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Concentration of alpha-defensins in the blood plasma of children with *Helicobacter pylori* – associated peptic ulcer of the duodenum

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ABSTRACT

Introduction. Various gastrointestinal and extragastric diseases are associated with H. pylori in children and adolescents, but the strongest testing and treatment guidelines are only for children and adolescents with peptic ulcer. The most promising developments now are the study of the antibacterial effect of endogenous antimicrobial peptides (AP), among which the most important are defenses 1-3 (human neutrophil peptides, HNPs1-3).
Objective. To investigate the concentration of HNPs 1-3 in children with H. pylori-associated peptic ulcer of the duodenum (DU).
Methods. A study of 65 children with DU (47 children with H.pylori-associated DU, 18 children with H.pylori-negative DU) and 25 healthy children aged 7-18 years with the determination of HNPs 1-3 levels in plasma.
Results. More common was *H. pylori* in the group of children with III grade of inflammation than in the group of children with II grade ($\chi 2$: $p<0.0001$). A significant association was found between mucosal inflammation activity and the presence of *H. pylori* ($r=0.66$, $p<0.0001$). The mean level of HNP1-3 in healthy children was 13.67±0.96 ng/ml, in children with DU it was probably higher and was 104.88±11.5 ng/ml ($p=0.005$). There is a direct correlation between the level of HNP1-3 and the severity of DU in children ($r=0.67$, $p>0.05$), as well as between HNP1-3 and the activity of the inflammatory process ($r=0.73$, $p>0.01$). In children with *H. pylori*-associated DU, HNP1-3 values were significantly higher (107.34±16.18 [98.45-119.22]) ng/ml than those in children with DU not associated with *H. pylori* infection (66.70±11.31 [59.54-73.29]) ng/ml and in healthy children (13.67±0.96 [7.27-18.91] ng/ml).
Conclusion. Elevated plasma HNP1-3 levels in children with DU in the presence of *H. pylori* are likely to be a protective response to limit infection that can be used as a potential biomarker of adverse events.

Keywords: children, *Helicobacter pylori,* duodenal ulcer, human neutrophil peptides (HNPs 1-3)

Abbreviations
Helicobacter pylori – H. pylori
DU – duodenum ulcer
AN – antimicrobial peptides
HNPs1-3 – human neutrophil peptides 1-3
IPI – determination of integrated pathology index

INTRODUCTION

Approximately one third of all children in the world are infected with *Helicobacter pylori* (*H. pylori*) (1-3). Bacterial transmission usually occurs from person to person, especially among family members (4). Various gastrointestinal and extragastric diseases are known to be associated with *H. py-*

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lori in children and adolescents, but the strongest testing and treatment guidelines are only for children and adolescents with peptic ulcer disease. Modern ideas about the formation and development of DU are based on the generalized concept of imbalance between the factors of aggression and defense and are considered in the context of persistence of *H. pylori*. Despite the introduction of a powerful arsenal of antisecretory and anti- helicobacterial agents (5,6), as well as pharmacogenetic studies that predict the effect of the drug in each patient, taking into account the ability of the macroorganism to respond to aggression, strategic and tactical issues of *H. pylori* infection therapy are not yet fully clarified (7,8). The direct association of *H. pylori* infection with the development of diseases of the stomach and duodenum, including in children, has led to the urgency of this problem, as resistance to the main antimicrobial drugs included in the first line of treatment is quite high (9-12). The most promising developments in this direction are the stady of the antibacterial effect of endogenous antimicrobial peptides (AP), which are currently considered as a new class of natural antibiotics and can replace traditional drugs (13). The most stadied and important for the human body are AP family of defensins 1-3 (human neutrophil peptides, HNPs1-3). Defensins are able to affect bacteria through a variety of antimicrobial mechanisms, inclDUing such as direct membrane destruction (14), inhibition of bacterial cell wall synthesis (15) and neutralization of secreted bacterial toxins (16). Crucial to the antimicrobial activity of human α-defensins is their hydrophobicity and selective cationicity, segregated on the dimeric structure, which is stabilized by intramolecular disulfides (17,18). Some recent studies have examined the role of the local innate immune system in responding to *H. pylori* infection (19,20), its clinical and epidemiological value, the possibility of using AP as biomarkers, and the methods used to determine their levels (21,22). However, as we know, this has not been studied in peptic ulcer of the duodenum associated with *H. pylori* in children.

