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Full Length Research Paper

Seroprevalence of *Helicobacter pylori* infection in patients suffering from gastric symptoms in the Northwest of Iran

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Helicobacter pylori **is a major gastroduodenal pathogen and its seropositivity is associated with increased risk of development of human active chronic gastritis, peptic and duodenal ulcer and gastric cancer. The aim of this study was to determine the seroprevalence of *H. pylori* infection among subjects with gastrointestinal problems. A total of 339 serum samples collected from 114 male and 225 female were screened for detection of anti *H. pylori* IgG, IgA and IgM using commercial ELISA tests. The overall seropositivity rates were as follows: anti *H. pylori* IgG 73%; anti *H. pylori* IgM 43% and anti *H. pylori* IgA 25%. Seropositivity of anti *H. pylori* IgG increased markedly with age and highest infection rate (96%) was seen in individuals 40 to 50 years old. Anti *H. pylori* IgA was also correlated with increasing age. No association was detected between *H. pylori* seropositivity, gender and inflammatory laboratory parameters. This study revealed the high prevalence of *H. pylori* infection among symptomatic subjects in northwest of Iran.**

Key words: *Helicobacter pylori*, seroprevalence, IgG, IgA, IgM.

INTRODUCTION

Helicobacter pylori is the most common cause of chronic gastritis, infecting more than half of the world's population (Torres et al., 2000). This bacterium is also associated with a variety of disease ranging from asymptomatic gastritis to severe gastric ulcer which can progress to a gastric malignancy. Numerous reports have confirmed an association between the presence of *H. pylori* on the gastric mucosa of patients and increased risk for gastric carcinoma (Peterson et al., 2000; Suerbaum and Michetti, 2002). Several seroepidemiological study have shown that the presence of serum IgG antibodies to *H. pylori* is related to an increased risk for developing peptic disease and duodenal ulcer (Peterson et al., 2000; Gdalevich et al., 2000) hence, detection of antibodies is a critical step in management and prevention of serious outcomes and complication of *H. pylori* infection.

The seroprevalence of *H. pylori* has been studied in healthy subjects and asymptomatic populations by ELISA methods, in both developed and developing countries (Best et al., 1994; Lindkvist et al., 1996) and high infection rates have been reported from developing countries (Torres et al., 2000). Moreover, several studies have shown a wide variation in the prevalence of *H. pylori* antibodies among age groups in different geographical regions (Atalay et al., 2003). In a cross-sectional study from Tehran, the capital city of Iran, the overall infection rate was 69% which increased positively with age, and

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the highest prevalence rate (79%) was seen in the 46 to 55 age group (Nouraie et al., 2009). No local studies are available regarding seroprevalence of *H*. *pylori* infection in North West of Iran. Simultaneous measurement of serum immunoglobulin G (IgG), M (IgM), and A (IgA) antibodies to *H. pylori* can be used to determine the prevalence of both acute and chronic infection. This study was conducted to evaluate the seroprevalence of *H. pylori* infection among symptomatic subjects using ELISA method to determine anti *H. pylori* IgG, IgA and IgM levels.

PATIENTS AND METHODS

Study population

From May 2010 to September 2010, a seroprevalence study was carried out among 339 subjects who had clinical gastric symptoms, with a mean age of 37.77 years (range, 2 to 80 years). All patients had blood drawn for serological testing at the time of attending in central laboratory. After collection, the serum was separated, aliquoted and frozen at -20°C until being tested. Demographic data including age and gender as well as Laboratory parameters such as WBC (White blood cell), ESR (Erythrocyte sedimentation rate), and CRP (C - reactive protein) were analyzed in all subjects.

