

Epidemiology of *H. pylori* and its Relation with Gastrointestinal Disorders, A Community-based Study in Dhaka, Bangladesh

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ABSTRACT

Helicobacter pylori infection is common worldwide and its prevalence is higher in the developing countries. Earlier studies in using urea breath test showed a high prevalence (67%) of *H. pylori* in early childhood

in Bangladesh. Data on *H. pylori* prevalence using bacterial culture is limited in Bangladesh. The aim of the study was to investigate the prevalence of *H. pylori* in Bangladeshi subjects using bacterial culture. We also determined the prevalence of infection among different age groups and find out the correlation between the prevalence of *H. pylori* and the related sociodemographic parameters. A cross-sectional study was conducted among randomly selected households from a peri-urban community in Dhaka, Bangladesh to get an idea about *H. pylori* status in the lower socioeconomic area. Sociodemographic and clinical information and stool specimen for screening *H. pylori* infection by stool antigen test were collected. Gastric biopsy was collected for culture from those positive in stool antigen test. A total of 287 subjects were screened by stool antigen test, of them, 92.7% were positive for stool antigen test. Of 259 stool antigen positive samples, 59.1% ($n = 153$) were *H. pylori* culture positive. Our data suggest that *H. pylori* infection is significantly associated with age and smoking habit (P value < 0.05). In addition, dyspeptic symptoms are significantly higher in *H. pylori* culture positive subjects than the *H. pylori* culture negative subjects. Endoscopic examination suggests that the gastroesophageal pathologies are significantly associated (P value < 0.05) and duodenal pathologies are moderately associated (P value 0.059) with *H. pylori* infection. So, *H. pylori* culture test is auspicious the significant colonization that might lead to pathological outcomes.

Key words: *H. pylori*; Bangladesh; Prevalence; Gastrointestinal disorders

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INTRODUCTION

Helicobacter pylori inhabit at least 50% of the world's population, the

prevalence of *H. pylori* infection appears to be higher in developing countries as compared to industrially developed countries and the prevalence vary between populations and between groups within the same population^[1,2]. The prevalence in Asia and Africa varies from 54.7% -79.1%, in North and Latin America the prevalence is 37.1% and 63.4% and in Europe, the prevalence is typically 47.0%^[3].

H. pylori infection may be acquired at any age but once acquired, the infection persists for years and often for the lifetime. Although the route(s) of transmission of *H. pylori* infection remains dubious, the gastro-oral, oral-oral and fecal-oral routes are being speculated^[4]. Recently, intrafamilial transmission of *H. pylori*, predominantly from mother to child has been reported^[5,6]. In both developing and developed countries, a high prevalence of *H. pylori* is seemingly related to poor socioeconomic conditions, such as overcrowded housing, low income, and the use of a stove for heating or cooking^[7]. A variety of clinical outcomes is associated with *H. pylori* infection has been reported including chronic gastritis, peptic and duodenal ulcer, gastric cancer, lymphomas, gastroesophageal reflux disease and adenocarcinoma of the gastric cardia^[8,9].

In a study conducted in 1995, 92% of the Bangladeshi population was found to be *H. pylori* positive by ELISA^[10,11]. In the same time, 67% of the children of a lower socioeconomic area was found to be *H. pylori* positive detected by urea breath test^[12]. It was found that re-infection of *H. pylori* was 5.02% per year in the *H. pylori* eradicated patients using urea breath test^[13]. 16s rRNA-based *H. pylori* specific PCR study showed that 67% of the dyspeptic patients in Chittagong were *H. pylori* positive^[14]. However, prevalence data in the lower socioeconomic area of Bangladesh was not available for a long time and existing prevalence studies were not confirmed by culture test, which is considered as gold standard for *H. pylori* detection.

The objectives of this study were to estimate the prevalence of *H. pylori* infection in Bangladeshi subjects in the lower socioeconomic area by *H. pylori* stool antigen and culture. We also analyzed the relationship of *H. pylori* prevalence with different sociodemographic parameters, dyspeptic symptoms as well as endoscopic findings.

MATERIALS AND METHODS

Study population

A cross-sectional study was conducted at Nandipara (a peri-urban community), 10 km from Dhaka, Bangladesh between July 2005 and November 2007. Since 1985, Clinical Services Divisions (CSD), ICDDR, B, maintained a household listing of Nandipara and a database of 3000 population and used to provide routine clinical services through outpatient clinic. We used the database and infrastructure of CSD, ICDDR, B, for selection and enrollment of patients during the study period. One hundred and twelve families from Nandipara community were randomly selected for the study; the selected families including the family head were (1) invited to visit the outpatient clinics at Nandipara; (2) approached to participate in the study; and (3) screened for inclusion/exclusion criteria (exclusion criteria includes concomitant disease; regular NSAID or corticosteroid use; treatment with antibiotics or proton-pump inhibitors for any household members in last 6 weeks; and pregnancy). Of the 112 families approached, fifty-five families (312 family members) were eligible for enrollment and agreed to provide a fecal sample for stool antigen testing. The sample collection from this population to culture *H. pylori* has been described in a flow diagram (Figure 1). The study was approved by Ethical review Committee (ERC) of icddr,b (Approval # 2005-010). Informed consent were obtained from the participants (individual consent for all members

above the age of 18 and parental consent for children below 18 were obtained) for enrollment. Families were compensated for wage loss and transportation^[15].

