Specific serum immunoglobulin G to *H pylori* and CagA in healthy children and adults (south-east of Iran)

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**AIM:** To evaluate the serologic IgG response to *H pylori* and CagA across age groups and in healthy children and adults.

**METHODS:** Totally, 386 children aged 1-15 years and 200 adults aged 20-60 years, were enrolled to study. The serum samples of participant were tested for presence of anti-*H pylori* and anti-CagA IgG by using ELISA method.

**RESULTS:** The seroprevalence of *H pylori* in adults was significantly higher than that observed in children (67.5% vs 46.6%; *P* < 0.000003). In children, the seropositivity rate in males (51.9%) was significantly (*P* < 0.05) higher than that observed in females (41.7%). The prevalence of serum anti-CagA antibody was 72.8% and 67.4% in infected children and adults, respectively. The mean titer of serum anti-CagA antibodies was significantly higher among children in comparison to adults (64.1 Uarb/mL vs 30.7; *P* < 0.03). In infected children and adults the prevalence of serum anti-CagA antibody was higher in males compared to females (78.4% vs 66.3%; *P* = 0.07 and 75.6% vs 54.71%; *P* < 0.04, respectively). The age-specific prevalence of anti-*H pylori* and anti-CagA antibody (in infected subjects) was 37.6% and 59.57% at age 1-5 years, 46.9% and 75% at age 6-10 years, 54.9% and 79.45% at age 11-15, 59.01% and 83.33% at age 20-30 years, 66.6% and 60.52% at age 31-40 years, 73.46% and 63.88% at age 41-50 years and 75.75% and 60% at age 51-60 years with mean titer of anti-CagA antibody of 75.94, 63.32, 57.11, 52.06, 23.62, 21.52 and 21.80 Uarb/mL, respectively. There was significant difference between mean serum anti-CagA antibody in age subgroups (*P* < 0.001).

**CONCLUSION:** These results showed that anti-*H pylori* and anti-CagA antibodies were common in the children and adults. The *H pylori*-specific antibodies influenced by age and sex of subjects. Moreover, it seems that males are more susceptible to infection with CagA+ strains compared to females. The seroprevalence of anti-CagA antibody was increased with age, up to 30 years and then decreased. It was also found that the magnitude of the IgG response to CagA decreased with advanced age.

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**Key words:** Seroprevalence; *H pylori*; Adults; Children; CagA; Iran

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**INTRODUCTION**

Epidemiologic studies have clearly demonstrated a major etiologic role of *H pylori* for several gastroduodenal diseases, including gastric ulcer, duodenal ulcer, gastric MALT lymphoma, and distal gastric cancer[3]. The prevalence of *H pylori* infection varies worldwide, but higher colonization rates are seen in developing countries compared to developed countries[3]. The infection is usually acquired during childhood, although expression of disease does not occur in most cases until adulthood. There has been evidence for both transient and persistent colonization in children[3], while colonization of adults with *H pylori* almost always persists[4].

*H pylori* strains are genetically diverse. *H pylori* strains may be divided into at least two subgroups based on the expression (type I) or nonexpression (type II) of CagA and the vacuolating cytotoxin. The cytotoxin-associated gene A (CagA) has been identified as a possible marker of virulence of *H pylori*.[6] In our previous study an association was observed between infection with CagA+ *H pylori* strain and peptic ulcer[4]. Moreover, we observed higher levels of serum inflammatory cytokine IL-18 in *H pylori*-infected subjects, especially in individuals infected with CagA strains[3].

There is no study regarding the immune response to *H pylori* and CagA antigen across age groups in the same population. This study conducted for the first time to evaluate the serologic immunoglobulin G (IgG) response to *H pylori* and its virulence factor, CagA protein,
across age groups and in children and adults with same population and similar socioeconomic levels.

