

Histomorphological study of gastric mucosal biopsy in dyspeptic patients and its association with *Helicobacter pylori* infection

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ABSTRACT

Background: The rationale of the study was to evaluate the morphological changes of chronic gastritis and its association with *Helicobacter pylori* infection and the efficacy of hematoxylin and eosin (H & E) and Giemsa techniques in a limited set up which helps in providing the vital data for subsequent treatment regimen. **Materials and Methods:** In this prospective study, 50 endoscopic biopsies were obtained from dyspeptic patients from January 2016 to June 2017. The samples were fixed in 10% buffered formalin and histopathological slides were prepared using paraffin blocks and subsequently stained with routine H & E and special stain with Giemsa. **Results:** There was a male preponderance in the study with a ratio of 2.3:1; mean age of patients was 37.59 years. Of the 50 patients, 47 patients (94%) had chronic gastritis. Twenty-one out of 47 samples showed positivity for *H. pylori* with routine H & E staining, which was confirmed with modified Giemsa staining method. **Conclusion:** Our study highlights the association of *H. pylori* in patients with functional dyspepsia and demonstrate the importance of cost effective Giemsa staining which can be practically applicable in Indian set-up for the detection of *H. pylori*.

Key words: Dyspepsia, Endoscopy, Giemsa, *Helicobacter pylori*, Immunohistochemistry

Dyspepsia refers to the constellation of upper gastrointestinal symptoms and it is one of the most common reasons for referral to the gastrointestinal unit [1]. Pathological lesions associated with *Helicobacter pylori* infection are chronic gastritis, atrophic gastritis, peptic ulcer disease, lymphocytic gastritis, intestinal metaplasia, gastric MALT lymphomas, and gastric adenocarcinoma [2]. Various technologies are used for the detection of *H. pylori* infection includes both invasive and non-invasive tests such as serology, stool antigen tests, 13C-urea breath test, culture, rapid urease test, histology, and PCR. Endoscopy is the most appropriate investigation to detect the pathological lesions and the presence of *H. pylori* in the alimentary tract so as to ensure that the patient receives appropriate treatment. With the advances in endoscopic techniques, biopsy can be easily obtained from the site intended with precision.

Biopsies are obtained using flexible fiber-optic endoscopes provide with an optical image or electronic video image which has aided in better diagnosis. *H. pylori* are found in the mucin covering the surface epithelium and within the foveolae, they could be detected by hematoxylin and eosin (H & E) stain when they are numerous but if they are sparse, special staining methods such as Giemsa, Genta, Warthin-Starry, triple stain, Alcian yellow, toluidine blue, and immunohistochemical antibody stain for *H. pylori* are advised. Immunohistochemistry is the gold standard for histology being a highly sensitive and specific staining method.

However, modified Giemsa stain can be the method of choice, because it is sensitive, cheap, easy to perform, and reproducible [3,4]. The present study was carried out with the objectives to study the morphological lesions associated with dyspepsia, to evaluate the common gastric pathological lesions associated with *H. pylori*, and to evaluate the efficacy of H & E and modified Giemsa technique in detection of *H. pylori* in the biopsy specimens.

MATERIALS AND METHODS

In the present study, a total of 50 endoscopic biopsies with clinical history of dyspepsia especially those with chronic gastritis obtained from January 2016 to June 2017 were evaluated. The study was approved by the Institutional Ethical Committee. Gastric biopsies from patients with clinical symptoms and signs of dyspepsia including epigastric pain/discomfort, bloating, anorexia, early satiety, belching, nausea, and heartburn were included in the study. Biopsies from patients on *H. pylori* eradication therapy and biopsy positive for malignancy in dyspeptic patients were excluded from the study.

Biopsy specimen was obtained from five sites in each case; two from antrum (one from distal lesser curvature and other from distal greater curvature), two from the corpus (one from the lesser curvature and other from the greater curvature), and one from the incisura angularis. The biopsies from the esophagus

and duodenum were not taken. The obtained specimens were fixed in 10% buffered formalin and histopathological slides were prepared using paraffin blocks. Routine H & E stain and modified Giemsa stain were used to study the specimens. The data obtained were analyzed using descriptive statistics including mean, percentage, Fisher's exact test, sensitivity, and specificity tests.

