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Histomorphological profile of Gastric antral mucosa in Helicobacter associated gastritis.

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Abstract

Background : *Helicobacter pylori* (*H. pylori*) infection has found to be associated with gastritis, non-ulcer dyspepsia, gastric adenocarcinoma and MALT associated lymphomas. Early detection and eradication of *H. pylori* infection is very essential to prevent pre-cancerous and cancerous lesions. **Aims:** The present study was planned to analyse the spectrum of the light microscopic histopathologic changes in gastric mucosa colonized by *H. pylori*. **Methods and Material:** Endocsopic biopsy samples of total 100 cases taken from the sites of antrum, were immediately fixed in 10% formalin and processed routinely. Special stains such as carbolfuchsin - light green and conventional Warthin – Starry silver stain were used for detection of *Helicobacter pylori*. The results and observations were tabulated and analysed for *H. pylori* with its histopathological significance. **Results:** Of all 100 cases, 64% which showed positivity for *H. pylori*. The significant histomorpholgical changes included epithelial degeneration, focal epithelial regeneration, mucosal hyperemia and edema and lymphoid. Inter-observer agreement was best with Light green stain when compared towarthin starry stain. The use of both the stains in combination increased the accuracy of *H. pylori* detection. The Light green stain was more economical and cheaper than the conventional silver staining techniques. **Conclusions:** A thorough clinical examination and history, followed by endoscopic evaluation and methods to detect presence of *H. pylori* organism becomes mandatory in the evaluation of case with symptoms of acid peptic disease. Use of combination of special stains increases the sensitivity of detection of the organism.

Keywords: Helicobactor, special stains, Gastric mucosa.

Introduction

Since 20th century, there were many reports of spiral organisms being isolated from the stomach of human beings and the possible role of these organisms in any human gastric disease. With the isolation of *H. pylori*, by J. Robin Warren and Barry J. Marshall, the flood gates opened to a new understanding of gastro duodenal pathology and its implications in the management of peptic ulcer disease.[1]It is a bacterial pathogen infecting the gastric antrum of half the population worldwide.

Helicobacter pylori (*H. pylori*) infection has found to be associated with acute and chronic gastritis,non-ulcer dyspepsia, peptic ulcers, gastric adenocarcinoma and gastric MALT associated lymphomas.[2] Early detection and eradication of *H. pylori* infection is very essential because it not only cause healing of gastric inflammatory lesion but also helps in reversal of precursor lesion which results in carcinoma such as gastric atrophy and intestinal metaplasia.[3]The Sydney system for

the classification of gastritis emphasises the importance of topographical, morphological and etiological information. This system was revised at the Houston Gastritis Workshop held in 1994. The histological severity of *H. pylori* density, inflammation, activity, atrophy and intestinal metaplasia were graded according to the updated Sydney system.[4]

The present study was planned to analyse the spectrum of the light microscopic histopathologic changes in gastric mucosa colonized by *H. pylori* in patients presenting to the outpatient department of medical gastroenterology.

Materials and Methods

This study was proposed and conducted in a Tertiary care hospital in Tamilnadu. A pilot study was done and approval of the ethical committee was obtained. Patients presented to the outpatient department of the Department of Medical Gastroenterology during the period of January 2010 to September 2012 were included in the study. A total of 100 cases presenting with features of acid peptic disease and GERD were examined. Exclusion criteria includes patients with acute gastritis, complications like bleeding and patient with gastric malignancy/esophageal malignancy.

Endoscopy was carried out using Olympus GIFSQ 30 video endoscopy system in the Department of Medical Gastroentrology. Endoscopy finding was recorded using a standard proforma. Biopsy samples was taken from sites of antrum of stomach .Concurrent examination of the esophagus was done in all these cases and biopsies were taken from them.

The specimens were immediately fixed in 10% formalin and processed routinely. Four microns sections were taken from both the tissues and stained with hematoxylin and eosin. Sections were also stained with special stain using carbol fuchsin and light green counter stain for detection of *Helicobacter pylori* and with the conventional Warthin –Starry silver stain for detection of Helicobacter pylori.

Each section was analysed for the presence of epithelial changes which includes surface irregularity, epithelial pits, drop out necrosis and micro-erosion. The histological changes such as inflammation, mucosal atrophy, neutrophilic activity, intestinal metaplasia and *H. pylori* status was graded according to the Sydney system. The results and observations were tabulated and analysed for their significance. An attempt was made to study the efficiency of a stain for demonstrating *Helicobacter pylori* in gastric biopsies using dilute carbol fuchsin-light green stain and comparison were made with that of warthin starry stain.

