Identification of helicobacter pylori infection in gastric biopsies: Prevalence and associated histopathology

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Abstract

Introduction: Helicobacter pylori (H. pylori) infection affects about 50% of the world population. It is considered as one of the most common chronic bacterial infection in human and a major concern today because of its relationships with gastrointestinal diseases.

Aim and Objectives: The study was conducted with the aim to determine the prevalence of H. pylori in gastric mucosal biopsies, to correlate the findings with age and sex, to identify the various types of histopathological changes associated with H. pylori positive gastric mucosal lesions.

Material and Methods: The retrospective study was conducted on 362 cases of gastric mucosal biopsy from January 2017 to December 2019. Histopathological examination of the gastric mucosal biopsies was done for presence of H. pylori and associated mucosal changes. The presence of H.pylori on haematoxylin and eosin was confirmed by giemsa stain.

Result: A total number of 362 gastric biopsies were analysed. H. pylori were positive in 148 cases of gastric biopsies, which were seen predominantly in males (58.11%). H.pylori was more prevalent in antral mucosal biopsies with 92.57% than other sites. The prevalence of H.pylori was high in third to fourth decades (38.51%). Morphological feature commonly associated with the organism was lymphoid follicle and the common gastritis was chronic active gastritis.

Conclusion: The study reveals that H. pylori infection was prevalent in 40.88 % of gastric mucosal biopsies. The presence of H. pylori was strongly associated with lymphoid follicles. Early identification and treatment for eradication of H.pylori is necessary to prevent gastric complications.

Keywords: Gastric mucosal biopsy, Giemsa stain, Histopathology, Helicobacter pylori, Lymphoid follicles

1. Introduction

Helicobacter pylori (H.pylori) is a spiral-shaped motile gram-negative and microaerophilic bacterium which colonizes the gastric epithelium [1]. The major cause of chronic gastritis is H. pylori infection [2]. It inhabits the stomach and affects almost 50% of the world’s population [3, 4]. This organism was first discovered by Dr. BarryMarshall and Robin Warren in the gastric mucosa of patients with gastritis and ulcers in 1982 [5, 6]. The low socioeconomic status, overcrowded living conditions and smoking are some of the risk factors associated with H. pylori infection [7, 8]. The production of urease by the microorganism helps it to proliferate and survive in the gastric environment and ability to adhere to the gastric epithelium [9]. Helicobacter pylori plays an important role in the pathogenesis of gastrointestinal diseases such as peptic ulcer disease, gastric adenocarcinoma and gastric lymphoma [10, 11, 12]. Infection with H.pylori may increase the risk of gastric lymphoma and 60% of cases evolve from chronic gastritis associated with H.pylori infection [2]. Its long-term colonization can provoke chronic inflammation and mucosal atrophy which can further lead to malignant transformation [13]. About 80% of population is infected with this bacterium in India and most of them are infected as early as 10 years of age [14, 15]. In India peptic ulcer disease especially duodenal ulcer disease is the most common manifestation of H. pylori infection [16]. Various diagnostic methods are available for the identification of H.pylori in gastric biopsies which aim for accurate detection of this organism. This includes non-invasive and invasive techniques [16]. Noninvasive methods include urea breath test (UBT), Stool antigen test and serology. Invasive techniques include Rapid Urea Test, histopathology, culture and Polymerase Chain Reaction (PCR) which
Require upper gastrointestinal endoscopy to obtain gastric biopsy samples [17]. Its presence can be easily diagnosed by rapid urease test at the time of upper gastrointestinal endoscopy. Rapid urease test is highly specific only when the bacteria density is high otherwise it produces false negative results [17]. But the histological examination is the gold standard for the diagnosis of H. pylori. [18,19] Culture can be done but it is a laborious and time consuming method. Histopathology is highly sensitive and specific especially in combination with special stains and immunohistochemistry [17]. The accurate detection of H. pylori is essential for managing infected patients and for eradicating the bacteria. This study was undertaken to find out the prevalence and various histopathological features associated with helicobacter pylori infection in gastric biopsies.

1.1 Aim and Objectives
1. To analyse the prevalence of H. pylori with the help of gastric endoscopic biopsies.
2. To correlate the values with age and sex of various H.pylori positive gastric mucosal biopsies
3. To identify the different histopathological features in gastric mucosa associated with H. Pylori positivity for better identification and treatment.

