Efficacy of eradication therapy in children with *H. Helicobacter pylori*-associated diseases depending on levels of nitric oxide and vitamin D

Tamila Sorokman¹, Nadiia Chernei¹, Snezhana Sokolnyk¹, Iryna Sokolnyk¹, Nataliya Popelyuk², Leonid Shvygar¹

¹Department of Pediatrics and Medical Genetics of Bukovinian State Medical University, Teatralna sq., 2, Chernivtsi, 58002, Ukraine
²Department of Pediatrics, Neonatology and Perinatal Medicine of Bukovinian State Medical University, Teatralna sq., 2, Chernivtsi, 58002, Ukraine

Corresponding author
Department of Pediatrics and Medical Genetics of Bukovinian State Medical University, Teatralna sq., 2, Chernivtsi, 58002, Ukraine; Email: t.sorokmam@gmail.com

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ABSTRACT
Introduction: Nitric oxide (NO) and vitamin D (25(OH)D3) plays a crucial role in many physiological processes in the human body. Aim: To investigate the effect of 25(OH)D3 and NO on the efficacy of eradication therapy in children with *H.pylori*-associated...
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Eradiative therapy, 12) It is believed that the main function of NO synthesized is to participate in immune processes. An important role is played by iNOS (type II), which is rapidly activated by bacterial products, inflammatory cytokines and mediators of immunoreactions (Fang et al., 1997). The antimicrobial effect of NO has been suggested by diverse observations. NO production by NO synthases (NOS)- inducible NOS (iNOS) has been stimulated by proinflammatory cytokines such as TNFα, IL-1, and IL-2 as well as by a number of microbial products like lipopolysaccharide or lipoteichoic acid (Fang et al., 1997). In vitro studies demonstrated that inhibition of NO synthases cells and reduction of bactericidal and bacteriostatic activity (Adams et al., 1990). An important role is played by iNOS (type II), which is rapidly activated by bacterial products, inflammatory cytokines and reactive oxygen species in immune, endothelial, smooth muscle cells, providing much more NO synthesis than other isozymes (Forstermann et al., 2012). It is believed that the main function of NO synthesized is to participate in immune processes. Studies (Vidmanova et al., 2014) have found an increase in the content of NO metabolites in children with duodenal ulcer. H. pylori eradication therapy is accompanied by a significant decrease in iNOS activity and catalase, which leads to a decrease in NO formation and can reduce its negative effect (Bondarenko et al., 1999). The antimicrobial activity of NO and its role in the infectious diseases. Methods: An observational prospective cohort study of 128 children with H. pylori-associated disease (endoscopic examination with verification of H. pylori in mucosal biopsies during primary endoscopy and 4-6 weeks after the end of treatment, the presence of the antigen CagA H. pylori in feces, levels 25(OH)D3 and NO). Results: The average of 25(OH)D3 was 25.7 ± 1.4 ng/mL, with H. pylori (+) - 1.6 times lower. In the group of children with vitamin 25(OH)D <20 ng/ml level, the eradication rate of H. pylori infection was 68.3%, while in the group of children with vitamin 25(OH)D>20 ng/ml – 84.6 %. The NO level in the blood plasma of the children was 9.78 ± 1.13 mmol/l, after treatment –11.09 ± 1.2 mmol / l. Conclusion: Vitamin D and NO deficiency is associated with worse rates of H. pylori eradication during treatment.

Keywords: H. pylori infection, Vitamin D, Nitric oxide.

