Histopathological Changes in *Helicobacter pylori* Associated Gastritis and Scope of Special Stain and Immunohistochemistry as Diagnostic Aids

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ABSTRACT

BACKGROUND

Helicobacter pylori associated chronic gastritis plays a vital role in the development of majority of gastric adenocarcinomas and most gastric MALT (Mucosa Associated Lymphoid Tissue) lymphomas. Many diagnostic methods are available for the identification of this organism. However, in gastroenterology practice, histopathological examination of biopsy samples provides visual identification of the pathogen and the associated mucosal changes with special stains like Giemsa. The aim of this study was to evaluate the efficacy of three stains H & E- (Haematoxylin and Eosin), Giemsa and IHC (Immunohistochemistry) in the identification of *H. pylori*. Associated histologic changes were noted and the relationship between the degree of colonisation and the activity and chronicity of gastritis were analysed.

METHODS

585 gastric biopsies taken from dyspeptic patients were evaluated for gastritis, based on updated Sydney System. In 250 randomly selected cases, three staining methods were used.

RESULTS

Out of 585 cases, 413 (70.60 %) had features of chronic gastritis. Mild chronic gastritis was the commonest finding and is seen in most cases of mild *H. pylori* colonisation. When activity was monitored, mild activity was the most frequent finding [225 (38.46 %)]. Majority of the severe activity cases showed severe *H. pylori* colonisation. 13.16 %, 4.79 % and 7.35 % showed intestinal metaplasia, atrophy and dysplastic changes respectively. Out of 250 cases, H & E and Giemsa stains showed 45.6 % and 57.2 % positivity while IHC demonstrated maximum number of positivity (156 cases - 62.4 %). Sensitivity and specificity of H & E was found to be 77.90 % and 98.95 %, positive predictive value was 99.13 % and negative predictive value was 69.18 %. For Giemsa stain, sensitivity was 91.67 %, specificity was 100 %, positive predictive value was 100 % and negative predictive value was 87.85 %.

DISCUSSION

H. pylori gastritis was a frequent finding in dyspeptic patients in southern part of India. When chi-square test was done, a significant statistical relationship between the severity of *H. pylori* colonisation, activity and chronicity of gastritis was noted. P value was < 0.001. With the use of special stain, Giemsa and ancillary techniques like IHC, the detection rate of *H. pylori* was enhanced considerably.

CONCLUSIONS

With increasing number of *H. pylori* in the mucosa, there was increase in the chronicity and activity of gastritis. Although immunohistochemistry revealed more cases of *H. pylori*, Giemsa can be a cost-effective substitute, because of its high specificity and positive predictive value.

KEYWORDS

H. pylori Gastritis, Giemsa, Haematoxylin and Eosin Stain, Immunohistochemistry

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BACKGROUND

Chronic gastritis is one of the commonest causes for upper gastrointestinal discomfort in patients attending gastroenterology outpatient department. This can be due to various causes like *Helicobacter pylori*, autoimmune disease, radiation, chronic bile reflux, mechanical trauma due to nasogastric tube and involvement by systemic diseases or graft versus host disease.[1] Among these, the most common cause is infection due to the bacillus, *Helicobacter pylori*. This organism was first discovered in the gastric mucosa of patients with gastritis and ulcers in 1982 by Dr. Barry Marshall and Robin Warren.[2] It is a spiral shaped, microaerophilic, gram negative bacterium measuring 2 to 4 µm in length and 0.5 to 1 µm in width. It has 4 - 6 sheathed polar flagella. The organism can live in the acidic environment of the stomach by its special structure and virulence factors. Among them the Cytotoxin-Associated Gene (cag-A), Vacuolating Associated Cytotoxin Gene A (vacA), outer inflammatory protein A, blood group antigen binding adhesin, lipases and Lipo-Poly-Saccharides (LPS) can cause inflammation in the host gastric tissues.[3] The infectivity of the organism is determined not only by the virulence of the infecting strain, but also by host genetics and environmental factors. Presence of *cagA* is associated with disease severity, but some studies indicated that this is not observed in Asian countries as majority of the *H. pylori* strains in this region carry *cagA* gene.[4]

The diseases caused by *H. pylori* are acute and chronic gastritis and gastric and duodenal peptic ulcers. Most patients will seek medical attention in chronic gastritis stage. In majority of patients, it is limited to antrum, with increased acid production giving rise to peptic ulcer disease of the duodenum or the stomach. Later it can involve the gastric body and fundus, leading to hypergastrinemia, as in autoimmune atrophic gastritis and intestinal metaplasia, dysplasia and increased risk of gastric adenocarcinoma over the course of many years. Approximately 60 % of gastric adenocarcinomas and most gastric MALT lymphomas are related to chronic infection with this organism. Considering all these findings and in correlation with epidemiological data, *H. pylori* was identified as a Class I (Definite) carcinogen by International Agency for Research on Cancer (IARC).

