Mucosal gastrin cells and serum gastrin levels in children with *Helicobacter pylori* infection

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Abstract

Purpose: Impaired control of gastric juice secretion is observed in chronic gastritis due to *Helicobacter pylori* (*H. pylori*) infection. G cells are stimulated by such cytokines as tumor necrosis factor (TNF-alpha), interferon gamma (IFN-gamma) and interleukin-8 (IL-8). The number of D cells producing somatostatin decreases simultaneously. An increase in gastrin levels could also depend on alkalization in G cell environment caused by bacterial urease. The aim of the study was to evaluate G cell counts in the antrum and gastrin levels in the serum of children with *H. pylori* infection and after bacterium eradication.

Material and methods: The study was performed in 106 patients. Children were divided into 3 groups with regard to the presence and course of *H. pylori* infection. Fifty nine children (55.7%) had chronic gastritis in the course of *H. pylori* infection with a positive titre of antibodies in IgG class against *H. pylori*; 29 children (27.3%) with past *H. pylori* infection, without bacterium colonization and gastritis but with a positive titre of antibodies in IgG class against *H. pylori*; 18 children (17%) with functional disorders of the gastrointestinal tract but without *H. pylori* infection.

Results: The quantitative analysis of gastrin cells in the antral mucosa of children performed by immunohistochemical method showed the highest gastrin cell count in group I with *H. pylori* infection (112.1±58.9 cell/mm^2) and in group II with past *H. pylori* infection (105.3±73.1 cell/mm^2). The serum gastrin level (92.9±41.6 μU/ml) was the highest in children with *H. pylori* infection. In controls, it was 70.0±15.3 μU/ml and could be compared to the results of children with past *H. pylori* infection.

Conclusions:

1. The *H. pylori* infection plays a significant role in the stimulation of G cells increase and gastrin release in the blood serum in children.

2. The eradication of *H. pylori* infection is probably a main factor in gastric secretion down-regulation during gastritis in children.

Key words: gastrin, gastrin cell, *Helicobacter pylori*, children.

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Introduction

The disorders of gastric juice secretion occur in the course of *Helicobacter pylori* infection, which contributes to the damage of gastric mucosa induced by an increase in gastrin production by G cells and a decrease in somatostatin secretion by D cells. Gastrin acting on the lining cells enhances the secretion of hydrochloric acid and increases the effect of histamine and acetylcholine. Few probable mechanisms are taken into consideration in the pathogenesis of increased gastrin secretion: antral mucosa inflammatory state per se (via IFN-gamma, TNF-alpha and IL-8 activity) activates the gastrin release by G cells, the antrum region alkalization with amonia stimulates directly G cells and increased leptin release induced by proinflammatory cytokines plays an important role in the control of gastrin production [1-3]. The decreased production and release of somatostatin play also an important role in this process. This is caused by a decreased count of D cells in the antrum, the activation of H_3 receptors on D cells due to N-alpha-metylhistamine production by *Helicobacter pylori* [4-7]. Hypergastrinemia observed in the course of gastritis and duodenal ulceration is caused by the hyperactivity of G cells, their multiplication (mucosa regeneration disorders present in the course of chronic inflammation) and their increased counts (most significant in atrophic inflammation with large changes) [1]. Two latter mechanisms seem to play a more important role in adults due to histopathological

changes characteristic of an inflammatory process persisting for a long time.

After effective bacterium eradication, D cell counts increase significantly, G cell counts decrease in the antrum and gastrin levels decrease in the antrum [8,9].

The aim of the study was to evaluate antral G cell counts and gastrin levels in the serum of children infected with *H. pylori* infection and after bacterium eradication.

Materials and methods

The study was carried out in 106 children divided into 3 groups with regard to the course of *Helicobacter pylori* infection.