1. INTRODUCTION

H. pylori are a clinically relevant pathogen responsible for a significant proportion of global morbidity and mortality worldwide (Hooi et al., 2017). The global Helicobacter pylori (H. pylori) prevalence in children varies significantly, from 2.5% in Japan (Kakiuchi et al., 2019) to 34.6% in Ethiopia (Shiferaw et al., 2019) and from 23.6% to 85.7% in Eastern Europe (Poland, Ukraine (Szaflarska-Popawska et al., 2019; Belousova et al., 2017). Although, in vitro H. pylori are highly sensitive to drug effects, H. pylori-associated disease treatment is a long process. More than 25 years of experience in the treatment of H. pylori infection has shown that eradication is becoming a more difficult task as the microorganism quickly acquires resistance to antibacterial drugs (Dang et al., 2017). In this regard, eradication therapy for H. pylori infection remains the focus of attention of world experts as well as the daily need of pediatricians and practicing gastroenterologists (Kakiuchi et al., 2019). Recently, a number of international consensus documents have substantially clarified the rules for the diagnosis, treatment and prevention of pathology associated with H. pylori (Maastricht V / Florence Consensus (Malfertheiner et al., 2017), Toronto Consensus (Fallone et al., 2016) American College of Gastroenterologists (Chey et al., 2017), Roman Recommendations (IV Consensus) (Drossman et al., 2016). The Kyoto consensus was important in establishing current views on the treatment of H. pylori-associated chronic gastritis and functional dyspepsia (Sugano et al., 2015). In addition, in 2017, the American College of Gastroenterologists and the Canadian Association of Gastroenterologists prepared a manual for the diagnosis and treatment of dyspepsia, which paid considerable attention to the treatment of Helicobacter bacterial dyspepsia (Moayyedi et al., 2017). The peculiarities of diagnosis and treatment of H. pylori infection in children have been noted (Koletzko et al., 2011). H. pylori are sensitive to many antibiotics. Different regimens of antibacterial therapy exceed the efficacy of eradication in vitro by more than 90%, however, in humans it is not possible to achieve such an eradication effect (Hu et al., 2020).

In the overall clinical experience, more realistic efficacy is 60-70%, while the achievement of H. pylori mucosal eradication cannot be explained simply by the patterns of antibiotic resistance (Chen et al., 2018). The pool of patients with persistent infection due to unsuccessful therapy is increasing and this is becoming a major medical problem. That is to say, the idea of H. pylori-associated disease in this case is too simplified. The unsatisfactory results of classic eradication regimens make it necessary to carry out repeated courses of treatment, and therefore there is a need not only to find new effective first line eradication regimens that work optimally in conditions of high antibiotic resistance, but also to develop new directions in carrying out antibacterial therapy, influencing other lines of H. pylori infection pathogenesis. To date, it is well known that an important role in the development of digestive diseases, including those associated with H. pylori, is played by disorders of the immune system that provide protection of the body and participate in the process of regeneration of the affected tissues (Dixon et al., 2019). Nitric oxide (NO) plays a crucial role in many physiological processes in the human body, including our immune system's response to pathogens.

This small molecule is a widespread and multifunctional cellular messenger (Forstermann U et al., 2012) and that is used by the human immune system as an antimicrobial agent. NO is considered as both a cellular messenger and as an antimicrobial agent of cell-mediated immunoreaction (Weller et al., 2001). The antimicrobial effect of NO has been suggested by diverse observations. NO production by NO synthases (NOS)- inducible NOS (iNOS) has been stimulated by proinflammatory cytokines such as IFNγ, TNF-α, IL-1, and IL-2 as well as by a number of microbial products like lipopolysaccharide or lipoteichoic acid (Fang et al., 1997). In vitro studies demonstrated that inhibition of NO synthases cells and reduction of bactericidal and bacteriostatic activity (Adams et al., 1990). An important role is played by iNOS (type II), which is rapidly activated by bacterial products, inflammatory cytokines and reactive oxygen species in immune, endothelial, smooth muscle cells, providing much more NO synthesis than other isozymes (Forstermann et al., 2012). It is believed that the main function of NO synthesized is to participate in immune processes. Studies (Vidmanova et al., 2014) have found an increase in the content of NO metabolites in children with duodenal ulcer. H. pylori eradication therapy is accompanied by a significant decrease in iNOS activity and catalase, which leads to a decrease in NO formation and can reduce its negative effect (Bondarenko et al., 1999). The antimicrobial activity of NO and its role in the infectious
process. Antibiotic inefficiency for the treatment of numerous antibiotic resistant infections requires the development of new agents, in particular, the natural antimicrobial agent, nitric oxide, is particularly hopeful. Activation of immunocompetent cells is known to be a calcium-dependent process. Vitamin D (25(OH)D3) deficiency in the body is considered as a predictor of the development of many chronic diseases as well as infections. The immunoregulatory effects of 25(OH)D3 deficiency are manifested in the form of enhancement of the proinflammatory vector of innate and adaptive immune responses. Moreover, the effect of 25(OH)D3 on these processes has a dose-dependent effect. In the absence or deficiency of 25(OH)D3, lymphocyte maturation is delayed in the body at the stage of lymphoblasts, which significantly affects the processes of the immune response to antigenic provocative. The confirmation of the 25(OH)D3 significance in immune regulation is the result of numerous experimental and clinical studies demonstrating the association between low levels of 25(OH)D3 and increased susceptibility to various infections (viral, bacterial, fungal etiology), as well as to development autoimmune and allergic pathology (Korf et al., 2014). Deficiency of 25(OH)D3 is thought to reduce the efficacy of eradication of H. pylori infection in infected patients.