Results

Age and Gender distribution

In hundred cases of gastric biopsies studied, sixty nine cases (69%) were males and thirty one cases (31%) were females with male to female ratio of 2.22:1.Mean age of presentation of gastric lesions was 35.5 years. Of all ages ranged from 11 to 84 years, the peak incidence was seen in the 4th decade of life. (Figure 1)

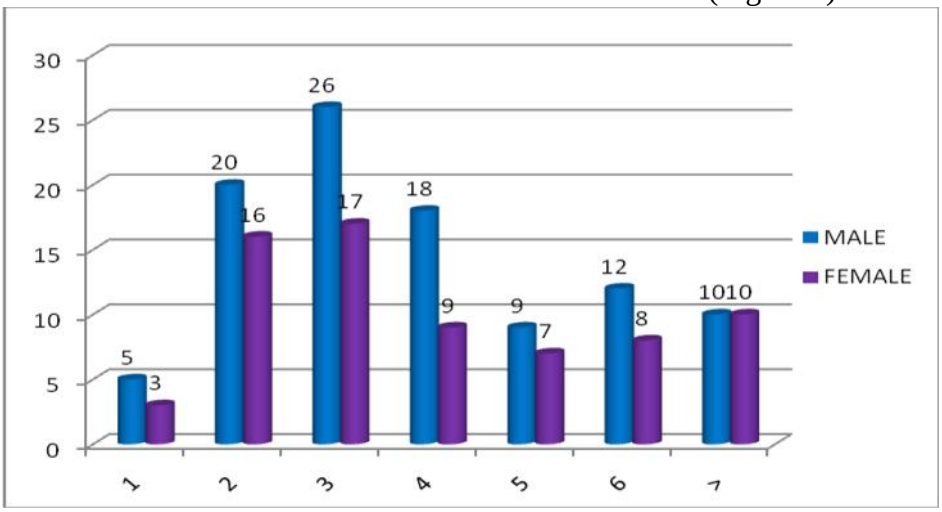

Figure 1: Age and Gender distribution in *H.pylori* induced Gastritis

H.pylori Gastritis

The age distribution of *H. pylori* showed peak incidence at fourth decade of life. Out of 26 patients seen in the 4th decade, 18 patients were found to be positive for *H. pylori* infection (Table 1). Among the 64 cases of Helicobacter pylori associated gastritis, 44 cases (68.75%) were males and 20 cases (31.5%) were females with a ratio of 2.2:1. This shows male predominance for *H. pylori* infection.

Table 1: *Helicobactor pylori* status in various age group

Age	No. of patient	*H. pylori* positive (%)	*H. pylori* negative
11-20	5	1	4
21-30	20	10	10
31-40	26	18	8
41-50	18	14	4
51-60	9	7	2
61-70	12	8	4
>70	10	6	4
Total	100	64	36

Histomorphological changes

In the present study, 27 cases with mild inflammation, 47 with moderate inflammation and 15 cases with severe inflammation were seen. In correlating the H pylori positivity status with that of the type of inflammation, there were 33.3% cases showing positivity in mild inflammation, 87.23% cases showing positivity in moderate inflammation and 93.33 % cases showing positivity with severe inflammation. This showed that there were increased positivity rate with increase in the density of inflammation.

In the present study, the activity status were noted in 65 cases of which 55 cases [84.60%] showed positivity for *H. pylori*. Among 22 cases with intestinal metaplasia 72.7% [16 cases] showed positivity for *H. pylori* organism.

Histomorphological Changes in H. pylori associated Gastritis

Percentage no of cases showing mucosal hyperemia, edema, surface epithelial degeneration, focal epithelial regeneration, surface erosion, foveloar hyperplasia, lymphoid aggregates, lymphoid follicles, eosinophilic infiltration, atrophy and intestinal metaplasia were found and tabulated (Table 2).