Group I – 59 children (55.7%) with chronic gastritis in the course of *Helicobacter pylori* infection with a positive titre of antibodies in IgG class against *H. pylor*i. The group consisted of 29 girls (49.2%) and 30 boys (50.8%). The age of children ranged from 2 to 18 years and the mean age was 12.2±4.6 years.

Group II – 29 children (27.3%) after past *Helicobacter pylori* infection, without bacterium colonization and gastritis but with a maintaining positive titre of *H. pylori* antibodies in IgG class. The group consisted of 14 girls (48.3%) and 15 boys (51.7%). The age of children ranged from 3 to 18 years and the mean age was 11.0±4.2 years.

Group III – 18 children (17%) with functional disorders of the gastrointestinal tract, without *H. pylori* infection and with normal IgG concentration against *Helicobacter pylori*. The group consisted of 12 girls (66.75%) and 6 boys (33.3%). The age of children ranged from 5 to 17 years and the mean age was 10.7±3.6 years.

Endoscopic and histological examinations of the upper gastrointestinal tract were performed in all children, due to chronic or recurring abdominal pains. Gastrin cells were counted in the antral mucosae and gastrin levels were measured in the serum. The endoscopic and histopathological evaluation was carried out basing on the Sydney System classification [10]. When estimating *H. pylori* colonization, the intensification of inflamed mucosa characterization and inflammation severity were expressed in a 4-grade scale (0-1-2-3), and gastritis activity was measured in quantity of infiltrating granulocytes. Two samples each were taken from the antrum and the sites changed pathologically. The samples were stained with hematoxyline and eosine. The Giemsa method was used to identify *H. pylori* infection. The quick urease test (CLO-test-*Helicobacter pylori*) was done using the kits of the Institute of Food and Nutrition in Warsaw.

Gastrin level was estimated using a radioimmunoenzymatic test (RIA test, CIS Bio International). The blood serum samples were prepared for the examination according to the manufacturer's instructions. The method was based on the assessment of gastrin stained with iodine 125 bound with anti-gastrin antibodies and its free fraction. In this method, the minimum reference limit equals 10 μU/ml.

Gastrin cells were counted using rabbit's monoclonal antibodies against human gastrin (Polyclonal Rabbit Anti-Human Gastrin, DAKO Cytomation, Denmark). The picture analysis on PC computer was done by means of Olympus BX50 light microscope and Lucia G (Nikon, Japon) programming. The concentration of particular cells was defined on 1 mm^2 of the sample. In children with gastritis and *H. pylori* infection, eradication treatment with three medications was applied according to the indications of the workshop of the Polish Association of Gastroenterology [11]. The rest of children were given appropriate pharmacological and diet treatment according to the ailments found and the abnormal results of the tests. Ethical approval for research was obtained from local Ethics Committee in the Medical University of Białystok (R-I-003/30/2002).

Statistical analysis

The following descriptive methods of statistics were used for each characteristic and group of patients: arithmetic mean ($\bar{x}$), median, mode, and scattering measure, measure of distribution, standard deviation, variance and the value of upper and lower quartile. Chi^2 and Kolmogorov-Smirnov tests were used to match the distribution of empirical data with normal distribution. Mann-Whitney U non-parametrical test was used to find significant differences in the parameters between groups, regarding differences at $p<0.05$ as statistically significant. Result correlation was calculated with the use of the Spearman correlation test. ROC (Receiver Operating Characteristic) curves were analyzed to evaluate the usability of diagnostic parameters. A computer program STATISTICA 5.0 in the version for Windows System was used to calculate the data.

Results

Endoscopic evaluation of gastric mucosa in children with *H. pylori* (group I) showed inflammatory changes in the antrum in 71.2% of children and in the corpus in 64.4% of the examined. In group II (after *H. pylori* eradication, with positive IgG against *H. pylori*), inflammatory changes in the antrum and corpus were found in 27.6% of children ($p<0.001$). Children in group III (control) with negative IgG against *H. pylori* had normal gastric mucosa in the antrum and corpus. When examining children with *H. pylori* (group I), according to the Sydney System, the severe degree of antral gastritis was found in 45.8% of the examined; moderate gastritis in 52.5% and mild gastritis in 1.7%.

