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Research Article

Prevalence of *Helicobacter pylori* infection and histomorphologic spectrum in endoscopic biopsies

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Abstract

Background and Objectives: Most of the cases of chronic gastritis are due to infection by *Helicobacter pylori*. *H.pylori* is also associated with peptic ulcer disease, gastric cancer and also with primary gastric mucosa-associated lymphoid tissue (MALT) lymphoma. This study explores the prevalence of *H.pylori* infection in endoscopically suspected cases of gastritis and correlates the endoscopic findings with the histomorphological findings.

Method: We conducted this cross-sectional study in a teaching hospital attached to a medical college in Bangalore from January 2010 to June 2011 at the Departments of Surgical Gastroenterology and Pathology. All the patients who had endoscopic features of gastritis were biopsied and these biopsies were studied for the presence of *H.pylori* infection. We routinely used Hematoxylin and Eosin staining along with the Giemsa for the identification of *H.pylori* in the biopsy specimens.

Results: Antral biopsy was performed in 400 dyspeptic patients. Of these biopsies, *H.pylori* was present in 150 cases (37.5%), with maximum prevalence in the 4th decade of life and higher preponderance in men compared to that in women (66% vs.34%). Endoscopically, findings noted were linear erythema (n =60, 40%), subendothelial hemorrhagic spots (n =50, 33%) and multiple erosions (n= 40, 27%). The present study revealed substantial prevalence of *H.pylori* (37.5%). Histopathologically, all the specimens displayed features of chronic gastritis with a varying degree of active inflammation (80%), regenerative atypia (72%), glandular atrophy (60%) and intestinal metaplasia (8%).

Conclusion: *H.pylori* infection was diagnosed in more than a third of endoscopically suspected cases of gastritis emphasizing the need for early detection and treatment. Awareness of histomorphological features that are typical to *H.pylori* gastritis would help clinical pathologists in identifying conditions such as atrophic gastritis and intestinal metaplasia which can later progress onto carcinoma.

Keywords: *Helicobacter pylori*, subendothelial hemorrhagic spots, chronic active gastritis, atrophic gastritis, intestinal metaplasia, upper GI endoscopy

1. Introduction

Ever since Warren and Marshall described curved bacilli in the gastric epithelium in chronic gastritis in 1983, the etiological relevance of *Helicobacter pylori* in many upper gastroduodenal diseases has been emphasized.[1-3] *Helicobacter*

pylori is responsible for a high proportion of peptic ulcers and possibly also gastric carcinoma and gastric mucosa-associated lymphoid tissue (MALT) lymphoma.[3-5] The bacteria are prevalent worldwide and more than half of the population are infected with H pylori.[5] However, the prevalence of *H.pylori* related diseases differ in various geographic regions of India.[6]

Helicobacter pylori are micro-aerophilic curved or spiral-shaped gram-negative bacteria measuring 2-4µm in length with marked urease activity that colonize the stomach. [6] *H.pylori* are found more frequently within the mucus layer and in the gastric pits; on electron microscopy, the organisms are adherent to the surface epithelial cells. The organisms are often visible on H and E staining, though they are more easily seen with a Giemsa stain, which is useful in detecting small numbers. The Warthin Starry Stain demonstrates *H.pylori* well but the procedure is tedious and expensive and seldom used in routine work.[7]

In developed countries, individuals with *H.pylori* infection have an active superficial gastritis, most prominent in the antrum, in which organisms are easily identified.[8] In contrast, *H.pylori* infected individuals in underdeveloped countries typically have atrophic gastritis, which is patchy in distribution and involves pyloric body and cardiac mucosa.[9,10] Ours being developing country, we see mixed type of responses ranging from superficial gastritis to atrophy with intestinal metaplasia.[11-13] Among patients with chronic active gastritis, approximately 50% will continue to have non-atrophic gastritis; the other half will eventually develop atrophic gastritis, often accompanied by intestinal metaplasia and is a precursor state for gastric cancer.[11-15]

It has been confirmed that the development of gastric cancer spans several decades sequentially starting from the *H.pylori* infection, development of chronic active gastritis to development of glandular atrophy and intestinal metaplasia in a subset of patients.[11] *H.pylori* infection occurring in childhood leads to an increased risk, in the order of 4 to 9 folds, of developing precancerous gastric condition. The International Agency for Research on Cancer (IARC) monograph committee (1994) has classified H pylori as a class I carcinogen to humans.[16] More than 80% of the MALT lymphomas are associated with chronic gastritis and *H.pylori* infection. The role of *H.pylori* infection as an important etiologic factor for gastric lymphoma is supported by the elimination of about 50% gastric lymphomas with antibiotic treatment for *H.pylori*.[17]

