

## Seroprevalence of Helicobacter Pylori Infection in Iranian Adolescents: the CASPIAN- III Study

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### Abstract

**Background:** Helicobacter pylori (*H.pylori*) is a common bacterial infection, with considerably high morbidity and mortality worldwide. This bacterium represents a key factor in the etiology of various chronic infections ranging from gastritis, peptic ulcer disease to gastric cancer; but the prevalence has large variations in different communities. The aim of this study was to estimate the prevalence *H. pylori* infection in a nationally representative sample of Iranian adolescents.

**Materials and Methods:** In this cross-sectional study, serum samples of 882 Iranian adolescents, aged 10-18 years, were examined for seroprevalence of *H.pylori*. They were randomly selected from the samples obtained in the third survey of a national surveillance program (the CASPIAN III study). Seroprevalence of *H. pylori* was examined by detection of *H. pylori* immunoglobulin A (IgA), immunoglobulin G (IgG) and immunoglobulin M (IgM) in sera by using Enzyme Linked Immunosorbant Assay (ELISA).

**Results:** The study participants had a mean age of  $14.82 \pm 2.77$  years. Overall, 51.7% of students were boys and 61.52% were urban residents. The *H. pylori* IGM and IGA seropositivity had no significant association with demographic characteristics ( $p>0.05$ ). The *H. pylori* IgG seropositivity were significantly different in boys and girls (69.7%, 95% confidence interval [CI] = 66.7-72.7 vs. 76.3%, 95% CI= 73.5-79.1, respectively,  $P=0.03$ ).

**Conclusion:** The seroprevalence of *H. pylori* IgG in Iranian adolescents is high, and girls had greater risk of *H. pylori* IgG seropositivity compared to boys. Preventive strategies and health education are recommended to reduce the prevalence of this infection in Iranian adolescent.

**Key Words:** Adolescents, Helicobacter pylori, Iran, Seroprevalence.

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## 1- INTRODUCTION

*Helicobacter pylori* (*H. pylori*) is a main cause of chronic active gastritis, and plays an important role in the etiology of peptic ulcer disease in humans (1). Evidence suggests that the communities with high prevalence of stomach cancer usually have a high rate of *H. pylori* infection (2). The seroprevalence rates of *H. pylori* infection have geographic variation; moreover the prevalence differs according to socio-economic factors, for instance: living conditions during childhood, poor sanitation, overcrowding, low income, and poor education (3). One probable cause for differences in outcomes of *H. pylori* infection might be linked to differences in the virulence of *H. pylori* strains (4).

*H. pylori* has been infecting humans for a long time (5-6). Serology examinations have been functional adjunct in improving our understanding of the coevolution of humans and infecting microorganisms (7). Using specific immunoglobulin G (IgG) and immunoglobulin M (IgM) antibodies as biomarkers, several prior studies assessed the prevalence of diverse infectious agents (7-8). By this test, the antigens found in the serum are detected against *H. pylori*; therefore, it is a reliable method to diagnose an active infection (9).

Previous studies at provincial level showed severe heterogeneity in reported seroprevalence of *H. pylori* in Iranian adolescents with a range of 13% to 82% (10-13). Most of these studies have been done in small sample size and in limited areas of the country. The aim of this study was to estimate the seroprevalence of *H. pylori* infection among a nationally - representative sample of Iranian adolescents.

## 2- MATERIALS AND METHODS

### 2-1. Methods

This survey was conducted as a sub-study of the 3rd round of the school-based surveillance program entitled "Childhood and Adolescence Surveillance and Prevention of Adult Non-communicable Disease (CASPIAN III)" study (2009-2010), in Iran. The aim and methodology of the CASPIAN- III study has been described previously (14).

In the CASPIAN- III study, 5,528 students, aged 10-18-years, were selected by multistage cluster random sampling from 27 provinces of Iran. Equal clusters at the level of school were included in each province, from which students were selected randomly. Information bank of the Ministry of Education was used for selection of schools. Stratification was done according to the school level and the living area with equal gender ratios. Under the supervision of health care experts, the students filled out the self-administered questionnaire at school.