Aim
To investigate the effect of 25(OH)D3 and NO on the efficacy of eradication therapy in children with H. pylori-associated diseases.

2. METHODS
An observational prospective cohort study of 128 children aged 7-18 years with H. pylori-associated diseases of gastroduodenal area (50 children with duodenal ulcer and 78 children with chronic gastrroduodenitis) who were treated at the Gastroenterology Department of Chernivtsi regional children's hospital during 2017-2019 years.

A comprehensive clinical-laboratory and instrumental examination was conducted (endoscopic examination to evaluate the mucous coat, according to the Sydney System (1990), taking into account the peculiarities in conducting the study in children, intragastric pH-metric, ultrasound of the abdominal cavity). Verification of H. pylori was performed in the mucosa biopsy (Sydney-Houston system) during primary endoscopy and 4-6 weeks after the end of treatment (eradication control), and was determined the level of specific immunoglobulins of classes M, A, and G for antigen CagA H. pylori in serum by enzyme-linked immunosorbent assay using the HelicoBest antibody test system (D-3752 series) and the presence of CagA H. pylori antigen in the feces using a Farmasco reagent kit (Sweden). CagA antibody titers (≥8 U/mL) were classified as positive, per manufacturer instructions. The patient was considered to be cured of H. pylori infection if all tests were negative.

Serum 25(OH)D3 levels were measured using an electrochemiluminescence method (Roche Diagnostics GmbH, Mannheim, Germany), with inter-assay and intra-assay coefficients of variation (CVs) of 2.4% and 5.7%, respectively. Sera obtained by centrifugation were stored at -20°C and analyzed simultaneously by technicians who were blind to group allocation.

The results were evaluated according to the recommendations of the International Society of Endocrinologists (Holick, 2011): deficiency of vitamin D - 25(OH)D less than 20 ng/ml (less than 50 nmol/l); vitamin D deficiency - 25(OH)D 21-29 ng/ml (51-75 nmol/l); normal vitamin D - 25(OH)D 30-100 ng/ml (76-250 nmol/l).

The plasma nitrite concentration was determined using a standard. Sodium nitrite, according to Golikov, was used as a standard (Golikov, 2004). 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 × E × 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. Before beginning H. pylori treatment patients were divided into 2 groups as follows: group 1 (vitamin D deficient) had a vitamin D level of <20 ng/mL, and group 2 (vitamin D sufficient) had a vitamin D level of ≥20 ng/mL.

The general clinical examination was supplemented by a multifaceted questionnaire of patients specifying the anamnestic social, household, environmental, hereditary and other characteristics of children to study the probable risk factors for influencing the effectiveness of H. pylori eradication.

Inclusion criteria for the study: (1) age 7-18 years; (2) examination and treatment by a single method for the determination of H. pylori (PPI + amoxicillin + clarithromycin as first-line therapy) and the level of 25(OH)D3.
Exclusion criteria for the study: (1) age less than 7 years and over 18 years; (2) major systemic diseases; (3) illnesses that may affect serum vitamin D levels (hyperthyroidism, malabsorption, rickets, hypercorticism, severe liver disease, kidney disease); (4) antibacterial and antisecretory medication usage, bismuth-based medication during the last 8 weeks; (5) allergy to drugs that are included in the eradication regimen; (6) usage of 25(OH)D3 drugs, non-steroidal anti-inflammatory drugs, glucocorticoids; (7) lack of informed consent for inclusion in the study.

The study results were statistically processed using the “STATISTICA” for Windows 8.0.0. (SPSSI.N.C.; 1989-1997), “STATISTICA V.6.0 (Stat Soft Inc; 1984-1996). Quantitative and ordinal parameters, which corresponded to the normal distribution, are presented as average (M) ± standard deviation (average square deviation) (s), qualitative - in the form of the absolute number of observations and the proportion (in %) of the total number of patients in the sample as a whole or the appropriate group. A one-sample Kolmogorov-Smirnov test was adopted to test the normality of continuous variables, which were expressed as medians and ranges for the variables with an abnormal distribution. Comparisons between the groups were performed using the Student's t-test, Wilcoxon signed-rank test, or the χ2 test, when appropriate. A two-tailed P < 0.05 was considered statistically significant.