2. Material and Method
This retrospective study was conducted at Sri Muthukumaran Medical college Hospital and Research institute over a period of three years from January 2017-December 2019 and 362 gastric biopsies were received during this period. Data analysis was done from all individuals referred to outpatient as well as inpatient department who had undergone gastric biopsy with the help of upper GI endoscopy for various dyspeptic symptoms. These patients did not respond to proton pump inhibitor. The gastric mucosal biopsy specimens were usually taken from the cardia, fundus, body and antrum areas showing abnormal mucosal changes with the help of endoscopy and sent for histological examination. The biopsy specimens were fixed in 10% buffered formalin then processed and embedded in paraffin. The paraffin sections were cut at 4 micron thickness and stained with Hematoxylin and Eosin (H &E). The upper gastrointestinal endoscopic findings were recorded and were correlated with histopathological findings. These biopsies were examined for presence of H. pylori using H&E and its associated features. The special stain modified Giemsa stain was used to confirm H. pylori infection. The tissues were also studied for associated features like gastritis, atrophy, lymphoid follicles, intestinal metaplasia, foveolar hyperplasia, lymphoid follicles, ulcerations of gastric mucosa and reactive atypia. The presence of lymphoid follicles was observed in 41.22% of the biopsies, (Fig: 5) the rest mucosal biopsies showed chronic active gastritis (52.70 %) is characterized on the presence of glandular atrophy, intestinal metaplasia, foveolar hyperplasia, lymphoid follicles, ulcerations of gastric mucosa and reactive atypia. The age range of the total samples varied from 15 to 74 years. Of the total samples, 148 biopsies showed H. pylori positivity (40.88%) and 214 biopsies were negative for H. pylori. (Table: 1) It was more prevalent between 31 and 40 years. Age range distribution of H. pylori positive cases 21 to 30 age group showed 14.86%, 31 to 40 showed 38.51% of cases, 41 to 50 had 29.05% of cases, 51 to 60. Least number of cases was seen in age group of 71 to 80 with 0.68% cases. (Fig: 3) H. pylori positivity was present in 86 (58.11%) males and 62 (41.89 %) females of the total cases. (Table: 2) 141 cases of H. pylori positivity was identified by Haematoxylin and eosin stain which was confirmed by Giemsa and 7 cases was identified with the help of special stain Giemsa stain. (Fig: 4) Among the various gastric site of H pylori positive mucosal biopsies, antrum showed maximum number of H. pylori infection with 92.57 % followed by fundus biopsies and least was from body with 38.51%. H.pylori infection was identified with the help of special stain Giemsa stain and reported depending on it. The results and observation were analysed.

2.1 Inclusion Criteria
1. All non-neoplastic gastric endoscopic biopsies received in our department were included in the study.
2. Gastric biopsies showing H.pylori positivity were included.

2.2 Exclusion Criteria
1. Biopsies received with incomplete history.
2. All lesions of the oral cavity, pharynx oesophagus and duodenum.
3. All neoplastic gastric mucosal lesions.

3. Result
Total number of gastric endoscopic biopsies sampled from January 2017 to December 2019 was 378. For data analysis 362 cases met the inclusion criteria. Most of the biopsy samples were from antrum of the stomach. Out of the various types of gastric mucosal biopsies samples, antral mucosal biopsy was more prevalent about 58% (208 cases), the next common site of endoscopy biopsy was body mucosa with 102 numbers of cases, 30 cases from fundus and least was from cardia with 22 cases. (Fig: 1)There was male predominance with 209 numbers of males and rest females. (Fig: 2) The age range of the total samples varied from 15 to 74 years. Of the total samples, 148 biopsies showed H. pylori positivity (40.88%) and 214 biopsies were negative for H. pylori. (Table: 1) It was more prevalent between 31 and 40 years. Age range distribution of H. pylori positive cases 21 to 30 age group showed 14.86%, 31 to 40 showed 38.51% of cases, 41 to 50 had 29.05% of cases, 51 to 60. Least number of cases was seen in age group of 71 to 80 with 0.68% cases. (Fig: 3) H. pylori positivity was present in 86 (58.11%) males and 62 (41.89 %) females of the total cases. (Table: 2) 141 cases of H. pylori positivity was identified by Haematoxylin and eosin stain which was confirmed by Giemsa and 7 cases was identified with the help of special stain Giemsa stain. (Fig: 4) Among the various gastric site of H pylori positive mucosal biopsies, antrum showed maximum number of H. pylori infection with 92.57 % followed by fundus biopsies and least was from body with 1.35%. H.pylori was not identified in cardia biopsies in our study. (Table: 3) The gastric mucosal lesions were histopathologically characterized on the presence of glandular atrophy, intestinal metaplasia, foveolar hyperplasia, lymphoid follicles, ulcerations of gastric mucosa and reactive atypia. The presence of lymphoid follicles was observed in 41.22% of the biopsies, (Fig: 5) the rest mucosal biopsies showed changes with foveolar hyperplasia in 40 numbers of the cases. Glandular atrophy was observed in 19.59% of cases. Intestinal metaplasia was mostly focal in type which was seen in 10 cases. Ulcerated lesions and reactive atypia were diagnosed in 2.70 % of cases. (Table: 4) Inflammatory activity was most commonly seen in antrum when compared to other sites of biopsy. Chronic active gastritis (52.70 %) is the common inflammatory pattern associated with H. pylori infection followed by chronic persistent gastritis with 31.76 % of cases. Least form of gastritis was chronic follicular gastritis. (Table: 5)
Table 1: Histopathologically positive H. pylori cases