Table 2: Distribution of Histomorphological Changes in *H. pylori* associated Gastritis

Nature of Lesion	No of cases
Mucosal Hyperemia	50
Mucosal edema	52
Surface epithelial degeneration	49
Focal epithelial regeneration	38
Surface erosion	54
Foveolar hyperplasia	38
Lymphoid aggregates	56
Lymphoid follicles	50
Eosinophilic infiltration	12
Intestinal Metaplasia	16
Atrophy	22
Total cases	64

Comparison of carbolfuchsin light green and warthin starry stain:

H&E stain is used conventionally to detect *H. pylori*. The present study experimented with simple carbol-fuschin light green staining and compared with the sensitivity and specificity with that of Warthin – Starry Silver stain. Of the total no of cases, 61 cases showed positivity by using Light green carbol fuschin staining technique and only 57 cases showed positivity by using Warthin –Starry staining technique (Figure 2 and 3). Also a comparison was made between Warthin starry and carbol fuschin light green stains about the relative cost, average time taken for staining and reproducibility of technique. It was found that Light green carbol fuschin was better than that of Warthin starry stain as it was cheaper, less time consuming, detection of the bacteria was also easier [pink spiral forms against green background].

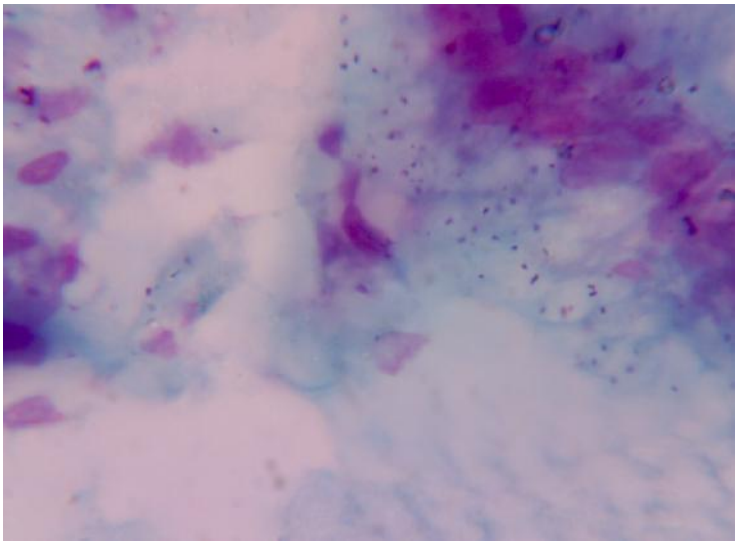

Figure 2: Carbol fuchsin and light green(X1000)- *H. pylori* infestation present in the epithelial surface

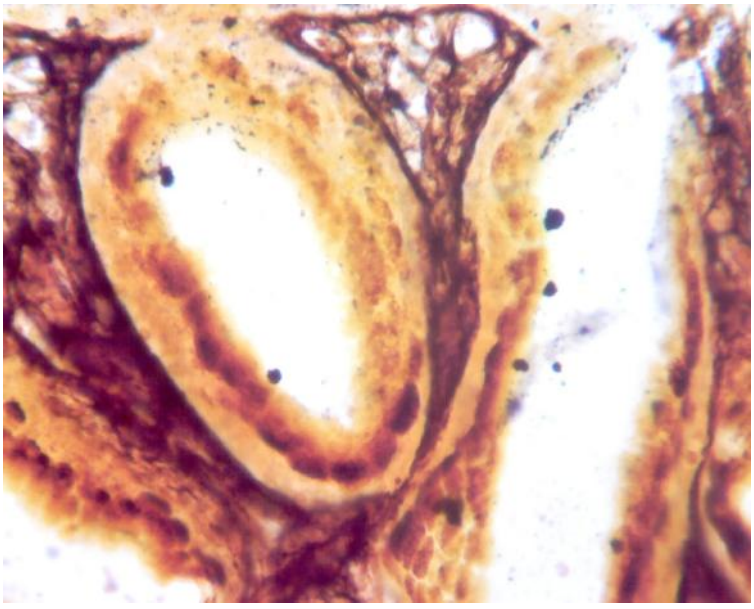

Figure 3: Warthin starry (X400) – *H. pylori* infestation present in the epithelial surface of the glandular lumen

Discussion

The present study was conducted on 100 cases presenting with symptoms of acid peptic disease to the out-patient clinic of Medical gastroenterology department in a tertiary care hospital. The endoscopic morphological studies and necessary biopsies were taken for the study.

The overall positivity of *H. pylori* in our study was 64% (64 cases out of 100 cases). This was close to the studies of Vijaya et al[5], Abbas et al[6] and Misra et al[7] where they found the positivity of *H. pylori* were 61.4%, 62.5 % and 62 % respectively. However Misra et al (2001)[7] had a higher positivity of 71% in his series of cases studied for the prevalence of *H. pylori* in gastric antral biopsies.

