ORIGINAL ARTICLE

HELICOBACTER PYLORI PREVALENCE AND HISTOPATHOLOGICAL FINDINGS IN DYSPEPTIC PATIENTS

Khalid Mehmood, Abdul Aleem Awan, Naveed Muhammad*, Fariha Hasan, Abdul Nadir*****

Department of Pharmacy, Hazara University Havelian Campus, Havelian, *Department of Pharmacy, Abdul Wali Khan University Mardan, **Department of Microbiology, Quaid-i-Azam University, ***Department of Gastroenterology, Shifa International Hospitals, Islamabad, Pakistan

Background: *Helicobacter pylori* (*H. pylori*) colonizes in half of the population of developed and nearly all inhabitants of developing countries. The infection is characterized by gastritis but can present more complicated disease states. We intended to report prevalence of *H. pylori* infection by histopathology and presence of gastritis, activity, atrophy and intestinal metaplasia in dyspeptic patients of Islamabad, Pakistan. **Methods**: Ninety four patients identified to be dyspeptic on the basis of Rome-III criteria were included in the study and diagnosed for *H. pylori* status by Histopathology. The grading and severity of gastritis was documented as nil, mild, moderate or severe, based on the Sydney system. Activity was recorded as present when an increase in the number of neutrophils was observed. Atrophic changes and intestinal metaplasia were also determined. **Results**: Eighty three out of total 94 (88.3%) patients were positive for *H. pylori* on histopathology. Out of total 94 patients, chronic gastritis was observed in 89 (94.6%), evidence of activity was found in 37 (39.4%), atrophic changes were observed in 66 (70%) and intestinal metaplasia was present in 4 (4.3%) patients. **Conclusion**: *H. pylori* infection in dyspeptic patients of Islamabad appears to be more related with gastritis.

Keywords: *Helicobacter pylori*, Histopathology, Dyspepsia, Gastritis, Pakistan

J Ayub Med Coll Abbottabad 2014;26(2):182–5

INTRODUCTION

The discovery of *H. pylori* and its association with gastric problems has revolutionized the perception and management of gastric disorders. Peptic ulcer disease is no more considered a chronic recurrent disorder; rather it is now an infectious disease curable by the use of antibiotics. *H. pylori* has been considered associated with acute and chronic gastritis, gastric and duodenal ulcers, lymphoma and even cancer.[1,2] Like all developing countries, Pakistan also has high *H. pylori* prevalence reported in the range of 50–90%.[3–7] The reason for such a huge variation in prevalence range may be use of different diagnostic techniques and population studied.

H. pylori infection can be diagnosed by invasive or non-invasive methods. Invasive methods require endoscopy and sampling of the biopsy tissues that can be used for histological examinations, culture or direct molecular examination. Non-invasive methods include antigen/antibody tests, urea breath test (UBT) or molecular detection techniques. Histological examination is the most commonly employed invasive method of *H. pylori* detection in clinical setting. Hematoxyllin and Eosin, Giemsa, and silver staining are commonly used for detection of *H. pylori* in the tissue sections of paraffin embedded gastric mucosa specimens.[8]

Despite very high prevalence of *H. pylori* infection, limited basic and applied data on *H. pylori* prevalence and its association with different clinical presentations is available from Pakistan. Genetic diversity of *H. pylori*, generally lower socio-economic status in Pakistan than that in the developed world and limited access to healthcare facilities in Pakistan warrant more research on different aspects of *H. pylori* infection. There is limited data available on morphological changes in gastric environment followed by *H. pylori* infection. We have previously reported the seropositivity of *H. pylori* infection and association of various risk factors with positive *H. pylori* status.[7,9] In this study, we intended to report prevalence of *H. pylori* infection and associated morphological changes in gastric mucosa that is gastritis, activity, atrophy and intestinal metaplasia in dyspeptic patients of Islamabad, Pakistan by histopathology.

MATERIAL AND METHODS

This cross-sectional study was conducted in Gastroenterology Clinic of Shifa International Hospital (SIH), Islamabad during the period from July 2007 to June 2008. Three hundred and eighty five consecutive dyspeptic patients were initially enrolled on the basis of qualifying Rome-III criteria after an interview by the gastroenterologist. Out of these, 116 patients were excluded due to presence of peptic ulcer disease, gallstones or positive HCV status. Remaining 269 patients were entered in the data collection forms after obtaining the informed consent. The study was approved by the institutional review board of SIH. The specific type of investigation tests (endoscopy with histopathology or ELISA) was determined by the gastroenterologist

on the basis of specific needs of the patient and affordability.