A team of trained health care professionals recorded information in a checklist. Questionnaires including demographic and socioeconomic variables were filled by one of the parents. Eligible students were referred to the laboratory, while one of the parents accompanied him/her. Then, 6 mL venous blood sample was collected after 12-h overnight fasting. All collection tubes were centrifuged at (2,500-3,000 x g) for 10 minutes at 4°C. Immediately after centrifugation, serum samples were aliquot into 200 microliter tubes and stored at -70° C. All samples were transferred by cold chain to Isfahan Mahdieh Laboratory.

To determine the seroprevalence of *H. pylori*, a minimum sample size of 882 was calculated by considering the expected prevalence of about 54 % (15), the level of confidence of 95%, and a precision of 3.3%. Therefore, 882 residual serum samples of CASPIAN-III along with their questionnaires were used in this cross-sectional study.

Demographic data such as age, gender, family size and living area as well as socioeconomic status (SES) were derived from the CASPIAN-III database. SES was calculated using principle component analysis (PCA). Using PCA, variables including parental education, parental job, possessing private car, school type (public/private), type of home (private/rented) and having personal computer in home were summarized in one main component to construct SES. This main component was divided into tertiles. The first tertile was considered as a "low", second tertile as a "middle", and third tertile as a "high" SES.

### 2-2. Serological methods

Serum was separated for measuring the IgM, IgG and IgA antibody by an Enzyme Linked Immunosorbent Assay (ELISA) method. Serum anti- *H. pylori* antibodies IgG and IgA were assayed by ELISA method supplied by ChoRus Company (Italy). Serum anti- *H. pylori* antibodies IgM were supplied by IBL Company (Germany). In present study for increase of test accuracy results was tested twice. IgG, IgA and IgM values above 12 AU/ml, 12 AU/ml, and 8 U/ml considered as positive, respectively.

### 2-3. Ethical considerations

The CASPIAN- III study was approved by ethical committees of Ministry of Health and Medical Education and Ministry of Education and Training of Isfahan and Tehran University of Medical Sciences. The current study was also approved by the Ethic committee of Alborz University of Medical Sciences.

### 2-4. Statistical analyses

Continuous and categorical variables are expressed as mean  $\pm$  standard deviation (SD) and number (%) respectively. The *H.*

*pylori* IgG, IgM and IgA seropositivity was reported as percentage (95% confidence interval [CI]). Association of *H. pylori* IgG, IGM and IGA seropositivity with demographic characteristics was assessed using Chi-square test. Statistical analyses were carried out using SPSS version 21.0. P-value less than 0.05 was considered as statistically significant.

## 3- RESULTS

Overall, in the present study, serum samples of 882 subjects were examined for seroprevalence of *H. pylori*. Participants had a mean age of  $14.82 \pm 2.77$  years without significant difference between boys ( $14.78 \pm 2.88$ ) and girls ( $14.86 \pm 2.65$ ). Overall, 51.7% of students were boys and 61.52% were from urban area. Totally, 31.75, 46%, 41.72% and 26.53% were in elementary, secondary and high school levels, respectively.

The *H. pylori* IgG, IgM and IgA seropositivity was 72.9% (95%CI= 76.5-81.8), 2.4% (95%CI=1.39-3.41) and 73.7% (95%CI= 70.9-76.6), respectively. **Table-1** shows the association of *H. pylori* IgG, IgM and IgA seropositivity with demographic characteristics. The *H. pylori* IgM and IgA seropositivity was not significantly associated with all demographic characteristics ( $P>0.05$ ).

The *H. pylori* IgG seropositivity in boys (69.7%, 95%CI= 66.7-72.7), and girls (76.3%, 95%CI= 73.5-79.1) was significantly different ( $P= 0.03$ ). The *H. pylori* IgG seropositivity in urban area was 72.5% and in rural area was 73.5%, that was not statistically significant ( $P=0.67$ ). The *H. pylori* IgG seropositivity was not significantly associated with age, level of education, family size and SES ( $P>0.05$ ).

