

Histopathological study of Helicobacter Pylori bearing Gastric Biopsies for Gastric Lesions

MULAZIM HUSSAIN BUKHARI, SAMINA QAMAR*, SHAHBAZ AHMAD QURESHI*, MALIK MAQSOOD ANWAR**

ABSTRACT

Background: *Helicobacter pylori*(*HP*) recently are associated with gastritis, dysplasia and gastric malignancies. The study was conducted to see the histopathological of *HP* bearing gastric biopsies for evaluate different gastric Lesions

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

Material and Methods: A descriptive cross sectional study was conducted on 760 gastric biopsy specimens collected from gastroenterology of Mayo Hospital Lahore. Biopsies were sent to the pathology lab of King Edward Medical University in 10 % buffered formalin filled containers. The tissues were processed in automatic tissue processor. The sections were stained with routine Haematoxylin and Eosin and Giemsa stain.

Results: Of the 760 patients, 527 (69%) were positive for *H. pylor* and 233 (31%) were negative for *H. pylori*. The gastric lesions were more common in males' 337/589:57% as compared to females' 252/589:43% with a significant difference ($p=0.000$). The males experienced gastric complaints and dyspeptic symptoms earlier than females (mean age 38.11 ± 16.66 years & 35.81 ± 14.19 years) with a positive association with patient's age ($p<0.05$: 95% confidence interval). Histological examination of 760 cases of gastric biopsies revealed acute and chronic gastritis in 89.5% of patients. A significant number of gastric lesions were seen in patients bearing *HP*, i.e., Intestinal metaplasia ($p=0.01$), formation of lymphoid follicles ($p=0.059$), However dysplasia was seen in (15/527; 2.8%) and malignancy (16/527:3%) in patients of gastritis infected with *HP* at non significant level. However, gastritis and gastric atrophy was seen in both types of gastritis.

Conclusion: A significant gastric lesions were seen in biopsies positive for *HP* as compared to *HP* free gastritis, however dysplasia and malignancies were non significantly associated with *HP* bearing biopsies vs *HP* negative ones.

Keywords: *Helicobacter Pylori*, Glandular Atrophy, Intestinal Metaplasia, Lymphoma, Malignancy.

INTRODUCTION

Helicobacter pylori was discovered in 1980s, a gram negative spiral bacterium that inhabits various areas of the stomach and duodenum, much has been learned about this bacteria and its associated disease states(1). "In 1994, the NIH Consensus Conference recognized *H. pylori* as a cause of gastric and duodenal ulcers. Later that year, the International Agency for Research on Cancer (IARC) declared *H. pylori* to be a group I human carcinogen for gastric adenocarcinoma"².

At least half of world's population is infected with *HP*, making it the most widespread infection in the world³. Third world has much infection higher rates than the west, where the rates are estimated to be

around 25% and the prevalence of *HP* is declining⁴.

Risk factors involved in the pathogenesis of *Helicobacter pylori* are low socioeconomic status, crowding, poor hygiene, diet, alcohol consumption, occupational exposure, smoking, family history of gastric diseases and poor water condition. Fecal-oral route is considered to be an important route for its transmission⁵. Infections are usually acquired in early childhood in all countries⁶. In Pakistan, it is reported to be present in 69% of population having gastritis. Out of those (69%) patients, superficial gastritis (Inflammation) was noted in 60.87% and atrophy of glands in 19.56% of patients⁷.

MATERIAL AND METHODS

An analytical cross sectional study was carried out on 760 gastric biopsies at the Department of Pathology, King Edward Medical University, Lahore in collaboration with Endoscopy unit of Medical Department of Mayo hospital Lahore. For this study

*Departments of Pathology and Medicine King Edward Medical University, Lahore

^{*}Dept. Of Gastroenterology, BV Hospitalk, Bahawalpur

^{**} Department of gastroentology, Ireland (Dublin)

Correspondence to Dr. Mulazim Hussain Bukhari Project Director and Professor of Pathology, Director PhD Program Email: nmulazim.hussain@gmail.com>

non probability purposive sampling technique was applied. Male and female patients with dyspeptic symptoms who underwent antral biopsies of 12-70 years ages were included in the study.