Ninety four dyspeptic patients underwent upper gastrointestinal endoscopy in SIH, Islamabad, Pakistan. The endoscopic examination was carried out by the gastroenterologist using Olympus CV-160, CLV-160 and GIF-160 system (Olympus, Japan). Four biopsies were collected from each patient (two each from body and antrum of the stomach). The histopathological confirmation of *H. pylori* infection was accomplished by H&E and where required by Giemsa staining. The presence of *H. pylori* was graded as absent or present. The grading and severity of gastritis was documented as nil, mild, moderate or severe, based on the Sydney system.[10] Gastritis was confirmed on finding increased number of mononuclear inflammatory cells in lamina propria. Activity was recorded as present when an increase in the number of neutrophils was observed. Atrophic changes (damaged gastric lining leading to loss of gastric glandular cells) and intestinal metaplasia (replacement of gastric mucosal cells by resembling intestinal mucosal cells) was also determined.

RESULTS

The antral biopsies of 94 dyspeptic patients were tested for *H. pylori* presence, graded for gastritis and other morphological changes like atrophy and intestinal metaplasia. There were 61 (64.9%) males and 33 (35.1%) females. The mean age and body mass index (BMI) of subjects were 45±6.3 years and 26.2±1.8 respectively. The status of *H. pylori* was positive if bacteria were seen in the tissue samples (Figure-1). The prevalence of *H. pylori* was 88.3% in these dyspeptic patients. Grading of inflammation was decided according to Sydney System. In the present study, histopathological examination of 94 patients revealed chronic gastritis in 89 (94.6%); mild in 51, moderate in 38 and severe in 4 patients. Evidence of activity was found in 37 (39.4%); mild in 16, moderate in 18 and severe in 3 patients. Atrophic changes were observed in 66 (70%); mild in 46, moderate in 15 and severe in 5 patients. Intestinal metaplasia was present in 4 patients (4.3%); mild in 1 and moderate in 3 patients (Table-1). Gastric atrophic changes were observed in 70% of the patients (66 out of 94), though sever atrophic changes could only be observed in 7.5% (5 out of 66) patients with atrophy.

Table-1: Histopathological findings and grading of gastric biopsy tissue samples (n=94)

Findings	Grading			Total (%)
	Mild	Moderate	Severe	
Gastritis	48	37	4	89 (94.6)
Activity	16	18	3	37 (39.4)
Atrophy	46	15	5	66 (70)
Intestinal metaplasia	1	3	0	4 (4.3)

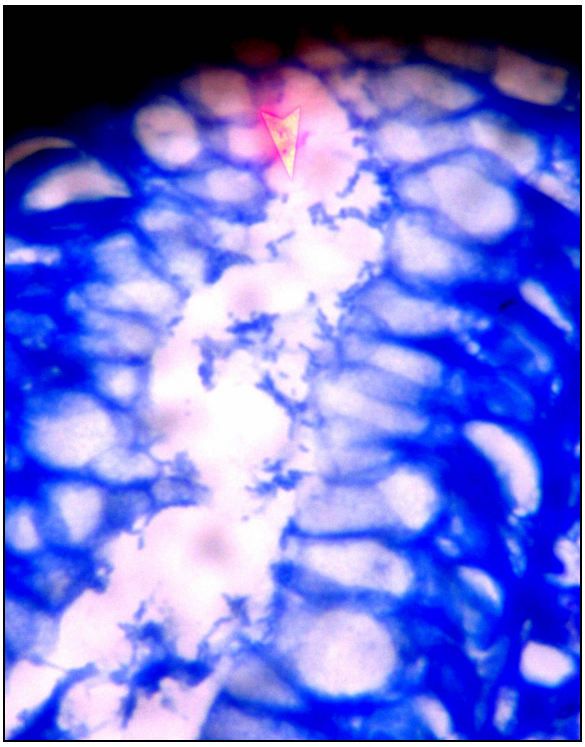

Figure-1: *H. pylori* observed in H & E stained gastric tissue.

