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Original Research Article

Study Of Histopathological Findings with Helicobacter Pylori Correlation in Upper GI Endoscopic Biopsies in A Tertiary Care Centre

M.S. Shruthi [1], R.D. Puvitha[2], G. Joshila Nandhini[3]

[1]Associate Professor, Department of Pathology, Government Dharmapuri Medical College, Dharmapuri

[2]Associate Professor, Department of Pathology, Government ESI Medical College, Coimbatore

[3]Assistant Professor, Department of Pathology, Government Dharmapuri Medical College, Dharmapuri

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Corresponding author: Dr.M.S.Shruthi
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Abstract:

Introduction: Dyspepsia is defined as upper abdominal and epigastric discomfort commonly after meals, most common symptom being indigestion. The most common investigations used are barium studies and endoscopy to know the diagnosis of dyspepsia, but these as such alone is not enough for final diagnosis. Hence biopsy and histological examination of such lesions will be helpful in identifying the cause. Based on the primary objective of this study is to detect Helicobacter pylori infection in the patients with features of dyspepsia who undergo upper gastrointestinal endoscopy.

Methodology: This prospective study was undertaken in Department of Pathology in a tertiary care centre. Hundred consecutive endoscopic gastric biopsies in patients presenting with symptoms of dyspepsia were included in the study. Various histopathological diagnoses in each case were noted down and were correlated with the incidence of H.pylori infection in each case. Density of H.Pylori was also assessed.

Results: Of the hundred cases, 74 cases of chronic superficial gastritis was seen, gastric ulcer in 7 cases (7%) and adenocarcinoma in 16 cases (16%).Three cases(3%) showed normal histological findings. Among the 74 cases of chronic superficial gastritis where H.pylori was studied, 40 cases were positive for Helicobacter pylori (54.05%). Of the 7 cases of Gastric ulcer 5 cases were positive for Helicobacter pylori (71.42%). In 16 cases of adenocarcinoma studied 9 cases were positive for Helicobacter pylori (56.2%). Of all cases positive for H.pylori in our study, 3 cases had a bacterial density of grade 3, fifteen patients had a bacterial density of grade 2 followed by 37 cases with a grade 1 bacterial density.

Conclusion: Early diagnosis and eradication of H.pylori not only improves symptoms but also helps to prevent complications associated with H.pylori infection. To conclude based on the findings in of the present study non- invasive tests for detection of Helicobacter pylori may be preferred choice for clinicians but histopathological demonstration has the advantage of accuracy. At the same time it gives us chance to study associated histopathological changes. Some of these changes may be of prognostic value.

Keywords: H.pylori, gastric ulcer, biopsy.

Introduction

Dyspepsia is defined as upper abdominal and epigastric discomfort commonly after meals, most common symptom being indigestion. Dyspepsia has a wide range of differential diagnosis and a diverse pathophysiology with a prevalence of approximately 25%. Dyspepsia contributes to 20-40 % of complaints in gastrointestinal OPD[1]. The most common investigations used are barium studies and endoscopy to know the diagnosis of dyspepsia, but these as such alone is not enough for final diagnosis. Hence biopsy and histological examination of such lesions will be helpful in identifying the cause. Cause may be multifactorial. Among various reasons of such non ulcer dyspepsia one important cause is Helicobacter pylori infection. In a recent systematic review of H.pylori prevalence rates in non-ulcer dyspepsia indicates that H.pylori is found more often in persons with non-ulcer dyspepsia than in asymptomatic individuals.[2] H.pylori infection is present in 30-60% of patients with non-ulcer dyspepsia in Western countries.[3]

Chronic and persistent infection with H. pylori is considered one of the earliest steps in human gastric carcinogenesis. It was also observed that there was an increased proliferative activity in H.pylori associated chronic gastritis in response to cell damage or injury. Increased cell turn over associated with incomplete intestinal metaplasia may result in DNA instability and subsequent development of intestinal type gastric adenocarcinoma in H.pylori infected human mucosa.

Eradication of H pylori is therefore considered to play important roles, not only in the treatment of gastritis and gastric ulcers, but also in preventing the development of gastric intestinal type carcinoma. [4,5] Eradication may suppress the DNA damage and subsequent increased cell proliferation of the gastric epithelium caused by H,pylori infection.[4]Dyspepsia presents with various histopathological findings ranging from gastritis to adenocarcinoma.

