Immunoglobulin G Antibody against Helicobacter pylori: Clinical Implications of Levels Found in Serum

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The clinical significance of high levels of antibody against Helicobacter pylori is still unclear. We sought to evaluate whether the serum antibody levels could predict the presence of macroscopic gastroduodenal disease, to identify factors that correlate with antibody levels in a multivariate context, and to determine the predictive value of antibody levels for diagnosing H. pylori infection. The grades of gastritis and density of H. pylori colonization were scored separately using the updated Sydney system for antral and body mucosa. An enzyme-linked immunosorbent assay (ELISA) for the quantitative detection in serum of IgG antibodies to H. pylori was performed. Of the 170 dyspeptic patients, 105 (62%) had H. pylori infection. There was no difference in antibody levels among endoscopic findings of normal mucosa, chronic gastritis, and duodenal ulcer. On multivariate linear regression analysis, the status of H. pylori infection, mononuclear cell infiltration of body mucosa, and age correlated with antibody levels. The positive predictive value for antibody levels of >70 U/ml is 98%, and the positive predictive value of antibody levels of >70 U/ml is 94%, and the positive predictive value of antibody levels of >70 U/ml is 98%. We conclude that serum antibody levels do not predict the severity of gastroduodenal diseases or the density of H. pylori colonization in H. pylori-infected dyspeptic patients. Higher levels are associated with the presence of H. pylori infection, the chronic gastritis score of the corpus, and older age. Setting a gray zone is necessary for ELISA, since the accuracy in this zone does not allow a precise determination of H. pylori status.

Helicobacter pylori has been established as an important etiological factor for chronic gastritis and duodenal ulcer (10, 15, 16). It is also associated with gastric ulcer and gastric cancer (4). As the test-and-treat policy for H. pylori infection is gradually being accepted by general practitioners, a simple, reliable, and noninvasive diagnostic test for H. pylori has become essential in clinical practice (5, 12). An easier and cheaper way to diagnose H. pylori infection noninvasively is to test for antibodies to the infection. Enzyme-linked immunosorbent assay (ELISA) has been the most commonly used serological test because it is suited for screening large populations (11, 13). Clinically, some patients are very concerned about contracting H. pylori infection when they are told they have high antibody levels. However, the correlation between anti-H. pylori antibody levels and the severity of histological gastritis or H. pylori density has been studied with conflicting results (8, 9, 14, 18, 19, 21). This discrepancy in results may arise from differences in classification and grading of gastritis, the numbers of subjects, consideration of confounding factors, and statistical methods. One study reported that immunoglobulin G (IgG) antibody levels correlated with the grades of antral polymorphonuclear leukocyte infiltration and antral bacterial density (8), while another study demonstrated that having higher levels implies advanced antral gastritis with either acute or chronic inflammation (18). Both studies concluded that levels in themselves do not provide a method for predicting the presence of an ulcer. However, the sample size was small in one study, and no body mucosa was evaluated in either study. Therefore, we conducted this study to identify which factors correlate with antibody levels in a multivariate context and to determine if antibody levels could predict the presence of macroscopic gastroduodenal disease. The predictive value of antibody levels for diagnosing H. pylori infection was also evaluated.

MATERIALS AND METHODS

Dyspeptic patients who were scheduled for upper gastrointestinal endoscopy were recruited into the study. Patients with any of the following conditions were excluded: (i) ulcer complications, such as bleeding, stenosis, or perforation; (ii) previous stomach surgery; (iii) intake of any substitute for benzimidazoles or preparations containing bismuth within 1 month prior to administration of the test; or (iv) having been treated with or currently on anti-H. pylori therapy. For serology studies, blood was drawn immediately after endoscopy, and sera were collected and stored at −70°C until they were assayed.

Histology. During endoscopy, two sets of biopsy specimens from the antrum and the greater curvature of the midbody were obtained for rapid urease testing (CLO Test, Delta West, Bentley, Australia) and histology. Hematoxylin and eosin stains were used to grade the gastritis. The grades of gastritis were assessed for infiltration of mononuclear cells or neutrophils, mucosal atrophy, and intestinal metaplasia using visual analogue scales described in the updated Sydney system on a four-point scale (0, normal; 1, mild; 2, moderate; and 3, marked). A modified Giemsa stain was used to assess the density of H. pylori (3). The number of lymphoid follicles was recorded. All histological sections were evaluated by the same pathologist, who was blinded to the patients’ clinical conditions.

