Relation between IgG and IgA antibody titres against *Helicobacter pylori* in serum and severity of gastritis in asymptomatic subjects

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Abstract

Aims—To investigate whether the absorbance index of IgG and IgA antibodies against *Helicobacter pylori* is related to a semiquantitative assessment of the density of *H pylori* colonisation in gastric biopsy specimens and to the severity of gastritis.

Methods—The grade of gastritis was scored separately for antral and fundic mucosa using three different classifications. Serum IgA and IgG antibodies against *H pylori* were measured by ELISA. The density of gastric *H pylori* colonisation was graded semiquantitatively from 0 to 3.

Results—Among 48 healthy volunteers studied, 17 were found to have gastritis according to Whitehead's criteria. *H pylori* was present in the biopsy specimens of 14 of 17 subjects with gastritis. The IgG *H pylori* antibody absorbance index was significantly ($p < 0.05$) correlated not only with the density of antral *H pylori* colonisation, but also with the degree of gastritis of the antrum, as assessed by the Whitehead score, activity, and the Sydney system ($p < 0.05$). The IgA *H pylori* antibody absorbance index was significantly correlated with the Whitehead score and Sydney system, but not with the activity score of the antrum or with the density of antral gastric *H pylori* infection. There were no significant correlations between the IgG *H pylori* antibody absorbance index and the gastritis scores of the fundus mucosa and the density of *H pylori* infection of the gastric body. The IgA *H pylori* antibody absorbance index was only significantly ($p < 0.05$) correlated with the density of *H pylori* colonisation and the Sydney system gastritis score of the corpus.

Conclusions—The serological absorbance index of IgG antibodies against *H pylori* is related to the severity of antral gastritis and the density of antral *H pylori* colonisation. Thus a high absorbance index of IgG antibodies against *H pylori* points to severe antral gastritis and dense *H pylori* colonisation of the antrum.

Chronic gastritis is associated with gastric *Helicobacter pylori* infection and increased serum antibodies against the organism.^{1,2} A previous study found a higher mean antibody titre in active chronic gastritis than in chronic gastritis,³ and we were interested to study whether the absorbance index of IgG and IgA *H pylori* antibodies was correlated with the severity of gastritis. If such a correlation existed, serology could be used to assess the severity of gastritis without endoscopy. To obviate possible confounding factors, such as gastric complaints, peptic ulcer, or use of drugs, we studied a group of healthy subjects without a history of gastrointestinal disorders or other chronic disease.

Methods

Forty eight asymptomatic volunteers, 29 of whom were members of the hospital staff, were examined. Their ages ranged from 19 to 58 years, with a mean age of 33 years. There were 22 women and 26 men. The mean age of the two groups was similar—33 for the women and 34 for the men. Volunteers with any clinical history of gastrointestinal disease or use of anti-ulcer drugs were excluded. The study protocol was approved by the ethics committee of the University Hospital Leiden. Informed consent was obtained from all volunteers before examination.

Endoscopy was performed after an overnight fast, with an Olympus GIF-K 30° forward-oblique viewing fiberoptic endoscope. Biopsy specimens were obtained from seven standard sites in the stomach (three from the gastric antrum and four from the corpus). The specimens were fixed immediately in 10% neutral buffered formalin. Before endoscopy, venous blood was withdrawn and the serum was stored at -70°C until assay.

HISTOLOGICAL ANALYSIS

The specimens were stained with haematoxylin and eosin, periodic acid Schiff, toluidine blue, and by James's reticulin method. All biopsy specimens were examined blind by an expert pathologist (JL). The specimens of antrum and corpus were classified as follows: (1) the criteria of Whitehead,⁴ (2) the Sydney system,⁵ and (3) an acute gastritis score.⁶ These classifications emphasise different

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features of gastritis: the Whitehead classification emphasises superficial and atrophic mucosal changes; the activity score the degree of polymorphonuclear and mononuclear leucocyte infiltration; and the Sydney system both infiltration and mucosal changes.

According to Whitehead *et al*⁷ the degree of gastritis is expressed in quantitative terms, so each degree of gastritis was scored from 0–4 according to the following scheme: normal = 0, superficial gastritis = 1, mild atrophic gastritis = 2, moderate atrophic gastritis = 3, severe atrophic gastritis = 4. In this analysis activity and metaplasia were not taken into account. Because the highest possible mean score is 4, multiplication of the mean scores by 25 yields a gastritis index ranging from 0 to 100.

Each parameter in the Sydney system— inflammation, activity, atrophy and intestinal metaplasia—was scored from 0–3 (0 is none, 1 is mild, 2 is moderate, 3 is severe). The total maximal score for these four parameters varied from 0–12. In the activity score the following parameters were scored: density of the inflammatory infiltrate in the lamina propria (0–2); density of polymorphonuclear leucocytes in the lamina propria (0–3); presence of intraepithelial polymorphonuclear leucocytes (0–3); and superficial erosions (0–2). The total score varied from 0–10. To compare the three different classifications the scores of the Sydney system and the activity score were multiplied by a factor of 8.3 and 10, respectively, to obtain a gastritis index from 0–100 for each score. Figures 1 and 2 show samples of the three different classifications.