Methods of detection are divided into invasive and non-invasive tests. Non-invasive tests available are serology and carbon-labelled urea breath test. The invasive tests available are the rapid urease test, histology, and culture. Among the invasive tests, histology is important in assessment of H.pylori status. Endoscopic biopsy allows detection of H.pylori, which determines treatment for peptic ulcer disease.[6]

For most studies histology is the gold standard for detection, because the objective is determination of whether the organisms are present and whether there is gastritis.[7,8] The Sydney system for the classification of gastritis emphasises the importance of topographical, morphological and etiological information.[6] The histological severity of H.pylori density, inflammation, activity, atrophy and intestinal metaplasia were graded according to the revised Sydney classification.[9,10] Based on above concept the primary objective of this study is to detect Helicobacter pylori infection in the patients who come to our Hospital with features of dyspepsia who undergo upper gastrointestinal endoscopy. Our other objectives were to study the various spectrum of pathological lesions in patients with dyspepsia and to study the incidence of H.pylori in various pathological lesions of dyspepsia.

Materials and Methodology

This prospective study was undertaken in Department of Pathology in a tertiary care centre. Hundred consecutive endoscopic gastric biopsies in patients presenting with symptoms of dyspepsia were included in the study. This study was conducted from August 2008 to August 2010.Biopsies were taken with a flexible fibreoptic

Gastroscope. Routine Haematoxylin and eosin stain and Giemsa stain for the demonstration of Helicobacter pylori were done in each case. Haematoxylin and eosin stained sections were examined for the histomorphological parameters associated with Helicobacter pylori infection namely lymphoid aggregates, chronic inflammatory infiltrate (mild, moderate and severe), activity (neutrophils), and intestinal metaplasia.

Various histopathological diagnoses in each case were noted down and were correlated with the incidence of H.pylori infection in each case. Density of H.Pylori was also assessed. Patients aged more than 18 years attending OPD with symptoms of dyspepsia (upper abdominal pain or discomfort, nausea, vomiting, bloating, postprandial fullness, belching and early satiety) were included. Patients whose age were less than 18 years, who had previous surgery of upper GI tract, treated for H.pylori during last one month, pregnant, patient with recent history of ischemic heart disease and patient who refused to participate in the study were excluded.

Results

In hundred cases of gastric biopsies studied, 69 cases (69%) were male and 31 cases (31%) were females with a male to female ratio of 2.22:1. Of the 100 patients studied, the age ranged from 25 to 85 years with a mean age of 53.45 years. Peak incidence of occurrence of gastric lesions were sixth and seventh decade with 29 cases (29%) and 21 cases (21%) respectively.

Of the hundred cases, 74 cases of chronic superficial gastritis was seen, gastric ulcer in 7 cases (7%) and adenocarcinoma in 16 cases (16%).Three cases(3%) showed normal histological findings. Among the 16 cases of adenocarcinomas studied, the peak incidence of occurrence was in sixth and seventh decade. (Table 1).

Table 1: Age distribution of gastric lesions

Age range	Normal	Chronic superficial gastritis	Gastric ulcer	Adenocarcinoma-diffuse	Adenocarcinoma- intestinal	Total
21-30	1	6	1	0	0	8
31-40	0	13	1	1	0	15
41-50	1	14	1	0	1	17
51-60	0	23	1	3	2	29
61-70	1	11	3	4	2	21
71-80	0	6	0	1	1	8
81-90	0	1	1	0	0	2
Total	3	74	7	9	7	100

Among the 74 cases of chronic superficial gastritis where H.pylori was studied, 40 cases were positive for Helicobacter pylori (54.05%). Of the 7 cases of Gastric ulcer 5 cases were positive for Helicobacter pylori (71.42%). In 16 cases of adenocarcinoma studied 9 cases were positive for Helicobacter pylori (56.2%). Of the 3 cases which showed normal histology, 1 case was positive for Helicobacter pylori (33.3%). In histopathological examination on microscopic view they were seen usually in close contact with the mucosa within gastric pits entrapped within the overlying mucus or in the lumen of the gastric glands. In Haematoxylin and Eosin stained sections they are faintly eosinophilic whereas in Giemsa stained sections they are better appreciated as blue or violet organisms. (Table 2)