Though there are various methods for detecting *H.pylori*, histological detection in a gastric biopsy is the commonest and the most sensitive.[11] Histopathologic examination of gastric biopsy specimen reveals that abnormalities are present in upto 2/3rd cases, but there appears to be a weak correlation between the endoscopic and histolopathologic findings.[4] Only histologic examination can provide an accurate diagnosis of various types of gastritis. For these reasons, it is recommended that endoscopic examination should always be accompanied by biopsy.[2]

H. pylori infection is a treatable condition but is often left undiagnosed. We feel that there is a need to identify the prevalence of *H.pylori* and associated the histomorphological changes in patients who routinely undergo upper gastrointestinal endoscopy and biopsy for dyspepsia. Early detection of *H.pylori* in any population and its eradication in such patients results in a significant reduction in usage of acid suppression and an improvement in overall quality and severity of dyspeptic symptoms.[12,13] Moreover, there is always a fear of resistance to anti- *H.pylori* treatment.

Further, awareness of histomorphological features that are typical to *H.pylori* gastritis would help clinical pathologists in identifying conditions such as atrophic gastritis and intestinal metaplasia which can later progress onto carcinoma. Thus, it is important to find out regional *H.pylori* prevalence and identify high risk population infected with *H.pylori* so that treatment strategies can be planned and implemented. The aim of this study was to identify the prevalence of *H.pylori* infection in dyspeptic patients undergoing endoscopic biopsy in a teaching hospital. The study also examined the correlation between endoscopic and histopathological findings.

2. Material and Methods

This cross-sectional study was undertaken at a teaching hospital attached to a medical college in Bangalore during January 2010 and June 2011. All the patients undergoing esophagogastroduodenoscopy at the Department of Surgical Gastroenterology for dyspeptic symptoms were included in the study. Gastric antral biopsies were taken from patients having endoscopic features of gastritis. These samples were referred to Department of Pathology for histopathological examination.

Gastric antral biopsies were taken from 400 dyspeptic patients with endoscopic features of gastritis. These were

subjected for routine histopathological analysis and a special stain Giemsa to confirm *H.pylori*. Biopsies thus obtained were oriented, fixed in 10% neutral formaldehyde and routinely processed. Approximately 5 µm - thick serial sections were cut and stained with hematoxylin and eosin. Special staining with Giemsa was also performed in addition to confirm the presence of *H.pylori*. Later, histomorphological features were studied in detail in patients with *H.pylori* positivity.

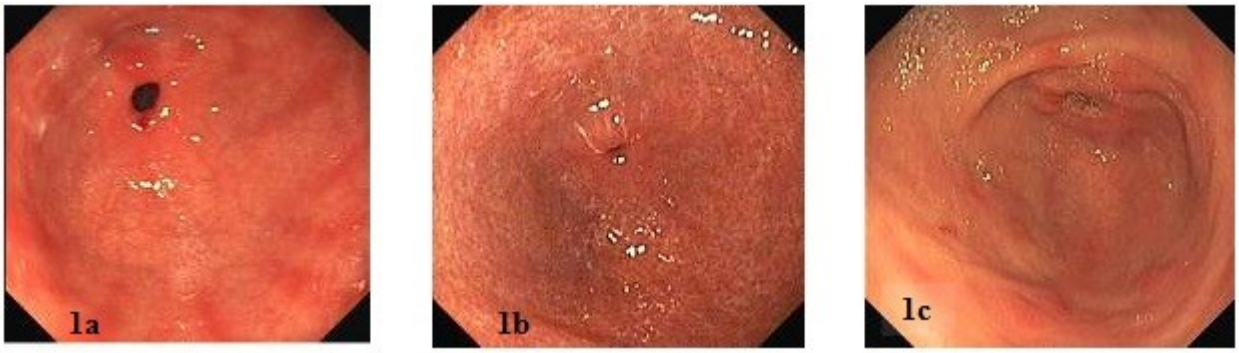
Patients with a history of previous treatment for *H.pylori*, NSAIDs, corticosteroids and previous gastric surgery were excluded from the study.

