Bangladesh J Med Microbiol 2009; 03 (01): 4-9

Bangladesh Society of Medical Microbiologists

Original Article

Helicobacter pylori Infection and Strain Types in Adult Dyspeptic Patients Undergoing Endoscopy in a Specialized Hospital of Dhaka City

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Abstract

***Helicobacter pylori* infection occurs worldwide with a high prevalence in developing countries. Virulence of *H. pylori* strains varies in different geographic regions. The aim of this study was to see *H. pylori* infection and its strain types in adult dyspeptic patients in Bangladesh and to analyze association of *H. pylori* strain types with clinical disease and severity of histological gastritis. Ninety consecutive adult dyspeptic patients undergoing diagnostic endoscopy were tested for *H. pylori* infection by culture, rapid urease test (RUT), histology and anti *H. pylori* IgG ELISA (Enzyme linked immunosorbent assay). *H. pylori* strain types were determined by Western Blot analysis. Association of strain types with clinical gastro-duodenal diseases and grades of histological gastritis were analyzed by χ^2 test. Among the selected patients, 53 (58.9%) were culture positive, 48 (53.3%) were RUT positive, 31 (34.4%) were histology positive and 82 (91.1%) were anti-*H. pylori* IgG ELISA positive. By Western Blot analysis of the 90 sera samples, 48 (53.3%) showed antibodies to Type I strain of *H. pylori*, 21 (23.3%) Intermediate strain and 3 (3.3%) Type II strain. Endoscopically, 20 (22.2%) patients were found normal, 27 (30.0%) had gastritis, 9 (10.0%) had duodenitis, 28 (31.1%) had peptic ulcer disease, 4 (4.4%) had gastric carcinoma, and 2 (2.2%) had reflux esophagitis. Histologically, 34.4% had *H. pylori*, 44.4% had polymorhonuclear neutrophil (PMN), 100% had mononuclear cell (MNC) infiltration of different grades, 1.1% had atrophic gastritis and 2.2% had intestinal metaplasia of moderate grade. *H. pylori* strain types was not associated with clinical gastro-duodenal diseases or grades of PMN or MNC infiltration ($p > 0.05$) in these patients.**

Key words: *Helicobacter pylori* infection, *H. pylori* strain types, gastro-duodenal diseases, grades of gastritis

Introduction

Helicobacter pylori cause gastritis,[1,2] that may progress to peptic ulcer disease (PUD),[3-5] gastric carcinoma,[6] and gastric lymphoma.[7] Its relation with non-ulcer dyspepsia has not been clear.[8] *H. pylori* infection is one of the most common infection worldwide.[9] The infection is acquired in childhood,[10] and persists despite local and systemic immune response.[9] Majority of the infections remain asymptomatic and only 10-20% progress to clinical disease.[11] This variable outcome may be due to difference in virulence of bacterial strains, host response or environmental influences.

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The fact that many *H. pylori* strains have disease-specific virulence factors, has prompted considerable research effort into whether there are such factors associated with the bacterium. Among the bacterial virulence factors, Cytotoxin associated antigen (CagA) and Vacuolating cytotoxin A (VacA) had been extensively studied. Depending on the expression of *cagA* and *vacA* genes, isolated *H. pylori* strains have been classified into: (i) Type I strains that produce both CagA and VacA antigens; (ii) Intermediate strains that produce either CagA or VacA; and (iii) Type II strains that do not produce either of the CagA or VacA.[12]

Relation of CagA and VacA with clinical disease and histological gastritis varies in different geographic regions.[13-19] Type I strain is more pathogenic in Western countries.[15] Prevalence of *H. pylori* infection and pattern of *H. pylori* gastritis varies between geographic regions.[10,20] Bangladesh is a developing country and epidemiological studies have shown 92% seroprevalence of *H. pylori* in asymptomatic adult population[21] and 85% by ^{C}13-Urea breath test among family members of *H. pylori* infected and uninfected children.[22] Prevalence of peptic ulcer is also high in Bangladesh. An endoscopic survey showed a point prevalence of 11.9% duodenal ulcer and 3.5% gastric ulcer among individuals above the age of 15 years.[23]

The aim of this hospital-based study was to see *H. pylori* infection by examining gastric biopsy and serological tests by finding out strain types of the isolates by Western Blotting, pattern of clinical gastro-duodenal diseases by endoscopy and grades of histological gastritis in adult dyspeptic patients and to analyze the association of *H. pylori* strain types with clinical gastro-duodenal diseases and severity of histological gastritis.