Binary logistic regression was applied to determine whether an independent risk factor of H. pylori eradication was the patient’s serum vitamin D level. The degrees of this association were measured using the odds ratio (OR) and 95% confidence interval (CI).

The study follows ethical principles for people who act as subjects of research, taking into account the main provisions of the GCR ICH and the Helsinki Declaration of the World Medical Association for Biomedical Research, where the person acts as their protective person, and other ethical principles for the child’s personality as a non-self-protective person, and other ethical principles for the children under study.

3. RESULTS

Of the 128 children surveyed, 86 (67.2%) were identified with H. pylori. There was a tendency to increase the number of people with Helicobacteriosis with age (Table 1).

Table 1 Distribution of sick children by age and sex (n = 128)

<table>
<thead>
<tr>
<th>Age (years)</th>
<th>Boys</th>
<th>Girls</th>
<th>H. pylori(+)</th>
<th>H. pylori (-)</th>
</tr>
</thead>
<tbody>
<tr>
<td>7-11</td>
<td>16/12.5</td>
<td>13/10.1</td>
<td>18/62.1</td>
<td>11/37.9</td>
</tr>
<tr>
<td>12-18</td>
<td>54/42.2</td>
<td>45/35.2</td>
<td>68/68.7</td>
<td>31/31.3</td>
</tr>
<tr>
<td>Total</td>
<td>70/54.7</td>
<td>58/45.3</td>
<td>86/67.2</td>
<td>42/32.8</td>
</tr>
</tbody>
</table>

Note. H. pylori – Helicobacter pylori.

Patients were divided into groups according to the nature of endoscopic changes in CO of stomach and duodenum: 34 children were diagnosed with antral superficial gastritis, including 12 H. pylori (+) and 22 H. pylori (-); 27 people have common superficial gastritis, including 12 H. pylori (+) and 15 in H. pylori (-); 23 children had nodular superficial gastritis characterized by hyperplasia of the lymphoid follicles in the antral gastric region, of which 14 had H. pylori (+) and 9 - H. pylori (-); 20 had flat erosion in the antral or duodenal bulb, including 14 H. pylori (+) and 6 H. pylori (-); 24 people had the ulcer of duodenal bulb, all children H. pylori (+). The pH-metrics had no significant group differences. In 54.6% of patients, chronic gastroduodenal pathology was accompanied by preserved acid-forming gastric function within the limits of normoacidity, in 43.1% it was increased and only in 2.3% - decreased.

In the analysis of 25(OH)D3 indicators content in the blood of children, a tendency to decrease in patients with chronic pathology of the gastroduodenal area was revealed (Table 2).

The average of 25(OH)D3 in these patients was 25.7 ± 1.4 ng/mL. In particular, the difference in rates was found in children depending on the presence of H. pylori - in patients with a positive test, the level of 25(OH)D3 was 1.6 times lower. The lowest level of 25(OH)D3 was observed in CagA-seropositive children. Among 86 children with H. pylori (+) in 60 (69.7%), the level of 25(OH)D was below 20 ng/mL with an average of 15.4 ± 1.1 ng/mL, whereas in children with H. pylori (-) - only 15 (35.7%) had its level below the lower limit of normal (Fig. 1).
Table 2 Levels of vitamin D in children depending on age sex presence of H. pylori infection

<table>
<thead>
<tr>
<th>Indicator</th>
<th>Age (years)</th>
<th>Gender</th>
<th>CagA seropositivity</th>
<th>H. pylori (+)</th>
<th>H. pylori (-)</th>
</tr>
</thead>
<tbody>
<tr>
<td>25(OH)D, ng/mL</td>
<td>7-11</td>
<td>girls</td>
<td>26.3± 1.2</td>
<td>12.1± 0.9</td>
<td>15.4± 1.1</td>
</tr>
<tr>
<td></td>
<td>12-18</td>
<td>boys</td>
<td>24.7± 1.1</td>
<td>1.3</td>
<td>1.1</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>25.8± 1.3</td>
<td>24.5± 1.1</td>
<td>25.3± 1.4</td>
</tr>
<tr>
<td></td>
<td>n=29</td>
<td>n=58</td>
<td>n=67</td>
<td>n=86</td>
<td>n=42</td>
</tr>
<tr>
<td>p-value</td>
<td>0.534</td>
<td>0.456</td>
<td>0.001</td>
<td>0.01</td>
<td>0.001</td>
</tr>
</tbody>
</table>