No severe gastritis was found in children with *H. pylori* eradicated (group II). The moderate degree of antral gastritis was proved in 6.9% of children and mild gastritis in 13.8%. Normal antral mucosa was found in 79.3% of children in group II. In controls, mild gastritis was observed in 15.4% and normal antral mucosa was found in 84.6% of children.

The evaluation of antral gastritis degree using Chi^2 test proved the statistically significant correlation in the study groups ($p<0.001$).

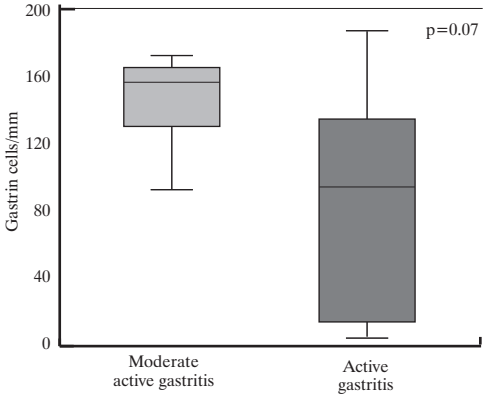
The analysis of antral gastritis activity indicated the most significant changes in children with *H. pylori* infection (group I). The severe active gastritis was found in 69.5% of children in this group; moderate active gastritis in 30.5% of the infected. No severe or moderate active gastritis was found in children with past infection after bacterium eradication (group II), but mild active gastritis was only in 20.7% of children in this group.

The analysis of antral gastritis activity using Chi^2 test proved

Table 1. **Gastrin cells in antral mucosa in children with *H. pylori* infection (I group), after eradication (II group) and in without infection *H. pylori* (III groups), Mann-Whitney U non-parametrical test**

Groups examined	Gastrin cells in antral mucosa/mm²								
	n	Min.	Max.	Arith. mean ($\bar{x}$)	Median (M)	Mode	Standard deviation (SD)	Lower quartile	Upper quartile
Group I	24	0	189	112.1	129.5	0	58.9	94.5	157
Group II	13	0	189	105.3	135.0	0	73.1	13.0	156
Group III	7	0	162	86.1	83.0	0	69.8	30.0	149

Figure 1. **Gastrin cell count and antral gastritis degree in children with *H. pylori* infection (group I), Mann-Whitney U non-parametrical test**



statistically significant results ($p<0.001$) in the groups examined. Histopathological evaluation of corpus gastritis was also performed basing on the Sydney System.

Corpus gastritis of mild and moderate degree (62.7% and 35.6% of the examined, respectively) predominated in children with *H. pylori* infection. Only 1.7% of children infected had corpus gastritis of the severe degree.

In children after *H. pylori* eradication (group II), normal corpus mucosa was observed in 82.1% of the examined, mild degree gastritis in 15.4% of children and moderate degree in 3.6%. In controls, only mild degree gastritis was observed in 15.4% and normal corpus mucosa was found in 84.6%. The results obtained in the histopathological evaluation of corpus mucosa changes were proved to be statistically significant ($p<0.001$).

Moderate active corpus gastritis was found in 55.9% and severe in 44.1% of children with *H. pylori* infection (group I). In children with past *H. pylori* infection after bacterium eradication (group II), mild active corpus gastritis was observed only in 17.9%. No moderate or severe activity was reported in this group. The results of histopathological evaluation of corpus inflammation were statistically different in each group ($p<0.001$).

The quantitative assessment of gastrin cells in the antral mucosa by immunohistochemical method showed the highest count in children with *H. pylori* infection (112.1±58.9 cells/mm²) and in the group with past *H. pylori* infection (slightly lower 105.3±73.1 cells/mm²). The results in both groups were higher in comparison with controls (86.1±69.8 cells/mm²) (*Tab. 1*).