3. Results

Age range of the patients in the study was between 20-80 years with a mean age of 48 years. The number of positive cases was highest in the age range 31-40 (25%), followed by 51-60 (23%), 41-50 (19%), 61-70 (15%), 21-30 (10%), > 70 (7%) and lowest in less than 20 years age (1.3%). Prevalence of *H.pylori* was 99 (66%) in males and 51 (34%) in females. Dyspepsia presenting with variable clinical symptoms were recorded. The commonest symptom was pain abdomen (100%) followed by vomiting (97%), abdominal discomfort (73%), retrosternal burning sensation (69%), distension of abdomen(63%) and bloating sensation(55%).

Endoscopic findings were also recorded in all the subjects. Findings are as following: multiple linear erythema (n=60, 40%) (Fig.1a), subendothelial haemorrhagic spots (SEHS) (n =50, 33%) (Fig.1b), and erosions (n= 40, 27%) (Fig.1c).

Fig 1: Endoscopic appearances. (a) Linear erythema (b) SEHS (c) Antral erosions



Of the 400 gastric antral biopsies, 150 (37.5%) proved positive for *H.pylori*. *H.pylori* organisms were seen as spiral, rod-like structures in the mucus layer covering the surface epithelium and within foveolae. When abundant, were readily detected on routine Hand E staining. When scanty, they were better visualized with a special stain, Giemsa.

All biopsies with *H.pylori* positivity showed varying degrees of chronic inflammation ranging from superficial to full-thickness gastritis. The other histopathological findings noted were chronic active inflammation (n=120, 80%) (Fig.2a), lymphocytic infiltration forming lymphoid follicles (n=115, 77%) (Fig.2b), regenerative atypia (n=108, 72%) (Fig. 3a), glandular atrophy (n=90, 60%) (Fig.3b) and intestinal metaplasia (n=12, 8%) (Fig.4) (Table 1).

Fig 2: (a) Chronic active inflammation showing lymphoplasmacytic and neutrophilic infiltrate forming pit abscess (*H&E 400X*). (b) Dense chronic inflammatory cell infiltrate forming lymphoid follicles (*H& E100X*)

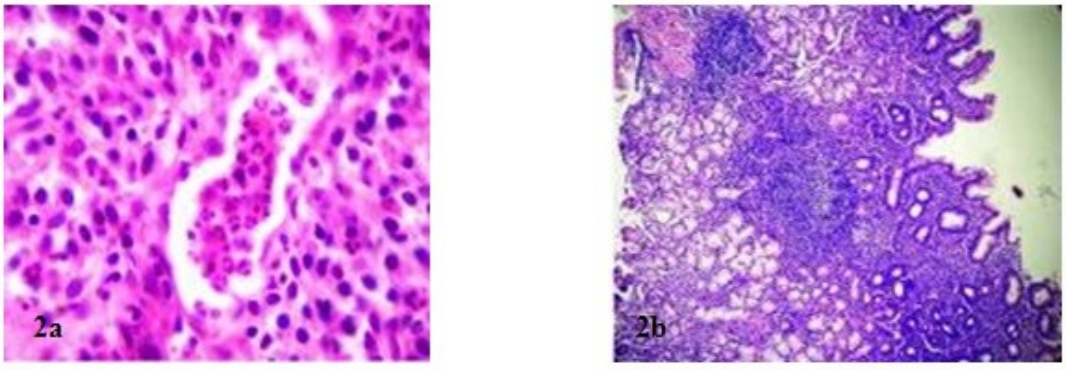


Fig 3: (a) Glands exhibiting regenerative atypia (*H&E 400X*). (b) Reduction in glands and thinned out mucosa suggesting atrophic gastritis (*H&E 100X*)

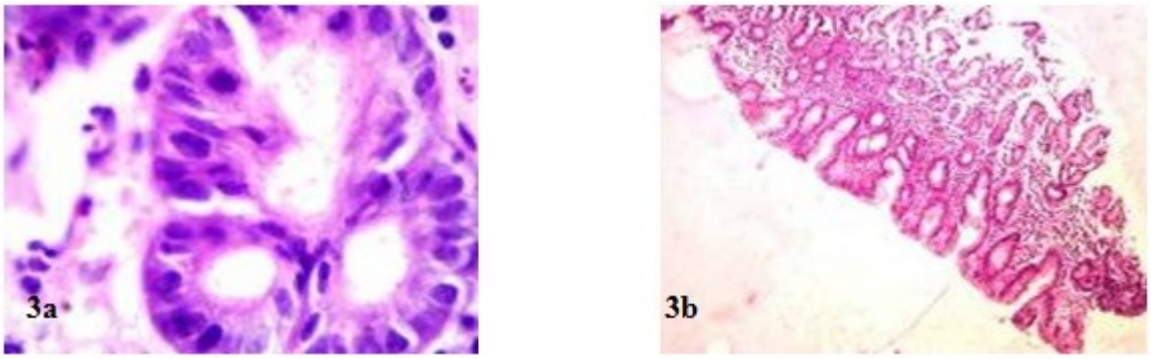


Fig 4: Intestinal metaplasia showing goblet cells (H & E 400X)

