

ORIGINAL PAPER

## Estimation of the invasiveness of *Helicobacter pylori* strains on the basis of serological investigations in blood

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### Abstract

**Introduction:** *Helicobacter pylori* is a recognized factor inducing gastritis. Chronic gastritis with *H. pylori* infection is a precursor of chronic peptic ulcer disease as well as gastric cancer and lymphoma. Alterations in mucous membrane leading to the development of a neoplasm can last for years or even decades. The most pathogenic *H. pylori* strains are *cagA+*/*vacA+* and infection with these strains causes particularly significant increase of gastric epithelial cells proliferation not accompanied by simultaneous intensification of apoptosis. The presence of antibodies against anti-*H. pylori* surface and specific antigens in blood of the infected subject corresponds to the infection with *H. pylori* strains of defined genotype. **Aim:** Estimation of the dependence between the occurrence and serum index of surface anti-*H. pylori* antibodies of IgG class, specific antibodies anti-CagA and the severity and activity of gastritis in the prepyloric part. **Material and methods:** The study comprised 154 children, aged 5–18 years (mean 13,6±3,6) with alimentary tract ailments. Basing on urease test, serological and histological findings two groups were distinguished: Group I – children with *H. pylori* infection (*Hp+*), N=99; Group II – children without current *H. pylori* infection (*Hp-*), N=55. ELISA was used to determine the serum level and index of anti-*H. pylori* antibodies of IgG class and anti-CagA antibodies. The presence, activity and severity of the inflammatory process were estimated in histological investigation of gastric mucosa specimens from prepyloric area. Pearson's correlation or Spearman's correlation coefficient was calculated to test the dependence. **Results:** Relatively high correlations were found between the value of the index of serum anti-*H. pylori* antibodies of IgG class and the occurrence of histological exponents of gastritis in prepyloric part (R=0,48, p<0,001). High positive correlation was demonstrated between the occurrence and index of anti-CagA antibodies in serum and the severity of gastritis in prepyloric part (respectively: R=0,51, p<0,001 and R=0,56, p<0,001) and the activity of the inflammation (R=0,48, p<0,001). **Conclusion:** There is a strong correlation between the level of anti-*H. pylori* antibodies of IgG class, the presence and the level of anti-CagA *H. pylori* antibodies in serum and the severity and activity of gastritis in prepyloric part. (*Clin Exp Med Lett* 2009; 50(3):137-140)

### Introduction

*Helicobacter pylori* (*H. pylori*) is a recognized factor inducing gastritis. The colonization of gastric mucosa by *H. pylori* concerns mainly the prepyloric part of the stomach, sometimes also its body, foci of gastric epithelium metaplasia in esophagus, duodenum and anus [1-3]. *H. pylori* infection is most frequently acquired in childhood. In majority of immune-competent children the infection stimulates the development of specific cellular and humoral resistance. Despite the formation of strong inflammatory reaction in mucous membrane colonized by *H. pylori* there does not come to spontaneous elimination of the bacteria from the host organism. Most frequently the infection becomes chronic. *H. pylori*-induced gastritis can last for years [4]. Chronic gastritis with *H. pylori* infection is a precursor of chronic peptic ulcer disease as well as gastric cancer and lymphoma. Complications of chronic *H. pylori* infection are usually related to the adult population [2].

*H. pylori* is characterized by high genetic diversity, susceptibility to mutations and ability to bypass host defense mechanism in an infected organism. Genome is the key to biological functions. The presence of antibodies against anti-*H. pylori* specific antigens in blood of the infected subject corresponds to the infection with *H. pylori* strains of defined genotype [5]. This is serological reflection of the expression of proteins coded by these genes.

Antigen diversity of bacterial *H. pylori* strains is assumed to be able to condition the clinical course of the infection [5,6]. The strongest antigen activity is demonstrated by antigen CagA coded by *cagA* gene and cytotoxin VacA coded by *vacA* gene. The quantity of vacuolating cytotoxin depends on *vacA* gene structure. In majority of strains *in vitro* the production of VacA cytotoxin is associated with the presence of *cagA* gene coding CagA protein. Antigen CagA is described as the main factor accompanying the intensified disease. Strains type I CagA+/VacA+ are considered to be the most cytotoxic [7]. *H. pylori* strains

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virulence, dependent on the capability of CagA protein synthesis, can be tested on the basis of the determination of the presence of specific antibodies against the above mentioned proteins in serum.

