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Original Article

Determination of *Helicobacter Pylori* Infection Prevalence by Non-invasive Methods

Mohammad Javad Gharavi[1], Javad Zarei[2], Parisa Roshani Asl[3], Zahra Yazdanyar[4], Masoud Sharif[4], Niloufar Rashidi[1*]

[1]Department of Medical Laboratory Sciences, Faculty of Allied Medicine, Iran University of Medical Sciences, Tehran, Iran
[2]Department of Health Information Technology, School of Allied Medical Sciences, Ahvaz Jundishapur University of Medical Sciences, Ahvaz, Iran
[3]Department of Microbiology, Faculty of Basic Sciences, Shahrekord Branch, Islamic Azad University, Shahrekord, Iran
[4]Department of Medical Laboratory Sciences, Faculty of Allied Medicine, Tehran University of Medical Sciences, Tehran, Iran

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***Corresponding author:**
Niloufar Rashidi,
Department of Medical Laboratory Sciences, Faculty of Allied Medicine, Iran University of Medical Sciences, Tehran, Iran.
Email: ni.rashidi@gmail.com

Abstract

Background: *Helicobacter pylori (H. pylori)* is identified as the most frequent agent of bacterial infections in humans which can cause various gastrointestinal diseases. This pathogen has infected approximately half of the world's population, and its outbreak has varied across different regions. The purpose of this study was to estimate the *H. pylori* infection prevalence amongst patients in Fardis county, Alborz province, Iran, using noninvasive methods.

Methods: A total of 5677 patients were analyzed from September 2020 to October 2021 to detect *H. pylori* by the use of enzyme-linked immunosorbent assay (ELISA) IgG, IgA, and IgM tests, stool antigen test (SAT), and urea breath test (UBT).

Results: Of 5677 patients, 3486 (61.4%) were female and 2191 (38.6%) were male with the mean age of 38.82 ± 18.289 years old. The overall rate of *H. pylori* infection positive was 31.46%, and the serological tests were the most prescribed types of tests. The IgG test and then SAT detected the most positive cases. Further, the infection rate was significantly associated with age. Except for the case of IgM, which was higher in females compared to males, no significant difference was found between gender and bacteria outbreak.

Conclusions: This study indicated a decline in *H. pylori* infection prevalence compared with the prior survey conducted at this center. However, its rate amongst the patients referring to Fardis laboratory is still high.

Keywords: *Helicobacter pylori*, Enzyme-linked immunosorbent assay, IgA, IgM, IgG, Stool antigen test, Urea breath test

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Background

Helicobacter pylori is recognized as the most prevalent pathogen responsible for chronic bacterial infection in humans and infects approximately half of the population around the world (1,2). *H. pylori* is a gram-negative, microaerophilic, spiral-shaped, and flagellated bacterium that is frequently found in the stomach (3,4). It contains microbiological features including urease, catalase, and oxidase activity that are crucial for facilitating the survival of the pathogen and colonization in the adverse acidic gastric environment (5).

Helicobacter pylori infection is extensively identified as the main cause of many gastro-intestinal diseases such as duodenal ulcer, peptic ulcer, chronic gastritis, mucosa-associated lymphoid tissue lymphoma, and especially gastric cancer (6,7). Moreover, several extra-gastric diseases like ischemic heart diseases, type 2 diabetes mellitus, and anemia are the outcomes of contamination with this microorganism (8). Not limited to gastric cancer, *H. pylori* infection also plays a major role in various cancer incidences including pancreatic and lung cancer (9). Consequently, the International Agency for Research on Cancer of the World Health Organization has classified *H. pylori* as a class I carcinogen in humans (10,11). Although the exact mode of bacterial transmission has not been clearly identified up to now, the scientific literature proposed that the infection transmission occurs person-to-person mainly through oral-oral, fecal-oral, and gastro-oral ways (12).

The *H. pylori* infection distribution varies all over the world, and it is influenced by socioeconomic conditions along with sanitation (13). In fact, in developing countries particularly those with substandard socioeconomic status, the *H. pylori* contamination rate is more than 80%; however,

this rate is less than 40% in developed countries (14,15).

