Original Article

Analysis of negative result in serum anti-*H. pylori* IgG antibody test in cases with gastric mucosal atrophy

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The purpose is to elucidate factors related to negative results of anti-*H. pylori* antibody test in cases with gastric mucosal atrophy. A total of 859 individuals without past history of eradication therapy for *H. pylori* (545 males, 314 females; mean age 52.4 years) who underwent an upper GI endoscopy examination and serological test were enrolled as subjects. Serological testing was performed using SphereLight *H. pylori* antibody J®, and endoscopic findings of gastric mucosal atrophy by the classification of Kimura and Takemoto and post-eradication findings were analyzed. The positive rates for the anti-*H. pylori* antibody test in subjects with and without gastric mucosal atrophy were 85.6% and 0.9%, respectively. In analysis of subjects with gastric mucosal atrophy, a low positive rate and serum titer was observed in subjects with C1, C2 and O3 atrophy. When the analysis was performed separately in male and female subjects, low positive rate was observed in males with O3 atrophy and females with C2 atrophy. Suspected post-eradication endoscopic findings were more frequently observed in cases with C2 atrophy. In conclusion, negative result of anti-*H. pylori* antibody test was frequently observed in middle-aged subjects with C1, C2 and O3 gastric mucosal atrophy.

***Key Words*: *Helicobacter pylori*, diagnosis, serologic tests, endoscopy, atrophy**

Helicobacter pylori (*H. pylori*) infection is known to cause several types of gastrointestinal diseases, such as gastritis, peptic ulcers and gastric cancer,[1–7] thus eradication therapy is widely recommended to prevent their occurrence.[7–12] As a result, it is very important to accurately diagnose *H. pylori* infection in clinical situations, with several different invasive and non-invasive methods available.[13,14] Among the available methods, a serologic test for *H. pylori* infection is easily performed using obtained serum samples for both epidemiologic studies involving large numbers of subjects as well as in clinical practice for individual patients. It has been reported that the sensitivity and specificity of serological methods for detection of *H. pylori* infection range from 80% to 90%.[14] On the other hand, the diagnostic accuracy of serological methods for diagnosis of *H. pylori* infection has been shown to vary based on the duration of exposure to *H. pylori*, cross-antigenicity with other prevalent antigenically related bacteria such as *Campylobacter*, the diversity of *H. pylori* strains in different regions, host immune response, the grade of histological gastritis, and the density of *H. pylori*.[14–20] The diagnostic accuracy of serological tests for *H. pylori* in Japanese subjects has been repeatedly demonstrated to increase when using kits derived from antigens of *H. pylori* strains obtained from Japanese patients.[17–20] SphereLight *H. pylori* antibody J® (Wako Pure Chem. Ind., Ltd., Osaka), a recently introduced anti-*H. pylori* IgG antibody detection kit, was developed using antigens from *H. pylori* strains derived from Japanese patients. This kit has been shown to have a high efficacy for diagnosis of infection,[21,22] and the serum titer of this test is nearly equal to that of another anti-*H. pylori* IgG antibody test (Eiken Chemical Co., Ltd., Tokyo) (unpublished data). In order to increase the sensitivity of diagnosis for *H. pylori* infection, an antibody titer of ≥4.0 U/ml is defined as positive in the SphereLight *H. pylori* antibody J test, while the cut-off value in the Eiken anti-*H. pylori* IgG antibody test is set at 10 U/ml. We have found that some patients without past-history of eradication therapy for *H. pylori* also show a negative result in the SphereLight *H. pylori* antibody J test, even though they have endoscopic evidence of gastric mucosal atrophy, which is mainly caused by long-term *H. pylori* infection.[23,24] Therefore, we performed the present retrospective study to elucidate factors related to a negative result in the SphereLight *H. pylori* antibody J test in cases with gastric mucosal atrophy by analyzing the presence of post-eradication endoscopic findings, based on several recent studies.[25–30]

Materials and Methods

The subjects were individuals who visited the Health Center of Shimane Environment and Health Public Corporation for a detailed medical checkup examination between April 2014 and March 2015. The majority were socially active and productive, and considered to be socioeconomically middle class. Those with a history of gastric surgery and eradication therapy for *H. pylori* infection, carefully confirmed by a public health nurse, were excluded. Those who had taken such medications as proton pump inhibitors and H2 receptor antagonists were also excluded. Finally, 859 subjects (545 males, 314 females; mean age 52.4 years) who underwent upper GI endoscopic examinations and serum anti-*H. pylori* IgG antibody testing on the same day were enrolled as subjects. None had severely abnormal findings in renal and liver function tests.