No statistically significant differences were found in gastrin cell counts in the antral mucosa with regard to the degree of antral gastritis ($p<0.07$). In children with *H. pylori* infection, the average count of gastrin cells in moderate degree gastritis was 145.4±29.20 cells/mm². In the same group, the average count of gastrin cells equaled 88.36±63.94 cells/mm² in severe degree gastritis (*Fig. 1*).

The average gastrin cell count in moderate active gastritis was 106.0±77.18 cells/mm² and in severe active gastritis – 113.35±57.01 cells/mm².

In children with *H. pylori* infection (group I), serum gastrin levels were highest and equaled 92.9±41.6 μU/ml. They were also significantly elevated when compared to the levels of gastrin in group II with past *H. pylori* infection ($p<0.01$). In controls, serum gastrin levels were 70.0±15.3 μU/ml and they could be compared to the levels in group II. *Tab. 2* presents the results of statistical analysis. In group I, a correlation between children's age and gastrin levels in the serum is non-linear and described by general formula $y=a+b/x$. This correlation is characterized by a decrease in gastrin levels in the serum together with an increase in the age of children (r -0.43, $p<0.001$).

A similar negative correlation (r -0.38) between age and gastrin levels was found in group II. It was also non-linear, described by a general formula $y=a+b/x$. This correlation is statistically significant ($p<0.005$) indicating a decrease in gastrin levels in the serum together with a increase in children's age. ROC analysis confirms a high usability of gastrin levels as a diagnostic parameter (AUC=0.85±0.05). The gastrin level of 61.38 μU/ml taken as a value criteria guarantees the highest accuracy of diagnosis. The sensitivity criteria equals 94.4% and specificity – 62.5% (*Fig. 2*).

Discussion

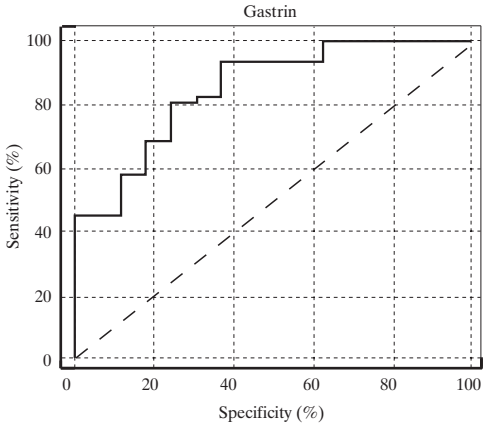
G cell counts in the antrum and gastrin levels in the blood serum were assessed in our study. A higher G cell count (mean value – 112.1±58.9/mm²) was established in children with gastritis in the course of *H. pylori* infection in comparison with children without infection (mean value – 105.3±73.1/mm²) and controls (mean value – 86.1±69.8/mm²). Gastrin cell count in the antrum was higher in moderate infection than in severe infection ($p<0.07$)

The findings on increased G cell counts in inflamed mucosa [12] with no significant difference of G cell counts between patients with and without infection [6] were presented in the

Table 2. **Gastrin levels in serum of children with *H. pylori* infection (I group), after eradication (II group) and in without infection *H. pylori* (III groups), Mann-Whitney U non-parametrical test**

Groups examined	Gastrin levels in serum (μU/ml)								
	n	Min.	Max.	Arith. mean ($\bar{x}$)	Median (M)	Mode	Standard Deviation (SD)	Lower quartile	Upper quartile
Group I	36	59	294	92.9	84.0	62	416	68	99
Group II	15	53	87	63.7	60.3	87	11.3	56	63
Group III	5	70	70	70.0	70.0	-	-	70	70