ELISA

Enzyme-linked immunosorbent assays (ELISA) were used for determination of anti *H. pylori* IgM, IgG and IgA in serum samples, according to previously reported methods (Pérez-Pérez et al., 1997). Assays were performed by a commercial ELISA kit, Helicobacter pylori IgG, IgM, and IgA (manufactured by IBL INTERNATIONAL GMBH) following the manufacturer's instructions. Conditions were the same for all assays. Briefly, frozen or fresh serums were diluted and then added into wells and incubated at room temperature for 60 min. After washing, diluted horseradish peroxidase-conjugated antihuman IgG, IgA, or IgM was reacted for 30 min at room temperature. After washing again, the wells were developed with tetra methyl benzidine (TMB) for 20 min and the optical densities were read at 450 nm with an ELISA reader. The cutoffs (in index values) for IgG, IgA and IgM were <8 for a negative result, 8 to 12 for an equivocal and >12 for a positive result. All equivocal results were repeated.

Statistics

Statistical analyses were performed using SAS software, version 9.1 (SAS Institute Inc., Cary, NC). The data has been analyzed by using CORR and Logistic procedures for each ELISA. p-value of 0.05 or less was regarded as statistically significant.

RESULTS

This study was performed in 339 subjects with gastric symptoms, including 225 female and 114 male with an age range of 2 to 80 years (mean age 37.77 years). All subjects were from Tabriz city, North-West of Iran, with a population of more than one and a half million.

Diagnosis of *H. pylori* by serological tests

Among 339 serum specimens tested for anti *H*. *pylori* IgG, 247 (73%) were positive and 92 (27%) were negative. Screening for anti *H. pylori* IgA revealed that 84 (25%) subjects were positive and 255 (75%) was negative. Examination for anti *H*. *pylori* IgM, showed that 145 (43%) cases were positive whereas 194 (57%) subjects were negative.

Correlation of age and sex with seropositivity of *H. pylori*

Analysis of results for gender showed that there were no significant differences in seropositivity to *H. pylori* between men and woman ($p>0.05$) but there was a significant correlation between anti *H. pylori* IgG and age of subjects ($p<0.01$). The rate of IgG seropositivity increased markedly with age, being maximum (96%) in 40 to 50 years old age group. After that, there was a small and steady decrease between age 50 and 70, but a slight arise was seen above 70 years old as shown in Figure 1. Anti *H. pylori* IgA seropositivity was increased significantly with increasing age from 10% in the first decade to 29% at age 40 to 50 ($p<0.01$). After fifth decade, the IgA seropositivity declined slightly and reached 24% in the 50 to 60 years old group thereafter peaked to 44% in the 70 to 80 year-old group (Figure 1). The seroposivity of IgM increased from 28% in the first age group to 78% in the second age group, then reduced to 56% in the third decade, following a fluctuation pattern with a steady rise and fall toward the last decade. Also, No statistically significant association was found between IgM seropositivity and age. There was a significant correlation between IgG and IgA levels ($p<0.01$), however, no significant correlation was found between IgM with IgG and IgA antibodies. Analysis of results for inflammatory markers (WBC, ESR, and CRP) showed normal mean of WBC count (7000 cells/ml), ESR rate (9 mm/h) and CRP levels in these subjects. Our data revealed no significant correlation between inflammatory markers and seroprevalence of *H. pylori* infection.

DISCUSSION

H. pylori infection is an important risk factor for development severe gastric problems (Malekzadeh et al., 2009). Detection of *H. pylori* infection with non invasive methods such as serological tests are useful, widely available and inexpensive (Ricci et al., 2007). These methods contribute an advantage to epidemiological research in which typically large numbers of subjects can be studied from easily obtained blood samples. The results of this study showed that the overall seroprevalence of *H. pylori* infection based on anti *H.*