The study was approved by ethical committee of the University. All patients with gastric complaints and attending for endoscopies at four medical wards of Mayo Hospital, Lahore were enrolled in the study from February 2010- Dec 2010. Before endoscopic examination were noted. Antral biopsies were taken by consultant physician in medical wards. Biopsies were then sent to the pathology lab of King Edward Medical University in 10 % buffered formalin filled containers. Slides were prepared for H& E, Giemsa and silver stains. All slides were examined by an experienced pathologist under light microscope. They searched for presence or absence of Helicobacter Pylori, Inflammation and atrophy of glands of stomach. The final results of study were compiled on the basis of histopathology.

The patients were considered *H. pylori* negative if the results of all tests were negative. Biopsies will be regarded as positive for *Helicobacter pylori* even if single curved bacteria is seen in the mucosal lining or in glands of stomach, under light microscope after staining the biopsy with Geimsa stain and Haematoxylin Eosin stain.

Presence of inflammation was confirmed after seeing the Giemsa and Haematoxylin/Eosin stained slides under light microscope and seeing the infiltration of lymphocytes, plasma cells, neutrophils in lamina propria. "The scoring was done by Sydney system for inflammatory cells none (score, 0), mild (score, 1), moderate (score, 2), or marked (score, 3). The numerical scores were derived by summing the single scores for mononuclear and polymorphonuclear cell infiltration (scores from 0 to 6)". Atrophy of gastric glands was labeled in a biopsy as positive when glandular thickness is decreased as compared to thickness of whole mucosa, as seen under light microscope. Grading for gastric atrophy was done as such 0, none; Mild (1-2), 1, foci where a few gastric glands were lost or replaced by intestinal-type epithelium; 2, small areas in which gastric glands had disappeared or been replaced by intestinal-type epithelium; moderate (3-4); 3, up to 25% gastric glands lost or replaced by intestinal type epithelium; 4, 25-50% of gastric glands lost or replaced by intestinal-type epithelium; sever (5-6) 5, more than 50% of gastric glands lost or replaced by intestinal-type epithelium; 6, only a few small areas of gastric glands remaining⁹.

Blood was sampled immediately before endoscopy; serum was immediately separated and was assayed for antibodies against *H. pylori* (HM-CAP, Enteric Products, and Westbury, N.Y.). A

positive serologic test for *H. pylori* was defined as one with a titer of 1.8 or more.

The data was entered and analyzed with SPSS version 16, a computer software & program for data analysis. For variables such as age of the patients, mean and standard deviation (SD) were calculated. Gender, *Helicobacter Pylori* (positive or negative), inflammation (Yes, No), and atrophy of glands (Yes, No) were calculated as frequency and percentage. Fisher Exact test was used to compare lesions in males and females. The Fishure exact test (for sex, diagnosis, grade of gastric mucosal atrophy, distribution of gastritis, and presence or absence of intestinal metaplasia).

RESULTS

The mean age of patients presenting with gastric complaint and undergoing endoscopic biopsy came out to be 37.38 ± 15.93 . Most of the patients ranged between 21-40 years of age. In our study, out of 760 biopsies, 427(56%) biopsies were from male patients and 333 (44%) were from females. Males ranged from 18-88 years of age with a mean of 42 ± 38.13 years. While, female ranged from 16-87 years with a mean of 42 ± 32.49 years. This showed that males experienced gastric complaints and dyspeptic symptoms earlier than females and presented to medical outdoors (Table 1-2). A total of 589(77.5%) patients turned out to be positive for presence of gastritis. Out of these biopsies, 427 (56%) 42 ± 38.13 were males and 333 (44%) 42 ± 32.49 were female patients. On the basis of gender separation 337 (57%) 30.75 ± 15.73 of males and 252 (43%) 19.42857 ± 23 of females were infected with *H.Pylori*. Statistically, there was significant difference in the number of infected male and females ($p=0.001$). But there was no difference in the age of patients in males and females (38.11 ± 16.66 years mean age of males and mean age of females that were infected with *H.Pylori* was 35.81 ± 14.19 years (Table I-2).