**Table-1:** Association of *H. pylori* seroprevalence with demographic characteristics

Characteristic	Category	IgM		IgG		IgA	
		Negative	Positive	Negative	Positive	Negative	Positive
Gender	Female	420(98.6)	6 (1.4)	101(23.7)	325(76.3)	103(24.3)	320(75.7)
	Male	441(96.7)	15 (3.3)	138(30.2)	318(69.8)	127(28.03)	326(71.97)
	p-value	0.06		0.03		0.21	
Age (years)	10-14	401(96.8)	13(3.14)	118(28.5)	296(71.5)	118(28.64)	294(71.36)
	14-18	460(98.3)	8(1.7)	121(25.85)	347(74.15)	112(24.14)	352(75.86)
	p-value	0.16		0.37		0.13	
Family size	≤ 4	267(96.74)	9(3.26)	79(28.62)	197(71.38)	68(24.91)	205(75.09)
	4>	488(97.79)	11(2.20)	138(27.65)	361(72.34)	134(28.75)	362(71.43)
	p-value	0.37		0.77		0.52	
Living area	Urban	530(97.78)	12(2.21)	149(27.49)	393(72.51)	137(25.51)	400(74.49)
	Rural	330(97.34)	9(2.65)	90(36)	249(64)	93(27.51)	245(72.48)
	p-value	0.67		0.76		0.52	
Level of education	Primary school	271(96.78)	9(3.21)	81(28.93)	199(71.07)	81(29.03)	198(70.97)
	Secondary	361(98.1)	7(1.9)	94(25.54)	274(74.45)	86(23.5)	280(76.5)
	High school	229(75.33)	5(24.67)	64(27.35)	170(72.65)	63(27.27)	168(72.73)
	p-value	0.55		0.62		0.26	
SES	Low	258(97.73)	6(2.27)	72(27.27)	192(72.73)	66(25.19)	196(74.80)
	Moderate	259(97.73)	6(2.64)	76(28.68)	189(71.32)	74(27.92)	191(72.07)
	High	257(97.35)	7(2.65)	65(25)	195(75)	72(27.69)	188(72.31)
	p-value	0.94		0.56		0.73	

Data are presented as number (%), SES: Socioeconomic status.

#### 4- DISCUSSION

The results of present study show that *H. pylori* IgG, IgM and IgA seropositivity was 72.9% (95%CI= 76.5-81.8), 2.4% (95%CI= 1.39-3.41) and 73.7% (95%CI: 70.9-76.6), respectively. Our findings about *H. pylori* IgG seroprevalence are consistent with the findings of Montazer et al. in a previous study in Iran (16). Similarly, in another study East of Iran, the overall prevalence of *H. pylori* colonization in students was 13.1% (13).

This rate of seroprevalence is reported as 66.9% in Korea, and 34% in India (17-18). In another study in Korea, the prevalence of *H. pylori* infection was 63% (19). These

findings confirm that the Seroprevalence of *H. pylori* infection has large variations in different communities. *H. pylori* is one of the most common human pathogens with a global infection rate of >50% (5); however, there are notable geographic variations in the frequency of (20). The present study is distinguished by its assessment of coexisting levels of IgG, IgA, and IgM antibodies to *H. pylori* in serum samples obtained from a large unselected population. Many scientists also believe that non-invasive detection methods are imperative when screening for *H. pylori* (21). There are several studies regarding the determinants of *H. pylori* infection in Iran (22, 15). Some studies

reported that lower levels of education, gender, age and SES is associated with the risk of *H. pylori* infection; however, our results did not show any association between SES, education level and age with the *H. pylori* infection. This finding is consistent with some other surveys from other countries that did not show significant difference between the prevalence of *H. pylori* infection and age. However, many investigations also reported that gender as a risk factor for the *H. pylori* infection (15, 23). The meta-analysis on studies conducted in Iranian adults- population showed that male subjects had higher risk of *H. pylori* infection (15).

#### 4-1. Limitations of the study

The first limitation is that we were not able to include all age groups in our study. In addition, we were not able to cover all provinces of Iran. However, the data of this multicentric study were obtained from a representative sample of Iranian adolescents, and this increases the generalizability of the present findings.

#### 5- CONCLUSION

The seroprevalence of *H. pylori* IgG in Iranian adolescents is high, with greater risk of *H. pylori* IgG seropositivity in girls compared to boys. The *H. pylori* seropositivity was not associated with age, SES, living area and family size. Preventive strategies and health education are recommended; health strategies should be planned to decrease the prevalence of this infection in Iranian adolescents. Further nationwide representative studies about *H. pylori* seropositivity and its potential risk factors are necessary to depict the clear picture of this infection in Iranian children and adolescents at national and subnational level.

**6- CONFLICT OF INTEREST:** None.

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