Methods

Ninety consecutive adult dyspeptic patients, attending at the Department of Gastrointestinal, Hepatobiliary and Pancreatic Diseases (GHPD) of Bangladesh Institute of Research and Rehabilitation in Diabetes, Endocrine and Metabolic Disorders (BIRDEM), Dhaka for diagnostic endoscopy during June, 2004 to January, 2005 were selected. Patients who had partial or complete gastrectomy, ever received *H. pylori* eradication therapy, taken colloidal bismuth compound, proton pump inhibitor, H_2-receptor blocker or NSAID in last four weeks were excluded. Approval of the Ethical Review Committee of BIRDEM was taken before initiation of the project work, and informed consent of the patients was taken prior to endoscopy and sample collection.

Endoscopy and biopsy

Endoscopy was done by the experts with Olympus EVIS 160 video Endoscope (Olympus Optical Company, Japan) after overnight fasting. Endoscopic diagnoses were grouped as Normal, Gastritis, Duodenitis, Peptic ulcer disease (PUD), Gastric carcinoma and Reflux oesophagitis (RE). From each selected patient, 6 gastric biopsy specimens, 3 from the antrum and 3 from the corpus were taken. Additional 5-6 biopsies were taken from margins of malignant looking gastric ulcers or proliferative growths for confirming the diagnosis histologically.

Collection of serum

After endoscopy, 3 ml of venous blood was collected from each patient. Serum was separated after 1 hour, kept at -70^{o}C and serological tests were performed.

Culture

Two gastric biopsy specimens, one from the antrum and another from the corpus, were inoculated separately into Stuart's transport media and were transported to the *H. pylori* Laboratory of Laboratory Sciences Division of International Centre for Diarrhoeal Diseases Research, Bangladesh (ICDDR,B) within 3-4 hours in a cool box, where culture was done as described previously.[19] Positive cultures were identified by colony morphology, Gram stain characteristics and positive catalase, oxidase and urease tests.

Rapid urease test (RUT)

Two gastric biopsy specimens, one from antrum and the other from corpus, were inoculated separately into Christensen's urea agar media (pH adjusted at 7.0) in screw-capped bottles. Change of colour from yellow to pink by any specimen within 2 hours was considered as positive.

Histology

Two gastric biopsy specimens, one from antrum and the other from corpus, were fixed in 10% formalin in separate containers and were sent to the Histopathology Laboratory of Ibrahim Medical College. If additional biopsy tissue was taken from a case of suspicious ulcer, the material was also kept in 10% formalin in a separate container and sent to the Histopathology laboratory of BIRDEM. Samples were embedded in paraffin wax, cut at 5 mm thickness and were

stained by modified Giemsa and Hematoxylin and Eosin (H&E) stains. *H. pylori* were identified by other characteristic appearance and distribution in histology slides. Gastritis was graded according to updated Sydney system. *H. pylori* density, polymorphonuclear neutrophil (PMN) infiltration (activity), atrophy and intestinal metaplasia were graded as absent, mild, moderate and marked and mononuclear cell (MNC) infiltration (chronic inflammation) was graded as normal, mild, moderate and marked using the visual analogue scale.[24] The participating Histopathologist was unaware of patients' clinical conditions and other test results.

Enzyme Linked Immunosorbent Assay (ELISA)

Anti-*H. pylori* IgG ELISA was done with a commercial ELISA test kit (AccuBind ELISA, Monobind, USA) according to instructions of the manufacturer.

Western Blot

Western Blot test was done with sera samples to detect antibodies against *H. pylori* antigens with a commercial kit Helico Blot 2.1 (Genelabs Diagnostics, Singapore) according to instructions of the manufacturer. The Western Blot positive patients were graded as: (a) High positive- both anit-CagA and anti-VacA positive; (b) Intermediate positive- either anti-CagA or anti-VacA positive; and (c) Low positive- at least two of 35 kD, 30 kD or 19.5 kD positive. This grading corresponds to infection with Type I, Intermediate, and Type II strains of *H. pylori* respectively.[14,19]

Data analysis

Data were analyzed using the Statistical Package for Social Science (SPSS) 12.0 for Windows. χ^2 test and Fisher's exact test were done where applicable. Value of $p < 0.05$ was considered as significant.

