Research Article

# Histological identification of *Helicobacter pylori*: comparison of staining methods

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## Abstract

**Introduction:** *Helicobacter pylori* has a high incidence world over and especially in developing countries. It causes many histological derangements apart from causing chronic gastritis. The study was done to compare Haematoxylin & Eosin (H&E) and Giemsa with Immunohistochemistry (IHC) for the detection of *Helicobacter pylori* and to correlate the *H. pylori* positivity with histological changes. **Methods:** This study was done in the Department of Pathology on gastric biopsies with clinical suspicion of *Helicobacter pylori* infection, received in the period between January 2013- December 2013. Sections were stained with H&E, Giemsa and IHC. Along with detection of the organism associated morphological changes were assessed. The stains were validated using IHC as gold standard. **Results:** A total of 58 samples were evaluated. Fourteen (24.1%) samples showed positivity with IHC, of which 11(19%) and 9(15.5%) were positive with H&E and Giemsa respectively. H&E had a high false positive rate (19%). Giemsa staining showed less sensitivity (64.28%) compared to H&E (78.57%). Morphological changes were assessed and organisms were noted in 14(25.5%) cases with inflammation, 8(30.8%) cases with activity and 2(28.6%) cases with atrophy. No organisms were seen associated with intestinal metaplasia. **Conclusion:** Giemsa was not found to be superior to H&E in detecting *Helicobacter pylori*. Use of IHC not only reduces rate of false positive results, but also diagnosis of any mild infection that is not detected on H&E can be made.

**Keywords:** Gastritis, *Helicobacter Pylori*, Haematoxylin & Eosin, Special Staining.

## Introduction

*Helicobacter pylori* is associated with chronic gastritis, ulcers and adenocarcinoma of stomach, [1, 2] more so in developing countries. Though *H. pylori* can be detected by invasive and non-invasive tests, [3, 4] histopathological evaluation is the gold standard. [4-6]. Detection in mucosal biopsy is done by staining with H&E, but is enhanced by use of histochemical and immunohistochemical stains [1, 7]. Many studies have found ancillary stains to be sufficient for diagnosis, [8-10] but Hartman and Owens [11] contradict this view and suggest the increased use of IHC for *H. pylori* [12, 13].

Therefore, the question of whether ancillary stains should be routinely used remains a subject of considerable debate.

Manuscript received: 14th Oct 2015
Reviewed: 19th Oct 2015
Author Corrected: 9th Nov 2015
Accepted for Publication: 18th Nov 2015

## Objectives

1. To compare Hematoxyline and Eosin (H &E) and Giemsa with immunohistochemistry for the detection of *H. pylori*.
2. To correlate the *H. pylori* positivity with histological changes.

## Materials and Methods

This study was conducted with a sample size of 58. In the present study, using published data of Tajalli et al., to get a relative precision of 13% and 95% confidence level in the result, the study required a minimum of 55 subjects.

Upper endoscopic biopsies received for dyspepsia and processed in the year 2013 were reviewed. All endoscopic biopsies which have been done for chronic upper abdominal symptoms such as abdominal pain, dyspepsia, heartburn, nausea and vomiting due to suspected *H. pylori* infection were included in the study. Inadequate, autolysed or necrotic tissues and those with extensive areas of haemorrhage were excluded.

Paraffin embedded blocks, of endoscopic biopsy, were retrieved from archives. 3 μm thick sections were made and mounted on slides for histochemistry and IHC. Sections were stained with H&E, Giemsa and IHC by standard procedures. Giemsa stain was used in 1:9 dilutions. IHC was performed using Primary Antibody HPYLORI-L-CE-S 0.1ml NCL-L-H pylori and Detection Kit RE7290-CE Novolink MinPolymer DS.

The H&E stained slides were reviewed by two pathologists.

**Detection of *H. pylori* and pathology**

1. Patients with positive result in immunohistochemistry were considered to be *H. pylori* positive.
2. Semi-quantitative method of scoring according to the Updated Sydney Classification System [14] was undertaken. The histopathological variables namely, *H. pylori* density and inflammation, were graded as absent, mild, moderate and severe with a scoring of 1 to 4 respectively. Other histopathological features (activity, atrophy and intestinal metaplasia) were not graded, but assessed in case of their presence or absence. *H. pylori* colonization was assessed and graded after careful search for focal or complete involvement of the gastric surface
3. The biopsy cases were analysed in an attempt to assess the major histopathological features of gastritis. The degree of inflammatory activity was investigated for involvement according to the density of neutrophils in gastric mucosal crypts, from one to all crypts. The presence of mononuclear infiltration was investigated. The degree of intestinal metaplasia was assessed according to the amount of glandular tissue replaced by intestinal type epithelium. Mucosal atrophy was defined as a loss of specialized gastric glands in mucosa, partly replaced by intestinal metaplastic epithelium. It was characterized by architectural changes manifested by variation in the volume and irregularity in the shape, branching, and spacing of the glands.

