Utility of immunohistochemistry in demonstrating *Helicobacter pylori*

**Abstract**

Background: *Helicobacter pylori* is the causative organism for chronic active gastritis, duodenal ulcer and also for malignancies like gastric adenocarcinoma and mucosa associated lymphoid tissue lymphoma. It is essential to mention the presence of *H. pylori* in gastric biopsies as it has an important role in patient care. Though there are several special stains to detect *H. pylori* in histological sections, their specificity and sensitivity vary greatly. Immunohistochemically *H. pylori* can be detected by using anti *H. pylori* antibody, which reacts with somatic antigens of the whole bacteria. The aim of this study was to compare the reliability of routine hematoxylin and eosin (H and E), Giemsa, Warthin–Starry (WS) silver stain and immunohistochemical technique in diagnosing *H. pylori*. **Materials and Methods:** In this retrospective 1-year (2009) study, endoscopic gastric biopsies taken from patients during gastrointestinal-endoscopy with histopathological diagnosis of gastritis were studied. Standard H and E staining was performed on 5-µm-sections from paraffin block of each specimen. Microscopic sections of biopsy specimens of patients showing features of gastritis histopathologically in routine H and E stain and where the presence of *H. pylori* was suspected were also stained with Giemsa, WS, and immunohistochemistry (IHC) using purified polyclonal *H. pylori* antiserum (BioGenex). We have not included gastric resection specimens in our study. **Results:** Of the 29 cases, 26 (32.9%) showed presence of *H. pylori* on H and E, Giemsa and WS stains, whereas 49 (62.0%) cases demonstrated *H. pylori* on IHC stain. **Conclusion:** We conclude that *H. pylori* detection by IHC has advantage over routine H and E staining. However, in the developing countries with financial constraints, routine H and E staining in combination with special staining are fairly reliable in demonstrating *H. pylori*.

**Key words:** Giemsa stain, *Helicobacter pylori*, hematoxylin and eosin stain, immunohistochemistry, Warthin–Starry stain

**INTRODUCTION**

*Helicobacter pylori* is the causative organism for chronic active gastritis, duodenal ulcer and malignancies like gastric adenocarcinoma and mucosa associated lymphoid tissue (MALT) lymphoma. It resides in the mucous layer of the gastric mucosa. It is essential to mention the presence of *H. pylori* in gastric biopsies as it has an important role in patient care. Due to the therapeutic implications, over the years pathologists have sought more reliable methods for detecting *H. pylori* in biopsy specimens, including immunohistochemistry (IHC), polymerase chain reaction (PCR) and more recently, in situ hybridization. Histopathological assessment of the antral biopsy specimens is an easy and cost-effective method for diagnosing *H. pylori* infection. Though there are several special stains to detect *H. pylori* in histological sections, their specificity and sensitivity vary greatly. Notable among these special stains are silver impregnated stains and modified Giemsa (MG) stain.

**MATERIALS AND METHODS**

In this retrospective 1-year (2009) study, endoscopic gastric biopsies taken from patients during gastrointestinal-endoscopy, with histopathological diagnosis of gastritis were studied.
The specimens were fixed in 10% formal saline for 24 h and then dehydrated in increasing concentrations of isopropyl alcohol followed by, clearing of alcohol by xylene before impregnating in paraffin wax. The specimens were subsequently embedded in paraffin wax in cassettes to facilitate tissue sectioning. Standard H and E staining was performed on 5-µm-sections from each specimen block. Histological sections of biopsy specimens of patients (formalin-fixed and paraffin-embedded), with histopathological evidence of gastritis or with the suspicion of presence of *H. pylori*, were also stained with the Giemsa, WS, and IHC using purified polyclonal *H. pylori* antiserum (BioGenex). Each biopsy section was carefully examined for the presence of *H. pylori*. The data obtained was given in the form of simple percentages. Statistics was done by using Chi-square method. We have not included gastric resection specimens in our study.