Results

The selected patients were aged between 18 to 75 years with a mean age of 47.4 years and standard deviation ±13.7 years. Fifty-three (58.9%) were males and 37 (41.1%) were females with a male female ratio of 1.4: 1. Twenty-seven (30.0%) patients were from low-income group, 50 (55.6%) from middle-income group, and 13 (14.4%) from high-income group. Nineteen (21.1%) patients had no education, 34 (37.8%) had primary education, 18 (20.0%) had secondary education and 19 (21.1%) had education higher than secondary. Majority (74, 82.2%) of the patients were diabetic and the others (16, 17.8%) were non-diabetic. Sixty-three (70.0%) patients were non-smokers and 27 (30.0%) were smokers. Eighty seven (96.7%) patients had never taken alcohol and only 3 (3.3%) patients had the habit of taking alcohol. Thirty-four (37.8%) patients presented with upper abdominal pain only, 37 (41.1%) presented with upper abdominal pain with other symptoms like anorexia, nausea vomiting and abdominal fullness, and 19 (21.1%) patients presented with one or more of the symptoms like anorexia, nausea, vomiting and abdominal fullness without abdominal pain.

Rate of infection varied depending on test applied for detection. Of them, the highest number of cases were identified by ELISA (82, 91.1%), followed by Western blot (72, 80.0%), culture (53, 58.8%) and RUT (31, 34.4%) Considering any of the gastric biopsy-based test-positive patients as infected, 69 (76.7%) were found infected with *H. pylori.* (Table I)

Table I: *Helicobacter pylori* infection detected by different methods (n= 90)

Method	Number of positive patients	Percentage
Culture	53	58.8
RUT	48	53.3
Histology	31	34.4
ELISA	82	91.1
Western Blot	72	80.0

Among the 72 Western Blot test positive patients, 48 (53.3%) had response to both CagA and VacA (infected with Type I strain), 21 (23.3%) had response to CagA but not to VacA (infected with Intermediate strain), and 3 (3.3%) had response to low molecular weight antigens only (infected with Type II strain). Remaining 18 (20.0%) patients were Western Blot test negative (uninfected). Response to VacA without response to CagA was not found. (Table II)

Table II: *Helicobacter pylori* strain types (n= 90)

Strain type	Number of patient	Percentage of patient
Type I	48	53.3
Intermediate	21	23.3
Type II	03	03.3
Uninfected*	18	20.0

* Seronegative by Western Blot test

At endoscopy, 20 (22.2%) patients had normal gastro-duodenal mucosa, 27 (30.0%) had gastritis, 9 (10.0%) had duodenitis, 28 (31.1%) had peptic ulcer disease, 4 (4.4%) had gastric carcinoma and 2 (2.2%) had reflux oesophagitis. Lesions having endoscopic appearance of gastric carcinoma were histologically adenocarcinomas.

Histologically, all patients had mild to marked mononuclear cell infiltration (chronic inflammation), but 40 (44.4%) had mild to marked polymorphonuclear neutrophil infiltration (activity). Prevalence of atrophy and intestinal metaplasia were very low. (Figures 1 and 2)

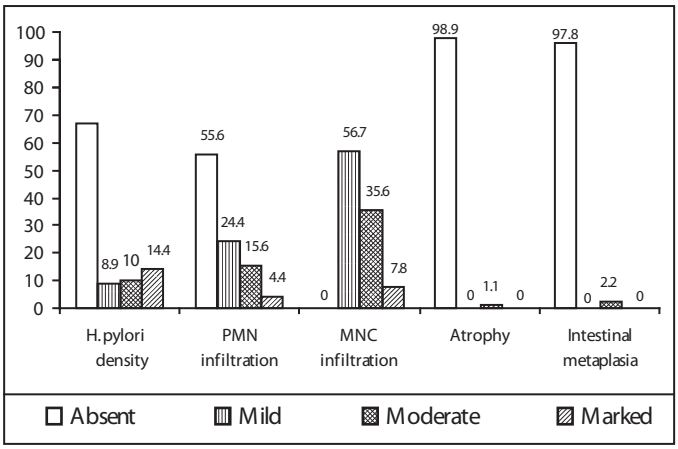

Figure 1 : Bar chart showing grades of histological gastritis in the antrum.