RESULTS

Out of 50 patients, 35 (70%) were male and 15 (30%) were female with M:F of 2.3:1. The age of the patients ranged from 21 to 70 years, with a mean age of 37.59 years. The most common chief complaint was pain in the abdomen (80%), followed by nausea and vomiting (72%), belching and epigastric discomfort (20%), and melena (4%). The most common endoscopic finding in dyspeptic patients was gastritis (60%), followed by gastritis with duodenitis (18%), gastritis associated with reflux (14%), esophagitis (4%), and hiatus hernia (4%). Most common biopsy site was antrum (80%) followed by corpus (12%) and both antrum and corpus (8%) (Table 1).

On histopathology, 47/50 specimens (94%) showed chronic gastritis with mild inflammation in 25 specimens (53.2%), moderate inflammation in 15 specimens (31.9%), and marked inflammation in 7 specimens (14.9%). Neutrophilic activity, atrophy of the mucosa, and lymphoid aggregates were observed. Out of 47 specimens, 21 specimens showed positivity with Giemsa stain (Fig. 1), whereas only 11 showed positivity with H and E stain. The grade of *H. pylori* colonization by Giemsa staining was graded 0–3; Grade 0 – absent, Grade 1 – one or few isolated group, Grade 2 – variable layer of *H. pylori* on the mucosal surface, and Grade 3 – more or less continuous. In our study, 13 specimens (61.9%) were Graded +1 and 8 specimens (38.1%) were Graded +2 (Table 2). There was highly significant association of chronic gastritis

with incidence of *H. pylori* using the Fisher's exact test ($p < 0.001$) (Table 3).

DISCUSSION

The present prospective study included 50 endoscopic gastric mucosal biopsies from dyspeptic patients. Endoscopic gastric mucosal biopsy provides information that cannot be obtained otherwise. Microscopic examination of the gastric biopsy gives, in addition to *H. pylori* status, information about the grade, extent, and topography of the gastritis and also atrophy-related changes in the gastric mucosa. The information obtained provides further possibilities for the assessment of risk and likelihood of various gastric disorders. In the study conducted

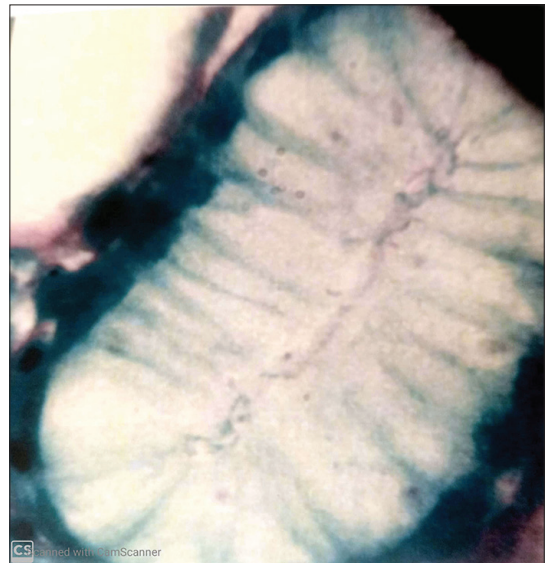


Figure 1: Grade 2 *Helicobacter pylori* – Giemsa stain ($\times 1000$)

Table 1: Clinical findings of the study population

Variables	No. of dyspeptic patients	Percentage
Symptoms		
Pain in abdomen	40	80
Nausea/vomiting	36	72
Belching/epigastric discomfort	10	20
Melena	2	4
Endoscopic finding		
Gastritis in patients	30	60
Gastritis with duodenitis	9	18
Gastritis associated with reflux	7	14
Esophagitis	2	4
Hiatus hernia	2	4
Site of biopsy		
Antrum	40	80
Corpus	6	12
Antrum and corpus	4	8

Table 2: Clinical findings of the study population

Histopathological features	No. of specimens	Percentage
Chronic inflammation		
Mild	25	53.2
Moderate	15	31.9
Marked	7	14.9
Neutrophilic activity	30	63.8
Lymphoid aggregate	15	31.9
Atrophy	1	2.1
<i>Helicobacter pylori</i> in Giemsa stain		
+1	13	61.9
+2	8	38.1

Table 3: Association of gastric biopsy findings with incidence of *Helicobacter pylori*