Serum anti-*H. pylori* IgG antibody detection was performed using SphereLight *H. pylori* antibody J®. The antibody titer was automatically measured using a chemiluminescent enzyme immunoassay method. An antibody titer ≥4.0 U/ml was defined as positive, according to the manufacturer's instruction sheet.

All upper endoscopic examinations were performed by licensed experienced endoscopists (K.A., T.M., S.T.) using an EG-530NW or EG-530NP endoscope (Fujifilm, Tokyo, Japan). When gastric

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mucosal atrophy was endoscopically observed, its degree was evaluated using the classification of Kimura and Takemoto, in which gastric mucosal atrophy is classified into 6 groups (C1, C2, C3, O1, O2, O3).[31] The cases without gastric mucosal atrophy was diagnosed as C0 in this study. The presence of gastric mucosal atrophy was carefully determined by the presence or absence of regular arrangement of collecting venules at angular portion and atrophic border in the cases with thin gastric mucosa. When cases with endoscopic evidence of gastric mucosal atrophy showed a negative result in the anti-*H. pylori* IgG antibody test, we investigated the existence of endoscopic evidence of post-eradication by examining for the presence of characteristic endoscopic findings in the stomach. For this study, we defined suspected post-eradication cases based on the presence of map-like redness or depressed patchy redness, as well as absence of diffuse redness, mucosal swelling, sticky mucous, and enlarged folds in endoscopic images.[25–30] The degree of endoscopically evident gastric mucosal atrophy and presence of suspected post-eradication findings in each study subject were simultaneously reviewed and determined by the same 3 licensed endoscopists.

Statistical analyses were performed using chi-squared, Kruskal-Wallis, and Mann-Whitney *U* tests. All calculations were done using the Stat View 5.0 software program (Abacus Concepts Inc., Berkeley, CA) for Macintosh and differences of $p<0.05$ were considered to be statistically significant.

This study was performed in accordance with the Declaration of Helsinki, and the protocol was approved by the ethics committee of the Shimane Environment and Health Public Corporation. Written informed consent indicating that clinical data would be used for a clinical study without release of individual information was obtained from all subjects before performing the medical checkup examinations.

Results

We found that 468 subjects were positive and 391 were negative for the anti-*H. pylori* IgG antibody. Furthermore, the positive rates for the anti-*H. pylori* IgG antibody in subjects with and without gastric mucosal atrophy were 85.6% and 0.9%, respectively (Table 1). The characteristics of our subjects without as well as with several degrees of gastric mucosal atrophy are shown in Table 2. Cases with higher grades of gastric mucosal atrophy were older as compared to those with lower grades or no atrophy. When the positive rate and serum titer of the anti-*H. pylori* IgG antibody were analyzed as variables, the subjects with C2 and O3 of gastric mucosal atrophy had a low positive rate. In addition, the serum titer was low in subjects with C1, C2 and O3 gastric mucosal atrophy, and the number of cases with serum titer of ≥40.0 U/ml was relatively small in these subjects. The serum titers of all cases without gastric mucosal atrophy (C0) were less than 10 U/ml (Table 2).

When the positive rate and serum titer of the anti-*H. pylori* IgG antibody were analyzed separately in male and female subjects, male subjects with O3 gastric mucosal atrophy and female subjects with C2 gastric mucosal atrophy had a low positive rate. In addition, low serum titer was observed in both males and females with mild gastric mucosal atrophy. When endoscopic post-eradication findings were investigated in 78 cases with gastric mucosal atrophy and negative result in the anti-*H. pylori* IgG antibody test, 52 cases had suspected post-eradication findings endoscopically. In 24 among these 52 cases, previous other diagnostic methods in our institute or other medical centers also showed negative results for *H. pylori* infection by their medical records. Interestingly, suspected post-eradication findings were more frequently observed in both females and males with C2 gastric mucosal atrophy (Table 3).

Discussion

In this study, we investigated the factors causing a negative result in the SphereLight *H. pylori* antibody J test in cases with gastric mucosal atrophy. Continuous *H. pylori* infection is a main cause of gastric mucosal atrophy, and nearly all Japanese individuals with gastric mucosal atrophy and without a past history of *H. pylori* eradication therapy are considered to be infected.[23,24] However, 78 of the present 543 study subjects with

Table 1. Results of serum anti-*H. pylori* IgG test and presence of gastric mucosal atrophy

	Serum anti-*H. pylori* IgG test	
	Positive	Negative
Cases with gastric mucosal atrophy	465	78
Cases without gastric mucosal atrophy	3	313

Data are expressed as number of cases.