## Aim

To estimate whether there is dependence between the presence and serum index of surface anti- *H. pylori* antibodies of IgG class, specific antibodies anti-CagA and the severity and activity of gastritis in the prepyloric part of the stomach.

## Material and methods

The study comprised 154 children, aged 5-18 years (mean  $13.6 \pm 3.6$ ) with alimentary tract complaints. Basing on gastroscopy with urease test, serological and histological (staining with Giemsa method) findings, two groups were distinguished: Group I – children with *H. pylori* infection (Hp+), N=99; Group II – children without current *H. pylori* infection (Hp-), N=55.

### Estimation of serum anti-*H.pylori* IgG antibodies

The investigations were performed with immunoenzymatic ELISA method on polystyrene plates coated with glycine extract (EG) (10 µg protein/ml) obtained from reference strain *H. pylori* CCUG 17874 and using reagents recomWell Helicobacter (Microgen, Germany). ELISA test index was calculated – the positive result was assumed for the value >1.2.

### Estimation of serum anti-CagA IgG antibodies

The investigations were performed with ELISA using reagents recomWell Helicobacter (Microgen, Germany). ELISA test index was calculated – the value > 1.2 was assumed as positive result.

### Statistical analysis

Means (x) and standard deviation (SD) were calculated from the obtained data. t-Student test for indepen-

dent samples or nonparametric U Mann-Whitney test for independent variables were used to calculate statistical significance;  $p < 0.05$  was considered significant. Comparison of the frequency and the investigation of the dependence between these parameters were performed with  $\chi^2$  test and when its assumptions were not fulfilled, exact Fisher's test was used. Pearson's correlation coefficient was calculated to test the dependences and Spearman's correlation coefficient when the assumption was not fulfilled.

Statistical calculations and graphics were performed with Statistica 6 computer program.

## Ethical issues

The study protocol was approved by the Bioethics Committee of the Polish Mother's Health Centre Research Institute in Lodz. Written consent was obtained from parents of the examined children and adolescents.

## Results

### Anti-*H.pylori* antibodies in serum and the prepyloric gastritis

Positive correlations were demonstrated: relatively high between the value of the index of serum anti-*H. pylori* IgG antibodies and low positive correlations between the presence of anti-*H.pylori* IgG antibodies in serum in the whole investigated group and the occurrence of histological exponents of severity of prepyloric gastritis, (respectively  $R=0.48$ ,  $p < 0.001$  and  $R=0.2$ ,  $p < 0.01$ ) and the activity of the inflammatory process (respectively  $R=0.44$   $p < 0.001$  and  $R=0.3$ ,  $p < 0.001$ ) (Table 1).

### Serum anti-CagA antibodies and prepyloric gastritis

Relatively high correlation was found between the presence of anti-CagA antibodies in serum, the value of anti-CagA index in serum and the severity of gastritis ( $R=0.51$ ,  $p < 0.001$ ) and ( $R=0.56$ ,  $p < 0.001$ ) and its activity ( $R=0.48$ ,  $p < 0.001$  and  $R=0.49$ ,  $p < 0.001$ ) (Figure 1, Figure 2), (Table 1).

Table 1. Correlations between the serum level of anti-*H. pylori* antibodies and the severity and activity of prepyloric gastritis

Prepyloric gastritis	Antibodies								
	anti- <i>H.pylori</i> IgG			Index anti- <i>H.pylori</i> IgG			anti-CagA		
	R	N	p	R	N	p	R	N	p
Severity of gastritis	0,22	154	<0,01	0,48	154	<0,001	0,51	61	<0,0001
Activity of gastritis	0,30	154	<0,001	0,44	154	<0,001	0,48	61	<0,0001

