Original Article

Correlation of Serology with Morphological Changes in Gastric Biopsy in *Helicobacter Pylori* Infection and Evaluation of Immunohistochemistry for *H. Pylori* Identification

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ABSTRACT

Background/Aim: *Helicobacter pylori* is implicated in various gastroduodenal diseases and many tests are available for its detection. The present study attempted to document the morphological changes in the gastric mucosa induced by *H. pylori* colonization and correlate them with the severity of the infection. The study also compared various diagnostic tests and evaluated the different staining methods used for *H. pylori* detection, especially immunohistochemical identification. **Patients and Methods:** One hundred and two patients with dyspepsia were included. Enzyme-linked immunosorbent assay (ELISA) for *H. pylori*-specific immunoglobulin G (IgG), immunoglobulin A (IgA), and immunoglobulin M (IgM) was used. Rapid urease test was performed on endoscopic biopsy and it was stained with hematoxylin and eosin (H and E), modified Giemsa, and immunohistochemical stains. **Results:** A significant correlation was found between the density of *H. pylori* and severity of gastritis. A significant correlation was observed between serology (especially when used in combination, IgG and IgA) and status of *H. pylori*. Immunohistochemical staining enhanced the diagnostic yield of *H. pylori* detection. **Conclusions:** Immunohistochemistry (IHC) should be used judiciously, whereas simple and economical tests like modified Giemsa should be used routinely for the detection of *H. pylori*. Combined ELISA (IgG and IgA) should be preferred over single ELISA. Simultaneous morphological and serological detection of *H. pylori* is preferable as *H. pylori* may not be detected on morphology alone due to its patchy distribution in the stomach.

Key Words: *Helicobacter pylori*, immunohistochemistry, serology

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Helicobacter pylori is a spiral Gram negative bacterium which was discovered by Marshall and Warren in 1982.[1] Studies have indicated that the presence of *H. pylori* is associated with a variety of gastrointestinal diseases including gastritis, duodenal and gastric ulcers, nonulcer dyspepsia, and gastric adenocarcinoma and lymphoma.[2-4] The removal of the organism by antimicrobial therapy is correlated with the resolution of symptoms and cure of diseases.[5]

The tests available for the diagnosis of *H. pylori* can be broadly divided into two types: invasive and noninvasive. Noninvasive tests include serological diagnosis, urea breath test (UBT), and stool antigen test. *H. pylori*-specific antibodies have been detected in the serum, saliva, and urine.[6,7] Invasive tests require an endoscopic gastric biopsy specimen and include rapid urease test, histological examination, and culture of the biopsy.

H. pylori can be seen in routine hematoxylin and eosin (H and E) staining, but many newer staining methods have been devised for better visualization of *H. pylori*, including immunohistochemical stains.[8,9]

The present study attempted to document the morphological changes in the gastric mucosa induced by the colonization of *H. pylori* and correlate them with the severity of the infection. The study also compared various diagnostic tests and evaluated the different staining methods used

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for the detection of *H. pylori* especially in relation to immunohistochemical identification.

PATIENTS AND METHODS

The present study was conducted in the department of pathology, Lady Hardinge Medical College and associated hospitals over a period of two years. All patients above 18 years of age, presenting with symptoms of dyspepsia and requiring an upper gastrointestinal endoscopy were included, comprising a total of 102 patients. Patients who had received antibiotics, proton pump inhibitors, H_2 blockers within the past two months, or patients with a history of gastric resection/vagotomy, and those with complicated peptic ulcer disease were excluded. The study was approved by the institutional ethical board, and written informed consent was obtained from all patients.

A blood sample of all patients selected for endoscopy was taken and serum was stored at –20°C for *H. pylori* serology (ELISA for specific IgG, IgA, and IgM). Antibody index of each sample was calculated by dividing the optical density (OD) value of each sample by cutoff value. Antibody index < 0.9 indicates no detectable antibody, 1.1 implies borderline positive, and >1.1 indicates *H. pylori* infection. Endoscopic biopsies from antrum and corpus of stomach (2 biopsies) were performed in all patients. One biopsy was immediately subjected to a rapid urease test (Pronto Dry Kit). The rest were preserved in 10% buffered formalin to be used for histopathological examination. Routine H and E staining, modified Giemsa staining, and immunohistochemistry were performed on tissue sections in each case.

