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THE INFLUENCE OF HELICOBACTER PYLORI ON THE LEVEL OF ENDOTHELINE-1 AND NITROGEN OXIDE IN THE BLOOD OF CHILDREN WITH THE DUODENAL ULCER

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Abstract

The most serious pathology of the of gastrointestinal tract in children remains duodenal ulcer (DU). Interesting is the study of the role of endothelin-1 and nitrogen oxide in the formation of DU.

The aim. To evaluate the nature of changes in the level of nitrogen oxide (NO) and endothelin-1 (Et-1) in the blood of children with H pylori-associated DU.

Methods. A single-center study was conducted in 42 children with DU aged 6–18 years and 20 children comparison group. Diagnostics of H. pylori was performed primary

endoscopy and 4 weeks after the end of treatment. Blood samples were collected in each visit to measure plasma Et-1 using an enzyme immunoassay kit and NO (nitrate/nitrite) levels Golikov's method.

Results. In 37 (92.5%) the ulcers went through re-epithelialization, while successful eradication of H. pylori was achieved in 32 people (80%). The NO level in the blood plasma of the children of the main group was 9.78 ± 1.13 mmol/l, after treatment –11.09 ± 1.2 mmol/l. After eradication of H. pylori, the level of Et-1 decreased from 2.39 ± 0.08 to 1.14 ± 0.07 pg / ml.

Conclusion. Levels of endothelin-1 (Et-1) and nitrogen oxide (NO) in children with duodenal ulcer vary depending on the stage of the disease. After H-pylori eradication, ulcer defect healing is associated with decreased of Et-1 in plasma and increased NO activity.

Key words: Children; H. pylori; Duodenal ulcer; Endothelin-1; Nitrogen oxide.

Abbreviation: H. pylori - Helicobacter pylori; Et-1 - Endothelin-1; NO - Nitric oxide, Nitrogen monoxide; DU - Duodenal ulcer; eNOS - Endothelial nitric-oxide synthase

Introduction. The most serious pathology of the upper section of gastrointestinal tract in children remains ulcer, which combines, as a rule, the highest manifestation of chronic inflammatory process with destruction of the mucous membrane of the stomach or duodenum [1, 2]. The primary importance in the formation of inflammatory-destructive diseases of the upper section of gastrointestinal tract is unjustifiably attached to the specific infectious agent Helicobacter pylori (H. pylori) [3-5]. According to various researchers, the infectivity of the pediatric population is 7.3 - 78.5% [6-9]. In recent years, there has been some evidence of a decrease in the prevalence of H. pylori. Estimated prevalence is almost 70% in developing countries and 30% -40% in the United States and other industrialized countries [10]. While there is a decline in the prevalence of H. pylori infection in northern and western European countries, the infection is still common in southern and eastern parts of Europe and Asia [11]. However, in erosive-ulcerative processes in the mucosa membrane of the stomach and duodenum, the number of persons with a positive test for H. pylori is much higher [12]. It is well known that the pathogenetic mechanisms of ulcer formation are multifaceted, and in childhood they have their own peculiarities. In particular, a large percentage of accompanying autonomic dysfunction are noticeable in children and adolescents. It is the disorders of the balance of the autonomic nervous system that contribute to ulcer formation [13, 14]. In

children with sympathicotonia there is a possibility of ulceration at normal gastric acidity, when reduction of the protective mechanisms, first of all, the disturbance of a microcirculation of a wall of stomach and duodenum, leads to sharp local hypoxia, ischemia with the subsequent ulceration of a mucous membrane.