Questionnaire

Subjects were interviewed by using a pre-structured standard questionnaire. Socio-demographic information (e.g. age, Gender, occupation, individual education level, size of family, approximate monthly family income, marital status, self-reported socioeconomic group, individual hygiene and smoking habits) medical history (hyperacidity, heartburn, acid eructation, epigastric and abdominal pain etc) and medication history (NSAID and proton pump inhibitors in last two months) for each family member were also collected.

Stool for antigen test

Morning stool specimen from each family member was collected and examined for *H. pylori* stool antigen using a commercial enzyme immunoassay (FemtoLab *H. pylori* Cnx, DakoCytomation Ltd, United Kingdom) as recommended by the manufacturers (the sensitivity and specificity of the test were 78.9% and 87.0%, respectively)^[16]. All *H. pylori* stool antigen-positive subjects (irrespective of participation to upper GI endoscopy) were offered treatment with standard triple therapy.

Endoscopic examination

All stool antigen test positive members of the enrolled household were invited for upper gastrointestinal endoscopy and/or gastric juice collection at ICDDR,B clinical research unit. Information on clinical symptoms related to gastric pathologies were collected by physician before endoscopy (epigastric pain, acid eructation, heartburn, anorexia, nausea, vomiting etc). Upper gastrointestinal endoscopy was conducted in a standard fashion with visualization of the esophagus, stomach, and duodenum using a short-acting sedative (10 mg lidocaine) and local anesthetic spray. Endoscopy examination was done by trained endoscopist using a sterile endoscope for each participant (GIF XQ 30, Olympus Optical Company, Japan). Inflammation of the esophagus, stomach, and duodenum were referred to as esophagitis, gastritis and duodenitis respectively. The esophageal mucosal erosion caused by the reflux of gastric component back to the throat through the esophagus was referred to as reflux esophagitis [Clinically this is known as Gastroesophageal Reflux Disease (GERD)]. If the stomach's lining was eroded, gets swollen or inflamed lesions was denoted as erosive gastritis. The condition in duodenum with inflamed duodenal wall becomes eroded and open sores formed was denoted as erosive duodenitis. Antral gastritis was denoted while inflammation occurred in the lower portion of the stomach (Antrum). Ulcer formation in stomach and duodenum referred to as ulcerated.

Culture of Biopsy samples and Gastric juice

Two biopsies were taken (antrum, corpus) using a sterilized endoscope in each adult patients. In younger children (Age ≤ 12 years), gastric juice samples were aspirated through a sterile nasogastric tube. The biopsy samples were placed in separate 1 ml Stuart transport media and transported on ice to the laboratory and processed within three hours of collection. Biopsy samples were vortexed vigorously for 5 min and the pH of gastric juice was adjusted to 7.4 using 1M Tris HCL. Both biopsy and gastric juice samples were plated on brain heart infusion agar (Oxoid, Ltd, Basingstoke, Hampshire, United Kingdom) supplemented with 7% sheep blood, 0.4% IsoVitaleX, and *H. pylori* Dent supplement

(Oxoid). Plates were incubated at 37°C in an atmosphere of 10% CO₂, 85% N₂ and 5% O₂, for 3 to 6 days. *H. pylori* colonies were identified based on their typical morphology, characteristic appearance on Gram staining and the production of oxidase, catalase, and urease according to the procedures described elsewhere^[17]. A pure culture of pooled isolates was stored at -80°C in 0.5 mL of brucella broth with 15% glycerol. The method of determining the mixed infection from *H. pylori* culture was described previously^[15].

Statistical analysis

The data were recorded and analyzed by using SPSS for Windows (Version 17, Chicago, IL, USA). Data were analyzed by chi-square (χ^2) test. The level of statistical significance was set at 0.05, and all tests were two-tailed. Odds ratio (OR) and 95% confidence intervals (CI) were calculated by unconditional logistic regression model to analyze the risk factors (*H. pylori* culture positive and smoking) and test the different dyspeptic symptoms as outcomes. We also performed logistic regression model to find the combined effect of *H. pylori* and smoking in case of dyspeptic symptoms.

RESULTS

Among 112 eligible households invited to participate the study 55 households agreed to participate the study. Stool specimen of 287 subjects from 55 households was screened by stool antigen test and of them, 266 (92.7%) was positive for *H. pylori*. All 266 *H. pylori* positive subjects were invited for upper gastrointestinal endoscopy and of them, 259 agreed to provide biopsy and/or gastric juice specimen (in children ≤ 12 Year $n = 49$) for culture. Of these 259 subjects, 153 (59.1%) were culture positive for *H. pylori* (Figure 1).

The Sociodemographic characteristics of the enrolled patients are presented in Table 1. Approximately half of the patients were male, 46.7% were children, and 53.3% were adult. Half of the patients had primary education and 15.3% of the patients reported smoking habit. 44.3% (77/174) were infected with mixed strains of *H. pylori*, 40.2% (104/259) reported dyspeptic symptoms and most of the patients 93.6% (150/160) had one or more gastrointestinal pathology.

The prevalence of *H. pylori* infection as determined by stool antigen test and by the culture of biopsy specimen among enrolled subjects (stratified by age, gender, income, education and smoking habit) is shown in Table 2. The stool antigen test detected *H. pylori* antigen in 92.7% of subjects. The prevalence of HP was 91.9% in subjects below 15 years of age. Such rate continued to be maintained with increasing age (Table 2). Among the subjects who provided biopsy samples, 59.1% were *H. pylori* culture positive. A significant relationship between the age and *H. pylori* infection (P value < 0.05) was observed (Table 2). *H. pylori* infection was significantly higher in patients with smoking habit compared to non-smokers (82.5%; 33/40 versus 54.8%; 120/219) (P value < 0.05). However, no significant difference in prevalence was observed when compared to gender, income, and education (Table 2). We also checked the prevalence of mixed infection in different age groups, smokers and genders. The result showed a significant difference (P value < 0.05) where the single and mixed infection was calculated to be increased with age and smoking habit but not significantly associated with gender (Table S3).