Histologic features such as gastric mucosal changes for any evidence of gastritis, and presence or absence of *H. pylori* and so on were studied on H and E-stained sections for all cases. These were also graded according to the updated Sydney system (1994) using the visual analog scale.[10] Tissue sections were stained with modified Giemsa, the method suggested by Gray *et al*.[11]

The tissue sections were also assessed for the presence of *H. pylori* infection by immunohistochemical staining using polyclonal anti-*H. pylori* antibody and polymer-HRP based (detection system). The slides were examined for the presence of *H. pylori* in the mucus and in the gastric pits and were also graded according to the following criteria: Grade 0 (0 bacteria/oil immersion field), Grade 1 (19 bacteria/oil immersion field), Grade 2 (20-29 bacteria/oil immersion field), Grade 3 (30-99 bacteria/oil immersion field) and Grade 4 ≥ 100 bacteria/oil immersion field).[12]

RESULTS

The study group comprised 102 patients with a mean age of 37.4 years (19-80 years) and male to female ratio of 1:1 approximately (52 males *vs.* 50 females). The most common symptom encountered was epigastric pain which was seen in 96% cases, followed by nausea, vomiting, or both.

Upper gastrointestinal biopsies were endoscopically normal in most of the cases (83%); 8% cases had mild hyperaemia of mucosa, 8% had mild antral gastritis, and 1% had severe antral gastritis.

For the purpose of analysis, a case was defined as positive for *H. pylori* if bacteria were seen on any of the following: H and E, modified Giemsa, and immunohistochemistry (IHC), of which IHC was taken as the gold standard. Of 102 cases, a total of 51 cases were positive for *H. pylori* on any one or more of the three tests. Of these, 37 had visible *H. pylori* on H and E, 41 had visible *H. pylori* on modified Giemsa, and all 51 were positive on IHC [Table 1].

Rapid urease test was positive in 70% cases. It showed a sensitivity of 74.5% and a positive predictive value of 54.3%.

Serum ELISA for *H. pylori* was positive in 68 patients. Out of these, IgG type was positive in 49 (72%), IgA in 56 (82.3%), and IgM in 25 (36.8%) cases. Serum ELISA for IgG antibodies against *H. pylori* correlated significantly ($P < 0.001$) with the presence of bacteria on histology (H and E, modified Giemsa, and IHC). Of 53 cases which were negative for IgG ELISA, only four showed *H. pylori*

Table 1: Comparison of modified Giemsa, H and E, and IHC

Test		*H. pylori*		Sensitivity (%)	Specificity (%)	PPV (%)	NPV (%)
		Positive	Negative				
H and E	Positive	37	0	72.5	100	100	78.5
	Negative	14	51				
Modified Giemsa	Positive	41	0	80.4	100	100	83.6
	Negative	10	50				
IHC	Positive	51	0	100	100	100	100
	Negative	0	51				

H and E: Hematoxylin and eosin, IHC; Immunohistochemistry, *H. pylori*: *Helicobacter pylori*, PPV: Positive predictive value, NPV: Negative predictive value

on H and E (false negatives)[Table 2]. The biopsy findings also correlate well with IgG status, as 43 (89.5%) of 48 cases with both gastritis and positive *H. pylori* had IgG antibodies to *H. pylori* in their serum. All the cases that did not have gastritis and which were negative for *H. pylori* had a negative ELISA for serum IgG antibodies to *H. pylori* ($P < 0.001$). IgG ELISA was found to have a sensitivity and specificity of 90.2% and 94.1%, respectively.

Serum ELISA for IgA antibodies also correlated significantly ($P < 0.001$) with H and E, modified Giemsa, and IHC. Of 44 cases which were negative for IgA ELISA, only two showed *H.pylori* on H and E (false negatives). Forty-five (93.75%) of 48 cases with both gastritis and positive *H. pylori* had IgA antibodies to *H.pylori* in their serum. Only five cases who did not have gastritis and who were negative for *H. pylori* had a positive ELISA for serum IgA antibodies to *H. pylori* ($P < 0.001$) [Table 3]. IgA ELISA was found to have a sensitivity and specificity of 94.1 and 84.3%, respectively. The present study found that the positive and negative predictive values of the combined IgG and IgA were higher than those of IgG or IgA alone, being 95.7 and 97.7%, respectively.

Serum ELISA for IgM antibodies against *H. pylori* did not correlate significantly with the presence of bacteria on H and E, modified Giemsa, and IHC. Of 25 patients positive by IgM serology, only 12 (48%) showed visible *H. pylori* on H and E.