MATERIALS AND METHODS

Subjects
From August 2005 to December 2005, a cross-sectional seroprevalence study was carried out among healthy subjects in Rafsanjan (a city that located in Kerman province, in South-East of Iran). In total, 586 subjects were studied, including 386 children (187 males; 199 females aged 1-15 years with a mean of 9.5 ± 3.9 years) and 200 adults (114 males; 86 females aged 20-60 years with a mean of 48.1 ± 15.9 years). All subjects were basically health, with no acute or chronic illnesses. The criteria for enrolment included no history of peptic ulcer disease, no abdominal surgery, no history of therapy for *H pylori* infection, and no symptoms of upper gastrointestinal disease such as indigestion, nausea, vomiting and epigastric burning pain.

The adults were recruited among blood donors of Rafsanjan Blood Transfusion Center. They were randomly selected according to registration number. Children were recruited from randomly selected schools and health centers. School students were randomly selected for blood samplings by their registration number and similar procedures were performed in health centers. Informed consents were obtained from parents of all the children before blood samplings. Children were recruited if their parents agreed with the study and signed the informed consents. Moreover, this study was evaluated and approved by the Ethical Committee of Rafsanjan University of Medical Sciences.

Two to three mL of peripheral blood was collected from each participant at the time of interviewing. The blood samples were centrifuged and the sera were separated and frozen at -20°C until analysis.

Determination of *H pylori*-specific antibodies in serum
The serum levels of anti-*H pylori* immunoglobulin G were measured by using the commercial enzyme-linked immunosorbent assay (Trinity Biotec, Ireland); previously the sensitivity of this method was estimated > 98% in Iranian subjects[8]. According to manufacturer guideline the results were obtained as Immune Status Ratio (ISR) and the values of ≥ 1.1 were considered as positive. Serum anti-CagA IgG antibody levels were also assayed by ELISA method using commercial kits (Dagnostic Bioprobes, Italy). The serum concentration of anti-CagA antibodies were expressed in arbitrary units per milliliter (Uarb/mL) as no International Standard is available. According to the manufacturer's guidelines the value of 5 Uarb/mL used to discriminate the negative from positive samples. Moreover, in each group the serum concentrations of anti-CagA antibody expressed as mean ± SD.

Statistical analysis
Differences in variables were analyzed using Kruskal-Wallis, Mann-Whitney U-test, Chi-square and Fisher exact tests as appropriate and *P*-values of less than 0.05 were considered significant. All the available data were analyzed by a computer program (SPSS, Chicago, IL, USA).

RESULTS

Anti-*H pylori* IgG seropositivity
The overall seroprevalence of anti-*H pylori* IgG was 53.75%. The seroprevalence of *H pylori* in adults was significantly higher than that observed in children (67.5% vs 46.6%; *P* < 0.000003). In children, the seropositivity rate in males (51.9%) was significantly (*P* < 0.05) higher than that observed in females (41.7%). Similarly, in adults, the prevalence of anti-*H pylori* IgG was higher in males compare to females but the difference did not reach statistically, significant (Table 1).

In children, the age-specific seropositivity rate of anti-*H pylori* IgG was 37.6% at age 1-5 years, 46.9% at age 6-10 years and 54.9% at age 11-15 years. Furthermore, in adults the age-specific seropositivity rate of anti-*H pylori* IgG was also 59.01% at age 20-30 years, 66.6% at age 31-40 years, 73.46% at age 41-50 years and 75.75% at age 51-60 years (Table 2).

Anti-CagA seropositivity
The overall seroprevalence of anti-CagA IgG was 70.47% in asymptomatic subjects. The prevalence of serum anti-CagA antibody was 72.8% and 67.4% in infected children and adults with mean titer of 64.1 ± 67.6 Uarb/mL and 30.7 ± 32.5 Uarb/mL, respectively (Table 1). There was no significant difference between children and adults regarding the prevalence of serum anti-CagA antibodies, although this parameter was higher in children than that in adults. However, the mean titer of serum IgG anti-CagA antibodies was significantly higher among children in adults. However, the mean titer of serum IgG anti-CagA antibodies was significantly higher among children in adults.

