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Original Article

Antibody Response to *Helicobacter pylori* among Patients with Suspected Gastric Disease

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Abstract

***Helicobacter pylori* infection is one of the most common infections worldwide. The prevalence of *H. pylori* infection varies widely by geographic area, age, race and socioeconomic status. The aim of this study was to determine the prevalence of anti-*H. pylori* IgG antibodies by ELISA method in common population in Bangladesh with respect to gender and age distribution. A total of 474 clinical samples were analyzed, of them 57.6% were positive and 42.4% were negative for anti-*H. pylori* IgG antibodies to *H. pylori*. There was significant association between *H. pylori* with gender and age. The seroprevalence was lower in females (53.5%) than in males (61.8%). Also, the anti-*H. pylori* positivity was higher in young age group (<15 years) both in males and females, and it was decreased with increasing age. The difference was statistically significant ($p > 0.05$).**

Keywords: *Helicobacter pylori*, seroprevalence, anti-*H. pylori* IgG

Introduction

Helicobacter pylori has been shown to play an important role in the development of gastritis and gastric ulcer. It is estimated that over 50% of the world population is infected with *H. pylori*[1]. *H. pylori* is a microaerophilic Gram-negative spiral-shaped bacterium which is causally related to chronic active gastritis, peptic ulcer disease[2], primary low-grade B-cell gastric lymphoma[3], and gastric carcinoma[4-5]. *H. pylori* infection is a common infection and is prevalent in both developing and developed countries. In contrast with industrialized nations, *H. pylori* infections occur early in life and with a higher frequency in the developing world. Also, while the prevalence of the infection has been dropped significantly in many parts of North America, Western Europe and Asia (especially Korea), no such decline has been noted in the developing world[6-7].

Epidemiological studies have revealed an association of *H. pylori* seroprevalence with increasing age[8], lower socioeconomic status[8-9] and crowdedness in the household[10] but not gastro-oesophageal reflux[11] or sex[12]. The prevalence of *H. pylori* shows large geographical variations. In various developing countries, more than 80% of the population is *H. pylori* positive, even at young ages[13]. The prevalence of *H. pylori* in industrialized countries generally remains under 40% and is considerably lower in children and adolescents than in adults and elderly people[14]. Within geographical areas, the prevalence of *H. pylori* inversely correlates with socioeconomic status, in particular in relation to living conditions during childhood[15]. In Western countries, the prevalence of this bacterium is often considerably higher among first- and second-generation immigrants from the developing world[16-17]. While the prevalence of *H. pylori* infection in developing countries remains relatively constant, it is rapidly declining in the industrialized world[18]. However, in industrialized countries the prevalence of *H. pylori* infection is low early in childhood and slowly rises with increasing age. This increase results only to a small extent from *H. pylori* acquisition at later age. The aim of the study was to evaluate the seroprevalence of *H. pylori* infection in Bangladeshi population.

Materials and Methods

Study population: The study population included patients with suspected gastric disease who were referred by practitioners, clinics and hospital throughout Dhaka City to Medinova Medical

Services Limited, Dhaka during January 2008 to August 2008. The personal information of patients was recorded.

Clinical specimen: For the detection of anti-*H. pylori* IgG antibodies to *H. pylori*, a total 474 blood samples were taken from the patients for culturing.

Determination of IgG of Helicobacter pylori: H. pylori IgG was done by enzyme-linked immunosorbant assay (ELISA). Blood samples were collected by venepuncture from patients and control. The samples were allowed to clot and the serum separated by centrifugation at 10,000 rpm for 15 min at room temperature. Serum samples were stored at –20°C until tested. Commercially available ELISA kit (*H. pylori* IgG EIA test kit, DRG International, Inc, USA) was used for the determination of IgG to *H. pylori* patients' sera. After performing the assay according to the supplied instruction absorbance of reaction mixture was measured at 450 nm using an ELISA reader (Organon Technika, Belgium). Absorbance was converted to values by plotting a standard curve to determine sample values. The anti-*H. pylori* IgG was determined by the corresponding concentration of *H. pylori* IgG in U/ml from the standard curve. Negative and positive controls were used for the test. A result less than 20 U/ml is considered to be the negative and indicates that *H. pylori* IgG antibodies were not detected in the sample. A result greater than 20 U/ml is considered to be positive and indicates that *H. pylori* IgG antibodies were detected in the sample. The sensitivity of the test was >99%, specificity >97% and accuracy was 97%.

