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Research Article

REVEALING THE FREQUENCY OF HELICOBACTER PYLORI INFECTION IN GASTRIC LESIONS BIOPSIES BY HISTOPATHOLOGICAL STUDY

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Abstract:

Helicobacter pylori (HP) has recently been associated with gastritis, dysplasia and gastric neoplasms. The study was performed to see histopathological study of gastric biopsies with HP to evaluate different gastric lesions.

***Objective:** To determine the frequency of H. pylori in patients with gastric disorders and to see the frequency of different morphological changes in gastric biopsies associated with H. pylori infection.*

***Study Design:** A descriptive cross-sectional study.*

***Place and Duration:** In the Pathology department of Mayo Hospital, Lahore in Collaboration with Gastroenterology department for One year duration from May 2018 to May 2019.*

***Methods:** The study was performed on 760 gastric biopsy specimens collected in the gastroenterology department. Biopsies were sent to pathology laboratory in containers filled with 10% buffered formalin. Tissues were processed in an automated tissue processor. Sections were stained with routine hematoxylin and eosin and Giemsa.*

***Results:** Of 760 patients, 527 (69%) were positive for H. pylori and 233 (31%) were negative for H. pylori. Gastric lesions were more common in males 337/589: 57%, 252/589: 43% compared to women, a significant difference ($p = 0.000$). In men, gastric complaints and dyspeptic symptoms were seen before females (mean age 38.11 ± 16.66 years and 35.81 ± 14.19 years), and a positive relationship with patient age ($p < 0.05$: 95% confidence interval). Histological examination of 760 gastric biopsies revealed acute and chronic gastritis in 89.5% of the patients. A significant number of gastric lesions, ie, intestinal metaplasia ($p = 0.01$), lymphoid follicle formation ($p = 0.059$), but dysplasia (15/527, 2.8%) were observed in patients with PD, and non-significant malignancy in patients infected with HP (16 / 527: 3%). However, gastritis and gastric atrophy were observed in both gastritis.*

***Conclusion:** Significant gastric lesions were observed in HP positive biopsies compared with HP-free gastritis, but dysplasias and malignancies were not significantly associated with HP biopsies versus HP negative biopsies.*

***Key words:** Helicobacter pylori, glandular atrophy, intestinal metaplasia, lymphoma, malignancy.*

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INTRODUCTION:

In the 1980s, *Helicobacter pylori* was discovered to be a gram-negative spiral bacteria that lived in various parts of the stomach and duodenum, and much was learned about this bacterium and its related disease states [1]. The NIH consensus conference in 1994 documented *H. pylori* as a cause of duodenal and stomach ulcers. Later the International Cancer Research Agency (IARC) announced that for gastric adenocarcinoma *H. pylori* was a group I human carcinogen [2]. "At least HP has affected 50 percent of population making it the maximum communal infection globally. The third world has much advanced infection ratio than in the West, with rates assessed to be around twenty percent and HP prevalence decreased [3]. Risk factors that play a role in the pathogenesis of *Helicobacter pylori* are: low socioeconomic level, agglomeration, lack of hygiene, diet, alcohol consumption, occupational exposure, smoking, family history of gastric diseases and poor water status [4]. The fecal-oral route is considered an important route for transmission [5]. In all countries; Infections are usually attained in early childhood. It is reported to be present in 69% of the population with gastritis in Pakistan [6]. Among these patients (69%), superficial gastritis (inflammation) was 60.87% and atrophy of the glands was 19.56% [7].

MATERIALS AND METHODS:

A cross-sectional analytical study was performed in cooperation with the Medicine department and Endoscopy Unit in 760 gastric biopsies at the Department of Pathology Mayo Hospital, Lahore in Collaboration with Gastroenterology department for One year duration from May 2018 to May 2019. Male and female patients aged 12 to 70 years with dyspeptic symptoms undergoing antral biopsy were included. The study was approved by the University ethics committee. All patients with gastric complaints and assisted in endoscopy were included in the study. It was observed before endoscopic examination. Antral biopsies were taken by a medical consultant in medical services. Biopsies were then sent to the pathology laboratory in 10% of formalin-filled containers. Slides were prepared for H & E, Giemsa and silver stains. All slides were examined by an experienced pathologist under a light microscope. They looked for the presence or absence of *Helicobacter Pylori*, inflammation of the gastric glands and atrophy. The final results of the study were compiled on the basis of histopathology. If the results of all tests were negative, patients were considered negative for *H. pylori*. After biopsy staining with Geimsa stain and staining with optical eosin, it will be considered positive for *Helicobacter*

