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ORIGINAL ARTICLE

Seroprevalence of *Helicobacter pylori* in a population of 15 to 35 years old in Mashhad

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ABSTRACT

Background and Objectives: *Helicobacter pylori (H. pylori)* is a bacterium involved in gastrointestinal disorders with a high prevalence in Iran. We have determined the seroprevalence of *H. pylori* in the young adult population of Mashhad city for the first time.
Materials and Methods: We carried out a cross-sectional study on 933 individuals between the ages of 15 and 35 in Mashhad. The serum level of IgG, IgM, and IgA *H. pylori* antibodies was determined using enzyme linked immunosorbent assay (ELISA) method.
Results: The average age of the participants including 290 (31.0%) male and 643 (68.9%) female cases was 25.47 ± 5.76. *H. pylori* IgG was seropositive in 365 (39.1%) of subjects. Occupation (p=0.002), body mass index (p=0.002), marital status (p<0.0001), and age (p<0.007) were meaningfully related to *H. pylori* IgG seropositivity. Other factors such as sex, educational attainment, history of chronic diseases, and smoking cigarette had no significant relationship with the presence of *H. pylori* antibodies.
Conclusion: The seroprevalence of *H. pylori* IgG antibodies in Mashhad's 15 to 35 years old citizens was determined 39.1%. We suggest further studies with larger sample sizes and different age groups as the target population.

Keywords: Prevalence; Seroepidemiologic studies; *Helicobacter pylori*; Iran; Young adult

INTRODUCTION

Helicobacter pylori (H. pylori) is a spiral, gram-negative, microaerophilic bacterium that causes gastrointestinal conditions such as gastritis, and duodenal and gastric ulcers, and can lead to gastric cancer. *H. pylori* is a bacterium principally found in the deeper sections of the gastric mucus and the gap between the mucus and the gastric epithelium. *H. pylori*`s structure helps it live in an unfavorable gas-

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tric environment (1, 2). Many methods for detecting the presence of the bacterium have been developed. Non-invasive tests include serology, C urea breath test (UBT), and stool antigen tests, are usually preferred in clinical examinations. *H. pylori*`s virulence potential is dependent on its ability to produce urease (3). *H. pylori* ranges over 50% in Asia. Its prevalence is estimated to be highest in Turkey, Egypt (>80%), Iran, and Oman (70% and 80%, respectively) (4, 5). It is currently unknown exactly how *H. pylori* infections spread. Researches have indicated that these bacteria can proliferate via oral-oral and oral-fecal pathways. As a result, the infection of one family member increases the other members at risk for infection (6). Numerous risk factors have been previously studied in *H. pylori* infection including body mass index (BMI) (7), gender, occupation, education (8), marital status (9), smoking cigarette, and age (6, 10). For instance, a case-control study in Saudi Arabia suggested that BMI remains a significant factor in *H. pylori* infection ($p<0.0005$) (7). The prevalence rate of *H. pylori* cases was higher in employed patients (8). According to another study, marital status impacts the prevalence of *H. pylori* infection. If one partner experiences reflux symptoms, the possibility of an *H. pylori* infection in the couple is four times higher (9).

There is a low prevalence of *H. pylori* infection in children in developed countries (10). However, given that this infection is often transmitted during infancy and spreads more widely in developing nations, the economic circumstances of childhood have been identified as a major risk factor for it (6).

A review and meta-analytic study showed that the average prevalence of *H. pylori* in Iran was 50.7% between 1994 and 2011, with the lowest and highest prevalence among the provinces of Tehran (19.2%) and Mazandaran (74.27%), respectively (4). An investigation in Tehran revealed that 87.6% of patients with dyspepsia were affected by *H. pylori* (11). A further consecutive study on 14,860 gastritis patients in Tehran between 2008 and 2014 revealed that 83.5% of cases were infected with *H. pylori* (12). Currently, there is no data on the prevalence of *H. pylori* infection in the young population of Mashhad city.

According to Iran's *H. pylori* infection rates, the condition needs to receive greater attention. The purpose of the current investigation was to evaluate the seroprevalence of *H. pylori* in a population aged 15 to 35 in Mashhad.

MATERIALS AND METHODS

The current cross-sectional epidemiologic survey was conducted in Mashhad from 2018 to 2019 to determine the prevalence of *H. pylori* infection. A total of 933 individuals who volunteered to participate in the study (between the ages of 15-35) were recruited from another study (13).

This study was performed in the Antimicrobial Resistance Research Center in Mashhad. Demographic and socio-economic characteristics, along with the history of various diseases, were recorded. Also, 10cc of brachial vein blood was obtained from all patients after giving informed written consent. Blood samples were centrifuged, and the obtained serum was divided into 0.5 cc microtubes and kept frozen at -70°C for further analysis.