Table 2: Helicobacter pylori positivity in different gastric lesions by Giemsa stain

	Chronic superficial gastritis (74 cases)	Gastric ulcer (7cases)	Adenocarcinoma (16 cases)	Normal	Total
No. of cases H.pylori positive	40	5	9	1	55
% of Positivity	54.05%	71.42%	56.2%	33.33%	55%

Of the 16 cases of gastric adenocarcinomas, 9 were cases of intestinal type and 7 were of diffuse type.

The intestinal type of adenocarcinoma had tumour cells arranged in form of glandular arrangement predominantly whereas in the diffuse type, the tumour cells were arranged predominantly in sheets, scatters with occasional poorly formed glands. Of the 9 cases of adenocarcinoma of intestinal type, 8 cases (88.88%) are positive for H.pylori, and of the 7 cases of adenocarcinoma of diffuse type, one was positive for H.pylori. (Table 2) In our study, among the 55 cases of Helicobacter pylori, 36 (65.45%) were males and 19 (34.54%) were females with a ratio of 1.89:1.

This indicates the preponderance of H.pylori in males. The age distribution of H.pylori shows a gradual increase in the H.pylori positivity with increasing age reaching a maximum at the 6th to 8th decade.

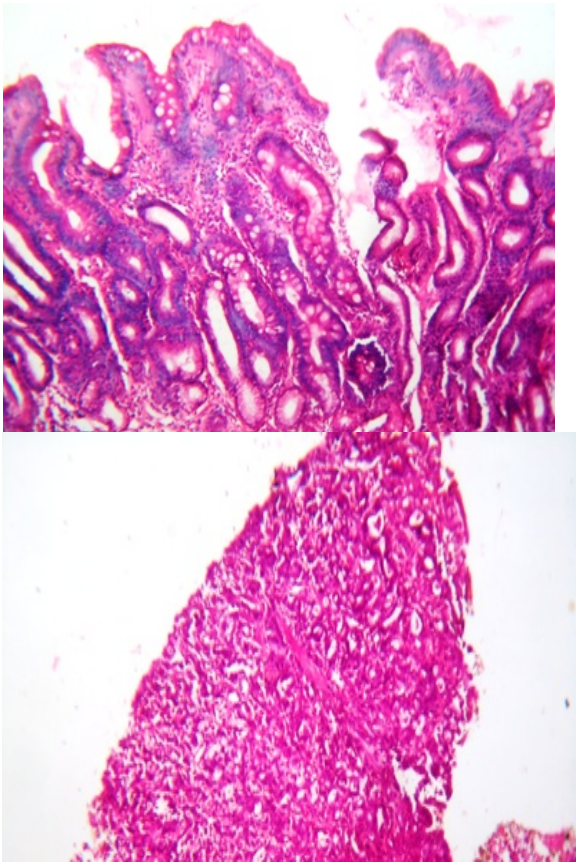

Figure 1 & 2: Chronic superficial gastritis with intestinal metaplasia and intestinal adenocarcinoma