pylori even if unique curved bacteria are observed in the stomach glands under an optical microscope. The slides were stained under optical microscope with Giemsa and hematoxylin / eosin and the presence of inflammation was confirmed after the infiltration of lymphocytes, plasma cells, neutrophils in the lamina propria. "Sydney system gave No (score, 0), mild (score, 1), moderate (score, 2) or marked (score, 3) score in inflammatory cells. Number scores were obtained by adding individual scores. For infiltration of mononuclear and polymorphonuclear cells (Points from 0 to 6) ". Atrophy of the gastric glands was marked as positive when the glandular thickness at biopsy decreased compared to the thickness of the entire mucosa, as observed on the optical microscope. Proficiency for gastric atrophy, 0; Slight focus (1-2), 1, where several gastric glands disappear or are replaced by the intestinal type epithelium; 2, small areas where the gastric glands disappear or are replaced by the intestinal type epithelium; medium (3-4); 3, up to 25% of gastric glands lost or altered with intestinal type epithelium; 4.25-50% of gastric glands were lost or altered with intestinal type epithelium; more than 50% of gastric glands lost or replaced by severe (5-6) 5, intestinal type epithelium; 6, there are only a few small areas of the stomach glands. Blood samples were taken just before endoscopy; Serum was immediately separated and analyzed for antibodies against *H. pylori* (HM-CAP, Enteric Products and Westbury, N.Y.). A positive serological test for *H. pylori* was identified with a titer of 1.8 or higher. Data were entered and analyzed with SPSS version 18, a software and computer program for data analysis. For variables such as the age of the patients, mean and standard deviation (SD) were calculated. Genus, *Helicobacter Pylori* (positive or negative), inflammation (Yes, No) and atrophy of the glands (Yes, No) were calculated as frequency and percentage. Fisher's exact test was used to compare male and female injuries.

RESULTS:

The mean age of patients with gastric pain and endoscopic biopsy was 37.38 ± 15.93 years. The majority of patients were between 21 and 40 years of age. In our study, 760 biopsies, 427 (56%) were male patients and 333 (44%) were female. The ages of the males ranged from 18 to 88 years with a mean age of 42 ± 38.13 . The ages of the women ranged between 16-87 years and the mean age was 42 ± 32.49 years. This shows that men experience stomach complaints and dyspeptic symptoms in women and present them to a doctor abroad (Table 1-2).

Table 1: Age and Gender Distribution of gastric lesions positive Helicobacter Pylori (n= 760)

| Age range in years Mean age=37.38±15.93 | Number of cases (n=760) | | Morphological Lesions n=589 (77.5%) | | | | |
|--|-------------------------|-----------------------|-------------------------------------|----------------------|------------|------------|--------|
| | Males | Females | Gastritis | | | Percentage | |
| | | | Male | Female | Total | Male | Female |
| 11-20 | 66 | 51 | 60 | 46 | 106(18%) | 91% | 90% |
| 21-30 | 126 | 97 | 121 | 92 | 213(36%) | 96% | 94% |
| 31-40 | 94 | 84 | 90 | 79 | 169(29%) | 96% | 94% |
| 41-50 | 54 | 42 | 34 | 16 | 50(8%) | 62% | 36% |
| 51-60 | 38 | 30 | 15 | 11 | 26(4%) | 39% | 36% |
| 61-70 | 33 | 18 | 12 | 5 | 17(3%) | 36% | 26% |
| < 70 | 16 | 11 | 5 | 3 | 8(1.5%) | 29% | 27% |
| Mean and SD Male=38.11333±16.66 Female=35.81±14.19 | 427 (56%) 42±38.13 | 333 (44%) 42±32.49 | 337(57%) 30.75±15.73 | 252(43%) 19.42±23 | 589(77.5%) | - | - |

A total of 589 (77.5%) patients were positive for gastritis. Of these biopsies, 427 (56%) were 42 ± 38.13 males and 333 (44%) were 42 ± 32.49 females. According to gender, 337 (57%) males were infected with 30.75 ± 15.73 and females 252 (43%) by 19.42857 ± 23 by H. Pylori. Statistically, there was a

significant difference in the number of infected men and women (p = 0.001). However, there was no difference between the ages of the patients in men and women (38.11 ± 16.66 years, mean age of men and 35.81 ± 14.19 years of H.Pylori-infected women (Table I-2).

