The Antibody Response to *Helicobacter pylori* in the Sera from a Rural Population in the Central Anatolia Region of Turkey

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***Helicobacter pylori* (*H. pylori*) infection is common worldwide. Although the seropositivity of *H. pylori* rates has been unclear in the Turkish population. In this study, anti-*H. pylori* IgG seroprevalence and anti-cytotoxin-associated gene A (CagA) IgG positivity were evaluated. The sera of 880 people without gastrointestinal symptoms (384 males, 496 females) were tested for anti-*H. pylori* IgG and anti-CagA IgG antibodies by enzyme linked immunoassay method. Anti-*H. pylori* IgG antibodies were positive in 263 sera (41%) and their rates increased with age. The seroprevalence of anti-*H. pylori* IgG was higher in females (43.8%) than in males (38%). Of the anti-*H. pylori* IgG positive sera, 194 (53%) were also positive for anti-CagA IgG. The anti-CagA IgG positivity did not significantly differ with age. However, the lowest rate (46.6%) was determined among individuals 20–29 years of age and the highest rate (62.5%) among individuals over 60 years age. Anti-CagA IgG positivity rates were higher in males (87.5%) than in females (37.5%).**

Key words —— *Helicobacter pylori*, cytotoxin-associated gene A, anti-*Helicobacter pylori* IgG

INTRODUCTION

Helicobacter pylori (*H. pylori*) infection is one of the most prevalent bacterial infections in human and has been associated with peptic ulcer disease and cancers of the human gastrointestinal tract.[1)] The infection is the major cause of peptic ulcer disease and all patients confirmed to have the disease associated with *H. pylori* infection should receive antimicrobial treatment. *Helicobacter pylori* infection is common everywhere in the world, and the prevalence of the infection increases with age.[2–4)] It affects more than 50% of the world's population. Seroprevalence of *H. pylori* varies from 20% in young adults in developed countries to 90% in developing countries.[5)] The environmental factors, such as race and socioeconomic and educational state, affect the prevalence of *H. pylori* infection.[2, 6–9)] The route of transmission of *H. pylori* remains unknown although most of the evidence supports person to person transmission with colonization occurring primarily in childhood. Under natural circumstances, transmission could be by the oro-oral or fecal-oral routes, but no strong evidence exists to support either route as the primary one, and both may be relevant depending on other factors.[10)]

Serology is now generally accepted as a valid noninvasive screening method for the detection of *H. pylori* infection. Enzyme-linked immunosorbent assay (ELISA) detects anti-*H. pylori* IgG antibodies, indicating current or past infection. Because *H. pylori* infection is not known to spontaneously resolve, a positive serologic test suggests active infection in patients who have not undergone eradication therapy. The serologic test results may or may not revert to negative once the organism is eradicated; therefore, the test is not used to identify persistent infection although a negative test result does reliably identify cure.[11)] The cytotoxin-associated gene A (CagA) has been identified as a marker of virulence of *H. pylori*; this gene has been detected in half of the *H. pylori* strains in western countries, and in almost all of the strains isolated in the Asian countries.[12–15)]

Turkey is a developing country, and *H. pylori* seroprevalence data obtained from Anatolia are not clear or exact. The aim of this study was to evaluate the seroprevalence of *H. pylori* infection in a Turkish population without symptoms in the city of Kırıkkale, Turkey. The seroprevalence was investigated through a cohort of randomly selected people from the Kırıkkale population between May 2002 and January 2003.

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MATERIALS AND METHODS

Samples —— The 880 sera (384 male and 496 female) were obtained from randomly selected people of Kırıkkale, Turkey who were at a middle socioeconomic level income. Distribution of the samples according to the specified age groups was as follows: 395 samples for the 0–9 age group, 134 for the 10–19 age group, 138 for the 20–29 age group, 98 for the 30–39 age group, 57 for the 40–49 age group, 32 for the 50–59 age group, and 26 samples the for 60+ age group.

All the subjects were questioned, and those who had suffered from health problems and were of low socioeconomic status were excluded from this study. All were basically healthy, with no acute or chronic illnesses. The criteria for enrollment included no history of peptic ulcer disease, no abdominal surgery, no history of therapy for *H. pylori* infection, and no symptoms of gastrointestinal system disease such as indigestion, nausea, vomiting or epigastric burning pain.