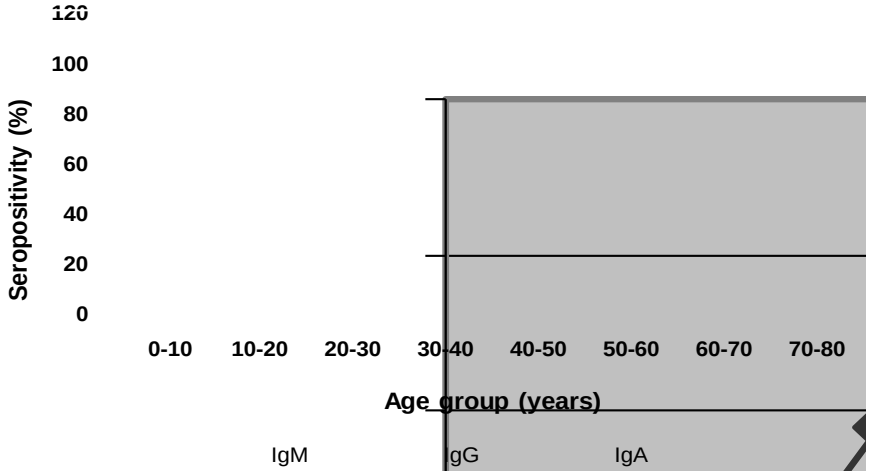

Figure 1. Distribution of immunoglobulinG (IgG), M (IgM), and A (IgA) antibodies to *H. pylori* in 339 subjects in different age groups. Age-related increase in IgG and IgA seroprevalence are seen in the figure.

pylori IgG was 73%. This result indicated that our seroprevalence is higher than many developed countries reported as 37% in United States (Graham et al., 1991) and 40% in Germany (Seher et al., 2000) but lower than some developing countries reported as, 85% in Nigeria (Oluwasola et al., 2002) and 90% in Bangladesh (Mahalanabis et al., 1996). It has been reported that IgA and IgG titers indicate chronic infection. Our result revealed an age-related increase of anti *H. pylori* IgG and IgA. The seropositivity of IgG varied in different age group, from 40% in 0 to10 years old group to 75% in the age group of 70 to 80 years. Additionally, anti *H. pylori* IgA reflected a similar pattern with advancing age from 10% in the first decade to 44% in the 70 to 80 year. In addition there was a slight decrease in the seropositivity of IgG and IgA over 50 years of age in our study, similar to results reported by Kate et al. (2001), and this may be related to eradication of the bacterium due to potentiation of immune response. The age-related increase in antibody prevalence in the present study was similar to results of previously published studies. A study conducted in southern of Iran (Shiraz) revealed that 82% of children aged nine months and 98% of two year old children were *H*. *pylori* infected (Alborzi et al., 2006). Also in Rafsanjan, another city of Iran, high infection rate of *H. pylori* in children and adults and its correlation with age has also been reported (Jafarzadeh et al., 2007). Our results also are in accord with many reports from other countries (Gisbert et al., 2001; Gill et al., 1994). A study by Fock (1997) showed that seroprevalence of *H. pylori* infection in Singapore increases with age from 3% in children under 5 years old up to 71% in adults above 65 years. The age-related increase of seroprevalence is attributable to the fact that *H. pylori* infection is usually acquired during childhood and carried for life. Our data showed no statistically significant correlation between increasing age and IgM antibody, presumably due to the fact that IgM titer rises early after acquisition of infection rather than reflecting chronic infections. IgM antibodies are usually directed against the bacteria in acute stage. Our findings on IgM seroprevalence do suggest that *H. pylori* infection can be acquired at all ages but the prevalence of primary infection seems to be high in second decade (78%).

Our study also demonstrated that gender had no influence on *H. pylori* infection rate. A number of other studies have yielded similar results and shown no significant correlation between *H*. *pylori* infection and gender (Jimenez-Guerra et al., 2000). However, there are few studies reported a significant association of *H. pylori* infection with male gender (Leandro et al., 2005). The results from this study, although indicated that inflammatory marker such as WBC (white blood cell), ESR (erythrocyte sedimentation rate), and CRP (C-reactive protein) were not correlated with seropositivity of *H. pylori* infection.

Conclusion

Our results showed high prevalence of *H. pylori* IgG antibodies in patients suffering from gastric symptoms suggesting necessity of screening for *H. pylori* infection in symptomatic patients. Our results also did not show any strict relations between *H. pylori* seropositivity, gender and inflammatory markers.

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