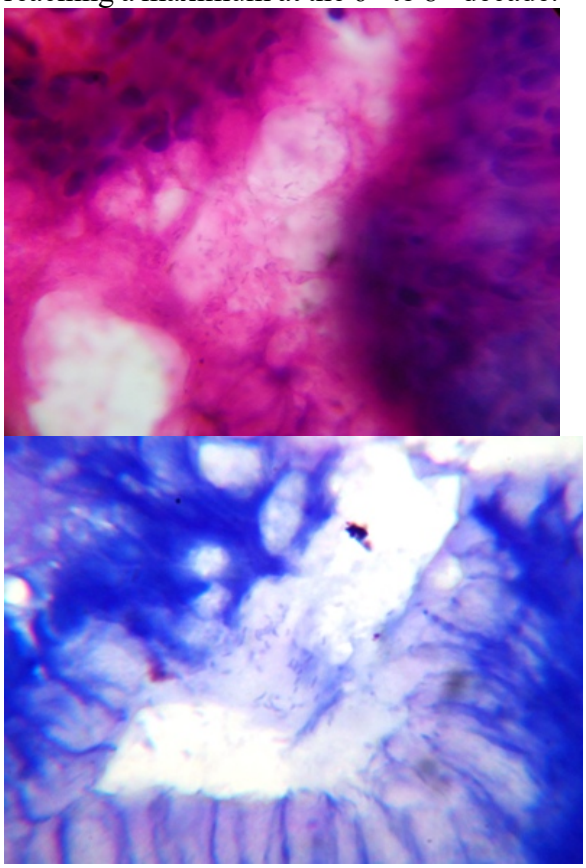

Figure 3&4: Photomicrograph showing Helicobacter pylori in gastric pit and gastric gland

Various pathological manifestations were seen which are discussed below. One such is chronic inflammatory infiltrate was

composed of lymphocytes, plasma cells and eosinophils, which was seen in the lamina propria in varying degree. Among the 74 cases of chronic superficial gastritis, mild degree of infiltrate was seen in 19 (25.67%) cases, moderate degree in 29 (39.18%) cases and severe in 26 (35.13%) cases. Activity is characterised by neutrophilic infiltrate and decreased mucus, were seen in 20 (27.02%) of the 74 cases of chronic superficial gastritis. Lymphoid aggregates and lymphoid follicles were noted in 24 (32.43%) cases. Intestinal metaplasia with goblet cells was seen in 17 (22.97%) cases. Atrophy with a decrease in the number of glands was noted in 10 (13.51%) cases. 7 cases (36.84%) of 19 cases of chronic superficial gastritis with mild inflammatory cell infiltrate were positive for H.pylori, whereas 15 (51.72%) and 18 cases (69.23%) of chronic superficial gastritis having moderate and severe inflammation respectively were positive for H.pylori. This observation indicates that H.pylori infection is associated with increase in the severity of chronic inflammatory infiltrate.

20 of 74 cases of chronic superficial gastritis showed activity. 17(85%) cases with activity were positive for H.pylori and was found to be statistically significant (P = 0.002). 24 of 74 cases of chronic superficial gastritis showed lymphoid aggregates. 17(70.83%) of these 24 cases with lymphoid aggregates were found positive for H.pylori.Among 74 cases of chronic superficial gastritis, 17 showed areas of intestinal metaplasia. 8 of these cases showed positivity for H.pylori but none of these cases were positive for H.pylori in the over the areas of intestinal metaplasia.

Among 74 cases of chronic superficial gastritis, 10 cases showed atrophic changes. 5 of these cases showed positivity for H.pylori. 40 of 74 cases of chronic superficial gastritis showed H.pylori. Of all cases positive for H.pylori in our study, 3 cases had a bacterial density of grade 3, fifteen patients had a bacterial density of grade 2 followed by 37 cases with a grade 1 bacterial density. (Table 3)

Table 3: Histological grading of chronic superficial gastritis by Sydney system

Mucosal changes	Number of cases	Percentage
Chronic inflammation		
Mild	19	25.67%
Moderate	29	39.18%
Severe	26	35.13%
Activity	20	27.02%
Lymphoid aggregates	24	32.43%
Intestinal metaplasia	17	22.97%
Atrophy	10	13.51%

Discussion

Helicobacter Pylori has been linked with many diseases, particularly several benign, premalignant, and malignant lesions of the digestive system including chronic gastritis, peptic ulcers, atrophic gastritis, intestinal metaplasia, gastric adenomas, gastric hyperplastic polyps, adenocarcinomas of the distal part of the stomach and lymphomas of mucosa-associated lymphoid tissue.[2, 3]

In our study; 100 patients with dyspepsia were evaluated on endoscopic gastric biopsies for histopathological gastric mucosal changes and H.pylori positivity. Of the 100 biopsies studied, 69 of them (69%) were male (n=69) and 31 were females (n=31) with male to female ratio of 2.22:1 which is similar to previous studies. There is no apparent reason as to why males would have greater exposure or greater susceptibility to infection than females. One reason suggested for the inconsistency

in results is that in certain populations H.pylori infections may be inadvertently eliminated because of more frequent antimicrobial treatment of women for urogenital tract infection.[11]

The age ranged from 25 to 85 years. The peak occurrence of gastric lesions was in sixth and seventh decades. Previous study reported showed that gastritis tends to increase with increasing age which is similar to our study where the incidence of H.pylori increased with age. A majority of our cases showed features of chronic superficial gastritis (n=74), a diagnosis of adenocarcinoma was made in 16 case. Among the rest, 3 cases were of normal histology and a diagnosis of gastric ulcer was made in 7 cases.