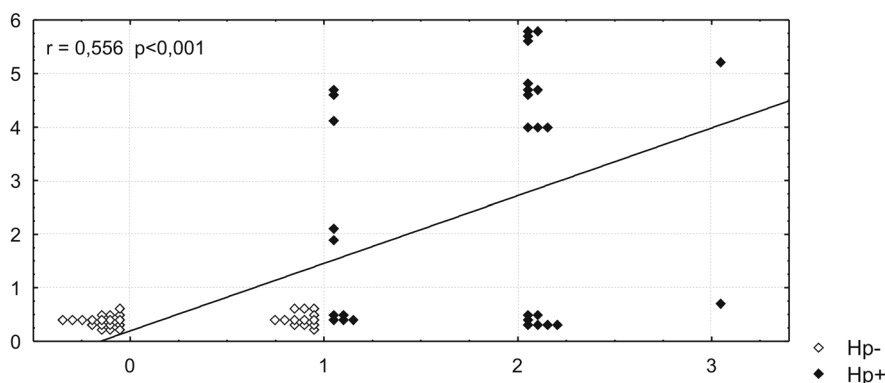


Figure 1. Severity of prepyloric gastritis and anti-CagA antibodies index

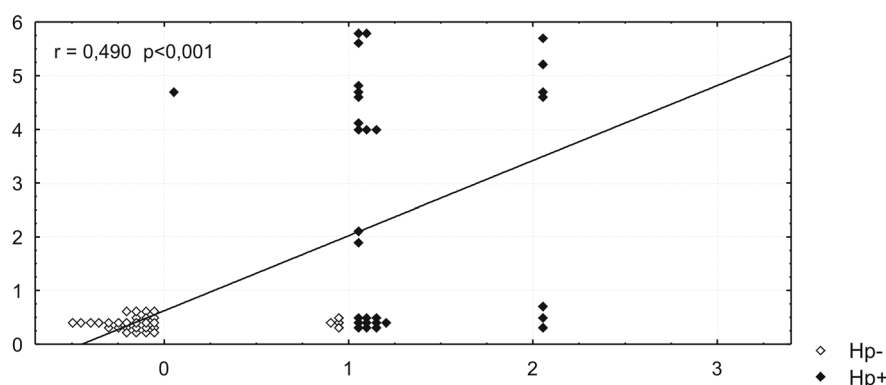


Figure 2. Activity of prepyloric gastritis and anti-CagA antibodies index

## Discussion

High correlation was found between the index of anti-*H. pylori* antibodies in serum and the prevalence and severity of prepyloric gastritis  $R=0,5$  ( $p<0,001$ ) and the occurrence of anti-CagA antibodies and anti-CagA antibodies index and severity and activity of inflammatory process  $R=$  from 0,4 to 0,5 ( $p<0,001$ ).

Dependences were weakly marked but positive in the case of the estimation of the presence of anti-*H. pylori* IgG antibodies in serum and the prevalence of prepyloric gastritis  $R=0,2$  ( $p<0,01$ ), severity and activity  $R=0,3$  ( $p<0,001$ ).

Studies concerning the associations between cytotoxic factors of the bacteria and histological manifestation of *H. pylori* infection in children are sparse. In the studies of Kohlo, similarly as in this study, the correlation coefficient between the serum level of antibodies against anti-*H. pylori* antigens and the intensification of inflammatory infiltration in prepyloric area was  $R=0,5$  [7]. Also Queiroz and Luzza found positive correlation between the presence of anti-CagA antibodies in serum with the severity of macroscopic and histologic traits of prepyloric gastritis as well as with the traits of degeneration and regeneration [5,8]. Similar observations were made by Polish researchers Dzierżanowska, Dzierżanowska-Faugrat and Iwańczak. In their studies they demonstrated positive correlations between the presence of anti-CagA antibodies and anti-CagA antibodies index and the severity of gastritis [6,9,10].

Plebani, who reported that *cagA* infection with positive *H. pylori* strains is accompanied by more severe gastritis and elevated level of anti-*H. pylori* IgG antibodies and pepsinogen C, concluded that the presence of anti-*H. pylori* antibodies and anti-CagA antibodies should be determined in epidemiologic investigations [11]. Also Luzza noted that the determination of anti-CagA IgG antibodies enables to detect a group of children with more severe gastritis and it can even serve for selecting children with *H. pylori/cagA+* infection for antibacterial treatment which will decrease the need for invasive endoscopic examinations with collecting gastric mucosa specimens in children [8].

Thus, serological response of a human organism to antigen CagA *H. pylori* can be an indication for determining the type of *H. pylori* strain with which it was infected. Higher level of anti-*H. pylori* and anti-CagA antibodies correlates with more severe gastritis of higher activity particularly in the prepyloric area. It seems that the mentioned serological diagnostic procedures will be basic in the future for establishing the infection and therapeutic management but putting them into practice in diagnosing children population requires further studies.

## Conclusions

1. There is a strong correlation between the serum level of anti-*H. pylori* IgG antibodies and anti-CagA

antibodies and the severity and activity of prepyloric gastritis.

2. Estimation of the serum level of antibodies against specific anti-CagA and serum level of anti-*H.pylori* IgG antibodies can be an indicator of the severity of gastritis resulting from *H.pylori* infection in children, but working out a table of dependences is necessary.

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