Among 259 subjects those agreed for upper GI endoscopy 160 had one or more pathological conditions as determined during endoscopic examination. Of them, 77% (123/160) were *H. pylori* culture positive. Among these 160 subjects, 75 (46.9%) have esophageal abnormalities, 149 (93.1%) have gastric abnormalities and 60 (37.5%) have duodenal abnormalities (Table 1). Patients with

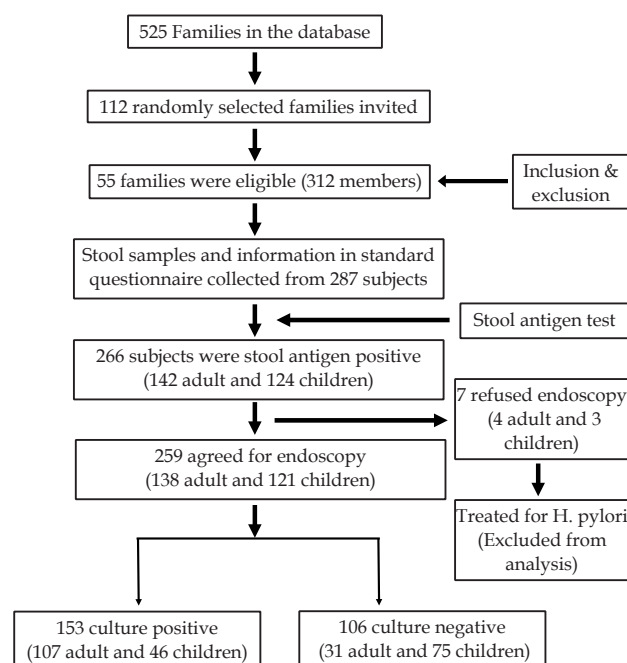


Figure 1 Flow diagram showing population selection and analysis of study.

esophageal and gastric pathology had significantly higher *H. pylori* culture positive (86.7% vs 68.2%; P value 0.02 and 78.5% vs 54.6%; P value 0.001). The esophageal, gastric and duodenal pathologies of the patients are presented in Table 3. We also examined the relationship between gastrointestinal pathology and stool antigen positivity and no correlation was observed (Table S1).

Binary logistic regression model was used to find the odds ratio for dyspeptic symptoms, where the dyspeptic symptoms were independent variable and *H. pylori* culture positivity as well as smoking habit was dependent variable. *H. pylori* culture positive subjects have 2.53 times more probability of epigastric and abdominal pain (OR 2.53; 95% CI 1.28-5.01), 2.53 times more probability of heartburn (OR 2.53; 95% CI 1.43-4.48), and 4.03 times more probability of nausea (OR 4.03; 95% CI 0.87-18.56) (P value < 0.05) (Table 4). *H. pylori* culture positive subjects have 3.46 times higher probability of dyspeptic symptoms (51.6% vs 23.6%; OR 3.46; 95% CI 1.99-5.99). These dyspeptic symptoms are also significantly higher in smokers compared to non-smokers (P value < 0.05) (Table S3). Whereas, Smoking habit was significantly associated with only the duodenal pathologies but not with esophageal and gastric pathologies (Table S4).

DISCUSSION

The prevalence of *H. pylori* infection varies worldwide, with major risk factors of the poor living condition and infected family members, have great public health implications^[18]. Nandipara is a periurban area with lower socioeconomic condition. Study of *H. pylori* in this lower socio-economic area adjacent to Dhaka city has an important implication as it might give a clear picture not only the other slum/periurban area of Dhaka city but also assume *H. pylori* condition of entire Bangladesh. *H. pylori* culture showed a high prevalence of *Helicobacter pylori* in the studied population. Approximately 93% of the population was *H. pylori* positive by stool antigen test and among those, 59.1% were *H. pylori* culture positive. High prevalence of *H. pylori* infection was found in every group based on age, gender, income, education and smoking habit while analyzed with stool

antigen test data. When we analyzed culture-confirmed cases, our data suggests a significant association of *H. pylori* infection with age and smoking habit. Whereas, there is no significant association was observed between *H. pylori* infection with gender, education and monthly income. This High prevalence may be the cause of frequent transmission of *H. pylori* in that lower socioeconomic area^[19]. Previously we showed that the transmission of *H. pylori* occurred in the intra-familial level in the same region^[5]. We also found frequent co-colonization by the mixed strains of *H. pylori* in that lower socioeconomic area^[15].

In recent years, risk factors for *H. pylori* infection have been investigated around the world. *H. pylori* infection was thought to be related to socioeconomic status, however, the results from different groups have been contradictory^[20]. Hence, the risk factors for *H. pylori* infection are still unclear^[21]. Malcolm *et al*^[22] reported that the *H. pylori* infection were associated with age, gender, and socioeconomic conditions. However, our findings suggested that there was no association between the *H. pylori* infection and gender but related to age. Previous studies showed that the prevalence of *H. pylori* infection increased with age, and the prevalence was lower

in subjects younger than 20 years old^[23]. Our data suggest a similar prevalence of *H. pylori* in different age groups in case of stool antigen report by ELISA, while the prevalence of *H. pylori* infection was increased with the age when considered the culture report. So, the colonization of *H. pylori* is a very slow process and emerge with the increased age. As age is a factor for having *H. pylori* culture positive, we compare the previous mixed infection data in different age groups, gender, and smokers^[15]. The data also showed a significant association of *H. pylori* mixed infection with age and smoking habit but not with gender.