Helicobacter pylori contamination commonly occurs at earlier ages. Only about 10% of the people infected with this bacterium exhibit clinical manifestations, while 90% of the patients are asymptomatic, and this infection would continue for all their life if the appropriate treatments were not going to be provided to patients (16). It has been demonstrated that *H. pylori* elimination can improve the consequences of those diseases caused by this bacterium and restitute a healthy microbiome in the stomach and intestines (17). Therefore, knowledge of the *H. pylori* situation through accurate diagnostic procedures is a significant step for effective management of its remedy. Nowadays, numerous methods are available for detecting the *H. pylori* infections, which are classified as invasive (e.g.,, endoscopy and endoscopic biopsy for histopathology, culture, and rapid urease test) and non-invasive (e.g., , stool antigen test (SAT), urea breath test (UBT), and serological tests) tests (18). It is also noteworthy to state that none of these tests alone is a definite method or a gold standard for detecting the *H. pylori* infection, and selection of the optimal technique depends on different variables, including the *H. pylori* spread in each area, patient's age, clinical outline, and test availability. However, the non-invasive methods are considered the preferred and recommended methods for the *H. pylori* infection screening in investigations with large populations (19,20). The basis of these tests is the tracing of the existence of bacterial enzymes, antigens, antibodies, or DNA sequences (21).

Given the above-mentioned discussions, investigating the spread rate of *H. pylori* in a geographical area is very important, leading to improvement in the clinical practice, development in prevention, and control of measures in that region. Therefore, this study aimed to determine the *H. pylori* infection prevalence using non-invasive methods.

Materials and Methods

Study Design and Population

This descriptive cross-sectional study was conducted to determine the *H. pylori* infection prevalence in patients suspected of being infected by *H. pylori* who were referred to the referral laboratory of Fardis county, Alborz province, Iran. A total of 5677 patients were examined from September 2020 to October 2021.

Inclusion/Exclusion Criteria

The only inclusion criterion to this study was living in Fardis, Alborz province, while exclusion criteria were history of *H. pylori* eradication therapy, use of any medication such as antibiotics, proton pump inhibitors, non-steroidal anti-inflammatory drugs, or H2-receptor antagonists within the past 30 days.

Serological Tests

About 5 mL of venous blood was obtained from each patient under sterile conditions and was transferred to a vacutainer tube without anticoagulant. These collected samples were placed at room temperature for 45 minutes to clot. After that, the test tubes were centrifuged at 3500 × g for serum isolation for 10 minutes. Afterward, the harvested serum was processed for *H. pylori* antibodies. Anti-*H. pylori* IgA, IgM, and IgG detection for each sera sample was accomplished using enzyme-linked immunosorbent assay (ELISA) kit (Pishtaz Teb Co, Iran) with a sensitivity of 95% and specificity of 98% according to the manufacturer's instructions. Any titer above 12 units was considered positive for IgA, IgM, and IgG (negative > 8, equivocal 8 to 12, and positive >12 U).

Stool Antigen Test

Stool samples were gathered from each participant and stored at -20°C until the time of analysis. The examination of stool samples for detecting *H. pylori* antigen was performed using ELISA by applying a commercially available kit (Astra SRL, Via Ciro Menotti, Milano, and Italy). At first, a small segment of the stool specimen was transferred to the sample diluent vial and mixed thoroughly. After that, the diluted stool sample was added to antibody-coated micro wells and was incubated at room temperature. In the next step, *H. pylori*-specific monoclonal antibodies conjugated to horseradish peroxidase were added and incubated again. After that, unbound material was removed by washing, and peroxidase was added. Accordingly, the absorbance was read at 450 nm using a spectrophotometer, a visible blue reaction indicated the *H. pylori* presence. The sensitivity, specificity, and accuracy of this kit have been validated to be 100%, 96.6%, and 96.7%, respectively.

Urea Breath Test

The UBT was performed using a commercial kit (Kibion, Sweden). After collecting a baseline breath sample, each patient received C-14 urea as a tablet that was dissolvable in water. Another breath sample was collected 30 minutes after the test solution administration. After that, the samples were measured for liberating CO2-14, which was detected by the Gamma counter apparatus. The urea hydrolysis with the rate of 50 counts/ minutes or higher was considered positive for *H. pylori* infection.

Data Analysis

The collected data were analyzed by using SPSS version 22 and by applying descriptive statistics (frequency, frequency percent, average, and standard deviation), one-way ANOVA, Independent samples *t* test, and the chi-square statistical tests. In addition, the confidence interval for statistical tests was considered significant at 0.95.

Results

Descriptive Information of the Study Population (the Patients With Suspected Helicobacter pylori Infection)

A total of 5677 subjects containing 3486 (61.4%) females and 2191 (38.6%) males were analyzed from September

2020 to October 2021. The mean age of subjects was 38.82 ± 18.289 years old within the age range of 1-92. Moreover, the participants mostly belonged to the age group of 31 to 45 years old (32.3%). The baseline demographic characteristics of participants are presented in Table 1.