**Statistical Tests done:** The statistics of *H. pylori* positivity with H&E and Giemsa have been analysed and presented in terms of percentage. Validity of H&E and Giemsa in detection of *H. pylori* was done using sensitivity, specificity, positive and negative predictive values by comparing with IHC as gold standard. Analysis was carried out using SPSS Version 17 software. Inter-observer variation was calculated using Kappa statistic.

## Results

In accordance with the updated Sydney system, the cases showing chronic gastritis were graded into mild 33 (56.9%), moderate 20 (34.5%) and severe inflammation 2 (3.4%). No inflammation was seen in the remaining 3 (5.2%) cases. Of the 58 cases 26 (44.8%) cases showed activity, glandular atrophy was noted in 7 (12.1%) cases and intestinal metaplasia in 12 (20.7%) cases.

With H&E (fig.1) *H. pylori* organisms were detected in 22 (38%) cases. 11 (19%) cases were found to be false positive and 3 (5.2%) cases were false negative. The sensitivity and specificity were 78.57% and 75% respectively. The positive and negative predictive values were 50% and 91.67% respectively with 75.9% accuracy. Statistically no significant difference was noted between H&E and IHC stains (p=0.057).

With Giemsa (fig 2) *H. pylori* organisms were detected in 13 (22.4%) cases. 4 (6.9%) cases were found to be false positive and 5 (8.6%) cases were false negative. The sensitivity and specificity were 64.28% and 90.9% respectively. The positive and negative predictive values were 69.23% and 88.8% respectively with 84.5% accuracy. However, statistically no significant difference was noted between Giemsa and IHC stains (p=1.000).

Of the 58 cases studied, H&E stained sections revealed mild colonization in 15 (25.9%), moderate colonization in 4 (6.9%) and severe colonization in 3 (5.2%). No colonization was detected in the remaining 36 (62.1%) cases. On correlating with IHC positivity it was seen that H&E detected all cases of severe colonization. However, there was disparity noted in the detection of mild and moderate cases of colonization. IHC confirmed the presence of the organism in 6 (10.3%) cases of mild colonization and 2 (3.45%) cases of moderate colonization, while H&E detected *H. pylori* in 15 cases and 4 cases respectively.

**Table 1: Pattern of gastritis of the studies cases**

| **Lesion** | **No. (%)** |
|---|---|
| Inflammation | |
| Absent | 3 (5.2) |
| Mild | 33 (56.9) |
| Moderate | 20 (34.5) |
| Severe | 2 (3.4) |
| Activity | 26 (44.8) |
| Glandular Atrophy | 7 (12.1) |
| Intestinal Metaplasia | 12 (20.7) |

**Table 2: Comparison of Results of H&E and Gold Standard IHC**

| | | **IHC** | | **Total (%)** |
|---|---|---|---|---|
| | | **+ (%)** | **- (%)** | |
| **H&E** | **+ (%)** | 11 (19) | 11 (19) | **22 (38)** |
| | **- (%)** | 3(5.2) | 33 (56.8) | **36 (62)** |
| **Total (%)** | | **14 (24.1)** | **44 (75.9)** | **58** |

**Table 3: Comparison of Results of Giemsa and Gold Standard IHC**

| | | **IHC** | | **Total (%)** |
|---|---|---|---|---|
| | | **+ (%)** | **- (%)** | |
| **Giemsa** | **+ (%)** | 9 (15.5) | 4 (6.9) | **13 (22.4)** |
| | **- (%)** | 5 (8.6) | 40 (68.9) | **45 (77.5)** |
| **Total (%)** | | **14 (24.1)** | **44 (75.9)** | **58** |

**Table 4: Validity of H&E and Giemsa**

| | **Sensitivity** | **Specificity** | **PPV** | **NPV** |
|---|---|---|---|---|
| **H&E** | 78.57% | 75% | 50% | 91.67% |
| **Giemsa** | 64.28% | 90.9% | 69.23% | 88.8% |

**Table 5: Comparison of degree of colonization in H&E and IHC**

| **Bacterial Colonization** | **H&E No. (%)** | **IHC** | |
|---|---|---|---|
| | | ***H. pylori* + No. (%)** | ***H. pylori* –No. (%)** |
| Mild | 15 (25.9) | 6 (10.3) | 9 (15.5) |
| Moderate | 4 (6.9) | 2 (3.45) | 2 (3.45) |
| Marked | 3 (5.2) | 3 (5.2) | 0 (0.0) |
| **Total** | **22 (37.9)** | **11 (18.95)** | **11 (18.95)** |