One of the factors, influencing the ulcer formation, is the representative of a new class of signaling molecules that perform intercellular communication and regulation of many functions in various tissues and systems of the body - nitrogen monoxide (nitric oxide, NO) [15]. The ability of NO to control the microcirculation in the stomach attributes it to the number of mucosal protection factors. The lack of NO impairs the blood supply to the organ, which indirectly affects the secretory function of the stomach and the ability of the CO to withstand the effects of aggression. A number of authors found increased expression of i-NOS in the mucosa during infection with H. pylori. NO is a powerful vasodilating agent that can provide a significant boost to the blood supply to the mucous membrane. An important role of this system is because through NO, as a secondary mediator, vasodilatory effects of the vagus nerve and many vasoactive substances are provided [16-17]. The literature data indicate that expression of iNOS in the mucous membrane of the gastrointestinal tract is one of the factors leading to trauma to the mucous membrane. In modern concepts of pathogenesis of the ulcer, many scientists emphasize the crucial role of the vascular wall in the regulation of the aggregate state of the blood, as well as the importance of its disorders in the development of diseases of the digestive system [9-13]. However, in the background compared to the severity of acid-peptic aggression in ulcer, the status of endothelial functions remains poorly understood. To date, there is no data on the role of H. pylori in the development of endothelial dysfunction and the possibility of its correction against antihelicobacter therapy. Endothelin-1 (Et-1) was initially found in vascular endothelium and is characterized by a very potent vasoconstrictor ability [18 - 20]. Submucosal injection of ET-1 into the gastric wall leads to trauma of the mucous membrane, since its integrity is destroyed by decreased blood flow [21]. In addition, a higher level of ET-1 in plasma is detected in patients with ulcer [22]. Et-1 may be one of the mediators contributing to the development of ulcer. It is interesting to study the role of endothelin-1 and nitrogen oxide synthase in the formation of duodenal ulcer [23]. Whether H. pylori infection affects Et-1 and NO release in patients with duodenal ulcer is unknown [24]. If such effect exists, then eradication therapy should lead to changes in the content of these biologically active substances during the course of the disease.

The aim. Assess the nature of changes in nitrogen oxide (nitric oxide, NO) and endothelin-1 (Et-1) levels in the blood of children with H. pylori-associated duodenal ulcer in the dynamics of eradication therapy.

Material and methods. A single-center study was conducted in 42 children with duodenal ulcer (DU) aged 6–18 years (main group, H-pylori (+) and 20 children of age without pathology of the digestive system (comparison group, 10 people with H-pylori (+) and 10 people with H-pylori (-)) by the simple randomization.

Criteria for inclusion: children with verified DU, associated with H. pylori, according to the order of the Ministry of Health of Ukraine No. 53 dated 29.01.2013; no eradication of H. pylori in the anamnesis; absence of symptoms of gastroesophageal reflux; active ulcer in the bulb of the duodenum with a minimal size of more than 2 mm, diagnosed on the basis of endoscopy; age of patients from 7 to 18 years; parental and patient informed consent for the scheduled examination.

Criteria for exclusion: the presence of DU complications; presence of concomitant inflammatory pathology of the upper section of the gastrointestinal tract, lungs, liver, kidneys, cardiovascular or cerebrovascular diseases; age of child up to 6 years; presence of other immuno-dependent diseases; children receiving antibacterial agents during the last 6 months; children receiving systemic glucocorticoid therapy for more than 14 days in the last three months; smoking, the presence of diseases of the oral cavity and teeth; children who have previously received ulcerogenic or acid-reducing drugs within 2 weeks before endoscopy; not signed parental and patient informed consent for the scheduled examination; hypersensitivity to the medicines proposed in the treatment regimen.

Criteria for patient's exit from the study: the decision of the patient and parents to discontinue their participation in research; not kept compliance during diagnosis and treatment; appearance in the process of examining exclusion criteria.

Assessment of the severity of clinical signs of ulcer was performed using the visual-analog scale (in points): «0» - no sign of symptom; «1» - weakly expressed; «2» - moderately expressed; «3» - significantly expressed). There was conducted an ultrasound examination of the abdominal organs with the «Aloca SSD-680» device.

The secretory and acid-forming functions of the stomach were investigated by topographic intragastric pH-metry. Endoscopic examination was performed according to standard methods using «Fuginon FG 12P» fibrogastroscope and a biopsy aiming for morphological examination to determine the variant and activity of the inflammatory process, the contamination of the H. pylori mucosa membrane. Cytoscopic diagnosis of H. pylori was