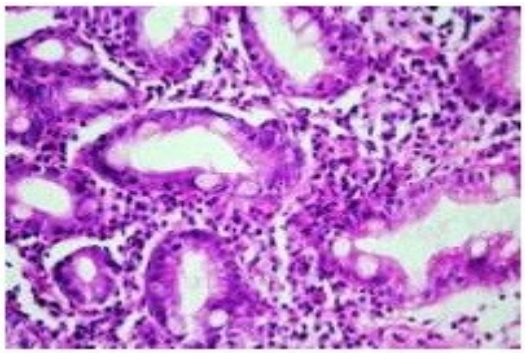


Table 1: Correlation between Endoscopic and Histopathological Findings

	Multiple Erosions N=111 (%)	Linear Erythema N = 135 (%)	SEHS N=140 (%)
Ch. Active Inflammation	28 (25.2)	41(30.3)	36(25.7)
Lymphoid Follicles	27(24.3)	37(27.4)	33(23.5)
Regenerative Atypia	27(24.3)	32(23.7)	36(25.7)
Glandular Atrophy	28(25.2)	21(15.5)	28(20.0)
Intestinal Metaplasia	1(0.9)	4(2.9)	7(5.0)

4. Discussion

The commonest indication for gastric biopsy is to identify if the patient is infected with *Helicobacter pylori* gastritis. Gastric antral biopsy at upper GI endoscopy provides information that cannot be obtained otherwise. It provides, in addition to *H.pylori* status, information about the grade, extent and topography of gastritis and also atrophy-related alterations in the gastric mucosa.[32] This information provides further possibilities for the assessment of risk and likelihood of various gastric disorders. Endoscopy with endoscopic biopsy is currently the major method of diagnosis of various gastrointestinal lesions.

The present study had 150 positive cases for H pylori of a total of 400 antral biopsies giving a prevalence of 37.5%. Our prevalence is comparable with the rate of 37.1% and 35% reported by Oksanen *et al*[18] and Kumar *et al*[19] respectively.

However, overall prevalence in our study is much lower compared to 54% - 100% reported in most previous studies.[8,10,18-22] This can be explained by the fact that the prevalence of *H. pylori* varies widely by geographic area, age, race, ethinicity, socioeconomic status and source of referral.[19] Moreover, variations in diagnostic methods may further

contribute to differing rates of detection.

Most of the *H. pylori* cases were seen in patients above the age of 20 years; only 1.3% was positive in those less than 20 years. Our finding is similar to those reported in previous studies. In the study by Gill *et al*, the maximum prevalence was in the age group 30-39 and minimum was in age group 10-19 years,[23] whereas in the study by Kumar *et al* the maximum prevalence was reported in the age group 36-45 and minimum in age group 66-75.[19] Similarly, the study done by Masood revealed peak prevalence in 3rd to 5th decade.[21] In the studies done by Hobsley and Tunio prevalence of *H. pylori* infection was more in age group 31-50 years.[24] Similarly, majority of the patients in a study done by Boixeda *et al* belonged to 4th and 5th decade.[25] Difference in prevalence with age suggests two possibilities. Either risk factor for infection in adults differ from those acting during childhood or most infections may be acquired before childhood and the observed increase in seroprevalence with age could be predominantly a cohort effect.[21] It is also possible that variations in prevalence across age groups may also be a function of case ascertainment methods.

In the present study among *H. pylori* positive patients, 99 (66%) were males and 51 (34%) were females. The male-female ratio in our study was 1.9:1. The study showed male preponderance (66%). Similar observations were noted in other studies, except for a study by Adisa *et al* which differed with female preponderance.[3]

In our study, endoscopic findings recorded were multiple linear erythema (40%), subendothelial haemorrhagic spots (33%) and multiple erosion (27%). Similar findings were noted in another study.[26] Of the patients having multiple linear erythema on endoscopy, histopathology showed chronic active inflammation (25.7%) and lymphoid follicles (27.4%). Similarly, regenerative atypia (25.7%) and intestinal metaplasia (5%) were the major findings histologically in patients having multiple SEHS. Whereas, glandular atrophy presented with both multiple linear erosion and multiple SEHS on endoscopy.