All biopsy specimens were also examined for the presence of *H. pylori*. If present, the density of mucosal *H. pylori* colonisation was graded semiquantitatively from 0 to 3.

Figure 1 Chronic active gastritis of antrum mucosa with severe atrophy: total score according to the Whitehead classification 4, gastritis index 100; total score according to the activity score 3, gastritis index 30; total score according to the Sydney system 6, gastritis index 50.

Figure 2 Chronic gastritis of fundic mucosa with moderate atrophy: total score according to the Whitehead classification 3, gastritis index 75; total score according to the activity score 2, gastritis index 20; total score according to the Sydney system 4, gastritis index 33.

SEROLOGY

Specific IgA and IgG antibodies against *H. pylori* were measured using a modified enzyme linked immunosorbent assay (ELISA). Microtitre plates were coated with a homogenate of six strains of *H. pylori*. Specific antibodies from diluted sera were detected by conjugates of horseradish peroxidase and goat antibodies specific for human IgA or IgG. The amount of bound peroxidase was measured through reaction of hydrogen peroxide and a chromogenic substrate, whose optical density (OD) was read by a photometer. Readings of unknown sera were compared with those of a high reference serum. The results were expressed as the absorbance index (AI):

$$AI = \frac{\text{mean OD reading (n=2) of patients' serum} - \text{mean OD of blank reading}}{\text{Mean OD reading (n=2) of reference serum} - \text{mean OD of blank reading}}$$

The procedure of the assay and determination of intra- and interassay variability of the ELISA has been described in detail by Peña *et al.*⁸ Serum with an AI of >0.35 for IgA antibodies to *H. pylori* and an AI of >0.32 for IgG antibodies to *H. pylori* were considered evidence of *H. pylori* infection.

Results were expressed as the mean (SE). The relations between the absorbance indices of IgG and IgA *H. pylori* antibodies, the degree of *H. pylori* colonisation of antrum and corpus, and the severity of gastritis according to the three different classifications were separately analysed by linear regression, and the correlation coefficient was inferred by two-tailed significance.

For obvious reasons these analyses could be performed only in subjects with histological evidence of gastritis.

Results

Seventeen of the 48 asymptomatic subjects had gastritis of the antrum and 16 gastritis of the corpus according to the criteria of Whitehead and the Sydney system. Results in these 17 subjects were further analysed. These 17 subjects comprised six men and 11 women with a mean age of 39 years. According to the activity score, 15 of these 17 subjects had gastritis of the antrum and corpus.

There were significant correlations in the

Figure 3 Individual scores of gastritis of the gastric antrum as assessed by the Whitehead classification (left panel), activity score (middle panel), and the Sydney system (right panel). Cross symbols denote *Helicobacter pylori* colonisation.

Figure 4 Individual scores of gastritis of the gastric corpus as assessed by the Whitehead classification (left panel), activity score (middle panel), and the Sydney system (right panel). Cross symbols denote *Helicobacter pylori* colonisation.

antral mucosa between the Whitehead classification and the Sydney system scores ($r = 0.80$; $p < 0.001$), and between the Sydney system and the activity scores ($r = 0.78$; $p < 0.001$). The individual histological scores of the antral mucosa are presented in fig 3. There were also significant correlations in the gastric corpus between the Whitehead classification and the Sydney system scores ($r = 0.85$; $p < 0.001$) and the activity score ($r = 0.75$; $p < 0.002$), and between the Sydney system and the activity scores ($r = 0.94$; $p < 0.001$). The individual histological scores of the fundus mucosa are shown in fig 4.

H pylori was observed in 10 subjects in the entire stomach and in four cases in the antrum alone. In 13 of these 14 subjects the absorbance index of IgG antibodies to *H pylori* was higher than 0.32, but only nine of these subjects had a raised absorbance index of IgA antibodies to *H pylori* above 0.35.

There was a significant correlation between the absorbance index of IgG antibodies to *H pylori* and the degree of gastritis of the antrum as assessed by all three gastritis scores (table 1). Individual data on the absorbance index of IgG antibodies to *H pylori* and the severity of gastritis according to the Whitehead score is shown in fig 5. The absorbance index of IgA antibodies was only significantly correlated with the Whitehead classification and Sydney system scores, but not the activity score (table 1).