performed after primary endoscopy and 4 weeks after the end of treatment for all patients (eradication control) [47]. The H. pylori CagA antigen determination in feces by enzyme-linked immunosorbent assay (ELISA) was performed, according to a conventional method using a set of reagents from «Farmasco» (Sweden); specific immunoglobulins of classes M, A and G to CagA H. pylori antigen in blood serum according to the conventional method using the «HelicoBest antibodies» diagnostic test-system (D-3752 series) and the set of «Vector BEST» reagents (Novosibirsk, Russian Federation). Endoscopy was performed twice - before and 1 month after eradication treatment. After each diagnostic endoscopy, plasma samples were collected from all infected patients with DU and stored at -80 ° C until measurement. The plasma nitrite concentration was determined using a standard. Sodium nitrite, according to Golikov P.P., was used as a standard. [14]. The reduction of nitrate to nitrite in the samples was performed using granular cadmium (mass fraction of granulated cadmium > 99.96%), aliquots of which were added to the centrifuge, and incubated at room temperature for 15 h. The pre-cadmium pellets were washed with 0.1 N HCl bidistilled water and bidistilled water again to neutral environment. The parameters of the completeness of recovery of cadmium granules added to the samples of nitrate in nitrite, were determined using the concentration dependence of nitrite verified in the Griss reaction. The resulting mixture was mixed with an equal volume of Griss reagent and incubated for 10 min at room temperature. The absorbance of the solution was measured on a spectrophotometer at a wavelength of 546 nm. The result obtained was compared with a calibration curve to determine the level of NO2– / NO3–, which is linear in the concentration range from 2.1 to 300 mmol / l. The NO level was determined by the formula: C (NO) = 227, 273 x E x K, where «E» is the data, obtained according to the comparison with the calibration curve, «K» is a coefficient of 1.18. The reference value was taken to be 15.84 ± 4.1 mmol / l. The study of the level of Et-1 in serum was performed by enzyme-linked immunosorbent assay kits from Biomedicagruppe (Germany). Antihelicobacter therapy included: nifuratel (15 mg / kg / day) + amoxicillin (25 mg / kg / day) for 7 days and children up to 12 years - famotidine (1-2 mg / kg / day), after 12 years - esomeprazole (0.5-0.8 mg / kg / day).

The design of the study envisaged compliance with the principles of confidentiality, the concept of informed consent, and the consideration of the main provisions of the GCR ICH and the Helsinki Declaration on Biomedical Research, where the person is a subject of the study and in their subsequent revisions (Seoul, 2008), the European Council Convention in Human Rights and Biomedicine (2007) and the positive conclusion of the local biomedical ethics committee.

The results of the study are represented by the number of observations in the group, percentages or the middle and root-mean-square deviation deviations. The probability of the difference between the relative values was determined by Fisher's angular transformation method "Pφ". To study the correlation coefficient between the two variables, linear regression was used. The «p» value, that was less than 0.05, was considered statistically significant.

Results. 40 individuals (95.2%) completed the study in full, in 37 (92.5%) the ulcers went through re-epithelialization, while successful eradication of H. pylori was achieved in 32 people (80%). Intra-group analysis in both groups of children examined showed no significant difference between NO content by blood and age ($p>0.05$). The level of NO in the blood plasma of children of the main group before treatment was significantly lower and was 9.78 ± 1.13 mmol / l, $p<0.05$, after treatment - 11.09 ± 1.2 mmol / l. According to the literature, NO is able to inhibit the activity of H. pylori, despite the fact that the latter has a number of adaptations to counteract it. Therefore, it was relevant to determine NO levels depending on the presence of this microorganism in the child's body (Fig. 1). In the control group, in the absence of H. pylori, NO blood levels were significantly higher than in the group with H. pylori (11.67 ± 1.2 mmol / l vs. 8.12 ± 1.2 mmol / l, $p <0.05$).