**Table 6: Comparison of degree of inflammation with presence of *H. pylori***

| **Degree of Inflammation (n)** | ***H. pylori* (IHC)** | |
|---|---|---|
| | **+ No. (%)** | **- No. (%)** |
| Mild (33) | 9 (27.3) | 24 (72.7) |
| Moderate (20) | 4 (20) | 16 (80) |
| Marked (2) | 1 (50) | 1(50) |
| **Total (55)** | **14 (25.5)** | **41 (74.5)** |

**Table 7: Colonization of *H. pylori* by parameters of gastritis**

| Pattern of Gastritis (n) | *H. pylori* | |
|---|---|---|
| | + No. (%) | - No. (%) |
| Activity (26) | 8 (30.8) | 18 (69.2) |
| Atrophy (7) | 2 (28.6) | 5 (71.4) |
| Metaplasia (12) | 0 (0.0) | 12 (100.0) |

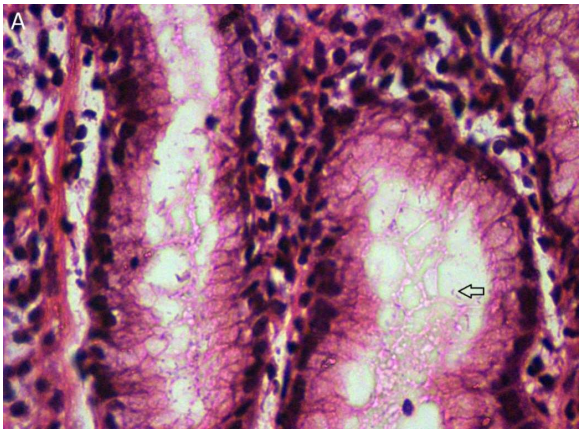


**Fig 1:** Microscopic appearance of *H. pylori* as seen with H&E staining

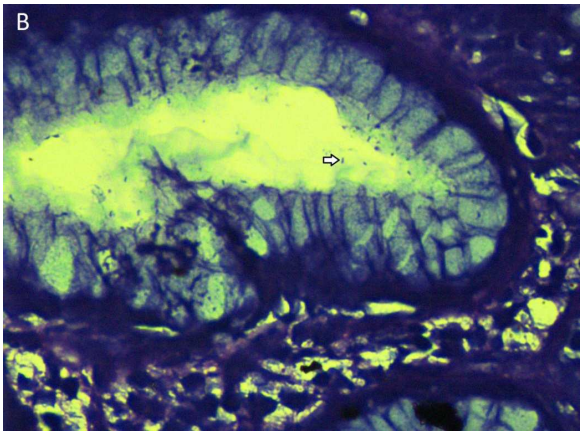


**Fig 2:** Microscopic appearance of H. pylori as seen with Giemsa staining

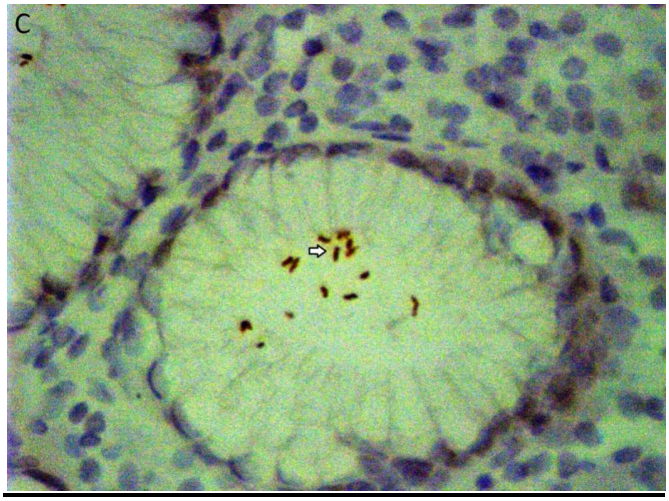


**Fig 3:** Microscopic appearance of *H. pylori* as seen with IHC staining

Most of the cases studied showed signs of inflammation. On correlating the grade of inflammation and *H. pylori* positivity with IHC the results obtained were such. The organism was found in 9 (27.3%) cases of mild inflammation, 4 (20%) cases of moderate inflammation and 1 (50%) case of severe inflammation.

*H. pylori* colonization, with IHC, was also found in cases showing activity and glandular atrophy. It was seen in 8 (30.8%) cases of activity, 2 (28.6%) cases of glandular atrophy. The organism however, was not seen to be associated with intestinal metaplasia. On the other hand, of the 14 IHC positive cases, 8 (57.1%) cases showed activity while no activity was noted in the remaining 6 (42.9%) cases. The inter-observer variation kappa (k) was found to be 0.47 which suggests moderate agreement.