There was no correlation between the absorbance index of IgG antibodies and the different gastritis scores of the corpus (table 2). For the corpus there was a significant correlation only between the absorbance index

Table 1 Correlations between serum absorbance indices of IgA and IgG antibodies against *H pylori* and degree of *H pylori* colonisation and severity of gastritis of the antrum

	IgA antibodies (AI)	IgG antibodies (AI)	Colonisation
Colonisation	$p > 0.10$	$p = 0.04$	
Gastritis (Whitehead score)	$r = 0.65$ $p = 0.005$	$r = 0.52$ $p = 0.03$	$r = 0.46$ $p = 0.07$
Gastritis (activity score)	$p > 0.10$	$r = 0.52$ $p = 0.03$	$r = 0.69$ $p = 0.002$
Gastritis (Sydney system)	$r = 0.62$ $p = 0.008$	$r = 0.61$ $p = 0.01$	$r = 0.63$ $p = 0.006$

Table 2 Correlations between serum absorbance indices of IgA and IgG antibodies against *H pylori* and degree of *H pylori* colonisation and severity of gastritis of the gastric body

	IgA antibodies (AI)	IgG antibodies (AI)	Colonisation
Colonisation	$r = 0.51$ $p = 0.04$	$r = 0.42$ $p = 0.10$	
Gastritis (Whitehead score)	$r = 0.47$ $p = 0.06$	$p > 0.10$	$p > 0.10$
Gastritis (activity score)	$r = 0.45$ $p = 0.07$	$p > 0.10$	$p > 0.10$
Gastritis (Sydney system)	$r = 0.55$ $p = 0.02$	$r = 0.42$ $p = 0.09$	$r = 0.46$ $p = 0.06$

Figure 5 Correlation between the serum absorbance index of IgG antibodies to *Helicobacter pylori* and the gastritis score of the antrum according to the Whitehead classification ($r = 0.52$; $p = 0.03$).

of IgA antibodies and the Sydney system gastritis score of the corpus (table 2).

The absorbance index of IgG antibodies to *H. pylori* was significantly correlated with the degree of *H. pylori* colonisation of the antrum ($r = 0.50$; $p = 0.04$), but not of the corpus ($r = 0.42$; $p = 0.10$). In contrast, the absorbance index of IgA antibodies was significantly correlated with the degree of *H. pylori* colonisation of the corpus ($r = 0.51$; $p = 0.04$), but not of the antrum ($p > 0.10$).

The degree of *H. pylori* colonisation of the antrum was correlated to the severity of gastritis in the antrum (table 1), but not to the severity of body gastritis. Furthermore, the density of *H. pylori* colonisation of the gastric body was not significantly correlated with the severity of body gastritis, but there was a significant correlation between the degree of *H. pylori* colonisation of the corpus and the severity of gastritis in the antrum according to the Whitehead classification ($r = 0.49$; $p = 0.045$) and the Sydney system scores ($r = 0.55$; $p = 0.02$).

Discussion

It has repeatedly been shown that the titres of IgA and IgG antibodies to *H. pylori* in serum can be used as non-invasive tests for the presence of gastric *H. pylori* infection and gastritis.¹² It is, however, not known whether the titres of *H. pylori* antibodies in serum are indicative of the severity of gastritis. If so, the severity of gastritis could be assessed and followed without endoscopy. Goodwin *et al.*³ reported a higher mean antibody titre in active chronic than in inactive chronic gastritis. In contrast, Booth *et al.*⁹ and Newell *et al.*¹⁰ did not find higher titres of *H. pylori* antibodies in severe gastritis, although in the last study a tendency to a higher concentration in more severe gastritis was observed.

In this study we therefore attempted to answer the question as to whether the titres of IgG or IgA antibodies to *H. pylori* are related

to the severity of gastritis. We were also interested to see if there was any association between the number of bacteria semiquantitatively assessed in the biopsy specimens, and the titres of the antibodies and the severity of gastritis. Endoscopic biopsy specimens of antrum and corpus were scored and analysed separately.

To obviate possible confounding factors, such as gastric complaints, peptic ulcer, or use of drugs, we studied a group of healthy subjects without a history of gastrointestinal disorders or other chronic disease. Seventeen of them had gastritis and they formed the study population for this investigation. Fourteen of them had histological and serological evidence of *H. pylori* infection. Thus the incidence of *H. pylori* infection in Dutch asymptomatic subjects is about 30%. Although the inclusion of a number of endoscopists as volunteers in this study may have affected this result, the percentage is in line with the results of Dutch blood donors of similar age.¹¹

Several interesting results were obtained. Clinically, the most important finding is that the concentration of IgG antibodies to *H. pylori* in serum correlates with the severity of gastritis of the antrum according to all three classification systems. The absorbance index of IgG antibodies in serum was correlated with the degree of colonisation of the antrum by *H. pylori*. Thus a high IgG antibody titre to *H. pylori* points to severe antrum gastritis with intense colonisation of the antrum. This finding may facilitate studies of the severity of gastritis without the need for endoscopic biopsies.

Little is known about the relation between serum *H. pylori* antibodies and the severity of gastritis in Western people. In Chinese subjects the serum antibody titres to urease were related to the bacterial numbers of *H. pylori* in gastric mucosa, but no relation with the severity of gastritis was found.¹² In Malawians with epigastric pain of more than two weeks, there was an association between the density of colonisation with *H. pylori* and the degree of polymorphonuclear and mononuclear cell infiltration, as found in our study, but the relation between the concentration of IgG antibodies to *H. pylori* in serum and the severity of gastritis was not assessed.¹³

In conclusion, this study shows that determining the absorbance index of IgG antibodies to *H. pylori* in serum could be used to assess the severity of gastritis of the antrum.

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