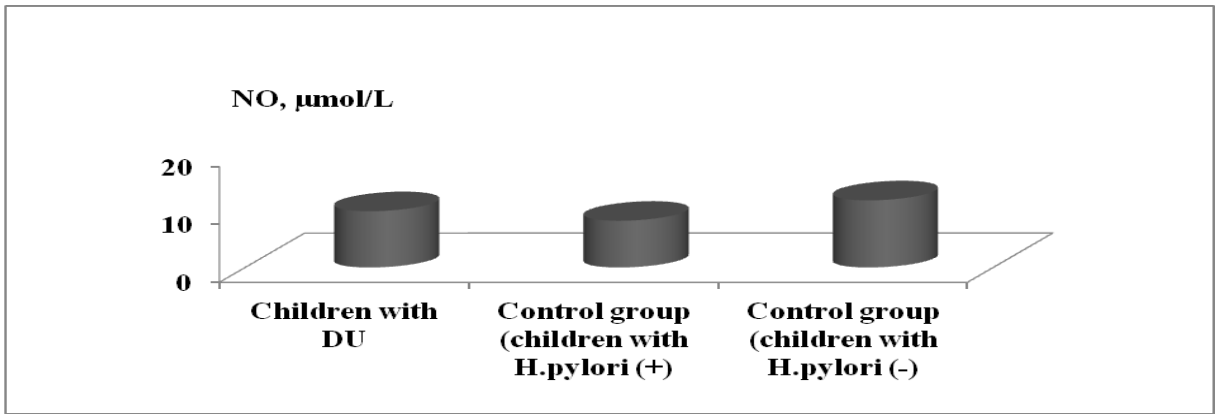

Fig. 1. Nitric oxide (NO) blood plasma levels of children

On average, in children with duodenal ulcer, the level of ET-1 in the blood was significantly higher than that in children of the comparison group (2.39 ± 0.08 pg / ml and 0.68 ± 0.01 pg / ml, respectively, $p <0.01$). The level of endothelin-1 in the blood of control children depended on the presence of H. pylori (Table 1).

The levels of Et-1 in the blood plasma of children with duodenal ulcer depended on the size of the ulcer: at the size of the ulcer defect from 0.2 cm to 0.8 cm (n = 12), the level of

Et-1 was 1.98 ± 0.12 pg / ml. , from 0.9 to 1.5 cm (n = 13) - 2.18 ± 0.11 pg / ml, from 1.6 to 2.2 cm (n = 8) - 2.34 ± 0.19 pg / ml, more than 2.3 cm (n = 7) - 2.44 ± 0.24 pg / ml.

Table 1

Levels of endothelin-1 in the blood plasma of children depending on the presence of H. pylori

Children (groups)	Ет-1, пг/мл
Comparison group (H.pylori +), n=10	0,71±0,02*
Comparison group (H. pylori -), n=10	0,66±0,01*
DU group (H. pylori +), n=40	2,39±0,08**

Note. * - probability values at p < 0.05; ** - probability values at p < 0.01; Et-1 - Endothelin-1; DU - Duodenal ulcer.

Changes in Et-1 and NO levels in the blood plasma of children with DU (Fig. 2) after triple therapy were noted in the dynamics of observation.

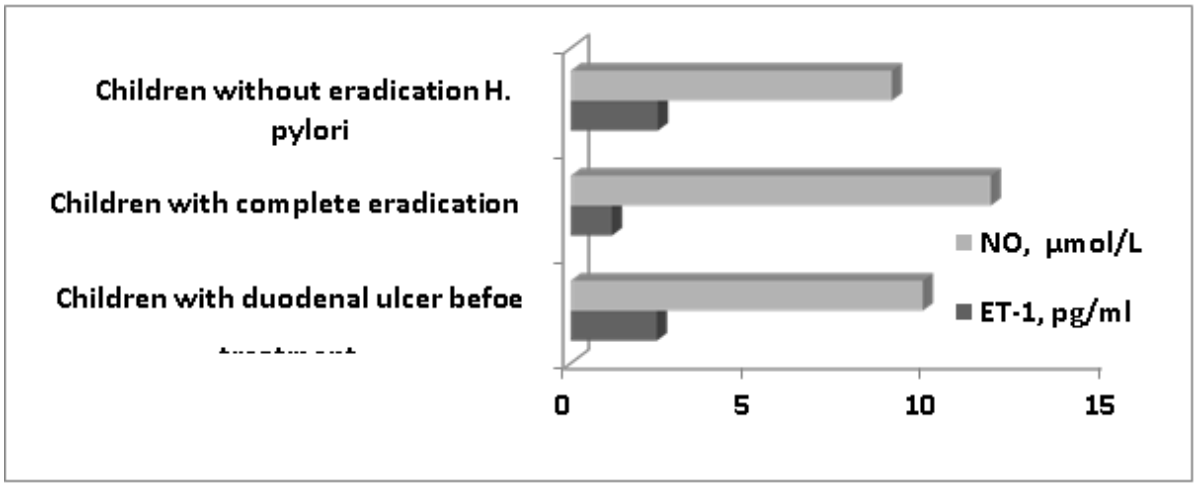

Fig. 2 Levels of Endothelin-1 (Et-1) and Nitric oxide (NO) in the blood plasma of children before and after eradication therapy

Among patients who underwent H. pylori eradication, Et-1 levels decreased during follow-up from 2.39 ± 0.08 to 1.14 ± 0.07 pg / ml, p <0.01), whereas this decrease was not observed in individuals without H. pylori eradication. The level of Et-1 in these patients was 2.42 ± 0.29 pg / ml. In children with duodenal ulcer with complete eradication of H. pylori, the NO level increased with a range from 9.14 to 14.32 mmol / l and averaged 11.67 ± 1.13

mmol / l, p <0.05. However, this increase was not found in patients without eradication of H. pylori (8.92 ± 1.33 mmol / l).