The prevalence of *H. pylori* in children varies in the developed and developing countries and in different age groups^[24]. In the present study, 92.5% children were found to be *H. pylori* stool antigen positive and 38.0% of them were *H. pylori* culture positive (Table 1). Among the 49 gastric juice samples from younger children (age < 12), 24 (48.97%) were culture positive. This result is alarming as they are culture positive at their early age and are at high risk of gastrointestinal disorders. This is to be noted that due to technical difficulties culture positivity from gastric juice might be low, so, even higher prevalence might exist. The reason for this high prevalence

Table 1 The characteristics of study sample with their demographic information. (n = 287, n = 259 for culture test, n = 160 for gastrointestinal disorders).

		Child (Age < 18)	Adult (Age > 18)	Total
Age	< 15	112 (100.0%)		112 (39.0%)
	16-25	22 (30.6%)	50 (69.4%)	72 (25.1%)
	26-35		24 (100.0%)	24 (8.4%)
	36-45		49 (100.0%)	49 (17.1%)
	> 46		30 (100.0%)	30 (10.4%)
		134 (46.7%)	153 (53.3%)	287
Gender	Male	71 (47.7%)	78 (52.3%)	149 (51.9%)
	Female	63 (45.67%)	75 (54.3%)	138 (48.1%)
Income	Lower (< Tk5000)	133 (48.9%)	139 (51.1%)	272 (94.8%)
	Higher (> Tk5000)	1 (6.7%)	14 (93.3%)	15 (5.2%)
Education	Illiterate	41 (32.8%)	84 (67.2%)	125 (43.6%)
	Primary education	92 (57.1%)	69 (42.85%)	161 (56.1%)
	Secondary education	1 (100.0%)	0 (0.0%)	1 (0.3%)
Smoking habit	Smoker	0 (0.0%)	44 (100.0%)	44 (15.3%)
	Non smoker	134 (55.1%)	109 (44.9%)	243 (84.7%)
<i>H. pylori</i> stool antigen test	Positive	124 (46.6%)	142 (53.4%)	266 (92.7%)
	Negative	10 (47.6%)	11 (52.4%)	21 (7.3%)
<i>H. pylori</i> culture	positive	46 (30.1%)	107 (69.9%)	153 (59.1%)
	Negative	75 (70.8%)	31 (29.2%)	106 (40.9%)
Types of infection	No infection	33 (71.7%)	13 (28.3%)	46 (26.4%)
	Single infection	13 (25.5%)	38 (74.5%)	51 (29.3%)
	Mixed infection	25 (32.5%)	52 (67.5%)	77 (44.3%)
Gastrointestinal disorder	Normal	7 (70.0%)	3 (30.0%)	10 (6.3%)
	Pathological condition	25 (16.7%)	125 (83.3%)	150 (93.7%)
	Esophagus	2 (2.7%)	73 (97.3%)	75 (46.9%)
	Stomach	25 (16.8%)	124 (83.2%)	149 (93.1%)
	Duodenum	3 (5.0%)	57 (95.0%)	60 (37.5%)
Dyspeptic symptoms	Normal	112 (72.3%)	43 (27.7%)	155 (59.8%)
	» Dyspepsia	9 (8.7%)	95 (91.3%)	104 (40.2%)
	Pain	5 (8.6%)	53 (91.4%)	58 (22.4%)
	Acid eructation	1 (5.6%)	17 (94.4%)	18 (6.9%)
	Heartburn	7 (7.9%)	82 (92.1%)	89 (34.4%)
	Anorexia	0 (0.0%)	20 (100.0%)	20 (7.7%)
	Nausea	0 (0.0%)	13 (100.0%)	13 (5.0%)
	Vomiting	0 (0.0%)	14 (100.0%)	14 (5.4%)

» The subject show any of the single or multiple dyspeptic symptoms.

in children might be the poor hygienic condition as well as higher intra-familial transmission from mother to child in that lower socioeconomic area^[19,5].

Multivariate logistic regression model analysis revealed that annual family income was an important risk factor for *H. pylori* infection^[23]. Our data is not showing any significant difference in the monthly income and *H. pylori* culture positive. There is a similar prevalence of *H. pylori* infection in both of the lower (57.4%) and comparatively higher (64.3%) income group (Table 1). In that low socioeconomic area, we found a small number of families who have a high income in respect with that area. So this may be limiting

Table 2 Prevalence of *H. pylori* infection among 287 subjects enrolled in the study. Subjects were screened for *H. pylori* by stool antigen test. Biopsy specimen from stool antigen test positive subjects were used for culture. *H. pylori* prevalence was compared among different age, gender, income, education, smoking behavior and gastric symptoms.