Of the samples of 880 people, anti-*H. pylori* IgG and anti-*H. pylori* IgG positive samples were determined and in these individuals, anti-CagA IgG was investigated through an ELISA test.

Assay for Antibodies to *H. pylori* —— The seroprevalence of anti-*H. pylori* IgG (code. K5HPG, Radim Diagnostics, Rome, Italy) and anti-CagA IgG (code K6HPG, Radim Diagnostics) (CagA-antigen specific IgG) antibodies were determined by a quantitative ELISA. Anti-*H. pylori* IgG and anti-CagA IgG value greater than 30 UR/ml was determined as positive, between 15 UR/ml and 30 UR/ml as suspicious, and lower than 15 UR/ml as negative. The suspected samples were reviewed again and determined to be positive or negative. All the assays were performed in duplicate and the intra-assay and inter-assay variations were less than 5%, as was estimated by the positive and negative control sera. As validated by the manufacturer, the results of this assay closely mirror those of a previously described anti-*H. pylori* IgG ELISA. In brief, serum specimens from patients were diluted 1:300 for use in the kit, and anti-*H. pylori* IgG levels of sera were determined according to the manufacturer's instructions (anti-*H. pylori* IgG antibodies for ELISA microplate, code K5HPG, Radim Diagnostics). Absorbance was read at 450 nm (reference filter at 620 nm).

Statistical Analysis —— Chi-square tests were used to test for an association between *H. pylori* infection with age groups and gender. $p < 0.05$ was considered statistically significant.

RESULTS

Involving the samples of 880 people from every age group, anti-*H. pylori* IgG antibodies were studied and were positive in those of 263 (41%). In these 263, anti-CagA IgG was investigated and were positive in the samples of 194 (53%, see Table 1).

The rate of anti-*H. pylori* IgG positivity increased with age. In the 0–19 year age group it was 30%, and in subjects over 19 years of age it was 58.1%. The difference was statistically significant ($p < 0.05$). The peak rate of anti-*H. pylori* IgG positivity (70.4%) was in the 40–49 year age group, slightly decreasing thereafter age limit. The rate of anti-*H. pylori* IgG positivity was 43.8% in females, which was higher than that in males (38.0%). Comparisons of the rates of anti-*H. pylori* IgG positivity for different age groups showed that it was higher in females up to 30 years of age and was higher in the males over 30. In the comparisons of anti-CagA IgG

Table 1. Anti-CagA IgG and Anti-*H. pylori* IgG Seropositivity according to Gender and Age

Age and sex		Anti-*H. pylori* IgG positivity				Anti-CagA IgG positivity*			
Age group	Male - female	Male	Female	*p* value	Total *n* (%)	Male	Female	*p* value	Total *n* (%)
n	*n*	*n* (%)	*n* (%)		(male and female)	*n* (%)	*n* (%)		(male and female)
0–9 (395)	188 - 208	42 (22.3)	56 (26.9)	0.291	98 (24.8)	29 (69.1)	24 (42.9)	0.106	53 (54.0)
10–19 (134)	76 - 58	32 (42.1)	29 (49.2)	0.366	61 (45.5)	24 (75)	7 (24.1)	0.000	31 (50.8)
20–29 (138)	44 - 94	21 (47.7)	54 (57.4)	0.285	75 (54.3)	16 (76.2)	19 (35.2)	0.001	35 (46.6)
30–39 (98)	35 - 63	23 (65.7)	31 (49.2)	0.115	54 (55.1)	19 (82.6)	14 (45.2)	0.007	33 (61.1)
40–49 (57)	16 - 41	11 (68.8)	29 (70.7)	0.833	40 (70.1)	8 (72.7)	13 (44.9)	0.115	21 (52.5)
50–59 (32)	13 - 19	9 (69.2)	10 (52.6)	0.348	19 (59)	6 (66.6)	5 (50)	0.463	11 (57.8)
60–+ (26)	13 - 13	8 (61.5)	8 (61.5)	1	16 (61.5)	7 (87.5)	3 (37.5)	0.039	10 (62.5)
Total (880)	384 - 496	146 (38.0)	217 (43.8)	0.099	263 (41)	109 (74.7)	85 (39.2)	0.000	194 (53)

* The seropositivity of anti-CagA IgG in anti-*H. pylori* IgG positive sera. *n*: number of people.

test results for different age groups, no statistically significant differences were found ($p > 0.05$). However, the 20–29 year age group had the lowest rate (46.6%) and the group over 60 years had the highest rate (62.5%). In the comparisons of anti-CagA IgG positivity, the males had a statistically higher rate than that of the females ($p = 0.000$).