PMN: Polymorphonuclear neutrophil, MNC: Mononuclear cell. In case of MNC infiltration, the term absent will be replaced by normal. Values are expressed in percentage.

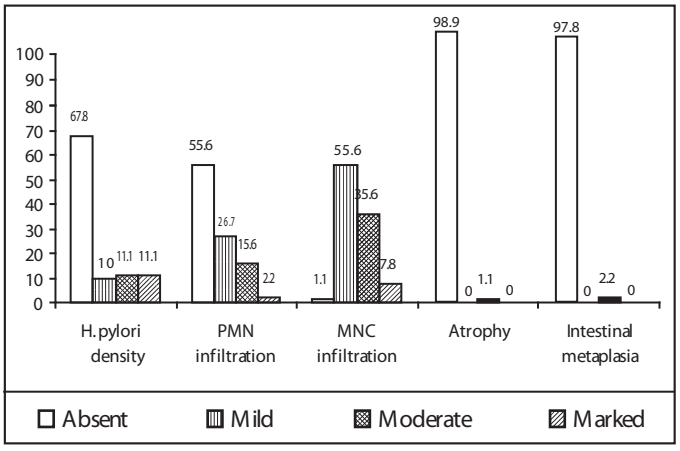

Figure 2 : Bar chart showing grades of histological gastritis in the body.

PMN: Polymorphonuclear neutrophil, MNC: Mononuclear cell. In case of MNC infiltration, the term absent will be replaced by normal. Values are expressed in percentage.

Relation of *H. pylori* strain types with endoscopic gastro-duodenal diseases shows that *H. pylori* strain types were not associated with gastro-duodenal diseases ($p > 0.05$). (Table III)

Relation of *H. pylori* strain types with grades of PMN infiltration and MNC infiltration in the antrum and body of the stomach shows that *H. pylori* strain types were not associated with grades of PMN and MNC infiltration in the antrum or body ($p > 0.05$). (Table IV)

Table III: *H. pylori* strain types and clinical gastro-duodenal diseases (N = 90)

H. pylori strain type	No (%) of patients showing Endoscopic gastro-duodenal diseases					
	Normal	Gastritis	Duodenitis	PUD	Gastric Ca	RE
Type I (n = 48)	8 (16.7)	13 (27.1)	4 (8.3)	19 (39.6)	3 (6.3)	1 (2.1)
Intermediate (n = 21)	6 (28.6)	8 (38.1)	3 (14.3)	4 (19.0)	0 (0.0)	0 (0.0)
Type II (n = 3)	0 (0.0)	1 (33.3)	1 (33.3)	1 (33.3)	0 (0.0)	0 (0.0)
Uninfected (n = 18)	6 (33.3)	5 (27.7)	1 (5.6)	4 (22.2)	1 (5.6)	1 (5.6)

PUD: Peptic ulcer disease, Ca: Carcinoma, RE: Reflux Oesophagitis

Table IV: *H. pylori* strain types and grades of Polymorpho nuclear Neutrophil (PMN) and Mononuclear Cell (MNC) infiltration (N = 90)

H. pylori strain type	Antrum				Body			
	Polymorphonuclear neutrophil (PMN) infiltration							
	Absent	Mild	Moderate	Marked	Absent	Mild	Moderate	Marked
	No. (%)	No. (%)	No. (%)	No. (%)	No. (%)	No. (%)	No. (%)	No. (%)
Type I (n= 48)	24 (50.0)	14 (29.2)	7 (14.6)	3 (6.3)	25 (52.1)	14 (29.2)	7 (14.6)	2 (4.2)
Intermediate (n=21)	10 (47.6)	5 (23.8)	5 (23.8)	1 (4.8)	10 (47.6)	6 (28.6)	5 (23.8)	0 (0.0)
Type II (n= 3)	2 (66.7)	0 (0.0)	1 (33.3)	0 (0.0)	2 (66.7)	0 (0.0)	1 (33.3)	0 (0.0)
Uninfected (n= 18)	14 (77.8)	3 (16.7)	1 (5.6)	0 (0.0)	13 (72.2)	4 (22.2)	1 (5.6)	0 (0.0)
	Mononuclear cell (MNC) infiltration							
	Normal	Mild	Moderate	Marked	Normal	Mild	Moderate	Marked
	No. (%)	No. (%)	No. (%)	No. (%)	No. (%)	No. (%)	No. (%)	No. (%)
Type I (n= 48)	0 (0.0)	29 (60.4)	14 (29.2)	5 (10.4)	0 (0.0)	29 (60.4)	14 (29.2)	5 (10.4)
Intermediate (n=21)	0 (0.0)	11 (52.4)	9 (42.9)	1 (4.8)	0 (0.0)	11 (52.4)	10 (47.6)	0 (0.0)
Type II (n= 3)	0 (0.0)	2 (66.7)	1 (33.3)	0 (0.0)	0 (0.0)	2 (66.7)	1 (33.3)	0 (0.0)
Uninfected (n= 18)	0 (0.0)	9 (50.0)	8 (44.4)	1 (5.6)	1 (5.6)	8 (44.4)	7 (38.9)	2 (11.1)