Quantitative ELISA. Serum specimens were tested for the presence of IgG antibodies against H. pylori using a quantitative ELISA (HEL-pTEST II; AMRAD, Kew, Australia). Reference standards were used to produce a standard curve to quantitate H. pylori antibody levels in patient samples. The results were expressed in arbitrary units per milliliter. The antigen was an inactive native antigen of H. pylori. On the day of testing, we added 100 μl of diluted specimens, diluted positive and negative controls, and duplicates of reference standards 1 to 4 to the appropriate wells of the microtiter plate. The plate was incubated for 15 min at room temperature and then washed six times with a wash buffer. After the washing, 100 μl of sheep anti-human IgG conjugated to horseradish peroxidase...
was added to each well. After a further 15 min of incubation, 100 μl of substrate reagent was added to each well and incubated in the dark for 15 min at room temperature. Then, stopping solution (100 μl of H2SO4) was added to each well to terminate the enzymatic reaction. The absorbance was read within 30 min using a 450-nm-pore-size filter with a 620-nm-pore-size filter as a reference.

**H. pylori status.** A patient was classified as *H. pylori* infected if both the CLO and histological tests were positive. A patient was classified as non-*H. pylori*-infected if both methods were negative. Patients who had only one positive CLO or histology test were considered to be of indeterminate status.

**Statistical analysis.** Continuous data were analyzed by the two-tailed Student’s *t* test or analysis of variance (ANOVA). When ANOVA of multiple groups revealed a difference at the 5% level, the post hoc multiple-comparison technique was used to determine which pairs of groups were responsible for the overall difference. Spearman’s rank correlation was used to examine the relationship between antibody levels and grades of gastritis. Multiple stepwise linear regression was also used to identify which factors were related to the antibody levels. The data were processed using SPSS software. Positive and negative predictive values were calculated for the ELISA (HEL-pTEST II), using gastric biopsy results as the “gold standard.”

**RESULTS**

One hundred and seventy patients (88 men and 82 women; mean age ± standard deviation [SD], 43.7 ± 13.5) were included in the study. There were 34 patients with normal endoscopic findings, 62 patients with gastritis, 57 patients with duodenal ulcers, 5 patients with gastric ulcers, 2 patients with combined gastric and duodenal ulcers, and 10 patients with other findings. Based on the proposed gold standard, 105 patients were infected, 64 were not infected, and 1 was of indeterminate status.

Anti-*H. pylori* antibody levels did not show a normal distribution (skewness, 1.888; kurtosis, 2.839), but its logarithm did (skewness, 0.033; kurtosis, −0.967), so logarithmic transformation was performed in the assessment of anti-*H. pylori* antibody levels during the two-tailed Student’s *t*-test or ANOVA by Scheffe’s method or a linear regression.

There was no difference in antibody levels between men and women or smokers and nonsmokers or among different blood groups. The influence of drinking habits on antibody levels was not evaluated because only six patients were regular drinkers.

For dyspeptic patients with *H. pylori* infection, there was no difference in antibody levels among the endoscopic findings for the normal mucosa (*n* = 12), chronic gastritis (*n* = 36), and duodenal ulcers (*n* = 46) (223 ± 220, 227 ± 223, and 228 ± 196 U/ml [mean ± SD], respectively; *P* = 0.99).

The mean serum anti-*H. pylori* antibody levels in relation to the grading of the four histological features of the antral mucosa and body mucosa are illustrated in Fig. 1 and 2, respectively. We see a trend for the mean antibody levels to rise as the grades of gastritis and *H. pylori* density increase.