The **aim** is to investigate the concentration of HNPs 1-3 in children with *H. pylori*-associated peptic ulcer of the duodenum (DU).

METHODS

A single-center comprehensive clinical and instrumental-laboratory study of 65 children with DU (47 children with *H.pylori*-associated DU, 18 children with *H.pylori*-negative DU) and 25 healthy children (comparison group) aged 7-18 years by simple randomization on the basis of the gastroenterology department of the Chernivtsi Regional Children's Hospital during 2020-2021.

Criteria for inclusion in the study:

- children with verified *H.pylori*-associated DU (25);
- no symptoms of gastroesophageal reflux;
- active ulcer in the bulb of the duodenum with a minimum size of more than 2 mm, diagnosed on the basis of endoscopy;
- age of patients from 7 to 18 years;
- informational consent of parents and patients to conduct the planned examination.

Criteria for non-admission of patients to the study:

- the presence of complications of DU;
- the presence of concomitant inflammatory pathology of the upper gastrointestinal tract, lungs, liver, kidneys, cardiovascular or cerebrovascular diseases;
- age of the child up to 7 years;
- the presence of other immune-dependent diseases;
- children who have received antibacterial drugs in the last 6 months;
- children who have received 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-restoring drugs for 2 weeks before endoscopy;
- the not-signed informed consent of the parents and the patient to conduct the planned examination.

Criteria for patient's withdrawal from the study:

- the decision of the patient and parents to stop their participation in research;
- observance of compliance during diagnostics;
- the emergence of exclusion criteria in the research process.

Assessment of the severity of clinical signs of DU was performed using a visual-analog scale (in points): 0 – no sign, symptom; 1 – weakly expressed; 2 – moderately pronounced; 3 – significantly expressed. Ultrasound examination of the abdominal organs was performed with the device «Aloca SSD-680».

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

was performed after primary endoscopy and 4 weeks after (eradication control). CagA *H. pylori* antigen in feces was determined by enzyme-linked immunosorbent assay (ELISA) according to standard methods using a set of reagents from «Farmasco» (Sweden); specific immunoglobulins of classes M, A and G to the antigen CagA *H. pylori* in blood serum according to the generally accepted method using the diagnostic test system «HelicoBest-antibodies» (series D-3752) and a set of reagents from the company «Vector BEST» (Novosibirsk, Russian Federation). After each diagnostic endoscopy, plasma samples were taken from all infected patients with DU and stored at -80°C until measurement. The level of HNPs 1-3 was determined in the serum by ELISA on the analyzer «TECAN SunRise» (Switzerland) test systems «Immundiagnostik», Germany. To stady the features of the clinic used the method of clinical assessment of the disease, developed (26), which includes the determination of integrated pathology index (IPI).

The design of the study included adherence to the principles of confidentiality, the concept of informed consent and taking into account the main provisions of the GCR ICH and the Helsinki Declaration on Biomedical Research, the Council of Europe Convention on Human Rights and Biomedicine (2007) and the positive conclusion of the local biomedical ethics commission.

The results of the study are represented by the number of observations in the group, the percentage or standard and standard deviation. The probability of the difference between the relative values was determined by the method of Fisher's angular transformation "Pϕ", criteria χ2. Linear regression was used to study the correlation coefficient between the two variables. A P<0,05 value was considered statistically significant.

RESULTS

The age of children with DU averaged 15.2±0.2 years, dominated by children of 13-17 years - 83.1%, the rest were children of 7-12 years – 17.9%. The share of boys was higher and amounted to 67.5%. 69.2% of children were diagnosed with ulcerative defects of small size (3-5 mm). Impaired motor function of the stomach was found in 32.3% of children. In 43.1% of children the second degree of inflammatory activity was established, in 29.2% - the third degree. Of the 65 children, 47 (72.3%) were infected with H. pylori. The mean age of children infected with H. pylori was 13.3±2.8 years, non-infected – 14.4±3.1, and the ratio of boys and girls was 28/19 and 11/7 in the groups with positive and negative H. pylori respectively. Thus, no significant difference was found in the age or sex of *H. pylori*-positive and -negative children. H. *pylori* was more common in the group of children with III grade of inflammation than in the group of children with II grade (χ2: p<0.0001).