Statistical Analysis: Chi-square tests were used to test for an association of *H. pylori* infection with age groups and gender. A *p*-value of >0.05 was considered statistically significant.

Results

The present study was conducted over a period of eight months (January-August 2008). A total of 474 individuals aged between 13 years to 78 years were recruited in this study. Blood samples were obtained from the individuals with suspected *H. pylori* infection. Anti-*H. pylori* IgG antibodies were positive in 273 (57.6%) cases. A slightly higher seropositivity was found among males (61.8%) than in females (53.53%) patients (Table 1).

Table 1: ***Immunological response of anti-Helicobacter pylori IgG according to sex (n = 474)***

Gender	Positive, No. (%)	Negative, No. (%)
Male (n = 233)	144 (61.8)	89 (38.2)
Female (n = 241)	129 (53.5)	112 (46.5)
Total (n = 474)	273 (57.6)	197 (42.4)

The seroprevalence of *H. pylori* was higher in early age group (0-15 years) both in males and females (Fig. 1). The seropositivity decreased with increasing age and the difference was statistically significant (p>0.05). In case of male, the seropositivity was 83.3% in early age group (<15 years). Then it was significantly decreased with increased age. The seropositivity was decreased to 60.2% in 16-25 years age group and 51.0% in 26-35 years age group. In the age group of 36-45 years, the prevalence was 69.1%. Thereafter, it was gradually decreased with increased age and in age group >65 years the seropositivity was 53.5%. However, in case of female the prevalence of anti-*H. pylori* IgG was decreased with increase of age with some fluctuations. In the early age group (<15 years) it was 75.0%, while in age group of >65 years it was 45.4%.

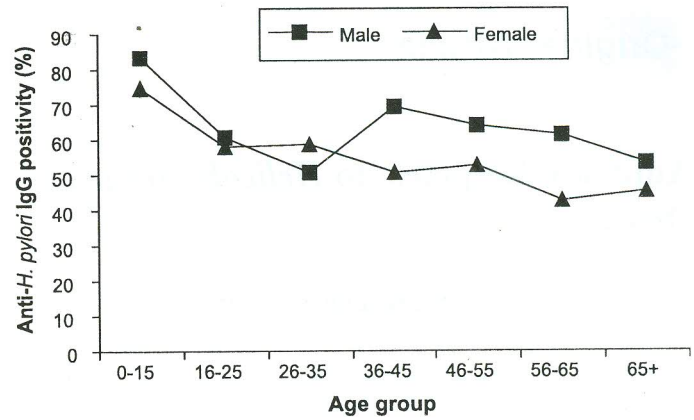

Fig. 1: *Prevalence of anti- Helicobacter pylori IgG in different age group.*

The antibody titre in different age groups varied considerably (Fig. 2). Based on anti-*H. pylori* IgG titre, the patients were divided into four groups: negative (0-20 U/ml), low (21-60 U/ml), moderate (61-100 U/ml) and high (>100 U/ml). In case of male, negative level of antibody titre was found in 38.2% cases, low in 39.1% and moderate in 15.0%, while high level of antibody titre was found in