Discussion

Prevalence of *H. pylori* infection varies between and within geographic regions depending on socioeconomic factors.[10] Its prevalence also varies depending on the method used to detect infection because gastric biopsy-based tests may give false negative results due to sampling error[25] and serological tests may give false positive results as they cannot differentiate current infection from past exposure.[26] Most of

the epidemiological studies involve either serological tests or ^{13}C-Urea breath test, as they are non-invasive. As cases included in the present study were symptomatic patients undergoing diagnostic endoscopy, *H. pylori* positivity was detected by both gastric biopsy-based and serological methods. Variable results were found with different methods. Among the gastric biopsy-based tests, culture showed the most positivity and histology showed the least. Low positivity by histology may be due to taking only two biopsy samples, one from the antrum and one from the body. Taking two biopsies from the antrum could give better result in histology.[25] Considering any of the gastric biopsy-based test positive result as the status of infection, 76.7% patients were found infected. This value is lower than previous studies in Bangladesh on asymptomatic population that used ELISA or ^{13}C-Urea breath test. ELISA positivity in our study was consistent with previous study in Bangladesh.[21]

This is the first study in Bangladesh that explored infection with Type I, Intermediate and Type II strains of *H. pylori* in adult dyspeptic patients from their serum IgG response by standardized commercial Western Blot test. Majority of the patients had response to Type I strain (response to both CagA and VacA) and response to Type II strain was very low. This reflects high prevalence of infection by Type I strain in adult dyspeptic patients. But this value is lower than another study in Bangladesh on asymptomatic children by Sarker *et al*,[22] who found 81% children seropositive for both CagA and VacA by an in-house Western Blot analyses. Response to VacA was lower in the present study. This may be due to different strains of *H. pylori* in the Western Blot tests. The commercial Western Blot kit of this study used ATCC 4950327 and the in-house Western Blot of Sarker *et al* used DH2 strain.[28] As DH2 is a local strain, possibly Bangladeshi population respond better to VacA of this strain than to VacA of ATCC 49503. Another important finding of this study was that no patient had response to VacA without response to CagA.

This study shows pattern of endoscopic gastro-duodenal diseases. This is the first study in Bangladesh that shows grades of histological gastritis according to updated Sydney system in adult dyspeptic patients. The MNC infiltration (chronic inflammation) in all patients and PMN infiltration (activity of inflammation) were found in less than half of the cases. Prevalence of atrophic gastritis and intestinal metaplasia that predispose to gastric carcinoma were very low.

In this study, no association of *H. pylori* strain types with endoscopic gastritis, duodenitis or peptic ulcer disease was found. This finding is consistent with studies carried out in East Asia, India and Bangladesh[16-19] but inconsistent with studies in Western countries.[13-15] As the number of patients with gastric carcinoma and reflux oesophagitis was very small, their association with strain types could not be analyzed.

In the present study, no association of strain types with PMN or MNC infiltration was found. It is consistent with Yamaoka *et al*[17] who did not find association of CagA and VacA with severity of histological gastritis in East Asian population but inconsistent with Warburton *et al*[14] who found CagA and VacA associated with severity of gastritis in Western population.

In summary, prevalence of *H. pylori* infection varied depending on the method used to detect it. Prevalence of atrophic gastritis and intestinal metaplasia were low. Majority of the dyspeptic patients were infected with Type I strain of *H. pylori* and this strain type was not associated with endoscopic gastritis, duodenitis, PUD or histological grades of PMN or MNC infiltration in these patients

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[Conflict of Interest: none declared]