The obtained results indicate the absence of a correlation between the severity of clinical symptoms and the degree of activity of the inflammatory process (p>0.05). A correlation analysis of morphological data revealed a significant association between mucosal inflammatory activity and the presence of *H. pylori* (r=0.66, p<0.0001).

The results of the study of HNPs 1-3 in the blood plasma of children with DU and healthy children are presented in Table 1. The average level of HNP1-3 in healthy children was 13.67±0.96 ng/ml, in children with DU it was probably higher and was 104.88±11.5 ng/ml (p=0.005).

TABLE 1. Levels of human neutrophil peptides 1-3 in the blood plasma of patients with DU (ng/ml)

Group	M±m	Me	Q25-75
Children with DU	104,88±11,5*	62,45	24,37-119,22
Healthy children	13,67±0,96	12,34	7,27-18,91

Note. * - p=0.005 (DU – duodenum ulcer)

The analysis of the dependence of the clinical picture of the disease on the level of defensins was carried out taking into account the total IPI and separately IPI pain, dyspeptic and astheno-vegetative syndromes (Table 2).

TABLE 2. Correlation analysis between clinical symptoms and levels of human neutrophil peptides 1-3 in children with DU

Clinical syndrome	Integral indicator of pathology	r	P
Pain	8,7	0,32	>0,05
Dyspeptic	6,6	0,37	>0,05
Astheno-vegetative	5,4	0,33	>0,05
Total	6,9	0,34	>0,05

There is a direct correlation between the level of HNP 1-3 and the severity of DU in children (r=0.67, p>0.05), as well as between HNPs1-3 and the activity of the inflammatory process (r=0.73, p>0.01).

The average level of HNPs1-3 among children with a low degree of inflammatory changes in the mucous membrane was 56.34±6.18 [46.23-59.87] ng/ml, while with moderate activity was determined in the range of 109.43±11, 76 ng/ml [95.43-113.56] (p>0.01).

In children with *H. pylori*-associated DU, HNPs1-3 values were probably higher (107.34±16.18 [98.45-119.22]) ng/ml than in children with *H. pylori*-not-associated DU (66.70±11.31 [59.54-73.29]) ng/ml and in healthy children (13.67±0.96 [7.27-18.91] ng/ml) (Fig.1).

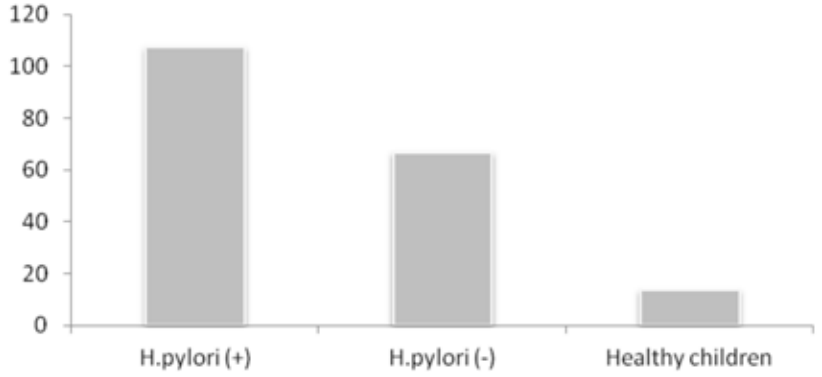

FIGURE 1. Levels of human neutrophil peptides 1-3 in the blood plasma of children depending on the presence of *H. pylori* infection

Neutrophil infiltration was not detected in *H. pylori* - negative children, but was found in the majority (95.7%) of *H. pylori* – positive children (P<0.001). Chronic inflammation was found to be more severe in the *H. pylori* – positive group (P<0.001). All children with *H. pylori* infection had moderate or severe chronic inflammation.