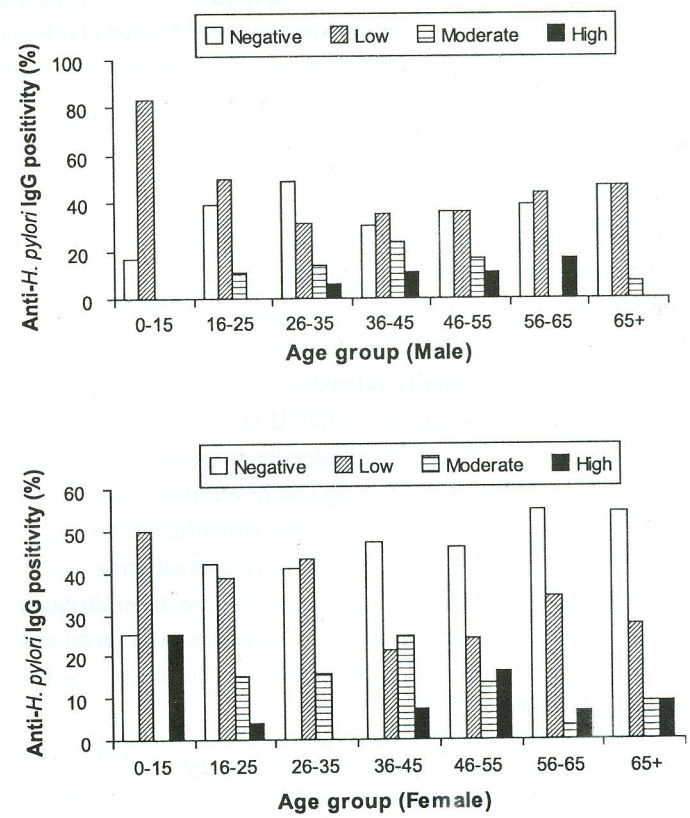

Fig. 2: *Titre of anti-Helicobacter pylori IgG in male and female patients of different age groups.*

only 7.7% cases. In early age group (<15 years) low level of titre was found in 83.3% cases, which was the highest among the different age group. Higher level of antibody was found in 5.9%, 10.3%, 10.64%, and 16.7% cases respectively in 26-35 years, 36-45 years, 46-55 years and 56-65 years age groups. There was no individual in the age group of 0-15 years, 16-25 years and >65 years who had high level of antibody. On the other hand, in case of female, negative level of antibody titre was found in 45.6% cases, low in 32.4%, moderate in 15.3% and high in only 6.6% cases. Therefore, higher level of antibody titre was found in lower percentage of women in different age groups.

Discussion

Helicobacter pylori is one of the world's most common infectious bacteria with more than three quarters of the population of the developing world being infected from an early age. Infection has been shown serologically after 6 months of age when the maternal antibody disappears. Numerous reports have confirmed an association between the presence of *H. pylori* on the gastric antrum and chronic antral gastritis and increased risk for gastric cancer[19-20]. In 1983, Waren and Marshal described a curved bacillus associated with gastric mucosa in cases of chronic gastritis, tentatively drawing comparisons between that organism and the genus *Campylobacter*[2]. The most recent clinical studies have confirmed *H. pylori* to be the causative agent for most cases of chronic gastritis and ulcers. Evidence has been presented that *H. pylori* is also associated with gastric carcinoma. A positive serological response to *H. pylori* antigens has been determined in individuals with duodenitis, chronic gastritis and gastric or duodenal ulcer. Further, many people without clinical symptoms are seropositive for *H. pylori* antibodies, with the prevalence increasing with age. The majority of individuals exposed to *H. pylori* posses IgG antibodies to the organism. In addition, the presence of *H. pylori* antibodies is a function of age, race, geography and clinical condition. Age specific rates are similar for males and females[21].

The prevalence of *H. pylori* varies worldwide according to country, region, and age. The prevalence of *H. pylori* infection is between 30 and 40% in the US and Canada, about 20% in Australia, 70% in Europe, and between 70 and 90% in Africa, South America and Asia[22]. In the United States, *H. pylori* positivity in children aged less than 10 years is lower than 5%, whereas it is 10% and 60% among individuals who are aged 20 years and above 60 years respectively[23]. In Japan, the prevalence of *H. pylori*-associated infection was 29% among persons aged 15-19 years[24]. The prevalence of *H. pylori* was 34% among children, aged less than 12 years, in Italy[25]. In more Westernized countries, the prevalence is low in children but increases with increasing age, paralleling the age related prevalence of chronic gastritis, found in 20% of those age 20 years old and 60% of those age 60 years old.