Diagnostic Tests

Detecting the bacterium for each one of the patients was accomplished using the method prescribed by their physician. For some patients, more than one test was performed. Totally, 8060 experiments were done on these patients. The highest performed diagnostic test was the anti-*H. pylori* IgA test (2539). Other experiments included IgG (2159), SAT (1964), IgM (1257), and UBT (141).

The Results of Helicobacter pylori IgG Antibody Test

The *H. pylori* IgG antibody test was performed on 2159 patients. The mean IgG antibody titer in these patients was 1.938 ±2.179, ranging from 0.1 to 11.5. The test results were positive for 1147 (53.1%) individuals with a mean of 2.282 ±3.218 antibody titer (Table 2). There was no statistically significant association between gender and the level of IgG antibody (*P* value = 0.802, 0.873). However, there was a significant association between the age of the patients and the level of *H. pylori* IgG antibody (*P* value = 0.00). Accordingly, the number of seropositive IgG cases was higher in those patients aged above 30 years old, and the mean IgG level was also higher among the group aged 31-75 years old (Table 2).

Table 1. Demographic Characters of the Study Population

Age Group	Gender		Total
	Male	Female	
Age (y)			
≤5	122	111	233 (4.1%)
6-15	214	279	493 (8.7%)
16-30	379	670	1049 (18.5%)
31-45	760	1073	1833 (32.3%)
46-60	449	896	1345 (23.7%)
61-75	221	394	615 (10.8%)
>75	46	63	109 (1.9%)
Total	2191	3486	5677

The Results of the Helicobacter pylori IgA Antibody Test

The *H. pylori* IgA antibody test was performed on 2539 patients. As indicated in Table 3, the average IgA antibody titer in these patients was 2.088 ± 2.285 ranging within 0.1-12.3. Furthermore, the test result was positive for 2 (0.2%) patients, and the mean antibody titer in these individuals was 12.1 ± 0.282. Further statistical analysis indicated no significant association between sex and IgA level (*P* value = 0.129, 0.793). However, in individuals aged over 31 years old, the number of equivocal cases for IgA antibody test was greater than others, and the IgA level average was also higher in the groups aged 31-75 years old. It means that there was a statistically significant association between age and the level of IgA antibody (*P* value = 0.00).

The Results of the Helicobacter pylori IgM Antibody Test

The results of this test which was performed on 1256 patients are reported in Table 4. According to the obtained data, the average level of IgM antibody was 2.259± 3.954, ranging from 0.1 to 25.7. The test was considered positive for 14 (1.1%) cases with an average antibody titer of 4.862 ± 16.136. Although there was no statistically significant association between gender and the result of the *H. pylori* IgM antibody test (*P* value = 0.827), there was a significant association between gender and IgM level (*P* value = 0.00), and the average level of the *H. pylori* IgM antibody in women was higher compared to men (4.207 to 3.551). Furthermore, there was a significant relationship between the age of the patients and IgM test results (*P* value = 0.00), and the number of positive and equivocal cases was higher in the age group between 30-45 years old. Along with that, the mean IgM level was higher in groups aged 16-30, 6-15, and 31-45 years old (4.258, 4.165, and 4.098), respectively, indicating a significant relationship between age and mean IgM level (*P* = 0.004).

Table 2. *Helicobacter Pylori* IgG Test Results

Criteria		*Helicobacter pylori* IgG Test Results			N
		Negative (>0.80)	Equivocal (0.80–0.89)	Positive (>0.89)	
Gender	Male	368(43.6%)	36(4.3%)	441 (52.2%)	845
	Female	554(42.2%)	54 (4.1%)	706 (53.7%)	1314
Age group	≤5	85	2	11	98
	6-15	171	12	32	215
	16-30	183	19	208	410
	31-45	219	29	424	672
	46-60	175	14	310	499
	61-75	81	11	139	231
	>75	8	3	23	34
Total		922 (42.7%)	90 (4.2%)	1147 (53.1%)	2159

SAT Results

SAT was performed on 1964 subjects. Of the tested patients, 673 (34.2%), including 275 (36.8%) males and 398 (32.7%) females, were positive for *H. pylori* antigens (Table 5). Although, no statistically significant association was found between SAT outcomes and sex (*P* value = 0.133), SAT was related to the age (*P* value = 0.00).

UBT Results

At this stage, the UBT was applied to 141 patients. Of the total number of the investigated subjects for this test, 40 (28.36%) cases were positive with the mean age of 38.15 ± 12.28 years old.