Prevalence of *H. pylori* varies depending on several factors like age of the individuals, socioeconomic status, sanitation facility etc. It is as low as 10 % in developed countries to as higher as 80 % in developing countries. In children, the prevalence is less than 15 % whereas in older individuals 50 – 60 %. The prevalence of *H. pylori* in India is as high as 80 percent.[5] In India the commonest manifestation of *H. pylori* infection is peptic ulcer disease, especially duodenal ulcer disease.[6]

Several diagnostic tests have been developed for identification of *H. pylori* such as carbon 13 urea breath test, serology, faecal bacterial detection, rapid urease test (RUT), histopathology, culture and polymerase chain reaction (PCR). The efficacy of these different methods was compared in numerous studies.[7]

Among these, histology with culture is considered to be the gold standard for direct demonstration of *H. pylori* infection. Disadvantages of culture is the limited sensitivity and it is a time consuming procedure[8]. In histologic sections, along with direct visualization of the bacteria, gastric mucosal changes can also be noted. The updated Sydney system recommend to take biopsy from five different sites. Expertise of the pathologist in picking up the bacilli in routine H & E stain is also of utmost importance. The organism may be missed in patients with absent or mild inflammation and patients on medications like proton pump inhibitors and antibiotics.

In order to reduce the subjective error, several special stains were described for the better identification of the organism. The stains include Warthin-Starry, Genta, toluidine blue, acridine orange and immunohistochemical stains. Compared to other special stains, Giemsa is cheaper, simple to perform, universally available and give consistently good results for *H. pylori*.[9] But there are certain problems with Giemsa stain. The lack of contrast is one among them. Another disadvantage is the failure to pick up the organism in patients on medications. The importance of ancillary techniques like IHC (Immunohistochemistry) in the identification of *H. pylori* is more important nowadays due to the injudicious use of proton pump inhibitors, which can cause reduction in the number and change in the morphology of *H. pylori*. The organism becomes coccoid and colonizes proximal stomach that too in deeper layers.[10] IHC has got high sensitivity and specificity. But cost effectiveness of it is still under debate, especially in developing countries like India. The aim of this study was to evaluate the utility of three stains (H & E, Giemsa and IHC) for the identification of *H. pylori*. The study also analysed the associated histologic changes seen in the gastric biopsies, based on Updated Sydney system. The correct diagnosis of *H. pylori* is necessary for the eradication of the organism, thereby reducing the incidence of gastric carcinoma and MALT lymphoma. A few studies regarding *H. pylori* gastritis were conducted in Kerala. But this study used immunohistochemistry for the precise identification of bacteria, which was a first of its kind done in this part of India.

METHODS

Endoscopic biopsies taken from antrum and body of patients who presented with dyspeptic symptoms were included in the study. This cross-sectional study was conducted in Department of Pathology, Government Medical College, Trivandrum, Kerala for a period of two years from December 2015 to December 2017 and was approved by the institutional ethics committee. Clinical details were obtained from the hospital records. Specimens were formalin fixed & paraffin embedded. 5-µm-sections taken from each specimen block and were stained with H & E. 585 gastric biopsies were studied using H & E stain for classification of gastritis and identification of *H. pylori*. Cases without adequate clinical data, biopsies showing gastric malignancy, polyps, and gastrectomy specimens were excluded from the

study. The histological findings were tabulated using Updated Sydney grading system based on activity, chronicity, metaplasia, and atrophy and *Helicobacter pylori* colonisation.

Normal gastric mucosa shows 2 to 5 lymphocytes, plasma cells and macrophages per high power field (HPF) in the lamina propria. In chronic infection lamina propria shows lymphoplasmacytic infiltration with foveolar predilection and lymphoid aggregate with germinal centre formation. Mild increase in inflammatory cells in the lamina propria without involvement of epithelium is regarded as mild chronic inflammation. Severe chronic inflammation is characterized by dense lymphoplasmacytic infiltration of the lamina propria with or without lymphoid follicles, with infiltration of epithelium. Inflammation intermediate between these is regarded as moderate degree of chronic inflammation.

