration – 10 ng/ml. LBP mean value in the blood serum of patients in this group was equal to 6,7±0,7 ng/ml, which was significantly (p<0,001) lower in comparison with children with erosive and ulcerative changes in the duodenal mucosa. LBP level in children with duodenal UD was significantly (p<0,01) higher (30,6±2,4 ng/ml) as compared to patients with EB (22,0±1,1 ng/ml). Analyzing the effect of Hp genotype virulence on LBP concentration, it is established that, in presence of virulent Hp strains in the child, LBP concentration in the blood serum was significantly (p<0,001) higher than (29,8±1,7 ng/ml) as compared to patients infected with the avirulent Hp strains (20,6±1,1 ng/ml).

Obtained results are likely to be explained by more evident inflammatory process in the lesion focus and deep disorders of microecology not only in the gastroduodenal area, but also within the all intestine in children on the background of erosive and ulcerative changes in the duodenal mucosa. Both Hp itself, as a representative of gram-negative microflora, and gram-negative opportunistic pathogenic microflora of the large intestine, as well as the bacteria in the state of overgrowth in the small intestine, may be the source of endotoxin on the background of increased permeability of the epithelial barrier of gastric and duodenal mucosa.

Conducted analysis of ​​antiendotoxin antibodies indices in children with erosive and ulcerative changes in the duodenal mucosa showed their low level on the background of evidence of the pathological process and endotoxinemia. Thus, anti-LPS-IgA mean value in children of the control group was equal to 104,9±6,7 EU/ml, which was significantly higher (p<0,05) as compared to children with destructive changes in the duodenal mucosa (Table 1). The mean value of anti-LPS-IgA level in the control group was significantly higher (p<0,001) in comparison with the first groups of children (69,3±4,4 EU/ml). The mean value of anti-LPS-IgA level in children of the second group was also lower as compared to the control group (90,0±5,4 EU/ml), but any significant differences weren’t detected (p>0,05).

Anti-LPS-IgG level depended on Hp virulence and degree of the destructive changes in the duodenal mucosa. Thus, anti-LPS-IgG level was significantly lower in statistical respect (p<0,001) in the blood serum of patients with CGDP as compared to the control group (Table 1). Anti-LPS-IgG mean value in the blood serum of patients infected with virulent Hp strains was equal to 73,1±4,2 EU/ml, with strains of low-virulent genotype – 90,1±4,6 EU/ml (p<0,01). Anti-LPS-IgG concentration in children of the control group was equal to 107,2±5,3 EU/ml, which was significantly (p<0,05) as compared to the Hp-infected children’s groups.

Anti-LPS-IgM concentration in the blood serum of children in the control group made up 110,2±4,3 EU/ml, which was significantly (p<0,001) higher as compared to children with destructive inflammatory changes in the mucosa (Table 1). Anti-LPS-IgM level in patients of the first group was 74,6±3,5 EU/ml, while in children of the second group ― 91,0±3,2 EU/ml, which was significantly (p<0,001) lower in comparison with the group of healthy children. Anti-LPS-IgM concentration in the blood serum of children with persistent virulent Hp strains was significantly (p<0,01) lower as compared to group of children infected with less virulent strains.

**Conclusions.** Therefore, stated results suggest that the erosive and ulcerative diseases of the duodenal mucosa occur on the background of disorders in the normal microflora of the various parts of the digestive system, which causes increased LPS absorption into the systemic blood flow and systemic endotoxin circulation. Long duration of the inflammatory process causes a gradual depletion of the organism’s compensatory abilities and the oppression of humoral antiendotoxin immunity. Virulent Hp infection of children with erosive and ulcerative processes in the duodenal mucosa leads to a more evident decrease in indices of antiendotoxin humoral immune protection on the background of increasing endotoxin concentration in the blood serum. Described pathological processes burden CGDP course, contributing to its progression, which should be considered upon the treatment of these patients.

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**Systemic endotoxinemia and tension of antiendotoxin immunity assessment in children with erosive and ulcerative changes in the duodenal mucosa**

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Parameters of systemic endotoxinemia and state of antiendotoxinhumoral immunity have been investigated in 60 children with erosive and ulcerative changes in the duodenal mucosa infected with different strains of Helicobacter pylori. Small intestinal bacterial overgrowth syndrome and systemic endotoxinemia, which exceeds the physiological norm, have been diagnosed in the majority of the examined patients. Systemic endotoxinemia was accompanied by the decrease of antiendotoxin immunoglobulins levels in all the examined patients. These changes indicate the depletion of compensatory abilities in the organism of these patients. The Helicobacter pylori virulent strains, which have cagA, vacAs1/m1 or vacAs1s2/m1m2 gens in the structure of their genotype, cause more significant inhibition of the antiendotoxin humoral immunity indices in the setting of the increased endotoxin concentrations.