Figure 2. **ROC curve for gastrin levels in serum of children with *H. pylori* infection**



known literature. Similarly to the study, Kozlowski et al. examining 40 children aged 5-17 years with *H. pylori* infection proved higher G and D cell counts in the antrum and a proportionally higher relation of G/D cells. The values of these parameters decreased after bacterium eradication [12]. However, Rindi et al. evaluating children with antral G cell hyperfunction with and without *H. pylori* infection did not confirm the bacterium influence on an increase in G cell counts [6]. Park et al. and Tzaneva et al. assessing G cell counts in adults with gastritis or gastric or duodenal ulcer in the course of *H. pylori* infection also did not discover significant differences in G cell counts between patients with or without infection. Although D cell count was lower in the infection, the relation of G/D was higher [5,7].

In our study, higher G cell counts in the antrum were found in children with *H. pylori* infection, though no correlation was revealed between G cell counts and the severity of inflammation. In the study, gastrin levels in the serum were significantly higher in group I with gastritis and *H. pylori* infection than in group II without infection ($p<0.01$). Other authors also observed higher gastrin levels in children with *H. pylori* infection [9,13,14,15].

Queiroz et al. examining children with active gastritis in the course of *H. pylori* infection found higher gastrin levels in the serum with accompanying lower somatostatin levels in the antrum when compared to controls [9].

Haruma et al. evaluating gastrin levels in children with duodenal ulcer and in children with antral gastritis demonstrated enhanced gastrin levels in comparison with healthy children.

Gastrin levels were higher in children with duodenal ulcer than in children with antral gastritis. According to the author, the measurement of gastrin levels in the serum can be a marker of subclinical duodenal ulceration, especially among patients predisposed genetically to digestive tract ulceration [13].

Kim et al. testing 51 children showed significantly higher gastrin levels in the serum of children with *H. pylori* infection in comparison with controls. The highest gastrin levels were found in children with duodenal ulceration, lower in children with gastric ulceration and chronic superficial gastritis, and the lowest in superficial gastritis [14].

In our study, no significant differences were found between gastrin levels with regard to the severity of the inflammatory process. Similarly, Oderda et al. observed no difference between gastrin levels in 44 children with *H. pylori* infection [16]. In contrast to our study, Oderda did not discover any correlation between gastrin levels in the serum and *H. pylori* infection (it was comparable in children with and without infection), but simultaneously she observed a significant decrease in gastrin levels after successful eradication [16].

In the study, a correlation between gastrin levels and the age of the examined with past *H. pylori* infection was observed ($p<0.05$). The levels of serum gastrin were higher in younger children and lower in older ones and they became stable between 58 μU/ml and 60 μU/ml. Mc Callion presented similar results confirming that gastrin levels depended on the age of patients [17]. He assessed gastrin levels in 134 children, aged 4-13 years with IgG antibodies against *H. pylori* and proved that the mean gastrin level was significantly higher in younger children (4-5 years, the gastrin level – 155 ng/l) than in older children (12-13 years, the mean gastrin level – 90 ng/l). Early childhood hypergastrinemia in the course of *H. pylori* infection can be associated with temporary achlorhydria accompanying an acute stage of the infection and stimulating gastrin production.

AUC below ROC curve was determined to evaluate the usability of gastrin levels as a diagnostic parameter ($AUC=0.85\pm0.05$ for gastrin in serum) The value of gastrin concentration at the level of 61.38 μU/ml taken as a criteria guarantees the accuracy of diagnosis. The sensitivity criteria was 94.45% and the specificity criteria was 62.5% at this gastrin level.

Summarising the results of our study, gastrin levels in the serum proved to be higher in children with *H. pylori* infection. G cell counts in the antral mucosa were enhanced in children with *H. pylori* infection as well as in children after eradication and they did not differ significantly in both groups.

Conclusions

1. The *H. pylori* infection plays a significant role in the stimulation of G cells increase and gastrin release in the blood serum in children.

2. The eradication of *H. pylori* infection is probably a main factor in gastric secretion down-regulation during gastritis in children.

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