**RESULTS**

There were 79 cases of histopathologically diagnosed gastritis in the study period. There was slight male predominance in our study group with M: F ratio of 1.2:1. The mean age was 47.2 years. The gastric biopsies were classified according to Sydney classification[^7] [Table 1]. *H. pylori* was detected in 49 (62%) cases. Routine H and E and special stains like Giemsa and WS detected *H. pylori* in 26 (32.9%) cases [Figures 1-3]. Statistical analysis done by Chi-square test showed both special stains and immunostains to be comparable and independently good. Immunostaining detected additional 29 cases which were not detected initially by routine H and E or special stains [Figure 4]. Here, however very few bacilli were detected by immunohistochemical method. Immunostains were negative in six cases where *H. pylori* was suspected by routine methods. The sensitivity and specificity for special stains was 100% and 90% respectively. IHC showed 100% sensitivity and 51% specificity. Positive predictive value for special stains and IHC was 77% and 41%, whereas negative predictive value for both was 100% [Table 2].

**DISCUSSION**

*Helicobacter pylori* infection is common in the Indian subcontinent. Exposure occurs in childhood and approximately 80% of Indian adults have been infected at some point in time.[^8] In a study from South India, the authors have concluded that *H. pylori* infection is very common in the South Indian population. A high prevalence is seen in all gastroduodenal diseases and more than half the population without any abdominal symptoms was colonized by the *H. pylori*.[^9]

In this study, *H. pylori* was detected in 62% of gastritis cases.

*Helicobacter pylori* is a Gram-negative, spiral organism, which colonizes the gastric mucosa.[^10] *H. pylori* infection is associated with gastritis, gastric ulcer, gastric adenocarcinoma, and MALT lymphoma.[^1,2] Therefore, it is useful to document the presence of *H. pylori* in a gastric biopsy for giving appropriate patient care. *H. pylori* survives in the acidic medium of stomach by a number of mechanisms. It secretes the urease enzyme, which converts urea to ammonia. The production of ammonia neutralizes the acidity of the stomach, making the

![Figure 1: Hematoxylin and eosin stained section showing suspicious organisms over the mucosal layer (H and E, ×400)](image1)

![Figure 2: Modified Giemsa stain showing few bacilli (modified Giemsa, ×400)](image2)

### Table 1: Histological findings and *H. pylori* detection

<table>
<thead>
<tr>
<th>Histology</th>
<th>Number of case (n=79)</th>
<th><em>H. pylori</em> detection (n=49)</th>
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<tbody>
<tr>
<td>Mild antral superficial gastritis</td>
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*H. pylori*: Helicobacter pylori

### Table 2: Study results and statistics

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<th>Stain</th>
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<td>H and E, MG, WS</td>
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H and E: Hematoxylin and eosin, MG: Modified Giemsa, WS: Warthin-Starry, IHC: Immunohistochemical stain, PPV: Positive predictive value, NPV: Negative predictive value

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medium alkaline, this being more suitable for its survival. In addition, because of its helical shape the *H. pylori* can burrow into the mucus layer, which is less acidic than the lumen of the stomach. Besides these, *H. pylori* have also developed means of interfering with local immune responses, making them ineffective in eliminating the bacteria.\(^{[11,12]}\)

Several methods have been described for the detection of *H. pylori* including both noninvasive and invasive diagnostic tests such as serology, culture, rapid urease test,\(^{[13]}\) C-urea breath test, and histology based on endoscopy. Though many of these tests have advantages over histopathology as being noninvasive, more rapid, and less expensive, still histological detection of *H. pylori* in a gastric biopsy remains the most common and the most sensitive test.\(^{[5,13]}\)

*Helicobacter pylori* infection is widely diagnosed by means of histopathological examination. For this, apart from the routine H and E, several special staining methods like MG, WS, Gimenez, Genta, and immunohistochemical *H. pylori* antibody stains are used. Only few studies have investigated the sensitivity and specificity of the different staining methods.\(^{[5,13,14]}\)

However, several studies found that none of these stains is specific for *H. pylori* and they may be difficult to interpret because of the nonspecific staining of mucus, debris and water bath contaminants. The WS stain, which is considered to be the most sensitive, is technically demanding and is often not reproducible. Optimal interpretation of these stains requires careful examination of the sections at high magnification. More recently, IHC, in *situ* hybridization and the PCR have been proposed as alternative specific detection methods.\(^{[15]}\) IHC is considered as the “gold standard” for histology, being a highly sensitive and specific staining method.\(^{[9]}\)