In developing countries, infection rates are higher in children; 85% of children below the age of 15 years old have been found as infected, and this chronic infection continues into adult life[26]. In Bangladesh, Mahalanabis *et al.* studied 469 children, aged 6-9 years, with urea breath test and found the prevalence of *H. pylori*-associated infection in 84% of them[27]. The infection is more prevalent in lower socioeconomic groups with poor living standards, such as crowded living conditions[28], those with lower levels of education and poor hygiene[26]. Similar findings were reported in Bangladesh previously. The infection rate of *H. pylori* in the general population of Bangladesh has been reported to be very high. Previous studies showed that *H. pylori* specific IgG antibody by ELISA was found in 92% among asymptomatic subjects who attended at the health check-up centre of Bangladesh Institute of Diabetes, Endocrine and Metabolic Disorders (BIRDEM)[29]. In 2008, Sumona *et al.*[30] evaluated the serologic (IgG) response to *H. pylori* in asymptomatic population in Bangladesh, a total of 45 asymptomatic healthy adults aged 18-65 years were included in that study. They found that seropositivity rate for anti-*H. pylori* IgG in asymptomatic adults was 88.89%[30]. Another study of 569 Bangladeshi children between 2 and 10 years of age, the prevalence of *H. pylori* was 42% by 2 years of age and 67% by 10 years of age[31]. In our study, the prevalence rate was average 80% in early age group (0-15 year) and 55% among the adult. Our study also found that the rate of prevalence was decreased with increased age.

On the other hand, many studies showed that *H. pylori* positivity increases with age in societies. While it is lower in paediatric age, it increases in young adults, peaking in adulthood and slightly decreases in old age. As the socioeconomic level increases, the rate of *H. pylori* seroprevalence decreases and peak is delayed to advanced ages. In a study conducted in China, this rate was 19-36% in 2003 up to 20 years of age, 53-54% for the 20 and 30 year age group, 63% for the 40 year age group, and 55% in the 50 year age group[32].

In our present study, a difference was found for antibody titre level in both males and female with different age group. In 2001, Yasmin *et al.*[36] found that among the 46 positive cases of duodenal ulcer patients in Bangladesh 20 had very high level of anti-*H. pylori* antibody (>141 AU/ml), 14 had moderately high (90-140 AU/ml) and 12 had low level (41-90 AU/ml) antibody titre. On the other hand, among 9 positive cases of non-ulcer dyspeptic patients, high level of antibody was found in six cases, only one had moderately high level and low in rest of the two cases. But no significant difference was found between the two groups ($p = 0.0947$)[33].

Previous study on assessing the clinical, endoscopic and histological importance of a positive ELISA IgG antibody to *H. pylori* in asymptomatic blood donors showed that duodenal ulcer is as common in *H. pylori* patients with dyspepsia as in the asymptomatic seropositive blood donors[34]. Measurement of IgG antibodies to *H. pylori* are helpful in detecting 'silent' peptic ulcer, which confirms previous reports that screening for *H. pylori* infection is an effective way of reducing the endoscopic workload[35]. *H. pylori* associated peptic ulcer occurs more frequently than previously understood and suggests that *H. pylori* infection, even in the absence of symptoms, is of far greater clinical relevance than originally thought. Another study showed that a relatively large proportion of patients who had positive levels of antibody were asymtomatic, even though they were colonized with the *H. pylori* organism. Therefore antibody levels did not necessarily correlate with the severity of clinical symptoms[21].

Conclusion

Helicobacter pylori is now considered as the major cause of gastritis in human being. *H. pylori* infections in different age group both in males and females in Bangladesh are still alarming position. So, different regimes are needed to be developed to eradicate the *H. pylori* infection in Bangladesh.

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