Discussion. *H. pylori* is a common gastrointestinal bacterial strain closely associated with the incidence of chronic gastritis and peptic ulcers [25]. Selected studies related to *H. pylori* prevalence conducted in Europe and analyzed the prevalence of *H. pylori* across 35 European countries and four European regions. Trends regarding *H. pylori* prevalence with respect to the incidence of gastric cancer were also analyzed. This investigation indicated that the prevalence of *H. pylori* infection ranged from 17% in Aarhus, Denmark to 88% in St. Petersburg, Russia. Compared with Southern or Eastern Europe, the prevalence of *H. pylori* was lower in Northern or Western Europe [26]. Ukraine is also a high prevalence country *H. pylori* [27]. In our study, the *H pylori* eradication rate in active DU patients undergoing this triple therapy was 80%. This result is comparable with others as well. The eradication rates of the first-line standard therapy are 55%-57% in Western Europe, 74.5% in China, 84% in South Korea and 87% in Nigeria [28-30].

H pylori infection induces inflammatory responses in mucosa including increased cytokines, activation of inflammation and stimulates the production of prostaglandins, gastrin and somatostatin, *etc*.[31].

Et-1 is a potent vasoconstrictive peptide present in the gastrointestinal tract [32, 33]. The effect of ET-1 is exerted predominately by paracrine and autocrine mechanisms through the stimulation of specific receptors. Et-1 has a major role in the development of gastric mucosal injury. Plasma and mucosal Et-1 levels are also elevated in patients with gastric ulcers, suggesting that the stomach is an important source of circulating ET-1. Our research shows indicated that the plasma Et-1 level in patients with active gastric ulcer is higher than that in these healed subjects and controls. Besides, the ulcer areas of these subjects have a significant correlation with plasma ET-1 levels. This data has been confirmed by other researchers [34]. Akimoto et al. [35] also confirmed that Et-1 levels are higher in healing ulcers than in active stage ulcers. They reported that, in addition to Et-1, during gastric ulcer healing, nitric oxide synthase, VEGF and some chemokines are induced. All these results indicate that Et-1 influences the healing of peptic ulcer disease. Although Akimoto et al. [36] claimed that a possible interaction between Et, nitric oxide, and VEGF during gastric ulcer healing was not confirmed that endogenous Et-1 promotes or delays ulcer healing. Our study found that Et-1 levels in patients with duodenal ulcers were also higher than in control group children, with Et-1 levels lower in H. pylori positive control group children. We suggest that effective eradication therapy contributes to the recovery of Et-1 in the blood plasma of

children. Ulcer sizes show little correlation with plasma Et-1 levels. Given these observations, we suggest that an increase in Et-1 production is one of the mechanisms leading to the formation of peptic ulcer disease, regardless of the location of the ulcer.

The enzymatic production of NO by endothelial NO synthase (eNOS) is critical in mediating endothelial function, and oxidative stress can cause dysregulation of eNOS and endothelial dysfunction [37]. NO is known as a vasodilatory molecule involved in control of the gastric blood flow and the maintenance of gastric mucosal barrier integrity in either healthy gastric mucosa or that damaged by strong irritants. The administration of NO-donors accelerated healing of gastric mucosa damage and experimental gastric ulcers [38].

Evidence indicates that NO acts as one of the endogenous vasodilators to regulate gastroduodenal mucosal blood flow and to maintain its integrity and defense. eNOS and its bioactive product, NO, mediate many endothelial cell functions, including angiogenesis and vascular permeability. Moreover, genetic disruption or pharmacological inhibition of eNOS attenuates angiogenesis during tissue repair, resulting in delayed wound closure [39].

The increase in NO levels in children with duodenal ulcers in our study may be explained by the protective response to ulcers. In addition, NO is able to inhibit the activity of H. pylori, despite the fact that the latter has a number of adaptations to counteract it. In view of the foregoing, it can be concluded that there is a need to further examine of the connection between the major etiopathogenetic mechanisms of duodenal ulcer development associated with H. pylori [40].

Conclusions. Levels of endothelin-1 (Et-1) and nitrogen oxide (NO) in children with duodenal ulcer vary depending on the stage of the disease. After H-pylori eradication, ulcer defect healing is associated with decreased of Et-1 in plasma and increased NO activity.

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