Neutrophilic infiltration of the lamina propria, pits, or surface epithelium is defined as activity. Mild activity denotes involvement of less than one third of pits and epithelium; neutrophilic infiltration less than or equivalent to two thirds of pits and epithelium are graded as moderate; more than two thirds are considered as severe activity. Presence of goblet cells in the gastric mucosa denotes intestinal metaplasia.

Helicobacter pylori is diagnosed when the characteristic curved organisms are identified in the mucin over the surface epithelium and within the foveolae. Mild colonization is indicated if scattered organisms are found in less than one third of the surface epithelium; If large clusters of *H. pylori* or a continuous layer of the organisms are seen over two thirds of the surface, it is classified as severe colonisation; moderate colonization indicates intermediate numbers between these.

2 additional sections were taken from randomly selected 250 cases. Giemsa stain and immunohistochemical study were done in these cases. *H. pylori* colonisation was graded and compared the three stains for the identification of *H. pylori*. Immunohistochemical study was done using purified rabbit polyclonal *H. pylori* antibody (BioGenex)... All the data obtained were analysed with SPSS 16. The results were compared and p value less than 0.05 was considered significant. Sensitivity, specificity, positive and negative predictive values were calculated.

RESULTS

Total of 585 cases was evaluated. The age of the patients ranged from 7 to 85 years. Among the *H. pylori* positive cases majority were in the age group of 41 - 60 years (41.54 %). Males were more affected than females (62 %). When histopathology of 585 cases were evaluated by routine H & E stain, 413 (70.60 %) had features of chronic gastritis. 172 (29.40 %) patients had no gastritis, activity was noted in 331 cases (56.58 %). 77 (13.16 %) had intestinal metaplasia and 28 (4.79 %) showed atrophy. *H. pylori* colonization was seen in 260 (44.44 %) and dysplastic changes in 43 (7.35 %) subjects.

Of the 413 patients with chronic gastritis, majority of the patients – 265 (45.3 %) had mild gastritis, 109 (18.63 %) suffered from moderate gastritis, and 39 (6.67 %) had severe chronic gastritis. When activity was monitored, most of the participants were in the mild activity group 225 (38.46 %). Only 39 cases (6.67 %) were in the severe activity group. Moderate activity was seen in 67 (11.45 %). In 260 participants showing *H. pylori* colonization, majority of the patients 134 (22.91 %) suffered from mild *H. pylori* infection, while 14.70 % had moderate infection and 6.84 % had severe *H. pylori* colonization. (Table 1)

		Chronic Gastritis				
		No inflammation	Mild	Moderate	Severe	Total
H. pylori colonisation	No organism	148 (45.54 %)	142 (43.69 %)	25 (7.69 %)	10 (3.08 %)	325 (100 %)
	Mild	22 (16.42 %)	89 (66.42 %)	17 (12.69 %)	6 (4.48 %)	134 (100 %)
	Moderate	1 (1.16 %)	24 (27.91 %)	51 (59.30 %)	10 (11.63 %)	86 (100 %)
	Severe	1 (2.5 %)	10 (25 %)	16 (40 %)	13 (32.5 %)	40 (100 %)
	Total	**172 (29.40 %)**	**265 (45.3 %)**	**109 (18.63 %)**	**39 (6.67 %)**	**585 (100 %)**

Table 1. Relationship between Intensity of H. pylori Infection and Severity of Chronic Gastritis

When the severity of gastritis was correlated with the degree of *H. pylori* colonization, mild gastritis was noted in most cases of mild *H. pylori* colonization (66.42 %). Moderate gastritis was noted in 51 out of 86 patients with moderate *H. pylori* colonization. Severe gastritis was seen in 13 out of 40 cases of severe *H. pylori* colonization. To know if there is any relationship between severity of chronic gastritis and level of *H. pylori* colonization, chi-square test was done, and p-value was found to be < 0.001. Hence, there found to be a significant relationship between severity of chronic gastritis and level of *H. pylori* colonization.