Inflammation comprising of neutrophils, lymphocytes and plasma cells was observed in 472/527 (89.5%) gastritis with *H.pylori* and 95%(59/62) in non infected gastritis of 589 biopsies, there was no statistical difference $p=0.074$. If we compare the presence of inflammation with presence of *H.Pylori* infection, we conclude that inflammation was present even in the absence of *H.Pylori* infection.

Atrophy of glands was manifested by decreased thickness of mucosa and increased distance between glands. It was seen in 5% (27/527) of biopsies with gastric lesion infected with *H.Pylori* while 5% (3/62) in non infected gastritis. Atrophy was seen in 18 males and 9 females showing positive *H.Pylori* bacteria.

Atrophy was more in males as compared to females ($p=0.014$) (Table II-III).

Lymphoid follicles are not a normal constituent of gastric mucosa. Follicles were appreciated in 5% (28/527) of biopsies. Out of these 18 were males and 10 were females. It was seen that there was significant difference in both genders ($p=0.03$). Most of the patients were above the age of 30. *H.Pylori* was present in all of these cases; no such histological lesions were seen in gastritis without *H.Pylori*. There was a highly significant difference in both types of gastritis. (Table II-III).

Intestinal metaplasia was characterized by presence of goblet cells and absorptive cells in the antrum of stomach (57/527=11%). Males constituted 37 (65%) and females 20 (35%) of biopsies that showed intestinal metaplasia. *H.Pylori* was found in 57 biopsies. Only 1 case showed intestinal metaplasia with *H.pylori* infection. The difference between both types of lesions was significant ($p=0.01$) (Table II-III).

Dysplasia: Pleomorphism, nuclear atypia and hyperchromasia of gastric mucosal cells were observed in 15/527 (3%) of patients and labeled as positive for dysplastic changes. Dysplasia was seen

in 9/15 (60%) of males and 6/15(40%) of females. *Helicobacter* was present in all (100%) of biopsies that showed Dysplasia (Table IV and V).

Malignancy was seen in 16/527 (3%) biopsies. Diffuse-type/ signet ring adeno -carcinoma was labeled due to presence of single dispersed signet cells with intracellular mucin. 9/16 malignant cases were of signet ring type. 6/16 cases were reported to be of Intestinal-type adeno-carcinoma. It showed pleiomorphic, hyperchromatic columnar mucus secreting cells. Some of the cells were ciliated. 4/16 cases were of Lymphoma that showed monotonous population of infiltrating, malignant lymphocytes. Interestingly, 6 (37.5%) of females and 10 (62.5%) male biopsy had malignant change. Only one case of malignancy was seen in non specific gastritis without *H.Pylori* means that 94% (16/17) malignant biopsies showed *Helicobacter* infection. Statistically it was highly significant (Table IV and V).

Only 13 (2.5%) biopsies showed ulceration of gastric mucosa, 160 were rendered unremarkable/normal and 11 were labeled as inadequate due to inadequate or insufficient tissue that showed either mucosa or submucosa alone (Table II).