In the present study, we prepared a questionnaire including data on age, gender, body mass index (BMI), education level, employment status, marital status, smoking, history of hypertension, diabetes, dyslipidemia, and cardiovascular diseases.

The level of *H. pylori* IgM, IgG, and IgA in serum samples was examined with an ELISA commercial kit (Padtan Elm Co., Iran). According to the results obtained from the optical absorption of the patient's serum, the samples were classified into three groups as follows. Titers higher than 30 units were considered positive for IgG, IgA, or IgM. Titers lower than 20 were considered negative and those between 20 and 30 were equivocal and were re-tested according to the kit's instruction. To investigate the relationship between the frequency of *H. pylori* antibodies and qualitative variables (such as education level, occupation, and gender) and quantitative variables (such as patient`s age), the Chi-square test and independent T-test were used, respectively. Furthermore, in order to examine the relevant variables, logistic regression was applied. The software used was SPSS 20, and the P value < 0.05 of the tests was considered statistically significant.

The current study protocol was approved by the Ethics Committee of Mashhad University of Medical Science, Mashhad, Iran (Approval code: IR.MUMS.MEDICAL.REC,1397.117).

RESULTS

Out of 933 participants, 290 were male and 643 fe-

males, with mean age of 25.47 ± 5.76 years. In the study population, 365 (39.1%) subjects were IgG seropositive against *H. pylori* (ELISA IgG-Ab+). IgM and IgA serum level was measured, but all cases were negative for *Helicobacter pylori*. There was no IgM or IgA antibody against *H. pylori* in this observation. The frequency of IgG antibody in the studied population was determined to be 39.1% (Fig. 1).

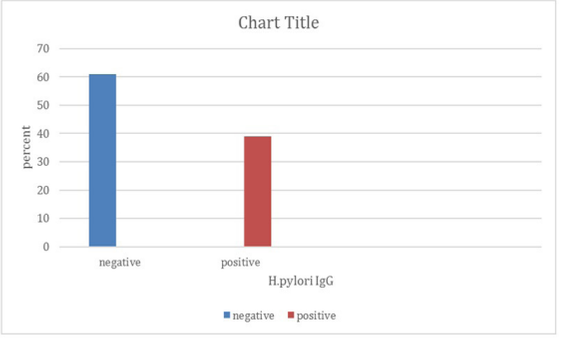

Fig. 1. Frequency distribution of anti-Helicobacter IgG antibodies in the 15-35-year-old population in Mashhad.

We found a higher prevalence of *H. pylori* seropositivity in older individuals as the mean age of IgG -positive subjects was 26.75 ± 5.13 and the mean age of IgG -negative individuals was 24.65 ± 5.63 ($p< 0.001$). There was also a significant positive relation between BMI and *H. pylori* infection (p=0.002). The mean BMI of the IgG positive group was 25.33 ± 4.46 compared to IgG negative subjects, which was 24.44 ± 4.02. The frequency of *H. pylori* infection considerably differed between the married group (positive n=234; 45.9% and negative n=276; 54.1%) and single individuals (positive n=127; 30.7% and negative n=287; 69.3%, $p<0.0001$). Our results indicated that the seropositivity rate is significantly related to job status (p=0.002) since 29.3% of the retired subjects were seropositive for *H. pylori* IgG, compared to 42.3% of unemployed individuals and 42.6% of the employed group.

The other factors, such as gender, education level, history of chronic diseases, and consumption of cigarettes were not significantly related to *H. pylori* seropositivity (Table 1).

Our logistic regression model showed a positive association between age and *H. pylori* prevalence: pooled odds ratio (OR) 1.04 (95% confidence interval [CI]: 1.1-1.08 and p=0.006).

DISCUSSION

Several reports have shown high prevalence of *H. pylori* in Iran (4). However, in the general population, the prevalence and onset of *H. pylori* is unclear because asymptomatic healthy individuals typically do not undergo diagnostic methods (14). The average prevalence of *H. pylori* was reported as 50.7% between 1994 and 2011 in Iran (9). To our knowledge, the present study was the first to evaluate the seroprevalence of *H. pylori* in a population of 15 to 35 years old individuals in Mashhad, which showed 39.1% seroprevalence in the preceding population. We also investigated the risk factors of *H. pylori*, such as gender, occupation, education, marital status, history of chronic diseases, age, and BMI.

More than 50% of people in Asian countries have *H. pylori* in their bodies. The prevalence might reach 80-90% in developing nations, compared to 40% in developed nations. However, there are specific differences in the incidence of *H. pylori* infection (8). Researchers have assessed the frequency of *H. pylori* infection in a Mashhad population aged 35 to 60. It has been revealed that 85.2% of the survey population had *H. pylori* infection (5).