Note. 25(OH)D, vitamin D3; H. pylori – Helicobacter pylori

In univariate logistic regression analysis, the presence of vitamin D deficiency (< 20 ng/mL) was significantly associated with increased odds of H. pylori infection (OR = 2.94 95% CI 1.22–6.99, p = 0.012). The efficacy of eradication therapy in children with gastroduodenal pathology associated with H. pylori averaged 73.2%. In the group of children with vitamin 25(OH)D <20 ng/ml level, the eradication rate of H. pylori infection was significantly lower (Fig. 2) and was 68.3%, whereas in the group of children with vitamin 25(OH)D >20 ng/ml – 84.6% (p <0.001).

Figure 1 Number of patients with serum vitamin D levels content depending on according to H. pylori infection status

Figure 2 Comparison of successful and failure rates of H.pylori eradication according to the vitamin D levels
Further multivariate analysis revealed that adequate dosage of medication (OR 0.263, 95% CI 0.139-0.513, P=0.001), duration of treatment (OR 0.259, 95% CI 0.129-0.499, P=0.001) and vitamin 25(OH) Serum D ≥20 ng / ml (OR 0.397, 95% CI 0.191-0.689, P=0.001) were independent predictors for successful eradication of H. pylori infection in children.

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 (Fig. 3). 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. 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). Thus changes in vitamin 25(OH)D and NO levels in the blood plasma of children with H. pylori infection after triple therapy were noted in the dynamics of observation.

![Figure 3 Levels of nitric oxide in the blood plasma of children before and after eradication therapy](image)

4. DISCUSSION

In this study, we assessed the association between vitamin D deficiency and H. pylori infection in children. Vitamin D deficiency was more prevalent among patients with H. pylori infection. There was an inverse linear trend between increasing 25(OH) vitamin D and frequency of H. pylori infection in children. H. pylori are of primary importance in the formation of inflammatory-destructive diseases of the gastroduodenal area in children (Jones et al., 2017). Today, it is known that the effectiveness of eradication of H. pylori infection is influenced by virulence and toxicity factors of the bacterium and host-related risk factors such as antibiotic resistance, immune system status and genetic disorders (Uotani et al., 2015).

In Europe, the highest clarithromycin resistance rates; more than 30%, have been reported in Austria, Hungary and Portugal. In contrast, low resistance rate of <10% have been observed in Northern Europe. A total of 2204 patients (1893 adults and 311 children) were included from 32 centres in 18 European countries. Of 2204 patients included, H. pylori resistance rates for adults were 17.5% for clarithromycin, 14.1% for levofloxacin and 34.9% for metronidazole, and were significantly higher for clarithromycin and levofloxacin in Western/Central and Southern Europe (>20%) than in Northern European countries (<10%) (Megraud et al., 2013). This large multicentre study performed in most of the European countries with a standardised protocol shows that resistance to certain antibiotics has reached a sufficient level not to prescribe them as an empirical therapy in some countries.

Most authors determine that the state of defense mechanisms should be considered as a factor that determines the realization of pathogenic properties of H. pylori (Dixon et al., 2019; Sanaii et al., 2019; Bagheri et al., 2019). Activation of immunocompetent cells is known to be a calcium-dependent process. Cholecalciferol deficiency in the body is considered as a predictor of the development of many chronic diseases as well as infections. The immunoregulatory effects of 25(OH)D3 deficiency are manifested in the form of enhancement of the proinflammatory vector of innate and adaptive immune responses (Aranow, 2011). Moreover, the effect of 25(OH)D3 on these processes has a dose-dependent effect. In the absence or deficiency of 25(OH)D3, there is a delay in the maturation of lymphocytes at the stage of lymphoblasts, which significantly affects the processes of immune response to antigenic provocative (Baeke et al., 2010). The link between vitamin D deficiency and the risk of H. pylori infection, the most common chronic
bacterial infection in humans, is discussed. According to a study (Yildirim et al., 2017), patients with H. pylori-associated gastritis had a lower serum vitamin D content. Other studies have also shown that vitamin D deficiency may be a contributing factor to autoimmune gastritis and gastric cancer (Dankers et al., 2016).