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%) 19.42857±2	589(77.5%)	-	-

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 adenocarcinoma 9/16 (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)

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%

Figure 1. Photomicrographs A showing H.Pylori (Giemsa 40 x), B. severe dysplasia (H&E 40x), C, Lymphoid hyperplasia and D, Intestinal metaplasia

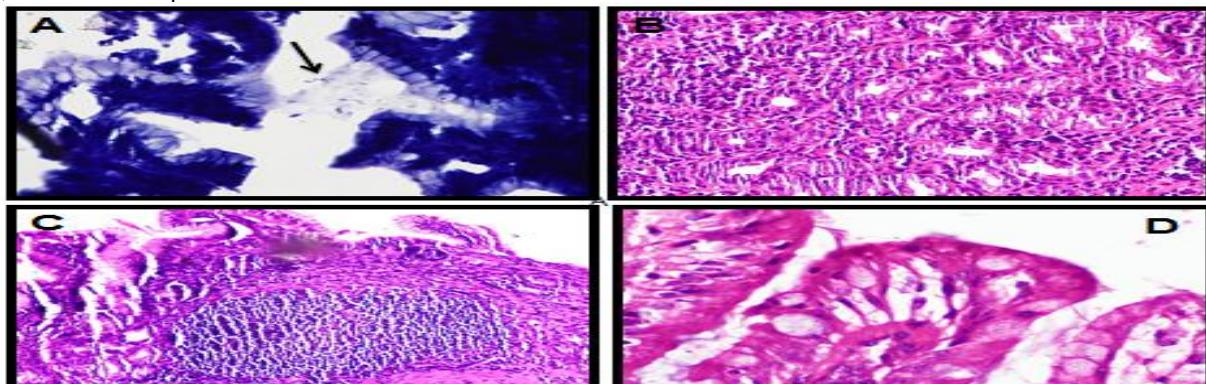
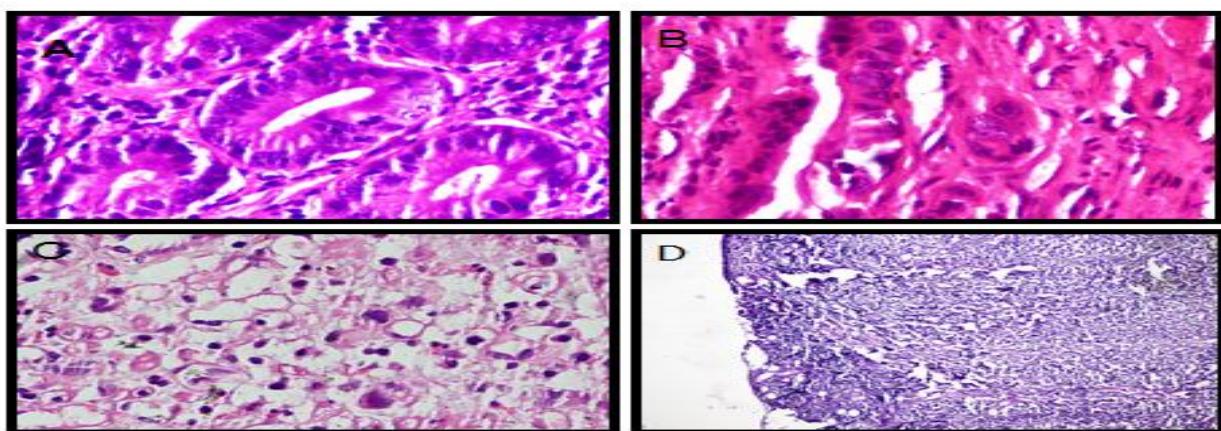


Figure 2. Photomicrographs A showing severe dysplasia (H&E 40 x), B. intestinal variant of gastric adenocarcinoma (H&E 40x), C, signet ring variant of gastric carcinoma and D, MALT lymphoma



DISCUSSION

In our study, the frequency of this infection is 69%. Out of which 58% (50/81) of males and 42% of females were infected with *H.Pylori*. It represents that males of our population might be more prone to *H.Pylori* infection than other societies. Percentage of infected male population is nearly similar to Korean subjects. Average age of infected male patients was 38.11 ± 16.66 years and mean age of females that were infected with *H.Pylori* was 35.81 ± 14.19 years. This is statistically significant ($p < 0.05$)

In the age group 21-30, 36% patients showed infection while in group of 31-40, 29% biopsies showed *H.Pylori*. These two age groups showed a relatively higher incidence of *H.Pylori* infection. It supports the fact seen in international studies that third world population acquires the infection in childhood and early adulthood⁶. As in our population, awareness about health and presenting to healthcare facility is not a priority; patients might be carrying the infection much earlier than being presented at hospitals. Acquiring the infection in early ages can

badly affect the outcome of infection as suggested by You et al in his study that was conducted in China¹⁰.