Variable	HP stool antigen positive (n = 287) (Percentage)	HP culture positive (n = 259) (Percentage)	P-value for HP culture
Age			
< 15	103 (91.9)	35 (35.0)	<0.05
16-25	68 (94.4)	48 (71.6)	
26-35	24 (100.0)	17 (73.9)	
36-45	45 (91.8)	33 (73.3)	
>46	26 (86.7)	20 (83.3)	
Gender			
Male	142 (95.3)	80 (57.6)	0.342
Female	124 (89.8)	73 (60.8)	
Income			
Lower (< Tk5000)	251 (92.3)	144 (57.4)	0.438
Higher (> Tk5000)	15 (100.0)	9 (64.3)	
Education			
Illiterate	117 (93.6)	67 (58.3)	0.463
Primary education	148 (91.9)	86 (60.1)	
Secondary education	1 (100.0)	0 (0.0)	
Smoking Habit			
Smoker	41 (93.2)	33 (82.5)	<0.05
Non-smoker	225 (92.6)	120 (54.8)	
Total	266 (92.68%)	153 (59.07%)	

Table 3 Prevalence of esophageal, gastric and duodenal pathological conditions as observed during endoscopic examination and prevalence of *H. pylori* (n = 160).

Variable	Participant (%)	HP positive (%)	P-value
Esophagus			
Normal	85 (53.1)	58 (68.2)	0.02
Esophagitis	73 (45.6)	63 (86.3)	
Reflux esophagitis	2 (1.3)	2 (100.0)	
Stomach			
Normal	11 (6.9)	6 (54.6)	0.001
Gastritis	94 (58.8)	68 (72.3)	
Erosive gastritis (EG)*	42 (25.6)	41 (97.6)	
Antral Gastritis	13 (8.1)	8 (61.5)	
Duodenum			
Normal	100 (62.5)	72 (72.0)	0.059
Erosive duodenitis	47 (29.38)	38 (80.9)	
Ulcerated	13 (8.1)	13 (100.0)	

*One sample was from a patient having erosive gastritis along with ulcer and gastric polyp. The subject was *H. pylori* culture positive.

factor in this case. It is generally considered that risk factors for *H. pylori* infection include sharing a bed with others in childhood, more siblings, more family members, and lower education status^[25]. We found a significant correlation between number of family members and *H. pylori* infection (*P* value 0.002, data not presented). In the families with more family members, there is a high rate of infection with *H. pylori*.

There was a general trend in which higher education status was associated with a lower prevalence of infection, and it was confirmed by multivariate logistic regression analysis^[26]. However, we did not find any significant difference between *H. pylori* infection and educational status.

Based on *H. pylori* culture data, we found a significant relationship between smoking and *H. pylori* infection (Table 2). So, smoking might be a risk factor for the *H. pylori* infection in Bangladesh. Several studies have reported the relationship between smoking and *H. pylori* infection, but none of these studies showed a positive association^[27-31]. Interestingly, all of the studies detected *H. pylori* from serum sample by ELISA, but not used culture as the gold standard for *H. pylori* detection^[32]. So, it might be possible that *H. pylori* find a suitable environment to colonize in the stomach of smokers leading to culture positive. It has been reported that *H. pylori* infection, smoking, and heavy drinking increase the risk of gastric cancer^[33]. The present analyses showed that smoking was associated with dyspeptic symptoms and duodenal pathology, however not related to gastro-esophageal pathology (Table S4 and S6). Surprisingly the outcome was supported by a previous study reporting smoking and *H. pylori* synergistically contribute to duodenal ulceration^[34].

The subjects with gastrointestinal signs have a high prevalence of *H. pylori*. We found a significant difference of *H. pylori* infection between subjects with gastroesophageal and gastroduodenal abnormalities and subjects with no abnormalities. The association between *H. pylori* infection and chronic gastritis was recognized early^[35]. A crucial role of the infection in peptic ulcer disease has been firmly established^[34] and accumulating data have also supported an association between *H. pylori* infection and gastric cancer^[33]. We found a relationship between esophageal and gastroduodenal abnormalities and *H. pylori* infection, providing evidence for the probable involvement of *H. pylori* in the progression of gastrointestinal abnormalities. Especially, 100% of the subjects with duodenal ulcer and reflux esophagitis as well as 97.6% subjects with erosive gastritis were *H. pylori* culture positive. Our data also suggest *H. pylori* may be a risk factor for dyspeptic symptoms.

Table 4 The dyspeptic symptoms in *H. pylori* culture positive subjects compared with *H. pylori* culture negative subjects.

Symptoms	<i>H. pylori</i> culture positive (n = 153)	<i>H. pylori</i> culture negative (n = 106)	P	P*	Odds ratio*	95% confidence interval*
Pain	40 (26.1%)	13 (12.3%)	0.004	0.008	2.53	1.28-5.01
Acid eructation	10 (6.54%)	6 (5.66%)	0.496	0.774	1.17	0.41-3.31
Heartburn	61 (39.9%)	22 (20.8%)	0.001	0.001	2.53	1.43-4.48
Anorexia	18 (11.8%)	0 (0.00%)	< 0.001	0.996	**	**
Nausea	11 (7.2%)	2 (1.9%)	0.046	0.074	4.03	0.87-18.56
Vomiting	11 (7.2%)	3 (2.8%)	0.104	0.141	2.66	0.72-9.77
» Dyspepsia	79 (51.6%)	25 (23.6%)	< 0.001	< 0.001	3.46	1.99-5.99

* values found from logistic regression analyses. ** irrelevant data may be due to absence of signs. » The subject show any of the single or multiple dyspeptic symptoms.

Table S1 Relationship between Stool antigen positivity and gastrointestinal symptoms. Most of the subjects with normal endoscopic examination were ELISA positive similar to the symptomatic subjects. Very low number of subjects were stool antigen negative.