Significant correlations were found between the antibody levels and the grades of gastritis in the antral mucosa (Spearman’s rank correlation: *r* = 0.586 and *P* < 0.001 for neutrophil infiltration, *r* = 0.620 and *P* < 0.001 for mononuclear cell infiltration, *r* = 0.429 and *P* < 0.001 for atrophy, *r* = 0.630 and *P* < 0.001 for *H. pylori* density, and *r* = 0.438 and *P* < 0.001 for lymphoid follicles) and in the body mucosa (Spearman’s rank correlation: *r* = 0.598 and *P* < 0.001 for neutrophil infiltration, *r* = 0.618 and *P* < 0.001 for mononuclear cell infiltration, *r* = 0.481 and *P* < 0.001 for atrophy, *r* = 0.680 and *P* < 0.001 for *H. pylori* density, and *r* = 0.190 and *P* < 0.05 for lymphoid follicles). However, if the calculation was restricted to *H. pylori*-infected patients, only the grades of neutrophil infiltration and mononuclear cell infiltration, atrophy, and the number of lymphoid follicles in the body mucosa retained significant correlation with antibody levels (*r* = 0.248 and *P* < 0.05, *r* = 0.292 and *P* < 0.005, *r* = 0.218 and *P* < 0.05, and *r* = 0.230 and *P* < 0.05, respectively). This is also noted in estimating correlation between antibody levels and age. Significant correlations were found when all subjects were considered (Spearman’s rank correlation: *r* = 0.200; *P* < 0.01), but there was no significant correlation in *H. pylori*-infected patients (Spearman’s rank correlation: *r* = 0.154; *P* = 0.117). However, if age is divided into older (>45 years) and younger (<45 years) groups, the difference in antibody levels between the two groups in *H. pylori*-positive patients approached significance (median, 164, and range, 38 to 823 versus 124 and 21 to 804; *P* = 0.09).

Intestinal metaplasia in antral specimens was observed in 18 patients (15 with score 2, 2 with score 3, and 1 with score 4), so scores 2, 3, and 4 were grouped together. There was no difference in the mean antibody levels between patients with and without intestinal metaplasia (*P* = 0.984). No intestinal metaplasia was observed in the body specimens.
A multiple stepwise linear regression was performed to analyze the relationship between antibody levels and the following predictors: sex, age, smoking, blood groups, grades of antral and body gastritis, number of lymphoid follicles, \textit{H. pylori} density, and status of \textit{H. pylori} infection. Table 1 shows that the status of \textit{H. pylori} infection, grade of mononuclear cell infiltration of body mucosa, and age were three independent factors correlated with antibody levels ($R = 0.780; R^2 = 0.608$).

Figure 3 shows the distribution of anti-\textit{H. pylori} antibody (IgG) levels in \textit{H. pylori}-infected and uninfected patients ($n = 169$). The negative predictive value of antibody levels of $<20$ U/ml is 100\%, and that for levels of $<30$ is 94\%. The positive predictive value of antibody levels of $>70$ U/ml is 98\%. There were 27 patients (16\%) whose antibody levels fell between 30 and 70 U/ml. Among them, 14 patients were \textit{H. pylori} infected and the rest were non-\textit{H. pylori} infected, judged by the reference standards. This suggests that little more than a guess is involved when making a positive-negative decision in this gray zone.

### DISCUSSION

In our present study, there was no difference in antibody levels of the normal mucosa, gastritis, and duodenal ulcers in \textit{H. pylori}-infected patients. This is in accordance with the study by Sharma et al., who reported that \textit{H. pylori} serology cannot be used to predict the presence or absence of gastroduodenal ulcer disease (17). Similarly, Sheu et al. reported no difference in antibody levels between nonulcer and ulcer subgroups (18), and Hsu et al. (8) reported that levels of anti-\textit{H. pylori} in serum are not predictive of the severity of macroscopic gastrointestinal damage.