When activity and the extent of *H. pylori* colonization were studied, out of 134 patients showing mild *H. pylori* colonization, 66.42 % showed mild activity. (Table 2)

		Activity				
		No activity	Mild	Moderate	Severe	Total
H. pylori colonisation	No organism	206 (63.38 %)	73 (22.46 %)	27 (8.31 %)	19 (5.85 %)	325 (100 %)
	Mild	34 (25.37 %)	89 (66.42 %)	7 (5.22 %)	4 (2.99 %)	134 (100 %)
	Moderate	12 (13.95 %)	46 (53.49 %)	21 (24.42 %)	7 (8.14 %)	86 (100 %)
	Severe	2 (5.0 %)	17 (42.5 %)	12 (30.0 %)	9 (22.5 %)	40 (100 %)
	Total	**254 (43.42 %)**	**225 (38.46 %)**	**67 (11.45 %)**	**39 (6.67 %)**	**585 (100 %)**

Table 2. Relationship between Intensity of H. pylori Infection and Activity

In patients showing moderate and severe *H. pylori* colonization also, mild activity was the commonest finding. While evaluating severe activity cases, it was noticed that majority of them showed severe *H. pylori* colonization. When chi-square test was done, a significant statistical relationship between severity of activity and severity of *H. pylori* colonization was noted. P-value was < 0.001.

Out of 250 randomly selected cases for comparison of three stains, H & E staining detected 114 cases (45.6 %) of *H. pylori.* With Giemsa stain, 143 cases (57.2 %) showed positivity for *H. pylori* while 107 cases were negative. IHC demonstrated positivity in maximum number of 156 cases (62.4 %). (Table 3)

Staining Method	*H. pylori* Colonisation				
	Negative	Mild	Moderate	Severe	Total Positive
H & E	136 (54.4 %)	65 (26 %)	39 (16 %)	10 (4 %)	114 (45.6 %)
Giemsa	107 (42.8 %)	74 (30 %)	57 (23 %)	12 (4.8 %)	143 (57.2 %)
IHC	94 (37.6 %)	81 (32 %)	61 (24 %)	14 (5.6 %)	156 (62.4 %)

Table 3. Comparison of Three Stains with Grading of H. pylori Colonisation

The sensitivity, specificity, positive predictive value and negative predictive value of H & E and Giemsa stains in comparison with immunohistochemistry were also calculated. (Table 4)

	H & E	Giemsa
Sensitivity	72.90 %	91.67 %
Specificity	98.95 %	100 %
PPV	99.13 %	100 %
NPV	69.18 %.	87.85 %

Table 4. Sensitivity, Specificity, PPV and NPV of H & E and Giemsa Stain

DISCUSSION

Helicobacter pylori gastritis is a major treatable cause of chronic gastritis in the developing nations. The identification and eradication of this organism not only alleviate the dyspeptic symptoms associated with chronic gastritis but also reduces the chance for development of gastric malignancies. This study was done to compare the three stains used in histopathology for the detection of *H. pylori*. The histologic changes seen in gastric biopsies were graded and the relationship between the severity of inflammatory changes and the degree of *H. pylori* colonization were studied.

In this study, out of 585 gastric biopsies studied, 413 (70.60 %) had features of chronic gastritis. This was similar to studies done in Saudi-Arabia and parts of India, where approximately 67 % of the cases showed chronic gastritis.[11,12]

In our study *H. pylori* colonization was seen in 260 (44.44 %) using routine H & E stain. This was lesser compared to study of Singh et al 12, where *Helicobacter pylori* was positive in 49 (61.3 %) symptomatic individuals. But with the use of IHC, *H. pylori* was detected in 62.4 %, Its slightly higher compared to other studies in South India, showing 59.4 % prevalence, which did not use immunohistochemistry.[13]

Majority of the *H. pylori* positive patients were in the age group of 41 - 60 years (41.54 %). This was in contrast to the finding of Jais et al where maximum prevalence in 30 - 39-year age group and then declined with advancing age.[14] but in another study by Genta et al the age-specific prevalence of *H. pylori* gastritis peaked in the 4th to 5th decades.[15] 62 % of *H. pylori* cases were males. Some studies showed male preponderance,[16,17] while females show high infectivity in others. Majority of the studies did not show any significant difference.