Gastric biopsy finding	<i>Helicobacter pylori</i> in Giemsa stain			p-value Fisher's exact test
	Present (%)	Absent (%)	Total (%)	
Gastritis	21 (44.7)	26 (55.3)	47 (100)	<0.001
Normal	0 (0)	3 (100)	3 (100)	
Total	21 (42)	29 (58)	50 (100)	

Table 4: Comparison of histopathological findings of chronic gastritis in various studies with the present study

Histopathological features	Suzana <i>et al.</i> (1999) [12] (%)	Alenezy and Hassan (2014) [10] (%)	Suzana <i>et al.</i> (2009) [13] (%)	Present study (%)
Mild inflammation	52.84	67.4	47.4	53.2
Moderate inflammation	40.46	16.80	40.9	31.9
Marked inflammation	6.68	15.80	11.7	14.9
Neutrophilic activity	42.47	10.53	73.38	63.8
Lymphoid aggregate	30	6.32	-	31.9
Atrophy	1.67	7.37	14.94	2.1
<i>Helicobacter pylori</i>	73.91	92	23.38	44.7
+1	-	-	22.2	61.9
+2	-	-	72.2	38.1
+3	-	-	5.5	-

by Godkhindi *et al.* [5], the maximum cases of dyspeptic patients were in 31–40 years followed by 41–50 years. In our study, maximum incidence was observed to be among patients in 41–50 years (n=23, 46%) followed by 31–40 years (n=15, 30%). Similar to the previous studies [5-8], we observed a male predominance in our study.

The most common chief complaint in our study was abdominal pain (n=40, 80%) followed by nausea and vomiting (n=36, 72%), which is in accordance with the studies by Khan *et al.* (1999) [9], Kumar *et al.* (2006) [8], and Godkhindi *et al.* (2013) [5]. With endoscopy, gastritis was observed in 47 (94%) patients while the findings were normal in 3 (6%) patients. In the study conducted by Alenezy and Hassan (2014) [10], gastritis was most common finding and was detected in 83 (83%) cases, duodenitis in 12%, while findings were normal in 5% of cases.

On histopathology, 47/50 specimens (94%) showed chronic gastritis with mild inflammation in 25 specimens (53.2%), moderate inflammation in 15 specimens (31.9%), and marked inflammation in 7 specimens (14.9%). The findings are similar reports by Adisa *et al.* [11] who observed gastritis in 95% of cases. Godkhindi *et al.* (2013) [5] have reported an incidence of *H. pylori* of 62.72% in dyspeptic patients and Shrivastava *et al.* [7] reported in 65% of cases. The incidence of *H. pylori* in dyspeptic patients in the present study was 44.7%. Among *H. pylori*-positive samples, 61.9% were Graded +1 and 38.1% were Graded +2; on the contrary, Suzana *et al.* (2009) [12] observed 22.2% Grade 1 *H. pylori* infection, 72.2% Grade 2, and 5.5% Grade 3 infection. Comparison of histopathologic findings between various studies is outlined in Table 4.

Kumar *et al.* (2006) [8] in their study observed that efficacy of Giemsa was better than Warthin-Starry and H and E stain. In the present study, Giemsa stain was used as a special stain for the detection of *H. pylori* and found to be efficacious as compared to H and E having a sensitivity and specificity of 96.9% and 92.9%, respectively. Suzana *et al.* (2003) [13] observed 95% sensitivity and 91.1% specificity with Giemsa stain and concluded that Giemsa staining was useful for *H. pylori* detection. Similarly, Kacar *et al.* (2003) [14] also

observed that 97% sensitivity and 90% specificity with Giemsa stain conferred that Giemsa stain was the most cost-effective and reliable stain.

CONCLUSION

H. pylori is strongly associated with dyspepsia and chronic gastritis. It was found that neutrophilic activity and lymphoid aggregate in chronic gastritis cases were associated with *H. pylori*-positive cases. Therefore, the presence of these features can be taken as an indirect evidence of *H. pylori* presence. Although non-invasive test is the preferred choice for the detection of *H. pylori*, histopathological detection has better accuracy which allows to study the histomorphological changes associated with it. It is important to find out regional *H. pylori* preference and identify high-risk population infected with *H. pylori* so that the treatment strategies can be planned and implemented, to reduce the complications associated the infection.

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