Esophagus	<i>H. pylori</i> ELISA positive (%)	<i>H. pylori</i> ELISA negative (%)	P value
Normal	86 (97.6)	2 (2.4)	0.885
Esophagitis	73 (98.6)	1 (1.4)	
Reflux esophagitis	2 (100.0)	0 (0.0)	
Stomach			
Normal	11 (90.9)	1 (9.1)	0.289
Gastritis	94 (98.9)	0 (1.1)	
Erosive gastritis (EG)*	42 (97.6)	1 (2.4)	
Antral gastritis	14 (100.0)	0 (0.0)	
Duodenum			
Normal	101 (97.0)	3 (3.0)	0.4
Erosive duodenitis	47 (100.0)	0 (0.0)	
Ulcerated	13 (100.0)	0 (0.0)	

*one sample was from a patient having erosive gastritis along with ulcer and gastric polyp. The subject was *H. pylori* ELISA positive.

Table S2 The prevalence of Mixed *H. pylori* infection in different age groups.

Age	Single infection	Mixed infection	<i>H. pylori</i> culture negative	P-value
< 15	10 (17.24%)	19 (32.76%)	29 (50.00%)	
16-25	15 (31.25%)	25 (52.08%)	8 (16.67%)	
26-35	6 (35.29%)	7 (41.18%)	4 (23.53%)	0.001
36-45	12 (37.50%)	16 (50.00%)	4 (12.50%)	
> 46	8 (42.11%)	10 (52.63%)	1 (5.26%)	
Gender				
Male	33 (35.86%)	36 (39.13)	23 (25.00%)	0.124
Female	18 (21.95%)	41 (50.00%)	23 (28.05%)	
Smoking				
Yes	13 (43.33%)	15 (50.00%)	2 (6.67%)	0.018
No	38 (26.39%)	62 (43.06%)	44 (30.56%)	

Our findings suggested that *H. pylori* culture data was associated with gastrointestinal pathologies (Table 3) but ELISA data was not associated (Table S2). ELISA is a sensitive technique, whereas culture is a confirmatory technique. So, the persons who are positive in ELISA but negative in culture test, might not be significantly colonized by *H. pylori*. This might be a cause that dyspeptic and gastrointestinal symptoms developed more in a culture positive subjects than the culture negative subjects (though ELISA positive). As age is a factor for culture positivity, it can be assumed that the subjects who were ELISA positive are at risk for future culture positive and consequently gastrointestinal abnormalities followed by an ulcer. So, the success rate of culture test is an indicator for significant colonization of *H. pylori* that might be resulted to pathological consequences.

In conclusion, the prevalence of *H. pylori* infection is high in that lower socioeconomic group in Bangladesh. As a developing country, Bangladesh is at high risk for Gastric diseases, such as gastric and duodenal ulcer and Gastric cancer. The prevalence of *H. pylori* infection was related to age, the size of family, smoking habit, but was not related to gender, education and family income. All the gastroesophageal and gastroduodenal abnormalities are majorly related with *H. pylori* culture test emphasizing the culture test as a marker for those symptoms while many asymptomatic subjects

Table S3 The rate of dyspeptic symptoms in smokers compared with nonsmokers in 287 participants enrolled in the study.

Symptoms	Smoker (n = 44)	Nonsmoker (n = 243)	P	P *	Odds ratio*	95% confidence interval*
Pain	20 (45.5%)	38 (15.6%)	<0.001	<0.001	4.49	2.26-8.94
Acid eructation	7 (15.9%)	11 (4.5%)	0.011	0.007	3.99	1.46-10.95
Heartburn	25 (56.8%)	64 (26.3%)	<0.001	<0.001	3.68	1.90-7.13
Anorexia	7 (15.9%)	13 (5.4%)	0.02	0.016	3.35	1.25-8.94
Nausea	5 (11.4%)	8 (3.3%)	0.034	0.026	3.77	1.17-12.11
Vomiting	5 (11.4%)	9 (3.7%)	0.046	0.039	3.33	1.06-10.47
»Dyspepsia	31 (70.5%)	80 (32.9%)	<0.001	<0.001	4.86	2.41-9.79

*values found from logistic regression analyses. »The subject show any of the single or multiple dyspeptic symptoms.

Table S4 The gastro-esophageal and gastro-duodenal abnormalities in smokers and nonsmokers.

	Smoker (%)	Nonsmoker (%)	P value
Esophagus			
Normal	13 (15.3)	72 (84.7)	0.078
Esophagitis	21 (29.2)	52 (70.8)	
Reflux esophagitis	1 (50.0)	1 (50.0)	
Stomach			
Normal	0 (0.0)	11 (100.0)	0.211
Gastritis	21 (22.3)	73 (77.7)	
Erosive gastritis (EG)*	12 (28.6)	30 (71.4)	
Antral gastritis	2 (15.4)	11 (84.6)	
Duodenum			
Normal	8 (8.0)	92 (92.0)	<0.001
Erosive duodenitis	19 (40.4)	28 (59.6)	
Ulcerated	8 (61.5)	5 (38.5)	

*One sample was from a patient having erosive gastritis along with ulcer and gastric polyp. The subject was Nonsmoker.

might be ELISA positive. Our suggestion will be to confirm *H. pylori* colonization by culture test of the subjects with gastrointestinal symptoms mostly ulcers and cancer. This study represents only the status of lower socioeconomic areas, so detailed studies will be required to understand the *H. pylori* status and their risk factors in nationwide to emphasize on *H. pylori* eradication strategy in Bangladesh.