<table>
<thead>
<tr>
<th>Histopathological findings</th>
<th>Total no of biopsies</th>
<th>%</th>
</tr>
</thead>
<tbody>
<tr>
<td>H. pylori positive cases</td>
<td>148</td>
<td>40.88</td>
</tr>
<tr>
<td>Negative cases</td>
<td>214</td>
<td>59.12</td>
</tr>
<tr>
<td>Total no of cases</td>
<td>362</td>
<td>100</td>
</tr>
</tbody>
</table>

Table 2: Sex distribution of H. pylori positive biopsies

<table>
<thead>
<tr>
<th>Gender of the patient</th>
<th>No of patients</th>
<th>Percentage</th>
</tr>
</thead>
<tbody>
<tr>
<td>Male</td>
<td>86</td>
<td>58.11%</td>
</tr>
<tr>
<td>Female</td>
<td>62</td>
<td>41.89%</td>
</tr>
<tr>
<td>Total no of H. pylori cases</td>
<td>148</td>
<td>100</td>
</tr>
</tbody>
</table>

Table 3: Prevalence of H. pylori positivity in Gastric Mucosa site

<table>
<thead>
<tr>
<th>S.no</th>
<th>Site</th>
<th>No of cases</th>
<th>Percentage</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.</td>
<td>Cardia</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>2.</td>
<td>Fundus</td>
<td>9</td>
<td>6.08</td>
</tr>
<tr>
<td>3.</td>
<td>Body</td>
<td>2</td>
<td>1.35</td>
</tr>
<tr>
<td>4.</td>
<td>Antrum</td>
<td>137</td>
<td>92.57</td>
</tr>
<tr>
<td>Total</td>
<td></td>
<td>148</td>
<td>100</td>
</tr>
</tbody>
</table>

Table 4: Histopathologic findings of the gastric mucosa in patients with H. pylori infection

<table>
<thead>
<tr>
<th>S.no</th>
<th>Histological features</th>
<th>No of cases present</th>
<th>%</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.</td>
<td>Lymphoid follicles</td>
<td>61</td>
<td>41.22</td>
</tr>
<tr>
<td>2.</td>
<td>Foveolar hyperplasia</td>
<td>40</td>
<td>27.03</td>
</tr>
<tr>
<td>3.</td>
<td>Glandular Atrophy</td>
<td>29</td>
<td>19.59</td>
</tr>
<tr>
<td>4.</td>
<td>Intestinal metaplasia</td>
<td>10</td>
<td>7.67</td>
</tr>
<tr>
<td>5.</td>
<td>Ulcerated lesion</td>
<td>4</td>
<td>2.70</td>
</tr>
<tr>
<td>6.</td>
<td>Reactive atypia</td>
<td>4</td>
<td>2.70</td>
</tr>
<tr>
<td>Total</td>
<td></td>
<td>148</td>
<td>100</td>
</tr>
</tbody>
</table>

Table 5: Histological inflammatory pattern in H. pylori positive mucosal lesions

<table>
<thead>
<tr>
<th>Inflammatory pattern</th>
<th>No</th>
<th>%</th>
</tr>
</thead>
<tbody>
<tr>
<td>Chronic active gastritis</td>
<td>78</td>
<td>52.70</td>
</tr>
<tr>
<td>Chronic persistent gastritis</td>
<td>47</td>
<td>31.76</td>
</tr>
<tr>
<td>Chronic follicular gastritis</td>
<td>23</td>
<td>15.54</td>
</tr>
<tr>
<td>Total</td>
<td>148</td>
<td>100</td>
</tr>
</tbody>
</table>