Our studies found significantly lower levels of 25(OH)D3 in children with H. pylori-associated pathology of gastroduodenal area, and we also obtained lower efficacy rates of eradication therapy in patients with 25(OH)D3 deficiency. The study (Yildirim et al., 2017) also showed that vitamin D deficiency was associated with worse H. pylori eradication rates during treatment. Therefore, vitamin D seems to be important not only to protect against H. pylori infection, but also to ensure a successful treatment. Preclinical studies have identified possible biological mechanisms by which vitamin D modulates the immune system. In particular, it is indicated that vitamin D exerts its physiological effect through the intracellular receptors of vitamin D, inducing the gene expression of antimicrobial peptides. These antimicrobial peptides include cathelicidin and β-defensin. Cathelicidins have an antimicrobial effect on gram-negative and gram-positive bacteria, as well as viruses, fungi and parasites. The active form of vitamin D (1,25-dihydroxyvitamin D) increases the expression of cathelicidin in epithelial cells of the stomach infected with H. pylori.

Another antimicrobial peptide, β-defensin, also decreases from H. pylori-infected gastric epithelium and exerts an antibacterial effect on the mucosal surface (Pero et al., 2017). Therefore, vitamin D deficiency can lead to a decrease in immunity due to the decreased secretion of both cathelicidin and β-defensin. This could at least partially explain the increased prevalence of H. pylori infection among patients with low vitamin D. In addition, vitamin D can support intracellular destruction of bacteria by inducing the secretion of nitric oxide inside macrophages. Several clinical studies have shown that vitamin D analogues may have antimicrobial effects (Gois et al., 2017), namely anti-H. pylori effects (Wanibuchi et al., 2018). Some studies have also found that serum vitamin D levels can have an effect on H. pylori eradication (Yildirim et al., 2017).

The increase in NO levels in children with duodenal ulcer in our study may be explained by the protective response to ulcer. 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. NO is a signaling molecule with powerful immunomodulatory potential, and we have directly studied it as an important protective component of the host in H. pylori infection. According to the literature, despite the induction of iNOS expression, H. pylori inhibits NO synthesis, limiting the availability of L-arginine and inhibiting iNOS translation through the formation of polyamines (Lewis et al., 2010). The experiment showed that restoration of NO production by gastric macrophages leads to a decrease in gastritis in H. pylori infected mice (Chaturvedi et al., 2010). To date, the cellular mechanism of this anti-inflammatory effect induced by NO remains unknown. There is evidence that H. pylori inhibits NO-dependent induction of heme oxygenase-1 (HO-1) in gastric epithelial cells by a CagA-mediated process. This is due to the activation of transcription factor of the heat shock factor-1 (HSF1). The dynamics of NO secretion can be used to detect the activity of the inflammatory process. There are many scientific papers on the subject, but they are mostly experimental in nature. At present, the clinical application of this knowledge remains relevant.

5. CONCLUSION
The immune response of the macroorganism is a determining factor in the clinical manifestation of the pathogenic properties of H. pylori. The effectiveness of eradication therapy for H. pylori infection is influenced by many factors, including the level of 25(OH)D3 and NO. In children with 25(OH)D3 deficiency in the body, a higher frequency of detection of CagA- H. pylori is found. Vitamin D deficiency is associated with worse rates of H. pylori eradication during treatment. Levels of NO in children with H. pylori infection vary depending on the stage of the disease. After H. pylori eradication, ulcer defect healing is associated with increased NO activity. It is obvious that further study of the role of NO in normal and pathological digestive tract will lead to the deepening of knowledge about the pathogenesis of various forms of pathology and the emergence of new criteria for their diagnosis and treatment.

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**Conflicts of Interest:** The authors declare no conflict of interest.

**Authors’ contribution**
All authors shared in this study design, they reviewed literature, and wrote the primary draft of the manuscript. All authors read and approved the final manuscript.

**Acknowledgements**
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List of abbreviations

<table>
<thead>
<tr>
<th>Abbreviation</th>
<th>Definition</th>
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<tbody>
<tr>
<td>NO</td>
<td>Nitric oxide</td>
</tr>
<tr>
<td>25(OH)D3</td>
<td>Vitamin D</td>
</tr>
<tr>
<td>H. pylori</td>
<td>Helicobacter pylori</td>
</tr>
<tr>
<td>NOS</td>
<td>Nitric oxide synthases</td>
</tr>
<tr>
<td>iNOS</td>
<td>Inducible nitric oxide synthases</td>
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</tbody>
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