Helicobacter pylori Infection Rate

Among the three methods used in this study, the most positive cases of *H. pylori* infection were identified by IgG, followed by SAT. The overall rate of *H. pylori* infection was 31.46%. The *H. pylori* infection rate was higher in the age group between 31-45 years old (37.92%), followed by 46-

Table 3. *Helicobacter pylori IgA* Test Results

Criteria		*Helicobacter pylori* IgA Test Results			N
		Negative (>0.90 >)	Equivocal (0.90–11.0)	Positive (>11.0)	
Gender	Male	445(46.8%)	516(53.1%)	1(0.1%)	962
	Female	715(45.6%)	851(24.4%)	1(0.1%)	1576
Age group	≤5	83	11	-	94
	6-15	186	32	-	218
	16-30	267	210	1	478
	31-45	276	489	1	766
	46-60	215	400	-	615
	61-75	114	193	-	307
	>75	29	32	-	61
Total		1170 (46.1%)	1367 (53.8%)	2 (0.1%)	2539

Table 4. *Helicobacter pylori* IgM Test Results

Criteria		*Helicobacter pylori* IgM Test Results			N
		Negative (>0.90)	Equivocal (0.90–11.0)	Positive (>11.0)	
Gender	Male	-	479(99%)	5(1%)	484
	Female	-	763(98.8%)	9(1.2%)	772
Age group	≤5	-	79	-	79
	6-15	-	138	1	139
	16-30	-	231	1	232
	31-45	-	391	7	398
	46-60	-	267	1	268
	61-75	-	115	3	118
	>75	-	21	1	22
Total		0 (0%)	1242 (98.9%)	12 (1.1%)	1256

Table 5. *Helicobacter pylori* SAT Results

Criteria		*Helicobacter pylori* SAT Results			N
		Negative	Equivocal	Positive	
Gender	Male	472 (63.2%)	-	275 (36.8%)	747
	Female	818 (67.2%)	1(0.1%)	398 (32.7%)	1217
Age group	≤5	84	-	6	90
	6-15	170	-	13	183
	16-30	268	-	109	377
	31-45	364	-	270	635
	46-60	274	-	191	465
	61-75	113	-	78	191
	>75	17	-	6	23
Total		1290 (65.7%)	1 (0.1%)	673 (34.3%)	1964

SAT: Stool antigen test.

60 years old (35.61%), 61-75 years (34.47%), 16-30 years (29.36%), age >75 (27.52%), 6-15 years (9.33%), and age ≤5 (6.87%).

Discussion

Helicobacter pylori is a frequent human pathogen with a global prevalence of 44.3% (22). Although, improvements in health conditions and premier human growth indices have generally reduced the *H. pylori* infection outbreak rate in developed nations, its rate is still high in developing countries (23). Accordingly, to attain further achievements in the prevention and treatment results, the infection diagnosis in different regions is very important.

This study attempted to establish the prevalence of *H. pylori* infection amongst those patients who referred to the Fardis central laboratory located in Fardis county, Alborz province, Iran, from September 2020 to October 2021. A total of 5677 participants including 3486 (61.4%) females and 2191 (38.6%) males with ages ranging from 1 to 92 years old were examined using ELISA IgG, IgA, IgM, UBT, and SAT. According to the results, the total prevalence of *H. pylori* infections among those investigated patients was 31.46%, which is in agreement with what was reported in Nepal (26.6%), Uganda (24.3%), Taiwan (29.51%), and France (36.5%) (24-27). Further studies published in all over the world indicated the different rates of *H. pylori* infection such as China with 63.80%, Iran with 64%, and Nigeria with 81.7% prevalence (28-30). These variations in prevalence could be attributed to different factors including geographical area, age, race, socioeconomic status, hygiene levels, life style, diagnostic method, type of study population, and eradication therapy (31,32).

Helicobacter pylori infection results in stimulation of local and systemic antibody responses. Furthermore, in the systemic reaction, an impermanent rise in IgM antibodies is detectable in the early stage of infection. After that, IgG and IgA antibodies will emerge and still remain high until the time the infection is removed. Serological detection of *H. pylori* specific antibodies using serum-based ELISA is the most common non-invasive method for this pathogen diagnosis (33,34).