Table 2: Comparison of *H. pylori* status with combined IgG and IgA serology

Serology	*H. pylori* status		Total
	Positive	Negative	
IgG + IgA+	44	2	46
IgG + IgA-	2	1	3
IgG – IgA+	4	6	10
IgG - Ig A-	1	42	43
Total	51	51	102

P<0.001; *H. pylori: Helicobacter pylori,* IgG: Immunoglobulin G, IgA: Immunoglobulin A

Chronic superficial gastritis was seen in 54 (53%) cases, of which 22 (40.7%) showed activity. Chronic atrophic gastritis was seen in nine (8.8%) cases. On scoring inflammation, acute inflammation was seen in 22 cases (19 mild and 3 moderate grade). However, this finding did not have a significant correlation with the presence of *H. pylori*, as only 12 cases (54.5%) of these 22 were positive for *H. pylori* ($P = 0.113$). Sixty-three cases showed chronic inflammation, of which 33 had mild, 24 had moderate, and six had marked chronic inflammation. Chronic inflammation score correlated significantly with *H. pylori* status ($P < 0.001$).

A total of 16 cases (15.7%) showed the presence of lymphoid follicles in addition to chronic inflammation. However, the presence of lymphoid follicles was not significantly correlated with the presence of *H. pylori* as only nine of these 16 patients were positive for *H. pylori* ($P = 0.393$). Intestinal metaplasia was seen in two cases (1.9%), both being mild. One case showed the presence of *H. pylori* with a positive serology. However, *H. pylori* were not seen overlying the metaplastic epithelium. The other case was serologically positive but did not show *H. pylori* on morphology. Glandular atrophy was seen in nine cases, of which eight were mild and one was moderate. Out of these nine cases, seven were positive on serology, whereas only five (55.5%) showed *H. pylori* on morphology.

H. pylori were seen in 37 cases on H and E [Figure 1], of which 29 showed mild (Grade 1), four showed moderate (Grade 2), and four showed marked (Grade 3) presence of *H. pylori*. H and E had a sensitivity and specificity of 72.5 and 100%, respectively. Modified Giemsa [Figure 2] was positive in 41 cases with a sensitivity and specificity of 80.4 and 100%, respectively. Modified Giemsa showed more concordance than H and E and rapid urease test with IHC.

Immunohistochemistry was positive in 51 cases, of which 42 cases had a grade of 1+. The remaining nine cases had grades between 2+ and 4+ [Figure 3]. Thus, IHC increased the diagnostic yield of modified Giemsa by a further 19.6% and of H and E by 27.5%, respectively. Comparison of modified Giemsa, H and E, and IHC are shown in Table 1.

Table 3: Correlation between biopsy findings and IgG and IgA status

Biopsy findings	No. of cases	IgG		IgA	
		Positive	Negative	Positive	Negative
Gastritis + *H. Pylori* +	48	43	5	45	3
Gastritis - *H. Pylori* -	36	0	36	5	31
Gastritis + *H. Pylori* -	15	3	12	3	12
Gastritis - *H. Pylori* +	3	3	0	3	0
Total	102	49	53	56	46

P<0.001; IgG: Immunoglobulin G, IgA: Immunoglobulin A

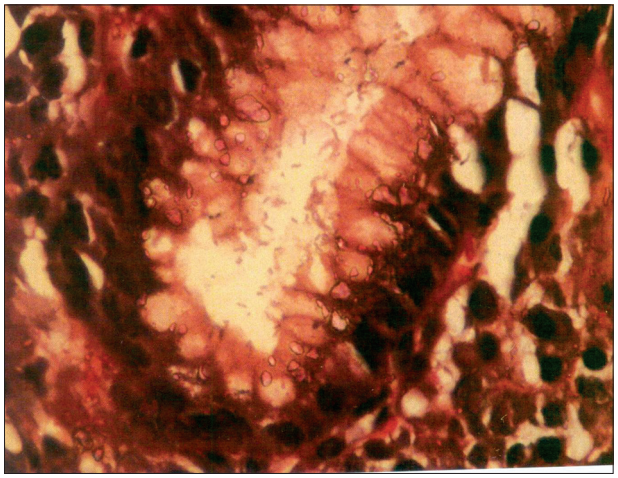

Figure 1: Photomicrograph showing numerous *Helicobacter pylori* within the gastric pit (H and E, 1000×)