According to our results and Hong et al. there is no clear association between gender and seroprevalence of *H. pylori* (15). However, in another study by Zhu et al., they found a significant relationship and the prevalence was higher among women. The mentioned study claimed that different hormones in the two sexes lead to this critical difference (12). A meta-analysis study also showed that gender is related to the prevalence rate (16, 17). One reason for the difference in the results of our study and the two mentioned studies is the high number of women participating in this study, while the sample size was not as large in other studies. A different diagnostic test to confirm *H. pylori* infection can also lead to this contrasting result. In this study, employment status was proposed as a risk factor. Our results, showed a statistically significant association between the prevalence of *H. pylori* and employment. The prevalence of *H. pylori* was higher in the employed subjects compared to unemployed individuals and students (8). One of the reasons for this is that the employed individuals have less time to eat at home and, as a result, they consume more fast food. According to studies, people who use fast food more often have a higher chance of contracting *H. pylori* infection

Table 1. Distribution of the assessed variables among the *H. pylori* IgG seropositive and seronegative individuals

Variable			*H. pylori* IgG antibody		P-value
			Positive	Negative	
Age			26.75 (± 5.73)	24.65 (± 5.63)	<0.001*
BMI			25.33 (± 4.46)	24.44 (± 4.02)	0.002*
Gender	Male		116 (31.7%)	174 (30.6%)	0.712**
	Female		249 (68.2%)	394 (69.3%)	
Smoking status	Non-smoker		300 (82.1%)	488 (85.9%)	0.125**
	Smoker		65 (17.8%)	80 (14%)	
Marital status	Single		127 (34.7%)	287 (50.5%)	<0.0001**
	Married		234 (64.1%)	276 (48.5%)	
	Divorced		4 (1%)	5 (0.8%)	
History of chronic disease	HTN	Yes	8 (2.1%)	15 (2.6%)	0.829**
		No	357 (97.8%)	553 (97.3%)	
	DM	Yes	8 (2.1%)	6 (1%)	0.264**
		No	357 (97.8%)	562 (98.9)	
	HLD	Yes	12 (1.3%)	14 (2.4%)	0.588**
		No	870 (98.6%)	554 (97.5%)	
	CVD	Yes	6 (1.6%)	8 (1.4%)	0.990**
		No	359 (98.3)	560 (98.5%)	
Education	Illiterate		6 (1.6%)	3 (0.5%)	0.272**
	Primary education		44 (12%)	67 (11.7%)	
	Secondary education		237 (64.9%)	359 (63.2%)	
	Tertiary education		29 (7.9%)	49 (8.6%)	
	Bachelor`s		38 (10.4%)	79 (13.9%)	
	Master`s and higher		11 (3%)	11 (1.9%)	
Employment	Student		68 (18.6%)	164 (28.8%)	0.002**
	Employed		106 (29%)	143 (25.1%)	
	Unemployed		191 (52.3%)	261 (45.9%)	

*independent T-test, **chi square test

BMI: Body mass index, HTN: Hypertension, DM: Diabetes Mellitus, HLP: Hyperlipidemia, CVD: Cardiovascular disease

(18). Although this relationship was not obtained in the logistic regression model, some other studies support this result, such as the one conducted by MH et al. in Afghanistan (8), with the difference that in the mentioned study, the prevalence among employed subjects was less, contrary to our results. One of the reasons that can justify this is better living conditions and access to a healthier environment if there is employment and a suitable job, while illiteracy and lack of employment, limit access to these resources. This relationship may also be linked to family income (19), highlighting the enduring influence of early-life circumstances on health outcomes and underscoring the crucial role of socioeconomic factors in health disparities (17).

According to the results obtained in our study, marital status was found to be one of the risk factors, similar to the study of Hadji et al. conducted in Tehran (20). The prevalence of *H. pylori* in married people was higher than in single or divorced individuals. According to another study by Sgambato et al., these two factors have a statistically significant relationship (9). However, the study conducted by Brenner et al. indicated that transmission between couples in populations with low prevalence is not considerable (21). Since the bacterium shows genetic heterogeneity, it would be recommended to conduct molecular typing to determine which result is more reliable. By examining the molecular typing in the microorganism, we can confirm whether each couple is infected with a single strain (9).