DISCUSSION

H. pylori is a gram-negative bacterium that inhabits the gastric environment of more than half of the world's population (27). Studies have shown that the prevalence of *H. pylori*-positive status varies depending on various factors, such as age, geographical area, living conditions and socio-economic status (28). Oral-oral transmission appears to be the major route of transmission of *H. pylori*. The infection is acquired mainly in childhood and remains the main cause of peptic ulcer, which is observed in some children, especially after 10 years. The diagnosis of infection should be based on endoscopy of the upper digestive tract with biopsy methods (29). In children, a comprehensive review and meta-analysis of original pediatric studies from 2011 to 2016 conducted on healthy children estimated an overall prevalence of 33% [95% confidence interval 27–38] (30). Another study indicates a much higher prevalence – from 42 to 85% (31). According to the results of (32), the prevalence of *H. pylori* infection in both children and adults is declining in developed countries. However, in Latvia over the last 10 years, no evidence of a reduce in the prevalence of it in children has been found (33). Our previous studies found that DU, which is associated with *H. pylori* in 67.2% (34) and 90% (35) of cases, is the most severe course and possibility of complications in children. In this study, the incidence of *H. pylori* in children with DU remained at the previous level (72.3%), which coincides with the results of other studies. Some recent studies have examined the role of the local innate immune system in response to *H. pylori* infection (19,20), in particular the group of AP, including defensins.

AP are endogenous polypeptides produced by multicellular organisms to protect the host from pathogenic microbes and act as effector molecules in wound healing, inflammation and activation of the immune system (36,37). The main mechanism of action of antimicrobial pathogens is associated with disruption of the cell membrane of pathogens (38), some may inhibit biofilm formation (39-41), some, such as defensins produced by epithelial cells to prevent pathogens and usually found in phagocytic cells, help destroy microorganisms when ingested[42]. Defensins have a broad-spectrum antimicrobial activity against gram-positive and gram-negative bacteria, viruses, fungi and protozoa (22). The antimicrobial activity of defensins is due to their unique amino acid sequence (43). Along with their direct antimicrobial properties, defensins play a role in cell-mediated immunity, being chemoattractants for immature dendritic cells (44,45). General studies of the structure-activity relationship (Fig. 2) have shown that total charge, hydrophobicity and amphipathicity are the most important physicochemical and structural determinants that determine their antimicrobial activity and cellular selectivity (46), as well as an important role in protection host from microbial invasion in inflammatory bowel processes (47,48). Human defensins are naturally expressed in the epithelial cells of the gastric mucosa and are actively involved in protecting the host from colonization by *H. pylori* (49).

Multilevel signaling pathways contribute to the molecular mechanism of defensin induction in response to the initial stages of *H. pylori* infection (50-51). Studies (52) showed a significant saturation of α-1-3-defensins of neutrophils in biopsies of the gastric mucosa of children with type B gastritis. Relationships between the nature of morphological changes in the gastric mucosa, the presence of *H. pylori*, gene polymorphism of macrophage inhibition factor and the level of defensin β2 in feces in children with chronic inflammatory diseases of the upper gastrointestinal tract have been found (53,54). Recent studies have shown that adults infected with

FIGURE 2. Generalized characteristics of defensins [48]

H. pylori have elevated levels of both α- and β-defensins, and that they decrease after treatment of the infection (55,56). We found significantly higher levels of HNPs1-3 in the plasma of children in *H. pylori* positive cases of DU. The results obtained in our work revealed an important multifaceted role of α-1-3-defensin in the development of the inflammatory process in the duodenal mucosa: HNPs1-3 determines the severity of endoscopic and morphological changes in DU in children. Direct correlations between the plasma levels of HNPs1-3 in children with DU with the presence of *H. pylori*, the degree of microbial seeding indicate the induction of defensin secretion in response to bacterial colonization, Dudnikova EV et al., also found relationships between the nature of morphological changes in the gastric mucosa, the presence of *H. pylori*, gene polymorphism of macrophage inhibition factor and the level of defensin β2 in feces in children with chronic inflammatory diseases of the upper gastrointestinal tract (57). Isomoto et al. showed that α-defensin expression positively correlates with neutrophil infiltration and chronic inflammation in the gastric mucosa (58). Because α-defensins are secreted mainly by neutrophils, there is a logical correlation between α-defensin and the severity of inflammation. The ability of α-defensins to attract T-lymphocytes and monocytes may play a role in this mechanism, especially in aspects related to chronic inflammation.

CONCLUSION

Elevated plasma HNPs1-3 levels in children with DU in the presence of *H. pylori* are likely to be a protective response to limit infection that can be used as a potential biomarker of *H. pylori*-associated destructive gastrointestinal damage. It can be assumed that AP analogues can be used in the fight against *H. pylori* infection.

Keywords: children, *Helicobacter pylori*, human neutrophil peptides (HNPs1-3), duodenal peptic ulcer.

Compliance with Ethics Requirements:

"The authors declare no conflict of interest regarding this article"

"The authors declare that 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"

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