Among 74 cases of chronic superficial gastritis, 40 cases (54.05%) were found to be positive for H.pylori in the present study. Various studies have shown H.pylori positivity in the same range like our study. Studies done in England, USA, and Indonesia shows prevalence ranging from 45- 68% which is similar to our study[7]. Coming to the grade of gastritis in a study done by Misra et al showed H.pylori positive cases increased with the increasing grades of gastritis and the association was found to be statistically significant.[6] ,91Similarly in our study, the number of cases of H. pylori increased as the severity of the gastritis increased but was found to be statistically insignificant. Of the 74 cases of chronic superficial gastritis, 20 (31.25%) cases showed activity. Among the 20 cases, (85%) cases showed H.pylori positivity. This was in concordance with the other studies.[9]The inflammatory infiltrate in H.pylori associated gastritis is usually mixed mononuclear and neutrophilic. In more severe cases intraepithelial neutrophils may be seen in the surface epithelium and in the gastric pits as microabscesses. In our study, intraepithelial neutrophils were observed but none of the cases with active gastritis showed microabscesses.

Many studies done previously[7] showed presence of lymphoid follicles in H.pylori associated gastritis, in our study too similar to other studies, among 18 cases, 13 (72.22%) cases with lymphoid aggregates or lymphoid follicles showed H.pylori positivity, which was found to be statistically significant

Atrophic gastritis and intestinal metaplasia are presumed to be important stages in the development of gastric adenocarcinoma.[8] In our study, 17 cases of intestinal metaplasia were diagnosed. Of them, 8 (47.05%) cases showed positivity for H.pylori. Coming to gastric ulcer our study showed a positivity of 5 cases (71.42%) out of the 7 cases of gastric ulcer which is in concordance with the above previous studies[12]. In our study, 16 cases of adenocarcinoma were reported among which 9 cases (56.2%) were reported to be H pylori positive. Studies have shown that 60% to 80% of gastric cancers are related to the long-term presence of H. pylori which is in concordance with our study.[13, 14] among these cases, most common was intestinal type while only a minimal where diffuse type.

The specificity of Immunohistochemistry, modified Giemsa, toluidine blue and H&E were 100%, 90%, 90% and 80% respectively. [15]

Immunohistochemistry is an expensive and time-consuming technique with procedure length ranging from 1 hour to 24 hours. Obviously, since organisms can be easily identified in the immunoslides, therefore sensitivity and specificity was high. Modified Giemsa is a cheap, easily applicable stain that can be performed in 15 minutes. The results are reliable and the sensitivity and specificity values are acceptable. The lack of contrast is a disadvantage of the Giemsa technique but careful observation should allow identifying the organisms. Due to its low cost and the short hands-on time required for staining and very high sensitivity and

specificity combined with a high interobserver agreement, Giemsa stain is the best stain for the detection.[15] The modified Giemsa stain provided distinctive shape and uniform staining of the bacteria making their identification easy. Modified Giemsa stain described by Gray et al (1986) has been favored by many researchers because of its easiness to perform and availability in most histopathology laboratories.[16] In our study, demonstration of H. pylori was done using modified Giemsa stain.

Conclusion

Early diagnosis and eradication of H.pylori not only improves symptoms but also helps to prevent complications associated with H.pylori infection. To conclude based on the findings in of the present study non-invasive tests for detection of Helicobacter pylori may be preferred choice for clinicians but histopathological demonstration has the advantage of accuracy. At the same time it gives us chance to study associated histopathological changes. Some of these changes may be of prognostic value. H.pylori may show patchy colonization of gastric mucosa, varying from one gastric pit to another and might even be absent if biopsied tissue consists of intestinal metaplasia. Hence multiple biopsies at least 2 should be taken to locate, identify and quantitate the bacterium correctly.

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