Another investigated factor was age. According to

our results, the probability of contamination by *H. pylori* increases with age. Most researchers agree with us on the positive effect of age on *H. pylori* prevalence (14, 15). Regarding the transmission route of *H. pylori*, we discussed earlier that its prevalence is higher among couples. Statistics of Iranian people show that in the age group of 15 to 35 years, the number of married people generally increases with age (22). We hypothesize that age, in combination with marriage, affects expanding the prevalence of *H. pylori*. A study stated that age significantly affects *H. pylori* infection, but unlike ours, it is declared that the prevalence of *H. pylori* decreases as people age. The results of this study indicated that the prevalence peaked at the age of 30-39 years (90.82%). This study was conducted on people aged 30 to 69, and perhaps the reason for the difference in our results is the age of the studied groups (14). Similarly, the logistic regression test results demonstrated that age is statistically related to *H. pylori* infection, among the other factors.

According to our results, we found a connection between BMI and *H. pylori* prevalence. One of the risk factors for *H. pylori* infection is abnormal BMI (7). A study by N. Lender et al. showed a significant inverse association between the prevalence of *H. pylori* and BMI in developed countries (23). So, the progressive decline in *H. pylori* infection was blamed for the obesity epidemic in the Western world, which differed with our study results. In contrast, based on the study conducted by Baradaran et al., the prevalence of *H. pylori* in people with higher BMI was higher than in people with normal BMI, and people with *H. pylori* infection suffered more from obesity than those without *H. pylori* infection (24).

Another factor that was examined as a risk factor in this study was the history of chronic diseases such as diabetes, hypertension, hyperlipidemia, and cardiovascular diseases. We found no statistically significant relationship between chronic diseases and the prevalence of *H. pylori*. However, some other studies reported a meaningful association between chronic diseases and the prevalence of *H. pylori*. Talebi Taher et al.`s study stated that the prevalence of *H. pylori* in a diabetic population is 60%, compared to 26.66% in non-diabetics (25). Bener et al. showed that the prevalence of *H. pylori* infection in diabetic and non-diabetic subjects was 64.1% and 40%, respectively (26). One of the reasons that these studies stated such difference is the autonomic neural pathway (27). The autonomic neural pathway in diabetics may be related to gastric emptying, so the absorption of carbohydrates and the time of insulin secretion are disturbed (28).

Some studies show a meaningful relationship between LDL and blood cholesterol levels with *H. pylori* infection (29). Also, according to Kwang et al., *H. pylori* infection is related to LDL in men and HDL in women, and according to these studies, *H. pylori* is effective on blood lipid levels (30). However, the exact reason still needs to be fully understood.

Some studies have shown that cardiovascular diseases such as high blood pressure are related to the incidence of *H. pylori*. However, the mechanisms that link the prevalence of *H. pylori* to these diseases are still unknown. The other reason is the consumption of salty foods that can lead to high blood pressure and increase the colonization of bacteria in the digestive system (31). Nevertheless, our study found no statistically significant relationship between cardiovascular disease and the prevalence of *H. pylori*. In the interpretation of the results of our research, we examined a low age range in which the probability of cardiovascular diseases is much lower.

In a study of the association between education status and *H. pylori* infection, one of the investigations by Hamrah et al. showed that since *H. pylori* infection is related to hygiene and the community members' education, there will be more hygiene awareness at the higher education level. Therefore, it is likely that people with higher education will be less affected by this disease, and it will be more prevalent in people with less education and knowledge. However, considering that the level of education in societies is increasing, it can be concluded that the prevalence of infection in the future will be reduced (8). Hojati et al., reported that education is one of the variables inversely associated with the prevalence of *H. pylori*. Those with greater education levels are less likely to suffer from this disease. Moreover, this study demonstrated that the prevalence of *H. pylori* is less prevalent in illiterate individuals than among educated participants. A small sample size can also cause this outcome (32).

Of course, our study may have different results than other studies conducted in Iran since it only examined the presence of this bacteria in serum samples, and we had a smaller sample size. We suggest that future researchers increase the sample size.

Strength and limitations. According to the population census of the Islamic Republic of Iran in 1395 AH, a large population of Iranians is aged 15 to 35 (44.66%). More than one-third of Mashhad inhabitants range in age from 15 to 35 (1059378 from 3012090 Mashhad residents, 35.17%) (9). That is why we decided to conduct the research on the 15 to 35-year-old Mashhad citizens. This study has some limitations. First, the study included a relatively small sample. In addition, our study relied only on serological testing to diagnose *H. pylori*. Although the province and patient population can be considered reasonably representative, but the patterns of complications in different regions of Iran might not be similar.

CONCLUSION

This pioneering study, was the first to focus on this age group in the city of Mashhad, and has represented similar results with another study on individuals aged 35-65 in the same city. The seroprevalence of *H. pylori* antibodies was found to be 39.1% among the participants, highlighting the unique and invaluable insights our research has provided.

Given the health implication of this infection, we suggest the implementation of hygiene methods and a concerted effort to raise awareness about the disease.

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