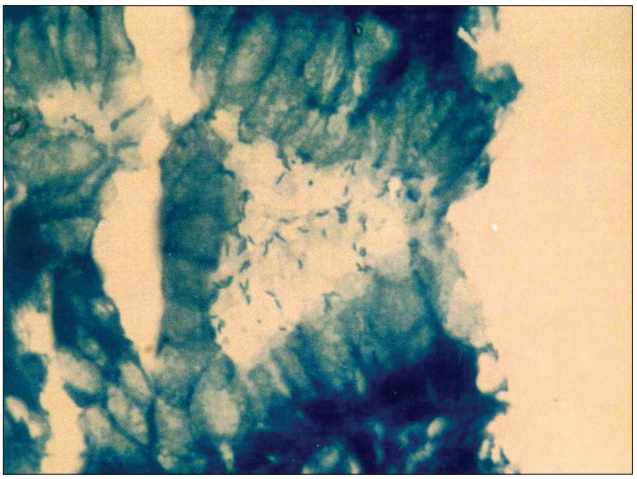

Figure 2: Photomicrograph showing numerous *Helicobacter pylori* within the gastric pit (modified Giemsa, 1000×)

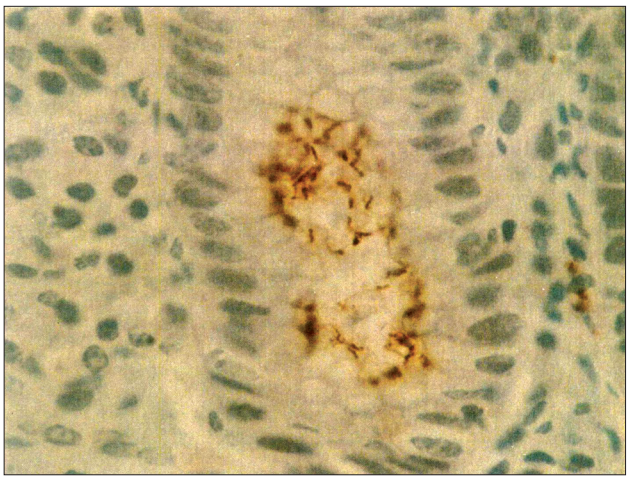

Figure 3: Photomicrograph showing *Helicobacter pylori* grade 3+ (IHC, 1000×)

DISCUSSION

H. pylori enjoys a worldwide distribution, though the prevalence strongly varies between developing and developed countries; it is more than 80 and 30%, respectively.[13] A recent report from India indicates that almost 80% of the population is infected with *H. pylori*.[14] Since its discovery, *H. pylori* has been implicated as a potential cause of nonulcer dyspepsia in a subset of patients.[15] Epidemiologic studies have clearly demonstrated a major etiologic role of *H. pylori* for several gastroduodenal diseases including gastric ulcer, duodenal ulcer, gastric MALT lymphoma (MALT: Mucosa-associated lymphotic tissue), and distal gastric cancer.[16]

Recently, we came across a few studies comparing the various diagnostic tests for *H. pylori*.

Peng *et al.*[17] reported that accuracy of capsule UBT was higher than conventional UBT and serology (98 vs. 93 and 88%, respectively). Similar accuracy for serological test was reported by Rahman *et al.*[18] Tzeng *et al.*[19] did not find any significant difference in sensitivity, specificity, and positive predictive value for the four diagnostic tests for *H. pylori*, namely, H and, Giemsa, rapid urease test, and imprint cytology ($P > 0.05$).

Very few studies[20,21] have correlated the serology of *H. pylori* with morphological changes and density of *H. pylori* on IHC with serology. In the present study, cases of nonulcer dyspepsia were taken and the morphological changes in the gastric mucosa induced by the colonization of *H. pylori* were documented and their correlation was done with anti-*H. pylori* serology and the severity of infection. The study also compared various diagnostic tests and evaluated the different staining methods used for *H. pylori* detection.

The rapid urease test was found to be of less value in diagnosing *H. pylori* infection in our study with a sensitivity of 74.5% which is comparable to the findings of Tokunaga *et al.*,[22] Malik *et al.*,[23] and Ceken *et al.*[24] On the contrary, Calvet *et al.*[25] and Redeen *et al.*[26] reported a much higher sensitivity of 94 and 90%, respectively. Moreover, Redeen *et al.*[26] recommended rapid urease test as the first choice as the result could be obtained within hours.

Siddique *et al.*[27] showed that increasing the number of gastric antral biopsies from one to four significantly improves the sensitivity of the CLO test (rapid urease test), eliminates sampling error, and hastens the time needed by the test to become positive for the diagnosis of *H. pylori* infection. About half of the patients (52%) had a positive CLO test in

group 1 (1 biopsy), compared to 68% in group 2 (2 biopsies), 76% in group 3 (3 biopsies), and 96% in group 4 (4 biopsies) (Group 1 vs. 4 $P < 0.01$).

