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Brief Communication

Anti-*Helicobacter pylori* IgG in Asymptomatic Population

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Abstract

To evaluate the serologic (IgG) response to *Helicobacter pylori* in asymptomatic population, a total of 45 asymptomatic healthy adults aged 18-65 years were included in the study. The study was carried out in the department of Microbiology, Mymensingh Medical College between July, 2006 to June, 2007. The sera samples of the participants were tested for presence of anti-*H. pylori* IgG by using Enzyme Linked Immunosorbent Assay (ELISA). Seropositivity rate for anti-*H. pylori* IgG in asymptomatic adults was found 88.89% at a cut-off level of 5 arbU/ml. The impact of changing cut-off level for IgG seropositivity was also examined in the present study. The seropositivity rate for anti-*H. pylori* IgG at 10 arbU/ml was found 71.11%.

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

Introduction

Infection by *Helicobacter pylori* is one of the most common chronic bacterial infections in human. It occurs throughout the world and causes several gastroduodenal diseases including gastric ulcer, duodenal ulcer, gastric mucosa-associated lymphoid tissue lymphoma and gastric adenocarcinoma.[1] In 1994, the World Health Organization has categorized *H. pylori* as a group I carcinogen.[2]

The prevalence of *H. pylori* infection varies worldwide, but higher colonization rates were seen among people in the developing countries compared to that in developed countries.[3] The infection was found to acquire during childhood and persisted throughout the life unless treated.[4] In most individuals, the *H. pylori* infection is asymptomatic. However, not all of the individuals but some with *H. pylori* infections were found to develop stomach and duodenal diseases due to other co-existing risk factors.[5] One of the major risk factors for acquiring this infection is low socioeconomic status, widely present in Bangladesh. Patients infected with this organism have been shown to produce serum antibodies to *H. pylori* antigens and several studies have implied that the immune response correlates with the histological findings in biopsy specimens.[6-8] This is of great importance, because it has been documented that patients with an untreated chronic active gastritis are more likely to develop an atrophic gastritis and may eventually progress to gastric cancer.[9]

In epidemiological studies, serological tests offer high sensitivity and specificity and serum assaying of anti-*H. pylori* IgG antibodies could be used as a marker for *H. pylori* infection.[10] No study report regarding the immune response to *H. pylori* in this locality is available. So, the present study was done to evaluate serologic IgG response to *H. pylori* in asymptomatic healthy adults.

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Methods

This observational study was carried out among 45 asymptomatic healthy individuals in the department of Microbiology, Mymensingh Medical College during July, 2006 to June, 2007. All of the included cases were healthy with no acute or chronic illness. The criteria for enrolment included no history of peptic ulcer disease, no history of therapy for *H. pylori* infection and no symptoms of upper gastrointestinal disease such as indigestion, nausea, vomiting and epigastric burning pain and did not undergo for any endoscopic examination.

Detection of anti-H. pylori IgG by ELISA

Enzyme Linked Immunosorbent Assay (ELISA) *H. pylori* IgG kit (Organics, France) was used to detect serum anti-*H. pylori* IgG antibodies in the samples. Microplates coated with *H. pylori* immunodominant antigen were used. Sera samples were diluted (1 : 101) with diluent provided with the kit and 100 µl was added to ELISA plate wells except the wells dispensed with 100 µl calibrators (provided with the kit). After incubation for 60 minutes at 37ºC, microplates were washed (4-5 times) with the wash buffer provided, followed by the addition of 100 µl of enzyme-conjugate (anti-human IgG conjugated with Horse Radish Peroxidase) and re-incubation for 60 minutes at 37ºC. Unbound antibodies were removed by washing and 100 µl chromogen/ substrate solution was then added. After another incubation for 20 minutes at room temperature, the reaction was stopped by adding 100 µl of Sulphuric acid into each well. The serum concentration of anti-*H. pylori* IgG antibodies was expressed in arbitrary units per milliliter (arbU/ml) as recommended by hte manufacturer and no International Standard was available. As the kit was originated in a developed country, a higher cut-off level than the original recommended value of 5 arbU/ml was set for the present study. Therefore, samples with a concentration above the cut off value of 5 arbU/ml were considered positive for anti-*H. pylori* IgG antibody and samples with a concentration lower than 5 arbU/ml were considered negative.