According to the results of this study, the seropositivity against *H. pylori* using ELISA kit for IgG, IgM, and IgA antibodies was 53.1%, 1.1%, and 0.2%, respectively. Some researchers reported 57.6% and 60.5% rates for IgG (35,36) that are in agreement with our results, while others reported 72.9%, 60%, and 21.2% rates which are inconsistent with our findings (29,37,38). The results of IgA were consistent with an earlier study which was 2.64% (39). However, other studies found 18% and 67.4%, which are inconsistent with our results (40,41). The IgM results were in accordance with those studies, reporting that 2.4%, 5.2%, and 5.81% of the subjects were positive for *H. pylori* (37,42,43). On the other hand, IgM seropositivity was 16.1% and 34.9% in some studies (44,41) that are inconsistent with our findings. The differences observed between various studies may be caused by the nature of the antigens used in the kits, infection stage, and sample size. The obtained results in this study demonstrated a significant association between serum levels of these antibodies and age increase. The IgG and IgA seropositive cases were significantly higher amongst the age group 31-75 years old, and the IgM seropositivity was significantly higher amongst the age groups 16-30, 6-15, and 31-45 years old, respectively. These findings are in agreement with several studies which found a significant effect of age increase on the levels of *H. pylori* seropositive cases (44,45), whilst others indicated contrasting results (38,46). In terms of gender, similar to an earlier study, no significant association was observed between IgG and IgA levels and sex, but positive cases of IgM were significantly higher in women compared to men (42). However, there is a conflict between our results and another study, which indicated higher IgG, IgM, and IgA seroprevalence cases in men compared with women (45).

SAT is a reliable test with high specificity and sensitivity that identify *H. pylori* antigens in stool (47). In this study, the total rate of stool antigen of *H. pylori* infection was 34.27%. This rate is comparable with the work of other researchers that reported 30.4% and 41% prevalence, respectively (36,48). However, some authors indicated 80.5% and 67.4%, which differs from results of the current study (49,50). Overconsumption of antibiotics is the most probable reason for the lower rate of infection amongst Iranian patients. The other causes of these differences might be attributed to the presence of patients with asymptomatic gastrointestinal bleeding in the investigated population and the condition of specimens like unformed or watery stool samples, preservation, and transportation. In this study, stool antigen detection did not indicate any significant association with gender, confirming an earlier report (51). However, it is in contrast with other investigations which indicated that females or males were more infected (50,52). Additionally, this study found a significant association with age that was similar to other published results by other studies (48,51), while contradictory results were reported elsewhere (52).

UBT is the most accurate and convenient non-invasive diagnostic assay for determining the *H. pylori* infection that is based on the *H. pylori* urease activity measurement (53). With respect to UBT results in this study, 28.36% with the mean age of 38.15 ±12.28 years old were diagnosed with *H. pylori* infection. This rate is consistent with a prevalence of 32.3 % from a recent study (54) and is also inconsistent with other investigations by prevalence rates of 17% and 52.2%, respectively (55,56). The history of proton pump inhibitors and antibiotic consumption are expected to influence the UBT test results.

In recent years, a reduction in *H. pylori* prevalence was observed in some parts of the world (57-59). In this study, it was also observed that the prevalence of *H. pylori* infection has significantly reduced in comparison with a previous similar study performed at the same laboratory in 2015 (83.77% vs 31.46%) (60). It appears that the bacterial outbreak reduction may be caused by increasing

the knowledge of people about this bacterium, hygienic condition improvement, as well as the development of appropriate treatment strategies by passing the time. Investigation of the studied methods in this research showed that serological tests (i.e., ELISA) are still the most commonly prescribed tests to detect the specific antibodies. Since antibodies, especially IgG, remain in the serum for a long time even after the bacteria have been suppressed, they do not differentiate between new and previous *H. pylori* infections and can yield false-positive results. Therefore, it is better to use serological tests for initial diagnosis and then confirm the results by another high-specificity test such as SAT.

Conclusions

The highest percentage of *H. pylori* was detected using by ELISA IgG (53.1%) and also the lowest ELISA IgA test (0.2%). There was a statistically significant association between *H. pylori* prevalence and age. No significant difference was found in *H. pylori* rate between the two genders, except in the case of IgM, which was higher in females compared to males. In addition, this study indicated a decline in the *H. pylori* infection prevalence compared to a previous study conducted at the same center. Despite the declining trend observed in this study, the outbreak of *H. pylori* amongst those patients who referred to Fardis laboratory is still high. Therefore, further preventive measures against *H. pylori* infection should be considered worthwhile. Moreover, we recommend conducting further studies with a larger sample size and sufficient time to evaluate the bacterium outbreak more precisely.

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Authors' Contribution

Study Design: MJG, NR. Data Collection: ZY, MS, and PRA. Statistical Analysis: JZ. Data Interpretation: NR, PRA. Manuscript Preparation: NR, PRA. Literature Search: PRA.

Conflict of Interests

The authors declare no conflict of interests.

Ethical Approval

Informed consent was obtained from all participants before the study. The patients' demographic characteristics were recorded in a questionnaire, and their information remained confidential. The Research and Ethics Committee of the Iran University of Medical Sciences approved all aspects of this study.

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