The commonly used H and E slide review had a very good sensitivity and specificity with all levels of observers.\(^{[10]}\) Its advantages include its adequacy for the initial assessment of gastric biopsies in symptomatic upper gastrointestinal patients. This is because it is a well-tested, inexpensive and easy staining method, requiring a relatively short period of time to perform, with highly reproducible results. In addition, assessment of morphological changes accompanying *H. pylori* infection can be done simultaneously.\(^{[10]}\) However, some authors are of the opinion that test performance characteristics of H and E stain are inferior to Giemsa, Genta, or silver stains.\(^{[10]}\) According to Rotimi *et al.*, the MG stain is the method of choice because it is sensitive, easy to perform, and reproducible.\(^{[15]}\) Sometimes, however, these bacteria were masked in both H and E and Giemsa stained sections by inspissated mucus or by being positioned flat, in close approximation to the epithelial surface. But in immunostained preparations, the organisms including coccoid forms, become more prominent.\(^{[11]}\) In their study Rotimi *et al.* they have found that by using heating method for antigen retrieval rather than trypsin, the problem of excessive background staining of epithelium and mucus, seen in HIC stain can be overcome. According to them, immunoperoxidase method is easy to use, less demanding than WS staining, and that it produces reliable results, which are easy to interpret. Low numbers or even single organisms, often difficult to detect using traditional stains, are easily identified in immunostained sections.\(^{[1,13]}\)

In our study, we have used heating method for antigen retrieval. Immunostains detected a total of 49 cases, including additional 29 cases, which were not detected initially by routine H and E or special stains. IHC was positive even when the number of bacilli was very low. This may be the reason for *H. pylori* not being initially detected by means of routine H and E stain in these specimens. On the other hand, immunostains were negative in six cases where *H. pylori* was detected by routine methods.

Recently, the detection of *H. pylori* is declining as a result of use and misuse of antibiotics. As a result, often pathologists face a scenario, where they are expected to find *H. pylori* based on history and histological findings, but the organism remains elusive in routine as well as in special stains. In these cases, IHC may play a role in detecting the organism.\(^{[16]}\)

The dilemma faced by the pathologists is well-analyzed by the article “*H. pylori* - To Stain or Not to Stain?” by Smith *et al.*\(^{[17]}\) They
concluded that identifying the organism on immunostain was much easier and less time-consuming. However, this may not be feasible in the presence of financial constraints. Another aspect is that, in their study, there was no significant difference between the resident and faculty member in identifying the organisms stained by IHC method.[19] By using immunostains, the interobserver variation, as reported by few other studies also can be reduced.[20]

Another study by Hartman and Owens has compared the routine stains and IHC.[21] The authors have noted that the sensitivity of special stains in their study was 62% and that of IHC was 97-100%. In our study, the sensitivity of both special stains and IHC was 100%. The specificity for special stains in their study was around 97-98% and for IHC it was 100%. We had observed 90% specificity for special stains and 51% specificity for IHC.[21]

One more study, using culture as a standard, has reported sensitivity for *H. pylori* as 90.0 ± 10.0% with MG, 70.0 ± 14.1% with WS, and 83.8 ± 11.1% with IHC using purified polyclonal *H. pylori* antiserum (DAKO B471). Specificity reported was 53.8 ± 19.3%, 82.5 ± 9.6% and 90.0 ± 0.0%, respectively.[21] In our study, we have taken IHC as standard and culture was not done in the cases included in the study.

Pathologists also should be aware of other causes of gastritis that may mimic *H. pylori* infection, which include reactive gastropathy with focal activity, focally active gastritis and carditis, autoimmune gastritis, granulomatous gastritis, lymphocytic gastritis, and other infections.[18] In these conditions, the stains for *H. pylori* help to rule out other similar conditions.

In our study, the clinical presentation was variable and in many cases the clinicians requested for *H. pylori* identification. We have not included data about previous treatment received by the patients, while evaluating these staining methods. A further study taking into account various clinical parameters and the association of *H. pylori* will be definitely helpful.

We conclude that routine H and E with the help of special stains reliably help in the detection of *H. pylori*. However, *H. pylori*, by means of IHC is more easily detected compared with the conventional methods. We agree with Wang et al., that IHC should be used judiciously for example in case of unexplained gastritis or in previously treated patients with low dose of organism, particularly in developing countries.[18]

**ACKNOWLEDGMENTS**

The authors would like to thank Dr. Aparna Bitla for her invaluable help regarding statistical analysis, Dr. V. Suresh for editing the article and senior technicians Mrs. Ushanandini and Mr. Ramana for their help.

**REFERENCES**


*Source of Support: Nil. Conflict of Interest: None declared.*