Table 2. Results of serum anti-*H. pylori* IgG test and degree of gastric mucosal atrophy

Gastric mucosal atrophy	C0	C1	C2	C3	O1	O2	O3
Number of subjects	316	27	162	139	93	75	47
Gender (male/female)	196/120	19/8	101/61	80/59	58/35	59/16	32/15
Age in years (mean ± SE)	49.2 ± 0.5	49.3 ± 1.9	51.8 ± 0.7	52.8 ± 0.7	55.4 ± 0.9	57.4 ± 1.0	62.4 ± 1.3
Positive of serum anti-*H. pylori* IgG test (%)	3 (1.0)	25 (92.6)	127 (78.4)	127 (91.4)	81 (87.1)	68 (90.7)	37 (78.7)
Titer of serum anti-*H. pylori* IgG test (U/ml) (mean ± SE)	0.8 ± 0.0	16.0 ± 3.3	23.4 ± 2.1	35.5 ± 4.7	32.4 ± 4.2	31.8 ± 3.5	26.3 ± 5.8
Distribution of titer of serum anti-*H. pylori* IgG test							
Number of subjects with titer of 0.0~0.9 U/ml (%)	226 (71.5)	1 (3.7)	9 (5.6)	6 (4.3)	4 (4.3)	0	2 (4.3)
Number of subjects with titer of 1.0~1.9 U/ml (%)	53 (16.8)	0	9 (5.6)	2 (1.4)	1 (1.1)	2 (2.7)	5 (10.6)
Number of subjects with titer of 2.0~2.9 U/ml (%)	26 (8.2)	0	7 (4.3)	4 (2.9)	4 (4.3)	3 (4.0)	2 (4.3)
Number of subjects with titer of 3.0~3.9 U/ml (%)	8 (2.5)	1 (3.7)	10 (6.1)	0	3(3.2)	2 (2.7)	1 (2.1)
Number of subjects with titer of 4.0~9.9 U/ml (%)	3 (0.9)	10 (37.0)	35 (21.6)	21 (15.1)	20 (21.5)	15 (20.0)	12 (25.5)
Number of subjects with titer of 10.0~39.9 U/ml (%)	0	13 (48.1)	64 (39.5)	72 (51.8)	34 (36.6)	32 (42.7)	16 (34.0)
Number of subjects with titer of ≥40.0 U/ml (%)	0	2 (7.4)	28 (17.3)	34 (24.5)	27 (29.0)	21 (28.0)	9 (19.1)

The degree of gastric mucosal atrophy was endoscopically evaluated using the classification of Kimura and Takemoto. There is significant difference in the distribution of titer of serum anti-*H. pylori* IgG test among the subjects with different degrees of gastric mucosal atrophy (C1~O3).

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Table 3. Gender and the results of serum anti-*H. pylori* IgG test

Gastric mucosal atrophy	C1	C2	C3	O1	O2	O3
Male subjects (number of subjects)	19	101	80	58	59	32
Age in years (mean ± SE)	50.3 ± 2.1	51.4 ± 0.9	53.4 ± 1.0	55.2 ± 1.2	57.7 ± 1.0$^{\#2,3}$	61.8 ± 1.4$^{\#1-5}$
Positive of serum anti-*H. pylori* IgG test (%)	18 (94.7)	86 (85.1)*	72 (90.0)	51 (87.3)	52 (88.1)	23 (71.9)$^{\#1,3}$
Titer of serum anti-*H. pylori* IgG test (U/ml) (mean ± SE)	14.8 ± 3.0	25.9 ± 2.7*	39.7 ± 7.7$^{\#2}$	37.8 ± 6.1$^{\#2}$	32.5 ± 4.1	24.8 ± 5.5
Cases with suspected post-eradication findings† (%)	0	12 (11.9)*	5 (6.3)	6 (10.3)	3 (5.1)	4 (12.5)
Female subjects (number of subjects)	8	61	59	35	16	15
Age in years (mean ± SE)	46.8 ± 4.7	52.5 ± 1.2	52.1 ± 1.0	55.6 ± 1.5$^{\#1,2}$	56.4 ± 2.5$^{\#1}$	63.9 ± 2.7$^{\#1-5}$
Positive of serum anti-*H. pylori* IgG test (%)	7 (87.5)	41 (67.2)*	55 (93.2)$^{\#2}$	30 (85.7)$^{\#2}$	16 (100)$^{\#2}$	14 (93.3)$^{\#2}$
Titer of serum anti-*H. pylori* IgG test (U/ml) (mean ± SE)	18.8 ± 8.8	19.1 ± 3.3*	29.7 ± 3.7$^{\#2}$	23.4 ± 4.5	29.4 ± 6.2$^{\#2}$	29.3 ± 14.0
Cases with suspected post-eradication findings† (%)	0	17 (27.9)*	2 (3.4)$^{\#2}$	3 (8.6)$^{\#2}$	0$^{\#2}$	0$^{\#2}$