The correlation between anti-\textit{H. pylori} antibody levels and the severity of histological gastritis or \textit{H. pylori} density has been studied with conflicting results (8, 9, 14, 18, 19, 21). Significant correlation was reported in studies in which non-\textit{H. pylori}-infected patients were included in the analysis (9, 14, 21). On the other hand, no such correlation was found in one report in which only \textit{H. pylori}-infected patients were analyzed (19). More than 90\% of \textit{H. pylori}-infected patients have detectable serum IgG antibodies, and the vast majority of \textit{H. pylori}-infected patients have chronic active gastritis in the antral biopsy specimens. Therefore, the status of \textit{H. pylori} infection is a crucial confounding factor in analyzing the relationship between antibody levels and histological gastritis. If non-\textit{H. pylori}-infected patients are included in the analysis, the correlation between antibody levels and gastritis scores or \textit{H. pylori} density may be misleading due to their common association with the status of \textit{H. pylori}. Our study also supports these observations. In this study, grades of active and chronic inflammation, atrophy, lymphoid follicles, and \textit{H. pylori} density were correlated with antibody levels in the antral mucosa but were not correlated if only \textit{H. pylori}-infected patients were analyzed.

Our results had some differences from the reports of Hsu et al. and Sheu et al. (8, 18). In the study by Hsu et al., antibody levels did not correlate with grades of antral mononuclear cell infiltration, mucosal atrophy, or intestinal metaplasia but did correlate with grades of antral neutrophil infiltration and antral bacterial density in \textit{H. pylori}-infected patients. The ELISA kit they used was the same as ours. Since anti-\textit{H. pylori} antibody levels do not show a normal distribution, logarithmic transformation is more appropriate when performing a parametric test. Therefore, the variance may result from the small number of cases in their study ($n = 36$) and different statistical methods. In the study by Sheu et al., antibody levels correlated with the severity of acute or chronic inflammation. However, the rate of absence of neutrophil infiltration (score, 0) in \textit{H. pylori}-infected patients in their study was 63\%. This is contrary to the well-established concept that the majority of \textit{H. pylori-}
infected patients have chronic active gastritis in the antral biopsy specimens. In addition, the ANOVA model with Duncan’s test is not an appropriate statistical method for data with unequal numbers in the groups.

In this study, grades of active and chronic inflammation, atrophy, and lymphoid follicles in the body mucosa remained significantly correlated with antibody levels in *H. pylori*-infected patients. The correlation coefficients, however, were reduced to half of those when non-*H. pylori*-infected patients were included. This indicates that the status of *H. pylori* infection is an important contributing factor, which has been demonstrated in the multivariate linear regression model. The data in this study were obtained from dyspeptic patients, so they may not generalize to asymptomatic subjects.

There has been a report showing that diffuse antral gastritis with prominent lymphoid follicles has the highest antibody levels (6). However, in that study, no statistical data were presented, so we calculated the difference between diffuse antral gastritis and chronic atrophic gastritis from the data presented in the table and found no statistical difference (0.274 ± 0.025 versus 0.266 ± 0.013 [mean ± SD]; *P* = 0.757).

In our study, antibody levels of <30 or >70 U/ml had good predictive value for the absence or presence, respectively, of *H. pylori* infection. For individuals having a value between 30 and 70 U/ml, the so-called gray zone, a different diagnostic method is recommended to assess *H. pylori* status more accurately. When antibody levels are >70 U/ml, higher levels do not improve the positive predictive value. However, our previous study of the rapid serological test has demonstrated that antibody levels in ELISA are correlated with the density of the reaction band in the rapid serological test (1). The higher the antibody levels, the more clearly the band is noted. The lower the antibody levels, the more likely that it will be regarded as negative in the rapid serological test due to a very weak color change of the reaction band. Thus, higher antibody levels have a favorable effect on the diagnosis of *H. pylori* infection in rapid serological tests.

There were several studies demonstrating that the sensitivity of serological diagnosis of *H. pylori* infection was higher in the older group (≥45 years) than in the younger group (<45 years) (2, 7, 20). Our study showing higher antibody levels in the older group in *H. pylori*-infected patients could explain this phenomenon, because the higher the antibody levels, the fewer false negatives there will be.

In conclusion, levels of IgG anti-*H. pylori* antibody in the serum do not predict the presence of macroscopic gastroduodenal diseases or the density of *H. pylori* colonization in *H. pylori*-infected dyspeptic patients. The presence of *H. pylori* infection, higher updated Sydney system chronic gastritis scores of the corpus, and older age are associated with higher antibody levels. Setting a gray zone is necessary for ELISA, since the accuracy in this zone does not allow a precise determination of *H. pylori* status.

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REFERENCES