The rates of anti-CagA IgG and anti-*H. pylori* IgG seropositivity according to gender and age are listed in the Table 1.

DISCUSSION

In the sera of the 880 individuals with no symptoms, anti-*H. pylori* IgG positivity was investigated and in 41% of the samples, it was positive. This indicates that 41% of the community has been affected by *H. pylori.* This rate varies worldwide according to country, region, and age. The rate of anti-*H. pylori* IgG positivity for Iran has been reported as 53.75%;[16] for China, as 47% in 2003;[15] for Finland as 56% in 1973 and as 31% in 1994.[7]

Helicobacter pylori positivity increases with age in all societies. While it is lower in pediatric 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 its peak is delayed to advanced ages. In a study conducted in China, this rate was 30–48% in 1993 up to 20 years of age, 65% in the 20 year age group, 72–76% in the 30 and 40 year age group and decreased to 68% in individuals over 50 years of age. The rates in 2003 were 19–36% for the 20 year age group, 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.[15] In Finland in 1973, *H. pylori* positivity increased from 38% to 57% between the ages of 15–55 and to 81% between the ages of 55–64, but then decreased to 73% between 64–75. In 1994, it increased from 7% to 30% between 15–55 years of age, to 63% between 55–64 and then to 68% between 65–74 years of age.[7] In two different studies conducted in Ankara, the capital city of Turkey, similar results were reported. In 1990, Özden *et al*. determined 78.5% prevalence of *H. pylori* for the 7–14 years of age group by ELISA, which decreased to 66.3% in 2000.[17] Us and Hasçelik, in their study conducted by ELISA in 1998, determined a rate of 15–30% for up to 9 years of age, 58% for teens and 62% and 67% for 20s and 30s, respectively. They determined a peak rate of 81% in the 40s and then 66% in the 50s.[18] Our results were parallel to theirs. The rate of *H. pylori* seroperevalence was 24.8% in the age group under 10 years, increased to 45.5% in the teen years and to 54–55% in the 20s and 30s. The peak rate was 70% in the 40s and after 50 years of age it decreased to 59–61%. The peak rate in this study is in conformity with those reported in the studies from China and Ankara: however, the rates of positivity are significantly lower than reported in those studies. Compared to the 1994 data from Finland, the positivity rates in our study were higher and the age at peak was younger.[7, 15, 17]

Comparisons for gender differences revealed that in our study, anti-*H. pylori* IgG positivity was slightly higher in females of pediatric and adolescent groups, and was higher in males 30 years of age or older. However, the difference between the rates of anti-*H. pylori* IgG positivity for males and females was not statistically significant. In the studies from Korea and Iran, higher rates in males than females.[16, 19] In two studies that were both performed via ELISA in Turkey, however, no differences were detected.[18, 20]

In our study, only half of the samples (53%) were positive for CagA antigen, a pathogenesis criterion for *H. pylori*. In earlier studies, nearly all of the strains isolated from the samples collected from East Asian countries were positive for CagA, while only half of those obtained from western countries were CagA positive.[14, 15] Our results obtained from the samples collected in Turkey as a passage country located between East Asia and Europe are compatible with those of European countries. In a study conducted in Iran,[16] a West Asian country, half of the *H. pylori* strains were positive for CagA, as was found in our study.

In this study, the rate of CagA positivity did not differ significantly with age. However, the CagA positivity of the males (74.7%) was statistically significantly higher than that of the females (39.2%). Similarly, in the study of Jafarzadeh *et al.*, the rate of CagA positivity was higher in males. Thus, the male sex is more susceptible to infection with $CagA^+$ strains compared to the female.[16]

To sum up, the seroprevalence rate of *H. pylori* increases with age, while CagA positivity is not affected by age. This finding is in conformity with some of the earlier reports. Nevertheless, unlike general data, anti-*H. pylori* IgG positivity was more common in females of younger ages (under 30). On

the other hand, the rate of CagA positivity, which has an important role in pathogenesis, was higher in the males (87.5%) than in females (37.5%).

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