4. Discussion

Helicobacter pylori infection is a major cause of many gastro-intestinal diseases [20]. Endoscopic examination followed by histopathology is now a day’s standard care for most patients with upper gastrointestinal symptoms. Helicobacter Pylori is one of the commonest bacterial infection in humans, affecting half of the world’s adult population [21]. It is the most common cause of chronic gastritis and has carcinogenic effect. It is high in developing countries and lower in the developed world. In this retrospective study, out of the total gastric biopsies received 362 cases of gastric mucosal biopsies were analysed. There was male predominance with 57.73% and female had 42.27% similar to study done by Kadam P.N et al. and Parvez Mujawar et al. which also showed male patients outnumbered females [22, 23]. Among the various gastric biopsies antrum was the common site for biopsy with 58%. Similar findings were found in studies done by Abilash SC et al., Krishnappa Rashmi et al. and Rupendra Thapa et al. [24, 25, 26]. The prevalence of H. pylori infection in gastric biopsies was 40.88% (148 cases). Parvez Mujawar et al. study showed 46.5% of positivity in their study [23]. Comparing the age distribution among the H. pylori
positive biopsies, third to fourth decade had more number of cases with 38.51% this finding correlated with the study done by Uppin MI et al., Parvez Mujawar et al. and Yakub MY et al.[7, 23, 27] Fourth to fifth decade of age was the second highest number of cases received. Of the H. pylori positive cases male patients were 58.11% which outnumbered the female cases. Parvez Mujawar et al. study also showed male predominance with 51.6% when compared to females[23]. Similarly Uppin MI et al., Shiva Raj et al., Raju Prasad et al. and Shrestha. R et al. study had more male cases than females[7, 11, 21, 28]. In our study H.pylori infection was seen commonly in antrum biopsies in about 92.57 % and second highest site of positivity was fundus. M Bhooma Reddy et al. study showed 85 % of H. pylori positivity in antral biopsies, next highest was fundus similar to our study[29]. Even in Trindade et al. study antrum was the commonest site for H pylori positivity[9]. Identification of H pylori was confirmed by histopathological examination using H and E staining and giemsa staining. (Fig 6) Haematoxyl and eosin staining showed 95.27 % positivity which was confirmed by giemsa staining. 7 cases were identified only with the help of giemsa stain. Similar findings were also made by Uppin MI et al. [7]. For the identification of H.pylori, H and E staining was more adequate but giemsa staining had advantage of identifying more organisms easily and number of cases identified was more. It is simple, easy and consistent staining method. Giemsa is superior to H and E in identifying H.pylori organism. Marcela S. Boldt showed findings similar to our study [30].

The data collected showed that the lymphoid follicles and collection of lymphocytes were present in 41.22 % of the H. pylori positive gastric biopsy samples. But 62 % of cases were present in Trindade et al; study [9]. Generally presence of lymphoid follicles is unusual in a normal or uninfected gastric mucosa. It appears within a week after the onset of acute infection by H. pylori[31]. So there is association between presence of lymphoid follicles and H. pylori infection in our study similar to Trindade et al. and Lee I. et al. study [9,32]. The presence of foveolar hyperplasia in the gastric mucosal biopsy was 27.03 % in our study commonly seen in antrum region. There is a link between foveolar hyperplasia and H. pylori in the presence of chronic inflammation in studies done by Trindade et al. [9] which was similar to our study. So presence of both lymphoid follicles and foveolar hyperplasia changes in the gastric samples has shown a significant association with H. pylori. Glandular atrophy was seen in 19.59 % cases in the present study but studies done by Poonam.S et al. and Garg B et al. showed only 12.36% and 12.33 % cases respectively which is less than our study [33, 34]. Intestinal metaplasia was present in 6.76% of gastric biopsies. Studies done by Poonam. S et al. and Aisook K et al. showed 7.87% cases and 8.2 % cases respectively [33, 35]. In the present study, ulcerative lesions and reactive atypia was found in 2.70 % of the H. pylori positive samples evaluated. Trindade et al. study showed higher prevalence of ulcerated lesions with 4.2% in their study [9]. Most of the biopsy samples from antrum and body showed chronic gastritis changes induced by H. pylori in concordance with Zhang RG et al. and Kusters JG et al. study [36, 37]. The gastric mucosal biopsy study showed chronic active gastritis with 52.70 % which was the most common histopathology findings. In KC Shiva Raj et al. and Shrestha. R et al study chronic active gastritis was common with 42.1%, 41.7% which is less prevalent than our study [11, 28]. Chronic persistent gastritis with 31.76 % was next common finding similar to KC Shiva Raj et al. and Shrestha. R et al. with 38.2% in their study [11, 23]. 15.54 % of chronic follicular gastritis cases were present in our study and Shrestha. R et al. study had 18.4% higher than our study [29]. Patients with chronic active gastritis had higher frequency of Helicobacter Pylori positivity in our study.

5. Conclusion

In our study, the prevalence of H. pylori was detected among the patients with gastrointestinal symptoms was 40.88%. H. pylori infection was higher in third to fourth age group patients. Male preponderance was seen in our study. The H. pylori colonization was seen more in antral mucosa than other sites. Histopathological finding commonly associated with H. pylori positivity was lymphoid aggregates and chronic active gastritis. All gastric mucosal biopsies should be routinely checked for presence of H. pylori and associated changes as they are prone to develop gastric cancer. Early and definitive diagnosis of H. pylori organism by histopathology is necessary which helps to initiate proper antibacterial therapy for the eradication of the bacilli. It also helps to control it and also for better patient recovery. The percentage of H. pylori infection present in our institution reflects that it is one of the major public health problems which have to be analysed for the benefit of patient’s wellbeing.

6. References


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