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REFERENCE

1. Frenck RW, Jr., Clemens J Helicobacter in the developing world. *Microbes Infect* 2003; **5(8)**: 705-713. [PMID: 12814771]; [DOI: S1286457903001126] [pii]
2. Eusebi LH, Zagari RM, Bazzoli F. Epidemiology of Helicobacter pylori infection. *Helicobacter* 2014; **19 Suppl 1**: 1-5. [PMID: 25167938]; [DOI: 10.1111/hel.12165]

3. Hooi JKY, Lai WY, Ng WK, Suen MMY, Underwood FE, Tanyingoh D, Malfertheiner P, Graham DY, Wong VWS, Wu JCY, Chan FKL, Sung JY, Kaplan GG, Ng SC Global Prevalence of *Helicobacter pylori* Infection: Systematic Review and Meta-Analysis. *Gastroenterology* 2017; **153**(2): 420-429. [PMID: 28456631]; [DOI: 10.1053/j.gastro.2017.04.022]
4. Kivi M, Tindberg Y *Helicobacter pylori* occurrence and transmission: a family affair? *Scandinavian journal of infectious diseases* 2006; **38**(6-7): 407-417. [PMID: 16798686]; [DOI: 10.1080/00365540600585131]
5. Nahar S, Kibria KM, Hossain ME, Sultana J, Sarker SA, Engstrand L, Bardhan PK, Rahman M, Endtz HP. Evidence of intra-familial transmission of *Helicobacter pylori* by PCR-based RAPD fingerprinting in Bangladesh. *Eur J Clin Microbiol Infect Dis* 2009; **28**(7): 767-773. [PMID: 19190943]; [DOI: 10.1007/s10096-008-0699-8]
6. Mamishi S, Eshaghi H, Mahmoudi S, Bahador A, Hosseinpour Sadeghi R, Najafi M, Farahmand F, Khodadad A, Pourakbari B Intrafamilial transmission of *Helicobacter pylori*: genotyping of faecal samples. *Br J Biomed Sci* 2016; **73**(1): 38-43. [PMID: 27182676]; [DOI: 10.1080/09674845.2016.1150666]
7. Moayyedi P, Axon AT, Feltbower R, Duffett S, Crocombe W, Braunholtz D, Richards ID, Dowell AC, Forman D. Relation of adult lifestyle and socioeconomic factors to the prevalence of *Helicobacter pylori* infection. *Int J Epidemiol* 2002; **31**(3): 624-631. [PMID: 12055165]
8. Pacifico L, Anania C, Osborn JF, Ferraro F, Chiesa C. Consequences of *Helicobacter pylori* infection in children. *World J Gastroenterol* 2010; **16**(41): 5181-5194.
9. Pilotto A, Franceschi M. *Helicobacter pylori* infection in older people. *World J Gastroenterol* 2014; **20**(21): 6364-6373. [PMID: 21049552]; [PMCID: PMC2975089]; [DOI: 10.3748/wjg.v20.i21.6364]
10. Ahmad MM, Rahman M, Rumi AK, Islam S, Huq F, Chowdhury MF, Jinnah F, Morshed MG, Hassan MS, Khan AK, Hasan M. Prevalence of *Helicobacter pylori* in asymptomatic population-a pilot serological study in Bangladesh. *J Epidemiol* 1997; **7**(4): 251-254. [PMID: 9465552]
11. Bardhan PK. Epidemiological features of *Helicobacter pylori* infection in developing countries. *Clin Infect Dis* 1997; **25**(5): 973-978. [PMID: 9402340]
12. Mahalanabis D, Rahman MM, Sarker SA, Bardhan PK, Hildebrand P, Beglinger C, Gyr K. *Helicobacter pylori* infection in the young in Bangladesh: prevalence, socioeconomic and nutritional aspects. *International journal of epidemiology* 1996; **25**(4): 894-898. [PMID: 8921472]
13. Ahmad MM, Ahmed DS, Rowshon AH, Dhar SC, Rahman M, Hasan M, Beglinger C, Gyr N, Khan AK. Long-term re-infection rate after *Helicobacter pylori* eradication in Bangladeshi adults. *Digestion* 2007; **75**(4): 173-176. [PMID: 17700024]; [DOI: 10.1159/000107046]
14. Habib AM, Alam MJ, Rudra B, Quader MA, Al-Forkan M. Analysis of *Helicobacter pylori* Prevalence in Chittagong, Bangladesh, Based on PCR and CLO Test. *Microbiol Insights* 2016; **9**: 47-50. [PMID: 27891051]; [PMCID: PMC5116947]; [DOI: 10.4137/MBI.S39858]
15. Kibria KM, Hossain ME, Sultana J, Sarker SA, Bardhan PK, Rahman M, Nahar S. The Prevalence of Mixed *Helicobacter pylori* Infections in Symptomatic and Asymptomatic Subjects in Dhaka, Bangladesh. *Helicobacter* 2015; **20**(5): 397-404. [PMID: 25827337]; [DOI: 10.1111/hel.12213]
16. Korkmaz H, Kesli R, Karabagli P, Terzi Y. Comparison of the diagnostic accuracy of five different stool antigen tests for the diagnosis of *Helicobacter pylori* infection. *Helicobacter* 2013; **18**(5): 384-391. [PMID: 23551920]; [DOI: 10.1111/hel.12053]
17. Perez-Perez GI. Accurate diagnosis of *Helicobacter pylori*. Culture, including transport. *Gastroenterol Clin North Am* 2000; **29**(4): 879-884. [PMID: 11190072]