Results

A total of 40 (88.89%) among 45 samples examined were positive for anti-*H. pylori* IgG by Enzyme Linked Immunosorbent Assay (ELISA) considering cut-off value of 5 arbU/ml. The seropositivity for anti-*H. pylori* IgG at 10 arbU/ml was found 71.11%. (Table I)

Table I: Anti-*H. pylori* IgG in asymptomatic population

Anti-*H. pylori* IgG cut-off value in ELISA	Asymptomatic population (n=45)	
	Positive serum	Anti-*H. pylori* seropositivity
5 arbU/ml	40	88.89%
10 arbU/ml	32	71.11%

Discussion

The present study, using an ELISA assay (cut-off =5 arbU/ml), showed 89% seropositivity for anti-*H. pylori* IgG in asymptomatic individuals aged 18 to 65 years. Similar, high infection rates have been reported from other developing countries including Thailand,[11] Mexico,[12] India,[13] Vietnam[14] and parts of other countries like Siberia.[15] 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).[16]

Jafarzadeh and Mehdi[17] from Iran reported anti-*H. pylori* IgG seropositivity of 73% in asymptomatic subjects and another study showed 77% serum antibody to *H. pylori* in a population in Bangladesh recruiting from an institutionalized area.[18] Similar findings in asymptomatic population were also noticed from other developing countries. The seroprevalence rate in lower socioeconomic class in India was found 79%.[19] Another study in asymptomatic children and adults from South India showed the anti- *H. pylori* IgG seroprevalence rate of 67%.[20]

Serological tests represent the most rapid and convenient way for diagnosing *H. pylori* infection in a population but the assays used need to be validated in the population studied.[21] A majority of serological studies are now conducted with commercial kits that have been evaluated in developed countries. It has been suggested that the usefulness and practicability of the tests are related to the background prevalence of *H. pylori* infection in a specific geographic area.[22] Serological assays developed from *H. pylori* strains of the West and validated in Caucasian populations might be different when applied in Asian patients. Most of these tests have limitations of a poor specificity due to frequent false-positive results.[11] In a study by Leung *et al*[23] evaluated three commercial ELISA tests (developed and validated in USA)

for *H. pylori* infection in Chinese population with disappointing results (sensitivities: 50-75%; specificities: 68-97%). On the other hand, Groves *et al*[24] from USA reported 100% sensitivity of an ELISA developed from a pool of *H. pylori* strains isolated from the Chinese and applied to the Chinese patients. Apart from the ethnic differences, genetic heterogeneity of *H. pylori* strains from different parts of the world seems to attribute to this discrepancy. Thus it is likely that antigenic profiles of bacterial strains from different regions vary and conventional serological tests, which are developed and validated for a specific population, may not be applicable universally.[25] In the present study, ELISA *H. pylori* IgG kit (developed in France) was used which was based on an immunodominant antigen derived from tissue culture of a virulent strain of *H. pylori*. The best strategy is to use the commercial ELISA-kits using local strains for the *H. pylori* antigen and the cut-off level used for serodiagnosis in the general population needs to be adjusted for better diagnostic performance.[21]

In the present study, at low antibody titre (cut-off level of 5 arbU/ml) anti-*H. pylori* IgG seropositivity was found high. The cut-off value (5 arbU/mL) recommended by the manufacturer was originally validated in French population with dyspepsia. At this low antibody titre, the test may show unfavourable result in a developing country like Bangladesh where the risk factors for *H. pylori* infection like overcrowding, poor hygienic condition, lack of safe water supply, inadequate sanitation practices are common. The impact of changing cut-off level for IgG seropositivity was observed in the present study and at higher cut-off value (10 arbU/mL), anti-*H. pylori* IgG seropositivity was also found high.

A study based 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.[26] 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.[27-32] *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.

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[Conflict of interest: None declared]