Histologic features of chronic gastritis are well known, but after the advent of *H. pylori* the various histologic components have a rational explanation.[26] The features comprise chronic inflammatory cell infiltration with lymphocytes admixed with plasma cells and neutrophils in active phase, epithelial degeneration with regenerative atypia, lymphoid follicles, glandular atrophy and intestinal metaplasia. All the cases in the present study had chronic gastritis (100%). It is now clear that *H.pylori* induces chronic gastritis in nearly all infected subjects.[27] A study by Adisa *et al* reported gastritis in 95% out of a total of 603 antral biopsies.[3]

Chronic active inflammation seen in 80% of cases was histologically characterized by neutrophilic infiltrate in the lamina propria, within the epithelium and within the foveolar lumen. A study done by Ohkuma *et al*. observed 90 fold increased risk of chronic active gastritis in patients infected with *H. pylori*.[28] Stolte *et al* compared the degree of gastritis, activity of gastritis and *H. pylori* colonization and found higher the grade of gastritis and activity, denser was the *H.pylori* colonization.[29] In a study done by Kuipers *et al* and Nai *et al*[10,20] chronic active inflammation was detected in 50% and 53% cases respectively which is less compared to our study, though study done by Kumar *et al* and by Ohkuma *et al*[19,28] showed activity in 95% and 100% of the cases respectively, higher in comparison to our study.

Regenerative atypia was present in 72% cases where epithelial cells of the surface mucosa and to a lesser extent the glands exhibit enlarged, hyperchromatic nuclei and high nuclear-cytoplasmic ratio.[30] A study done by Nai *et al* had regenerative atypia in 27% which is comparatively less than our study. Successful eradication of infection will rapidly revert these changes, lending support to a direct role of bacterial products in their causation.[20]

Lymphoid follicles represent a relatively common histological feature of *H. pylori*.[31] In a study by Stolte *et al*[29] lymphoid follicles were detected in 54% of the cases; they also found the prevalence of lymphoid follicles correlated significantly with the activity of gastritis. In other studies, the rate of lymphoid hyperplasia ranged from 13% to 54%, much lower than the 77% found in this study.[20,22,29] Our study showed comparatively higher percentage of lymphoid follicle formation. If sufficient biopsies and sections are examined it is claimed that lymphoid follicles will be found in most *H. pylori* positive cases.[33]

Glandular atrophy was seen in 60% of the cases which is higher than what has been reported in many previous studies (6-40%).[10,18,26] with the exception of one study which reported a very high rate of 96%.[21] The rate of gastric atrophy is higher in infected gastritis compared to non-infective gastritis. In one study the rate of atrophy in infected gastritis was 83% [34] and in the other it was 39% compared to 10% and 9% respectively in the non-infected group.[28] They

also assessed the frequency of gastric atrophy in different age groups with and without *H.pylori* infection. There was significant increase in the frequency of atrophic gastritis with advancing age in patients with *H.pylori* infection with no significant difference in the prevalence in those patients without *H.pylori* infection. Greater the intensity of *Helicobacter pylori*, the greater is the degree of chronic gastritis and atrophy.[35] It has been established that *H.pylori* infection as a definite risk factor for the development of gastric cancer.[36,37] Hence, it is important to diagnose atrophy in *H.pylori* infected cases.

The rate of intestinal metaplasia in our study is 8%, similar to 11% reported by Nai *et al*[20] but much less compared to the 33% reported by Ohkuma *et al*[28] Intestinal metaplasia is a common finding in chronic gastritis; prevalence increases in accordance to the duration of infection. A significant relationship between *Helicobacter pylori* infection and the grade of gastritis, atrophy and intestinal metaplasia has been demonstrated.[28]

5. Conclusion

Though the *H.pylori* infection is prevalent worldwide, its incidence is more commonly seen in the developing nations like ours. Early detection of *H.pylori* infection aids in the treatment and eradication. Also, the awareness of histomorphological features that are typical to *H.pylori* gastritis would help clinical pathologists in identifying conditions such as atrophic gastritis and intestinal metaplasia which can later progress onto carcinoma. Thus, it is important to find out regional *H.pylori* prevalence and identify high risk population infected with *H.pylori* so that treatment strategies can be planned and implemented in such patients to reduce the menace of this disease.

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