Table 2: Relationship between variables related to the patients studied for H.Pylori Induced Gastritis in both sexes

| Characteristic | Patients | | |
|--|----------------------|--------------------|----------------------------|
| | Male (n = 427) | Female (n=333) | P value |
| No. of men: no. of women | (56%) | (44%) | 0.000 (Highly significant) |
| Age range (yr) | 18-87 | 16-85 | |
| Mean ± SD age (yr) 37.38±15.93 | 38.11±16.66 | 35.81±14.19 | <0.05 (significant) |
| No of patients with Gastritis (589: 77.5%) | 337(57%) 30.75±15.73 | 252(43%)19.42857±2 | 0.000(Highly significant) |
| No.(%) of patients with H.Pylori (527=69%) | 306 (58%) | 221 (42%) | 0.00 (Highly significant) |
| Inflammation (472/527: 89.5%) | 252 (54%) | 218 (46%) | 0.161 (Significant) |
| Gastric Atrophy (27/527= 5%) | 18 (66.6%) | 9 (33.4%) | 0.014 significant |
| Intestinal Metaplasia (57/527=11%) | 37 (65%) | 20 (35%) | 0.001(Highly significant) |
| Lymphoid follicles (28/527= 5%) | 18 (64%) | 10 (36%) | 0.030 (Significant) |
| Dysplasia 15/527 (2.8%) | 9 (60%) | 6 (40%) | 0.233 (Not significant) |
| Malignancy 16/527 (3%) | 10 (62.5%) | 6 (37.5%) | 0.144 (Not significant) |
| Signet ring variant adenocarcinoma9/16 (56%) | 4(44.5%) | 5(55.5%) | 0.408 (Not significant) |
| Intestinal Variant Adenocarcinoma 3/16 (19%) | 3(100%) | 0 | 0.608 (Not significant) |
| MALT Lymphoma 4/16 (25%) | 3(75%) | 1(25%) | 0.511 (Not significant) |

Inflammation involving neutrophils, lymphocytes and plasma cells was observed with H. pylori in gastritis 472/527 (89.5%), and 95% (59/62) of 589 biopsies were observed in uninfected gastritis, with no statistically significant difference p = 0.074. If we compare the presence of inflammation in the presence of H. pylori infection, we concluded that inflammation is present even in the absence of H. pylori infection.

Atrophy of the glands manifested by a decrease in the thickness of the mucosa and an increase in the distance between the glands. It was observed in 5% (27/527) of biopsies with gastric lesion infected with H. Pylori and in 5% (3/62) of non-infected gastritis. Atrophy was observed in 18 men and 9 women with H. Pylori positive bacteria. Atrophy was higher in males than in females (p = 0.014) (Tables II-III).

Table 3: Frequency and percentage of different pathologies in gastric biopsies (n=760)

| Lesions (589) | In <i>H.Pylori</i> Positive cases | | In <i>H.Pylori</i> Negative cases | |
|-----------------------|-----------------------------------|------------|-----------------------------------|-------------|
| | Cases | Prevalence | Cases | Prevalence |
| H.Pylori infection | 527/760 | 69% | 62 | 8% |
| Inflammation | 472/527 | 89.5% | 59 | 95% P=0.074 |
| Atrophy of Glands | 27/527 | 5% | 3 | 5% 0.610 |
| Intestinal Metaplasia | 57/527 | 11% | 1 | 1.6% 0.01 |
| Lymphoid Follicles | 28/527 | 5% | - | 0% 0.041 |
| Dysplasia | 15/527 | 3% | - | 0% |
| Malignancy | 16/527 | 3% | 1 | 1.6% 0.000 |
| Ulcers | 13/527 | 2.5% | - | 0% |