Maximum number of patients 134 (22.91 %) in our study showed mild *H. pylori* infection. A similar observation was observed in a study conducted by Priyadarshini in the neighbouring state of Tamilnadu where most of the cases expressed low grade *H. pylori* positivity.[18]

4.79 % showed atrophy and dysplastic changes were noted in 7.35 % subjects. This was in contrast to the findings of study conducted by Hashemi et al where atrophic changes were in a higher number of patients (25 %) and glandular dysplasia in only 0.1 %.[19] This may be due to ethnic difference and the duration of *H. pylori* infection. In our study, 13.16 % had intestinal metaplasia. Prevalence of intestinal metaplasia in a study conducted in Iraq was 15 %.[20] when the severity of gastritis was correlated with the degree of *H. pylori* colonization, mild gastritis was noted in most cases of mild *H. pylori* colonization (66.42 %). About 70 % of patients with *H. pylori* infection experienced chronic gastritis with highest incidence of mild gastritis. In a study conducted by Hamid et al, 90 % of people infected with *H. pylori* showed chronic gastritis with majority showing moderate gastritis.[21] A positive correlation between severity of chronic gastritis and level of *H. pylori* colonization was observed in our study. Similar correlation was observed by and Hamid et al.

But study conducted by Ardakani and another one by Park et al in Korea did not show significant relationship between *H. pylori* colonization and degree of chronic gastritis.[22,23] When activity and the extent of *H. pylori* colonization were studied, out of 134 patients showing mild *H. pylori* colonization, 66.42 % showed mild activity. Mild activity was the most frequent finding in patients showing moderate and severe *H. pylori* colonization. Majority of the severe activity cases showed severe *H. pylori* colonization. This was in concordance with the study of Sonam et al in which all cases having marked neutrophilic activity showed *H. pylori* positivity.[24] Alagoz et al also noted a correlation between activity and *H. pylori* positivity.[25]

In this study, 45.6 % of gastric biopsies revealed the presence of *H. pylori* by H & E stain, while Giemsa and IHC stain showed 57.2 % and 62.4 % positivity respectively. This result is comparable to findings in other studies which showed positivity of 54.5 % by the routine H & E stain and 68.7 % by Giemsa stain.[26] In the present study sensitivity and specificity of H & E was 72.90 % and 98.95 % respectively. Sensitivity and specificity of Giemsa was 91.67 % and 100 % respectively. Kacar N et al observed that the sensitivity and specificity for H & E, Giemsa and IHC in detection of *H. pylori* was 97 % / 80 %; 97 % / 90 %; 100 % / 100 % respectively.[27] HR. Wabinga et al in his study evaluated the staining ability of Giemsa and IHC in gastric biopsies and inferred that the sensitivity of Giemsa stain was 85 %, specificity was 89 %, positive predictive value was 93 % and negative predictive value was 74 %.[28] Sensitivity of detection of *H. pylori* in gastric biopsies and resected specimens using modified Giemsa and IHC were compared by Babic et al which revealed the sensitivity of Giemsa to be 73.3 % and 90 % for IHC.[29]

Hartman and Owens review showed 83.3 % sensitivity of H & E stained sections.[30] Compared to that review, the sensitivity of H & E in this study was less, may be because organisms with typical morphology only were included in this study. Sensitivity of H & E stain is low due to lack of contrast between the bacteria and the surrounding tissue. The specificity of H & E is also low due to its nonspecific staining of non *H. pylori* bacteria in the stomach and in cases showing

low density of organisms.[31] Modified Giemsa is a cheap, easily applicable stain that can be performed in 15 minutes. It is sensitive, easy to perform, and reproducible. The sensitivity and specificity values are acceptable.

Lack of contrast is the disadvantage of the Giemsa technique, but careful observation allows identification of the organisms correctly. Immunohistochemistry is an expensive and time-consuming technique. Sensitivity and specificity are high for the detection of *H. pylori* using IHC. But some studies found that IHC staining may not be necessary for cases without active gastritis.[32] they advocated IHC in special situations like 1) Giemsa-negative cases, if marked superficial mucus and acute inflammatory exudates are present 2) when the density of *H. pylori* is expected to be low, as may occur in post treatment biopsies. Regarding limitation of this study biopsy sites and numbers, staining methods and intake of drugs like proton pump inhibitors (PPI) can influence the results.

CONCLUSIONS

H. pylori was frequently seen in patients presenting with dyspeptic symptoms in southern part of India. Severity of *H. pylori* colonisation showed good correlation with the chronicity and activity of gastritis. The study also found out that immunohistochemistry demonstrated more number of *H. pylori* cases than special stain Giemsa and routine H & E stain. The cost of IHC is a major challenge in developing countries. Giemsa is a good alternative because of its high specificity and positive predictive value as seen in this study. The use of IHC can be limited to special situations like post treatment biopsies in which number and morphology of the organism is altered.

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