| Group | Sex | Anti-*H pylori* seropositivity | Anti-CagA seropositivity | Mean titer of anti-CagA (Uarb/mL) |
|-------|-----|-------------------------------|--------------------------|---------------------------------|
| Children | Male | 97/187 (51.9%) | 76/97 (78.4%) | 69.24 ± 70.83 |
| | Female | 83/199 (41.7%) | 55/83 (66.3%) | 58.08 ± 63.58 |
| Total | | 180/386 (46.6%) | 131/180 (72.8%) | 64.1 ± 67.63 |
| Adults | Male | 82/114 (71.9%) | 62/82 (75.6%) | 31.6 ± 31.6 |
| | Female | 53/86 (61.6%) | 29/53 (54.7%) | 29.2 ± 34.1 |
| Total | | 135/200 (67.5%) | 91/135 (67.4%) | 30.7 ± 32.5 |

![Table 1 Seroprevalence of *H pylori*-specific antibodies in children and adults](www.wjgnet.com)
comparison to adults ($P < 0.03$).

In infected children, the prevalence of serum anti-CagA IgG antibodies was markedly higher in males compared to females (78.4% vs 66.3%; $P = 0.07$). Moreover, in infected adults, statistical analyses showed that the prevalence of anti-CagA IgG antibodies was significantly higher in males in comparison to females (75.6% vs 54.71%; $P < 0.04$). No significant differences were observed between males and females regarding titer of serum anti-CagA antibodies.

In infected children, the age-specific seropositive rate of anti-CagA IgG was 59.57% at age 1-5 years, 75% at age 6-10 years and 79.45% at age 11-15 years, with mean titer of 75.94, 63.32 and 57.11 U arb/mL. Furthermore, in infected adults the age-specific seropositive rate of anti-CagA IgG was also 83.33% at age 20-30 years, 60.52% at age 31-40 years, 63.88% at age 41-50 years and 60% at age 51-60 years, with mean titer of 52.06, 23.62, 21.52 and 21.80 U arb/mL (Table 2). Accordingly, the seroprevalence of anti-CagA antibody increased with age, up to 30 years and then decreased. Collectively, the seroprevalence of anti-CagA antibody in those with age of $< 30$ years was significantly higher than that observed in those with age $> 30$ years (74.53% vs 61.61; $P < 0.02$). However, the mean titer of serum anti-CagA antibodies declined with age and statistical analysis showed that there was significant differences between mean serum anti-CagA antibodies in age subgroups ($P < 0.001$).

**DISCUSSION**

*H. pylori* infection is thought to play an etiologic role in several gastroduodenal diseases. In epidemiological studies, serum assaying of anti-*H. pylori* IgG or IgA antibodies could offer high sensitivity and specificity and could be used to determine prevalence of infection. The results of the present study showed that the overall seroprevalence of *H. pylori* infection was 46.6% in children at age 1-15 years and was 67.5% in adults at age 20-60 years. In different studies, the prevalence of *H. pylori* is variable in children such that it has been reported to be 60% at age 4 years in Ethiopia, 7.5% at age 2-18 years in Czech, 44% at age 6 mo to 17 years in Turkey, 8% at age 1-3 years in USA, 50% at age 1-9 years and 80% at age 10-19 years in Libya, 56% at age 1-14 years in Brazil, 96% at age 1-14 years in Saudi Arabia and 80% at age 1-5 years in Bangladesh. Moreover, It has been reported that children in Gambia and Nigeria are almost all infected by *H. pylori* at age of 5 years. On the other hand, Heuberger et al. reported the prevalence of *H. pylori* infection among adolescents between 15-16 years of age, living in Switzerland. They found one of the lowest prevalence of *H. pylori* infection among adolescents in Europe (7.3%). In developing countries, more than 80% of adults are colonized with *H. pylori* compared to 30% of the adults in developed countries. This discrepancy may be attributed largely to differences including race and ethnic background and socioeconomic status such as family income, size of the family, type of housing, location of housing, water supply, health and education level, and keeping pets.