DISCUSSION

H. pylori is considered to be the most important cause of human gastritis, duodenal and gastric ulcers and has been classified as class-1 human carcinogen.[11] The usual disease progression following *H. pylori* infections is gastritis followed by atrophy, intestinal metaplasia and dysplasia that can lead to carcinoma of gastric mucosa. Gastritis is developed in almost all individuals infected with *H. pylori* whereas gastric atrophy and intestinal metaplasia appear more often in *H. pylori* positive than in negative patients.[12,13] Prevalence of 88.3%, though seems on the higher side as compared to generally reported, however corresponds to some reports like 84.6%, by another group using PCR in the same region.[6] It is generally accepted that prevalence varies with the method of investigation employed.

Histological examination is the most commonly employed invasive method of *H. pylori* detection in clinical setting. Hematoxyllin and eosin, Giemsa, and silver staining have been used for detection of *H. pylori* in the tissue sections of paraffin embedded gastric mucosa specimens.[8] Accuracy of histology for *H. pylori* detection may depend on the adequate number of gastric biopsies, rightly chosen biopsy sites or skill of the pathologist. The advantages of histology are detection of *H. pylori* and its colonization density, and information

about morphological changes in the gastric mucosa demonstrating gastritis, atrophy, intestinal metaplasia, dyspepsia or malignancies.[14]

The finding that gastritis was present in almost all infected patients is in complete agreement with other reports where gastritis was found present in more than 90% of *H. pylori* infected patients.[15,16] Presence of intestinal metaplasia in only 4% of the patients is an interesting finding. *H. pylori* infection has been considered to be most important but not the only risk factor for atrophy and intestinal metaplasia, the key changes leading to gastric cancer development. A comparative study conducted in China reported a generally higher rate of atrophic changes and presence of intestinal metaplasia in gastric ulcer patients than that in gastritis patients.[17]

Genetic diversity and origin of the infecting *H. pylori* strain are the major factors that determine the outcome of disease in most cases. Virulent *H.* pylori cytotoxin-associated gene A *(cag*A) positive strains are considered to be associated with more severe disease state as patients infected with *cag*A positive strains had higher risk of gastric cancer development than those *cag*A negative strains.[18] Various studies reported that 60–70% of *H. pylori* strains isolated from North American and European populations had *cag*A gene[19,20,] whereas, over 90% of strains isolated from China and Asia-Pacific have been reported *cag*A positive.[21,22] Ahmad *et al*, (2009) however reported lower prevalence (24%) of *cag*A positive *H. pylori* strains from Pakistani dyspeptic patients than that reported from other countries of the region.6 This lower prevalence of highly virulent *H. pylori* strains may be attributed to less severe gastric atrophy and generally lower prevalence of intestinal metaplasia in Pakistani, however more focused studies are required to confirm such findings. There has been reported at-least another *H. pylori* genetic marker associated with cancer development[23] that is being developed besides *cag*A to identify patients at risk for cancer development presenting advanced *H. pylori* disease states.[24,25]

CONCLUSION

Prevalence of *H. pylori* infection in Pakistani population is alarming. Gastritis is the major condition associated with Pakistani dyspeptic patients. Low prevalence of severe atrophy and intestinal metaplasia predict low risk of gastric cancer.

ACKNOWLEDGMENTS

We are thankful to Pakistan Higher Education Commission (HEC) for funding this research work.

REFERENCES:

1. Atherton JC, Blaser MJ. Coadaptation of *Helicobacter pylori* and humans: ancient history, modern implications. J Clin Invest 2009;119(9):2475–87.
2. Kusters JG, van Vliet AH, Kuipers EJ. Pathogenesis of *Helicobacter pylori* infection. Clin Microbiol Rev 2006;19(3):449–90.
3. Hassan SR, Abbas Z: Presence of *Helicobacter pylori* in dyspeptic patients with endoscopically normal stomach. Pak J Med Sci 2007;23(3):335–9
4. Nizami SQ, Bhutta ZA, Weaver L, Preston T. *Helicobacter pylori* colonization in infants in a peri-urban community in Karachi, Pakistan. J Pediatr Gastroenterol Nutr 2005;41(2):191–4.
5. Abbas Z, Jafri W, Khan AH, Shah MA. Prevalence of *Helicobacter pylori* antibodies in endoscopy personnel and non-medical volunteers of Karachi. J Pak Med Assoc 1998;48(7):201–3.
6. Ahmad T, Sohail K, Rizwan M, Mukhtar M, Bilal R, Khanum A. Prevalence of *Helicobacter pylori* pathogenicity-associated cagA and vacA genotypes among Pakistani dyspeptic patients. FEMS Immunol Med Microbiol 2009;55(1):34–8.
7. Mehmood K, Hameed Z, Shoukat S, Hasan F, Alam AY, Hameed A, Nadir A. Predictors of depression in patients presenting with dyspeptic symptoms in a GI clinic. J Ayub Med Coll Abbottabad 2011;23(4):49–52
8. Rotimi O, Cairns A, Gray S, Moayyedi P, Dixon MF. Histological identification of *Helicobacter pylori*: comparison of staining methods. J Clin Pathol 2000;53(10):756–9.
9. Mehmood K, Nadir A, Hasan F. Lower education status predicts higher seropositivity for *Helicobacter pylori* infection in Pakistan. Annual Research & Review in Biology 2014;4(24):3734–41
10. Price AB. The Sydney system: histological division. J Gastroenterol Hepatol 1991;6(3):209–22.
11. IARC. Infection with *Helicobacter pylori*. IARC Monogr Eval Carcinog Risks Hum 1994;61:177–240.
12. Uemura N, Okamoto S, Yamamoto S, Matsumura N, Yamaguchi S, Yamakido M *et al*. *Helicobacter pylori* infection and the development of gastric cancer. N Engl J Med 2001;345(11):784–9.
13. Kuipers EJ. Review article: Relationship between *Helicobacter pylori*, atrophic gastritis and gastric cancer. Aliment Pharmacol Ther 1998;12(1 Suppl):25–36.
14. Megraud F. How should *Helicobacter pylori* infection be diagnosed? Gastroenterology 1997;113(6 Suppl):93–8.
15. Galbán E, Arús E, Periles U. Endoscopic findings and associated risk factors in primary health care settings in Havana, Cuba. MEDICC Rev 2012;14(1):30–7.
16. Ekesbo R, Toth E, Fork FT, Held M, Nilsson I, Wadstrom T *et al.* K. Chronic *Helicobacter pylori* infection in a population in southern Sweden analysed by histopathology, immunoblot and ELISA serology. Eur J Gastroenterol Hepatol 2006;18(6):589–3.
17. Zhang C, Yamada N, Wu YL, Wen M, Matsuhisa T, Matsukura N. Comparison of *Helicobacter pylori* infection and gastric mucosal histological features of gastric ulcer patients with chronic gastritis patients. World J Gastroenterol 2005;11(7):976–81.
18. Parsonnet J, Friedman GD, Orentreich N, Vogelman H. Risk for gastric cancer in people with CagA positive or CagA negative *Helicobacter pylori* infection. Gut 1997;40(3):297–301.
19. Tummuru MK, Cover TL, Blaser MJ. Cloning and expression of a high-molecular-mass major antigen of *Helicobacter pylori*: evidence of linkage to cytotoxin production. Infect Immun 1993;61(5):1799–809.
20. Covacci A, Censini S, Bugnoli M, Petracca R, Burroni D, Macchia G *et al*. Molecular characterization of the 128-kDa

immunodominant antigen of *Helicobacter pylori* associated with cytotoxicity and duodenal ulcer. Proc Natl Acad Sci U S A 1993;90(12):5791–5.
21. Yang H, Wu SV, Pichuantes S, Song M, Wang J, Zhou D,*et al*. High prevalence of *cagA*-positive strains in *Helicobacter pylori*-infected, healthy, young Chinese adults. J Gastroenterol Hepatol 1999;14(5):476–80.
22. Ito Y, Azuma T, Ito S, Miyaji H, Hirai M, Yamazaki Y,*et al*. Analysis and typing of the vacA gene from cagA-positive strains of *Helicobacter pylori* isolated in Japan. J Clin Microbiol 1997;35(7):1710–4.
23. Wang JT, Chang CS, Lee CZ, Yang JC, Lin JT, Wang TH. Antibody to a *Helicobacter pylori* species specific antigen in patients with adenocarcinoma of the stomach. Biochem Biophys Res Commun 1998;244(2):360–3
24. Mehmood, K, Hasan F. Construction and use of a prokaryotic expression system for *Helicobacter pylori* AhpC. BMC Research Notes 2012;5:328.
25. Huang CH, Chuang MH, Lo WL, Wu MS, Wu YH, Wu DC *et al*. Alkylhydroperoxide reductase of *Helicobacter pylori* as a biomarker for gastric patients with different pathological manifestations. Biochimie 2011;93(7):1115–23.

Address for Correspondence:

Khalid Mehmood, Department of Pharmacy, Hazara University, Havelian Campus, Abbottabad, Pakistan.
Cell: +92-333-5516552
Email: khalidadc@yahoo.co.uk