## Discussion

The study was started with the aim of comparing, the easily available and widely used staining methods H&E and Giemsa against the gold standard IHC among patients with clinical suspicion of *H. pylori* infection.

The study showed sensitivity of 78.57%, specificity of 75% and a false positive rate of 19% for the routine H&E staining method. The low sensitivity was also noted by Shukla et al [6] and Intisar et al [13] in their studies. It was suggested that the low sensitivity and high false positive rate was due to the lack of contrast between the organism and the surrounding gastric mucosa. [13] This could also be because of the confusion which arises due to gastric secretions or eosinophilic debris seen in H&E stained sections.

Giemsa staining showed low sensitivity (64.28%) but a high specificity (90.9%). Similar results were obtained in the study by Laine et al. [15]; while the study by H. R. Wabinga [9] showed higher sensitivity (85%). In our study, Giemsa stained sections revealed organisms in 13 (22.4%) cases compared to the 22 (38%) cases detected by H&E. Giemsa had a lesser rate of false positives (6.9%) than H&E. In the present study Giemsa showed higher specificity than H&E which was also noted by other studies [3, 6, 9]. The low sensitivity, also noted by Tajalli et al. [3], makes it less reliable for detection of *H. pylori* infection. The low sensitivity could be due to the fact that the bacilli adherent to the glandular epithelium, especially in mild degree of colonization could be missed as both appear blue on Giemsa staining.

In this present study the association of *H. pylori* with inflammation (mild: 27.3%, moderate: 20%, severe: 50%) [Table 5] was not in accordance with the association seen in the study conducted in Africa (mild: 55%, moderate: 63.2%, severe: 42.9%) [9]; or that conducted in Duhok, Iraq (mild: 14%, moderate: 72.6%, severe: 36.8%). [13] Activity was seen to be associated with *H. pylori* positivity in 30.8% of cases. Also 57.1% of *H. pylori* positive cases showed evidence of activity. Such significant association between activity and presence of *H. pylori* infection was also noted in study by Toulaymat M et al. [5] Studies have suggested activity to be almost always associated with *H. pylori* infection. [16, 17] In our study the organism was seen to be associated most, with severe inflammation. Hence, the presence of severe form of inflammation and activity should prompt a careful scrutiny of the sections for the presence of the organisms.

*H. pylori* infection is an important risk factor for the development of gastric atrophy and intestinal metaplasia. [18] In this study [Table 7] glandular atrophy was noted in 28.6% of cases with *H. pylori* colonization. The association between these parameters has been shown in other studies. In the study conducted by Toulaymat et al [5] it was 69.4% and it was found to be 48.3% and in the study done by Intisar et al [13]. This variation may be explained by the finding of another study which showed a poor inter-observer variation value (k=0.31) for the evaluation of atrophy [19]. In contrast to the findings of Toulaymat et al [5] and Shukla et al [6] of finding intestinal metaplasia in *H. pylori* positive cases, no such association could be established by our study. It is known for the organisms to be absent in the region of metaplasia and must be looked for in other parts of the section.

The study also considered the inter-observer variation between two observers for H&E stained sections which was found to be k=0.47, suggesting moderate agreement. Study by Aydin et al [19] also suggested moderate agreement with k=0.56. The organism is known to have a patchy distribution, especially in mild infection. Therefore this variation may be reduced by screening multiple sections for the organism.

## Conclusion

Giemsa staining showed a lower sensitivity compared to H&E. However, Giemsa staining showed a high specificity and lower false positive rate than H&E. Therefore, Giemsa may be used complementary to H&E. Use of IHC further reduces the rate of false positive results. There is a strong association between activity and the presence of organisms, especially with mild infections. Considering this and the high cost of IHC, IHC can be restricted to cases showing activity, cases without clear diagnosis with ancillary stains and those cases with a strong clinical suspicion.

**Acknowledgements:**
Grants from:
- Indian Council of Medical Research
- Medical Education Research Trust
- M S Ramaiah Medical College

**Samples:** Dr. Umesh Jalihal, Professor and HOD, Dept. of Gastroenterology, M S Ramaiah Hospitals.

**Statistical Analysis**: Mr. Shivraj, Ms. Radhika, M S Ramaiah Medical College

**Funding:** Nil,**Conflict of interest:** None.
**Permission of IRB:** Yes

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**How to cite this article?**

Pranjal Sharma R, Kalpana Kumari M.K. Histological identification of *Helicobacter pylori*: comparison of staining methods. *Int J Med Res Rev* 2015;3(10):1167-1173. doi: 10.17511/ijmrr.2015.i10.211.

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