In the present study, there was an increasing in prevalence of *H. pylori* infection with age, reached to a maximum of 75.75% at 51-60 years of age, suggesting a steady colonization rate through the different age groups. This overall infection rate curve shared common patterns with other reports, although considerable differences exist between developing and developed countries.

The results of the present study showed that prevalence of anti-CagA antibody was 72.8% and 67.4% among asymptomatic infected children and adults, respectively. The cagA has been identified as a possible marker of virulence of *H. pylori*. Since the cytotoxin-associated gene product (CagA, 120 to 140 kDa) encoded by cagA is immunodominant, therefore, serum IgG antibodies to the CagA antigen may be a reliable marker of carriage of a CagA* H. pylori* strain. In other studies the seroprevalence of anti-CagA antibody in *H. pylori*-infected asymptomatic subjects was evaluated, so that it has been reported to be 56.7% at age 1-14 years in Saudia Arabia, 82% at age 1.5-5 years in Bangladesh, 46.9% at age 1-15 years in Mexico, 88.5% and 81.3% at age 3-12 years in two counties of China, 83% at age 20-65 years in Turkey, 95.3% at age 20-60 years in Nigeria. Accordingly, our observation confirms that CagA seroprevalence varies geographically.

Another interesting result observed here was that the seroprevalence of anti-cagA was correlated with age, increasing with age up to 30 years and then decreasing. Moreover, the prevalence of anti-CagA was higher in children compared to adults, although the difference was not significant. Thus, susceptibility to colonization by a CagA-positive strain seems to be linked to age. This differential prevalence may be related to differential expressions of gastric mucosa adherence molecules, which may be modified by age or may be dependent on differential expression of bacterial adhesin molecules. It has been reported that the fucosylated blood group antigens Lewis b (Leb) and H-1 (the precursor for Leb) involve in adherence of *H. pylori* to human gastric epithelial cells in situ. Ilver et al. showed that 66% of clinical isolates of *H. pylori* bound the Leb antigen. These authors demonstrated that bacterial adhesin to the Leb antigen is coded by the babA2 gene and is associated with the presence of the cagA gene. An interesting and surprising finding, demonstrated by Çelik et al., is that children probably have few Leb receptors on surface mucous cells, which may explain the fact that susceptibility to colonization with a CagA-positive strain is linked to age.

An interesting finding was also that the mean titer of the IgG response to CagA were significantly ($P < 0.05$) higher in children compared to adults. Another result observed here was that the decrease of antibody response to CagA through the different groups of ages. Accordingly, there is a reverse association between levels of anti-CagA antibody with advanced age and it seems that the age of the subjects may also influence the antibody response to CagA. These observations are difficult to interpret and may attributed to differential CagA expression and/or changes in the host immune response with aging. Moreover, one possibility would be that at older ages ( $> 30$ years) the bacterial colonization may gradually shift.
from cagA-positive strains to cagA-negative strains and accordingly, in some adult subjects cagA-positive strains may disappear. Based on these observation it seems that colonization of some younger subjects (< 30 years) with CagA-positive strains is a transient phenomenon. The accumulating evidence regarding CagA-positive strains are more susceptible than CagA-negative strains to eradication treatment[13] is consistent with our observation.

Our result showed that the prevalence of anti-\textit{H pylori} antibody was significantly higher in males compared to females. Although, similar results reported from other countries[24,30]. Our results were inconsistent with finding recently, reported in Iranian children from Tehran city, so that higher prevalence of \textit{H pylori} were found in females compared to males[31]. However, in some studies no significant statistical differences observed between both sexes[25]. Our results for the first time showed that the prevalence of anti-CagA was markedly higher in males compared to females. Accordingly, it seems that the male gender are more susceptible to infection and colonization by CagA-positive strains of \textit{H pylori}. This differential susceptibility may be directly related to the long-term clinical outcome. It has been reported that the males are at a greater risk of \textit{H pylori} clinical manifestations [34,35]. These observation may account for higher prevalence of duodenal ulcer and gastric cancer in males. More studies should be conducted to document that this differential susceptibility in males and females can cause the male preponderance to peptic ulcer disease and gastric cancer.
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