Previous studies indicate that the prevalence of *H. pylori* is high in developing countries like Pakistan. Its seroprevalence exceeds 51-58% of our general population. In Karachi, 67% of Infants at 9 months of age showed infection with *H. pylori*^{9,11}. A study conducted by Yakoob J et al on *H. Pylori* positive patients concluded that 63% were males and 37% were females with a mean age of 45±16. However, he also noted that prevalence of CagA (virulent strain) positive *H. pylori* infection is low, 56% compared to over 70% noted in neighboring countries like Iran, India and Bangladesh. It is now well known that strains that possess the Cag secretion system, which translocates the bacterial effector CagA into host cells, augment cancer risk¹². In Georgia, a developing country, 70% of adult population is infected¹³.

In our study we found out that 88% of our total subjects showed inflammation in *H.Pylori* infected biopsies. Males with chronic inflammation constituted 70% and females were 63% of infected population. This might indicate that in our region, factors other than *H.Pylori* are responsible for producing chronic gastritis. Egi Y et al found *H. pylori* in 95% patients. Inflammation was present in all cases with *H. pylori* infection¹⁴. This finding is comparable to our present study.

We observed atrophy in 5% of cases infected with *H.Pylori* and dysplasia in 2.8% of *H.Pylori* infected cases. Our findings are not consistent with international studies regarding these histopathologic changes. Gastric atrophy was also seen in non infected gastritis, therefore this lesion was not statistically significant ($p=0.610$), however it was significant among both genders (0.014). Dysplastic lesions were statistically not important in both sexes ($p=0.233$) and but significant among histological variants of gastritis.

In our study Intestinal Metaplasia was seen in 9.3% of cases. *H.Pylori* was seen in 92% of cases of intestinal metaplasia. Georgian population had *H.Pylori* in 35% of cases of metaplasia. This is a significant percentage. Yakoob J et al found intestinal metaplasia in 2% of population in Karachi at Agha Khan Hospital.(12).This number is very low when compared with a study conducted on 611 *H. Pylori* positive patients in Taiwan which showed 21% patients to be positive for intestinal metaplasia. This could be due to larger sample size taken or lower risk in our population¹⁵. Another study showed metaplasia in 24% of subjects, in Japan.(14). In our study Intestinal Metaplasia (57/527=11%) was more common in males 37 (65%) as compared to females 20 (35%) and there was statistically highly significant

difference between both sexes ($p=0.001$). this difference was also significant between both types of gastritis ($p=0.01$).

No lymphoid follicle was seen in *HP* negative gastritis but formation of lymphoid follicles was seen in 28/527 (5%) *HP* positive gastritis with a positive statistic difference ($p=0.041$). This difference was also prominent between two genders ($p=0.03$)

Malignant cases associated with *HP* were 16/527 (3%). No statistical difference was seen in males and females. There were 13 cases of gastric carcinoma and 03 biopsies representing lymphoma. When we compare different types of cancers, we found that diffuse type of carcinoma held 9/16 (56%) share of malignancy and thus predominated.

CONCLUSION

Helicobacter pylori induced gastritis is much more common in our population. Moreover, significant gastric lesions were seen in biopsies positive for *HP* as compared to *HP* free gastritis. Dysplastic and malignant gastric lesions were non significantly associated with *HP* bearing biopsies vs *HP* negative ones.

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