We found the seroprevalence of *H. pylori* infection to be 66.7%, which was comparable to two studies by Kate *et al*.[28,29] Serum ELISA for IgG antibodies against *H. pylori* correlated significantly with the presence of bacteria on histology (H and E, modified Giemsa, and IHC) which is in accordance with Booth *et al*.[30] and Perez-Perez *et al*.[20] Hashemi *et al*.[31] studied the diagnostic accuracy of four different staining methods on touch cytology, and stated that rapid urease test should still be acknowledged as the primary test for diagnosing *H. pylori* following upper gastrointestinal endoscopy.

In the present study, the sensitivity, specificity, positive predictive values and negative predictive values of IgG ELISA and IgA ELISA were similar to Urita *et al*.[32] and Martin-de-Argila *et al*.[33] On the contrary, She *et al*.[34] found much lower values for sensitivity, specificity, and positive predictive values as they used stool antigen test as the gold standard. Lindsetmo *et al*.[35] also found a much lower specificity of anti-*H. pylori* IgG and IgA (32-50% among the peptic ulcer patients and 58-71% among the controls). The specificity of combined IgG and IgA ELISA (82.4%) in our study fell somewhere between the specificity found by Martin-de-Argila *et al*.[33] (85.3%) and De Wouw *et al*.[21] (79%). We found that 72.1% cases positive for *H. pylori* on serology had gastritis on morphology which is comparable to Perez-Perez *et al*.[20] and Booth *et al*.[30]

Although in adults, ELISA has proven to be highly accurate in diagnosing *H. pylori* infection, it has demonstrated variable accuracy in children. Leal *et al*.[36] conducted a systematic review and meta-analysis to assess the accuracy of antibody-based detection tests for the diagnosis of *H. pylori* infection in children. In-house ELISA with whole-cell antigen tests showed the highest overall performance: Sensitivity, 94% [95% confidence interval (CI): 90.2–96.7] and specificity, 96.4% (95% CI: 94.2–97.9), whereas ELISA commercial tests varied widely in performance (test for heterogeneity $P < 0.0001$). Me Graud[37] compared four diagnostic tests, that is, UBT, stool antigen test, and antibody detection in serum and urine in comparison with biopsy based tests in children and adolescents. The positive and negative predictive values for the serological tests were 76.4 and 98.3%, respectively, comparable to results in adults.

In the present study, there was a significant correlation between the severity of gastritis and the grade of *H. pylori* infection on IHC ($P < 0.001$) which is in accordance with several other authors.[12,20,22]

Acute inflammation was seen in 22 cases in our study. However, this finding did not have a significant correlation with the presence of *H. pylori* as only 12 cases (54.5%) were positive for *H. pylori* ($P = 0.113$). On the contrary, Perez-Perez *et al*.[20] and Shafii *et al*.[38] found a significantly higher activity in *H. pylori*-positive cases as compared to negative cases.

Although *H. pylori* can be visualized in H and Estained sections in most of the infected gastric biopsies, when the bacterial load is low, they can be missed. In such cases, other stains like modified Giemsa, Wright's, Warthin–Starry, and so on can be useful. Immunohistochemical detection of *H. pylori* in cases with very low density of infection has proved to be a very effective diagnostic modality in recent years. In our study, modified Giemsa fared better than H and E and was positive in 41 (80.4%) of 51 cases which were positive for *H. pylori*. Loffeld *et al*.[39] found Giemsa to be positive in 78% patients and IHC in 89% and recommended Giemsa staining as a routine detection method. Similar results were obtained by Tokunaga *et al*.[22]

We recommend that IHC should be judiciously used, and simple and economical tests like modified Giemsa should be used routinely for the detection of *H. pylori*. IHC should be used if there are no constraints of resources and it should be used only in cases where other staining modalities have failed to detect *H. pylori*. Combined ELISA (IgG and IgA) should be preferred over single ELISA for the detection of *H. pylori* infection. IgM ELISA was found to be of little diagnostic utility and it is recommended that the use of IgM serology can be avoided. Multiple biopsies should preferably be taken because of the highly patchy distribution of *H. pylori* due to which its presence can be underestimated. Simultaneous morphologic and serological detection of *H. pylori* is preferable as it may not be detected on morphology alone due to its patchy distribution in the stomach. Secondly, ELISA if done alone, may overestimate the presence of active *H. pylori* infection as antibody titers can remain elevated even after the eradication of *H. pylori*. Moreover, precancerous morphological changes associated with *H. pylori* infection may be missed if serology alone is performed.

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