The degree of gastric mucosal atrophy was endoscopically evaluated using the classification of Kimura and Takemoto. †Suspected post-eradication findings: presence of map-like redness or patchy redness, and absence of diffuse redness, mucosal swelling, sticky mucous, and enlarged folds. *Significant difference between males and females. $^{\#1,2,3,4,5}$Significant difference vs C1, C2, C3, O1, O2 gastric mucosal atrophy, respectively.

evidence of gastric mucosal atrophy were not positive in results of anti-*H. pylori* IgG antibody testing of their serum. Male subjects with O3 grade of gastric mucosal atrophy showed a lower positive rate in antibody test, and subjects with with C2 gastric mucosal atrophy showed a low positive rate and titer of the antibody in this study. There are several possibilities to explain why our subjects with gastric mucosal atrophy had negative results in the anti-*H. pylori* IgG antibody test, including the antigens used to produce the anti-*H. pylori* IgG antibody test kit did not match those possessed by the subjects. However, the kit employed for this study was produced using antigens from *H. pylori* strains derived from Japanese patients and its good accuracy has been demonstrated.[21,22] Low serum titer of the anti-*H. pylori* IgG antibody easily induces to a negative result in an anti-*H. pylori* IgG antibody test, although the cut off value for the SphereLight *H. pylori* antibody J test is set at 4.0 U/ml to increase sensitivity for diagnosis of *H. pylori* infection. The disappearance of *H. pylori* in the stomach is well known to occur due to intestinal metaplasia after long-term infection, while several investigators have also reported that a lower serum titer of the antibody is correlated with the progression of gastric mucosal atrophy.[32–35] Indeed, a relatively low positive rate in antibody test was observed in subjects with O3 gastric mucosal atrophy in the present study, especially in male. On the other hand, we could not clearly explain the lower positive rate and titer of the antibody in subjects with mild gastric mucosal atrophy. The titer of the antibody has been shown to vary based on the duration of exposure to *H. pylori*, the grade of histological gastritis and the density of *H. pylori*.[9,15–20] A majority of our study subjects were middle-aged, and the subjects with *H. pylori* infection are considered to have long exposure duration to *H. pylori*, since *H. pylori* infection generally occurs during childhood.[23,36] Therefore, the lower titer of the antibody in subjects with mild gastric mucosal atrophy may be caused by the low grade immune response to *H. pylori*, low grade of histological gastritis and low density of *H. pylori*. In addition, unplanned natural eradication is considered to correlate with low positive rate and titer of the antibody in subjects with C2 gastric mucosal atrophy, since suspected post-eradication was more frequently observed in cases with C2 atrophy. When serum antibody test is negative in middle-aged cases with mild gastric mucosal atrophy in clinical practice, we should carefully examine the presence of *H. pylori* infection by other diagnostic methods, since low titer of antibody test could cause the negative results. In addition, the possibility of unplanned eradication should be considered in these cases.

Our study has several limitations. We only utilized one type of serum anti-*H. pylori* IgG antibody test to evaluate the status of *H. pylori* infection and did not employ other diagnostic methods, as the study was a retrospective examination of individuals who visited a medical center for a detailed medical checkup. In addition, a majority of our subjects were socially active, productive, and socioeconomically middle class, thus young and elderly individuals were relatively few. Additional large-scale investigations employing other anti-*H. pylori* IgG antibody tests are needed to clarify the present observations, including our findings that subjects, especially females, with a mild degree of gastric mucosal atrophy had a low positive rate and serum titer in the anti-*H. pylori* IgG antibody test.

In summary, we investigated the factors causing a negative result in anti-*H. pylori* IgG antibody testing in subjects with evidence of gastric mucosal atrophy. We found that the middle-aged subjects with a mild degree of gastric mucosal atrophy had a low positive rate and titer in serum, and endoscopic suspected post-eradication findings was more frequently observed in these cases.

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Conflict of Interest

No potential conflicts of interest were disclosed.

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