Lymphoid follicles are not a normal component of the gastric mucosa. Follicles were seen in 5% (28/527) of biopsies. 18 of them were men and 10 were women. There was a significant difference in both sexes ($p = 0.03$). Most of the patients were over 30 years old. In all these cases *H.Pylori* was present; No such histological lesion was seen in gastritis without *H.Pylori*. There was a significant difference in both types of gastritis. (Tables II-III). Intestinal metaplasia was characterized by the presence of goblet cells and absorptive cells in the antrum of the stomach (57/527 = 11%). There were 37 (65%) female and 20 (35%) men with biopsies with intestinal metaplasia. 57 biopsies found *H.Pylori*. Only one case had intestinal metaplasia with *H. pylori* infection. The difference between the two types of lesions was significant ($p = 0.01$) (Tables II-III). Pleomorphism, nuclear atypia and hyperchromasia of gastric mucosal cells were observed in 15 of 527 patients (3%) and classified as positive for dysplastic changes. Dysplasia seen in 9/15 (60%) of males and 6/15 (40%) of females. *Helicobacter* was present in all (100%) biopsies showing dysplasia. Malignancy was observed in 16/527 (3%) biopsies. Gasket ring / diffuse type adenocarcinoma was marked due to the presence of scattered isolated seal cells containing intracellular mucin. 9/16 malignant cases were seal ring type. 6/16 cases of intestinal type adenocarcinoma were reported. It showed pleomorphic, hyperchromatic cells secreting mucus. Some of the cells calcified. 4/16 cases were lymphomas showing a monotonous population of infiltrating malignant lymphocytes. Interestingly, 6 of the women (37.5%) and 10 (62.5%) had a malignant change in male biopsy. Only one case of malignancy without *H. pylori* was observed in nonspecific gastritis; 94% (16/17) of malignant biopsies showed *Helicobacter* infection. It was statistically significant. Only 13 (2.5%) of the biopsies showed ulceration of the gastric mucosa, 160 were normal and 11 were labelled as insufficient due to insufficient tissue showing mucosa or submucosa (Table II).

DISCUSSION:

In our study, the frequency of this infection was 69%. Of these, 58% (50/81) and 42% of women were infected with *H.Pylori*. It suggests that men in our population may be more susceptible to *H. pylori* infection than other populations [8]. The percentage of infected male population is almost the same as that of Korean subjects. The mean age of the infected male patients was 38.11 ± 16.66 years and the mean age of the women infected with *H. pylori* was 35.81 ± 14.19 years. This was statistically significant ($p < 0.05$) [9]. In the 21-30 age group, infection was present in 36% of the patients, and *H.Pylori* was present in 29% of the biopsies in the 31-40 group [10]. These two age groups showed a relatively high incidence of *H. pylori* infection [11]. International studies are consistent with the fact that the third world population has acquired infection in childhood and early adulthood. As in our population, health awareness and presentation to health facilities are not a priority; Patients may be carriers of infection long before they are presented in hospitals. Early onset of infection can seriously affect the outcome of the infection, as recommended by Yous and colleagues in China¹². Previous studies have shown high prevalence of *H. pylori* in developing countries such as Pakistan. Seroprevalence exceeds 51-58% of our general population. In Karachi, 67% of 9-month-old infants showed *H. pylori* infection. A study by Yakoob J et al. On patients positive for *H. pylori* concluded that 63% were males and 37% were females with a mean age of 45 ± 16 years. However, the prevalence of *H. pylori* infection with positive CagA (virulent strain) was 56%, although more than 70% observed in neighboring countries such as Iran, India and Bangladesh [13]. It is now well known that strains having a Cag secretion system that converts bacterial effector CagA into host cells are known to increase the risk of cancer. Georgia, a developing country, is infected with 70% of the adult population. In our study, intestinal metaplasia was observed in 9.3% of the cases. *H.Pylori* was observed in 92% of intestinal metaplasia cases. The Georgian population had *H. pylori* in 35% of metaplasia cases [14]. This is a significant percentage. Yakoob J et al. found

intestinal metaplasia in 2% of the population in Karachi at the Aga Khan Hospital. This number is very low compared to a study conducted in Taiwan on 611 patients who were positive for *H. pylori* and 21% were positive for intestinal metaplasia. This may be due to a larger sample size or a lower risk in our population. Another study showed that 24% of the subjects in Japan had metaplasia. In our study, intestinal metaplasia ($57/527 = 11\%$) was more common in male 37 (65%) than 20 female (35%) and there was a statistically significant difference between both genders ($p = 0.001$). This difference was significant between the two types of gastritis ($p < 0.01$). No lymphoid follicles were observed in negative HP gastritis, but formation of lymphoid follicles with positive statistical difference was observed in 28/527 (5%) positive HP gastritis ($p = 0.041$)¹⁵. This difference was significant between the two genders ($p = 0.03$), and the HP-related malignant cases were 16/527 (3%). There was no statistical difference between men and women. Thirteen patients had 03 biopsies representing gastric cancer and lymphoma. When we compare different types of cancer, we found that the common type of carcinoma has 9/16 (56%) malignancy and is therefore predominant.

CONCLUSION:

Gastritis caused by *Helicobacter pylori* is more common in our population. In addition, HP-positive biopsies showed significant gastric lesions when compared with HP-free gastritis. Dysplastic and malignant gastric lesions were not significantly associated with HP-carrying biopsies versus HP-negative ones.

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