18. Goh KL, Chan WK, Shiota S, Yamaoka Y. Epidemiology of *Helicobacter pylori* infection and public health implications. *Helicobacter* 2001; **16** Suppl 1: 1-9. [PMID: 21896079]; [PMCID: PMC3719046]; [DOI: 10.1111/j.1523-5378.2011.00874.x]
19. Sarker SA, Rahman MM, Mahalanabis D, Bardhan PK, Hildebrand P, Beglinger C, Gyr K. Prevalence of *Helicobacter pylori* infection in infants and family contacts in a poor Bangladesh community. *Dig Dis Sci* 1995; **40**(12): 2669-2672. [PMID: 8536529]
20. Koch A, Krause TG, Krogfelt K, Olsen OR, Fischer TK, Melbye M. Seroprevalence and risk factors for *Helicobacter pylori* infection in Greenlanders. *Helicobacter* 2005; **10**(5): 433-442. [PMID: 16181354]; [DOI: 10.1111/j.1523-5378.2005.00351.x]
21. Perez-Perez GI, Rothenbacher D, Brenner H. Epidemiology of *Helicobacter pylori* infection. *Helicobacter* 2004; **9** Suppl 1: 1-6. [PMID: 15347299]; [DOI: 10.1111/j.1083-4389.2004.00248.x]
22. Malcolm CA, MacKay WG, Shepherd A, Weaver LT. *Helicobacter pylori* in children is strongly associated with poverty. *Scott Med J* 2004; **49**(4): 136-138. [PMID: 15648706]; [DOI: 10.1177/003693300404900406]
23. Aguemon BD, Struelens MJ, Massougbodji A, Ouendo EM. Prevalence and risk-factors for *Helicobacter pylori* infection in urban and rural Beninese populations. *Clin Microbiol Infect* 2005; **11**(8): 611-617. [PMID: 16008612]; [DOI: 10.1111/j.1469-0691.2005.01189.x]
24. Yucel O. Prevention of *Helicobacter pylori* infection in childhood. *World J Gastroenterol* 2014; **20**(30): 10348-10354. [PMID: 25132751]; [PMCID: PMC4130842]; [DOI: 10.3748/wjg.v20.i30.10348]
25. Farrell S, Doherty GM, Milliken I, Shield MD, McCallion WA. Risk factors for *Helicobacter pylori* infection in children: an examination of the role played by intrafamilial bed sharing. *Pediatr Infect Dis J* 2005; **24**(2): 149-152. [PMID: 15702044]
26. Moreira ED, Jr., Santos RS, Nassri VB, Reis AT, Guerra AL, Alcantara AP, Matos JF, Carvalho WA, Moura CG, Silvani CS, Sant'Ana CS. Risk factors for *Helicobacter pylori* infection in children: is education a main determinant? *Epidemiol Infect* 2004; **132**(2): 327-335. [PMID: 15061508]; [PMCID: PMC2870109]
27. Reshetnikov OV, Denisova DV, Zavyalova LG, Haiva VM, Granberg C. *Helicobacter pylori* seropositivity among adolescents in Novosibirsk, Russia: prevalence and associated factors. *J Pediatr Gastroenterol Nutr* 2003; **36**(1): 72-76. [PMID: 12499999]
28. Malaty HM, Kim JG, Kim SD, Graham DY. Prevalence of *Helicobacter pylori* infection in Korean children: inverse relation to socioeconomic status despite a uniformly high prevalence in adults. *Am J Epidemiol* 1996; **143**(3): 257-262. [PMID: 8561159]
29. Tsugane S, Tei Y, Takahashi T, Watanabe S, Sugano K. Salty food intake and risk of *Helicobacter pylori* infection. *Jpn J Cancer Res* 1994; **85**(5): 474-478. [PMID: 8014104]; [PMCID: PMC5919501]
30. Shinchi K, Ishii H, Imanishi K, Kono S. Relationship of cigarette smoking, alcohol use, and dietary habits with *Helicobacter pylori* infection in Japanese men. *Scand J Gastroenterol* 1997; **32**(7): 651-655. [PMID: 9246703]
31. Shibata K, Moriyama M, Fukushima T, Kaetsu A, Miyazaki M, Une H. Green tea consumption and chronic atrophic gastritis: a cross-sectional study in a green tea production village. *J Epidemiol* 2000; **10**(5): 310-316. [PMID: 11059513]
32. Andrews J, Marsden B, Brown D, Wong VS, Wood E, Kelsey M. Comparison of three stool antigen tests for *Helicobacter pylori* detection. *J Clin Pathol* 2003; **56**(10): 769-771. [PMID: 14514781]; [PMCID: PMC1770087]
33. Massarrat S, Stolte M. Development of gastric cancer and its prevention. *Arch Iran Med* 2014; **17**(7): 514-520. [PMID: 24979566]; [DOI: 10141707/AIM.0013]

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34. Malfertheiner P, Chan FK, McColl KE. Peptic ulcer disease. *Lancet* 2009; **374**(9699): 1449-1461. [PMID: 19683340]; [DOI: 10.1016/S0140-6736(09)60938-7]
35. Marshall BJ, Warren JR. Unidentified curved bacilli in the

stomach of patients with gastritis and peptic ulceration. *Lancet* **1984; (8390):** 1311-1315. [PMID: 6145023]

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