Present study population comprised of patients with age ranging from 11 to 84 (Mean age was 35.54%) and the maximum number of cases were belonging to 4th decade (26%), followed by 3rd decade with 20 cases. This can be comparable with the observations of Abbas et al[6] who had a mean age of 45 years and Csendes et al[8]with a mean age of 43 years. In the present study, in relation to gender distribution, men out-numbered women which can be comparable to the study of Regev et al[9] where the male female ratio was found to be 1.6:1.

Correlating the *H. pylori* positivity with the age of the individuals, there is a gradual increase in the incidence of *H. pylori* status with the age of the individuals. The positivity is 50% in the age group of 21-30 years, and it gradually ascends to 69% in the age group of 31-40 years with much higher percentage of 77.7% seen in age group of 41- 60 years. There is a slight fall in the positivity rate in individuals more than 60 years. This is similar to the observations of Vijaya[5],who found a peak positivity rate of 67.2% in age group of 31-40 years, followed by 63.8% in 50 to 59 years of age. She also had reported the fall in the positivity rate after 60 years. In her study she observed a very high percentage of 69.3% in patients belonging to age group of 20 to 29 years, which was not observed in the present study.

Activity was seen in 65 % of patients on gastric biopsy and it was made out that the positivity of the *H. pylori* with the activity status was statistically significant (84.5%). This was similar to the observations of Ahluwalla et al and Vijaya et al.[5,10]

Intestinal metaplasia was seen in 22 % of patients of dyspepsia on gastric biopsy. The positivity of the *H. pylori* in these group was found to be 72.7% which was in contrast to the study of Ahluwalla et al (25%).[10]

Glandular atrophy was seen in 33% of patients, of which 23 (69.6%) cases showed positivity for *H. pylori.* This can be correlated with the studies of Vatsala et al and Vijayaet al.[5,7] Many authors have observed that development and extension of atrophic gastritis was more commonly seen in *H. pylori* positive cases than in negative cases.[11-13]

Lymphoid aggregates were significantly more frequent in *H. pylori* positive cases (87.5%). This is in contrast to the study conducted by Amarapurkar et al[14], which failed to show a significant association between *H. pylori* infection and lymphoid aggregates. The present study, however, supported the findings of other studies on the subject.[15]

H. pylori may produce gastric epithelial cell damage directly or by stimulating host immune response. Adherence of the organism produces loss of microvilli and irregularity of the luminal border. It is also known to release vacuolatingcytotoxins and enzymes, including urease, which have toxic effects on the epithelium. *H. pylori* induces epithelial cells to release chemokines including GRO- and IL-8 which are chemotactic for neutrophils.IL- also causes neutrophils to produce reactive oxygen radicals (ROR) which cause tissue damage. Other factors released (RANTES and MIP-1) recruit monocytes and lymphocytes which also release pro-inflammatory mediators and act as antigen presenting cells to initiate specific immunity (T-lymphocytes and plasma cells).[16,17]

Comparison of staining Methods

Histological detection of *H. pylori* is considered as the gold standard test. Many investigators followed different staining methods to detect the organism such as modified Giemsa, Warthin – Starry, Gimenez, Genta and latest immunohistochemical H pylori antibody stains.[18] In the present study, two stains were used for identifying the bacteria in the endoscopic biopsies namely the conventional Warthin Starry silver stain and Light Green carbol fuschin stain. Similar to the studies of Young and Rotimi, the present study found that the Light Green Carbol fuschin stain could be used as an alternate for the conventional Warthin Starry silver stain or it could be used as an additional staining method for the detection of *H. Pylori*, as it was inexpensive, less time consuming and detection of the bacteria was easier.[18,19]

Summary and Conclusion

Infection with *Helicobacter pylori* has been established as the major cause of chronic gastritis and is important in the pathogenesis of other gastroduodenal diseases such as peptic ulcerations, gastric adenocarcinoma and gastric lymphoma. In view of this significance, an accurate diagnosis of the infection becomes essential to institute, eradication treatment in appropriate cases. Histological diagnosis of gastric lesions along with detection of *H. pylori* becomes a part of the protocol of work up in patients with symptoms of acid peptic disease. A thorough clinical examination and history, followed by endoscopic evaluation and methods to detect presence of *H. pylori* organism becomes mandatory in the evaluation of case with symptoms of acid peptic disease. Use of combination of special stains increases the sensitivity of detection of the organism. In conclusion, we have confirmed that the Light Green Carbol fuschin stain is a cheap, easy to perform and convenient histological means of identifying *H. pylori* in gastric biopsies.

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