Journal of Medical Bacteriology

Application of a Stool Antigen Test to Evaluate the Prevalence of *Helicobacter pylori* Infection in Qazvin, Iran, and Necessity of Implementing New Therapeutic Strategies to Combat Bacterial Infections

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ARTICLE INFO

Article type:
Research Article

Article history:
Received: 17 Apr 2020
Revised: 22 May 2020
Accepted: 18 May 2021
Published: 06 July 2021

Keywords: *Helicobacter pylori, Stool Antigen Test, Therapeutic strategies*

ABSTRACT

Backgroun: *Helicobacter pylori (H. pylori)* infection is one of the most common infections acquired in the community. Study after study has shown that, *H. pylori* plays a major role in gastritis and gastric ulcer which is result in gastric cancer. Gastrointestinal diseases are major reasons for morbidity and mortality throughout the world. Many epidemiological studies have been done up to now indicated the predominant serious prevalence of *H. pylori.* The aim of this retrospective cross-sectional survey is to assess the incidence of *H. pylori* in Qazvin province, Iran.

Methods: A sero-epidemiological study was carried out in Qazvin, Iran. *H. pylori* was diagnosed using a commercially available stool antigen test (HpSA). The *H. pylori* CARD Test is a rapid immune chromatography assay (ICA) test that uses a monoclonal anti-*H. pylori* antibody on a strip for the detection of *H. pylori* infections in stool specimens.

Results: In the current study the overall infection rate was calculated approximately near to 52% that is remarkable (224 out of 434 patients).

Conclusion: Accordingly, dedicating attention to further studies on the development of new strategies in order to treat and manage this bacterium through methods like Phage therapies, clustered regularly interspaced short palindromic repeats (CRISPR) and focusing on the use of microbiomes are suggested.

- ***Please cite this paper as:*** Rahimi S, Sadeghi H, Gholamzadeh Khoei S, Bakht M. Application of a Stool Antigen Test to Evaluate the Prevalence of *Helicobacter pylori* Infection in Qazvin, Iran, and Necessity of Implementing New Therapeutic Strategies to Combat Bacterial Infections. *J Med Bacteriol.* 2021; **10** (1, 2): pp.53-62.

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Introduction

Understanding of the important role of *H. pylori* infections as a leading factor of gastric ulcer and gastric cancer is from the discovery of these bacteria in 1984 by Marshall and Warren (1). *H. pylori* is a gram-negative microaerophilic spiral shaped bacterium which resides in the human gastric mucosa (2). This microbial agent creates the basis of pathogenesis of numerous gastric diseases including duodenal ulcers, gastric and gastritis, mucosa associated lymphoid tissue (MALT) lymphoma and gastric adenocarcinoma through both, colonizing on mucosa layer and various mechanisms in order to survive in the harsh acidic environment (3). *H. pylori* have different kinds of virulence factors that Causes infections and stomach inflammation (4). Based on various epidemiological studies *H. pylori* has been classified as a class I carcinogen in humans by a working group of the World Health Organization International Agency for Research on Cancer (IARC) (5). The burden of *H. pylori* infections goes beyond the gastrointestinal tract and is related to different complications such as hyperemesis gravidarum (6), coronary heart disease (7), anemia (8), diabetes mellitus (9). There is a correlation between Failure to treatment of *H. pylori* eradication and bacterial resistance and poor patient compliance (10). In the 2017, the prevalence of *H. pylori* infection in developed countries was 25%-50% and 70%-90% in developing countries (11). For all these reasons, in the near future the eradication of *H. pylori* may will become a routine measure in the management of patients. Although the person-to-person transmission is the most way for spreading, oral-oral and fecal-oral passing have also been reported (12). The diagnostic tests for *H. pylori* can be classified into two categories: Invasive and noninvasive tests. The invasive methods consist of histology, culture, and rapid urease test. Direct diagnosis with invasive methods requires an endoscopy and biopsy specimens from antrum and stomach body, Endoscopic and biopsy specimens directly detect active *H. pylori* infection. Although these methods are highly specific and high positive predictive value, on the other hand the cost and discomfort to the patients are very high (13). Noninvasive procedures have been developed to detect *H. pylori* infection that is based on the analysis of samples of breath, blood, or stool (14). A non-invasive test which is widely available, probably is a serologically based test. Serologic testing detects the specific IgG antibody of *H. pylori* in the serum of the patient with current or prior infection. Serology test is a simple, convenient test with relative high sensitivity. The most important limitation of serology test is the inability to distinguish between current and past infections.

antibody may be present in the diseased person for a long time after the organism is eradicated. The urease breath test (UBT) with C or C labeled urea, is a noninvasive test based on the urease activity of the organism. UBT method is able to detects active *H. pylori* infection and is highly sensitive and specific. The UBT requires a high density and active bacteria and it should not be performed until 4 weeks after treatment so that the remaining bacteria can be increased sufficiently to detect (13). All the tests have advantages and disadvantages. The rapid urease test (RUT) is known as a gold standard method for the diagnosing of *H. pylori*, and it is faster and cheaper in comparison with other invasive tests (14). Stool antigen tests (SATs) are non-invasive, simple and inexpensive test for detecting active *H. pylori* infections. This test consists of two versions: enzyme immunoassay and immune chromatography. Eradication of *H. pylori* infection is evaluated by SATs. For this reason this test is so useful before and after *H. pylori* therapy (15). The incidence of *H. pylori* infections is widely different by age, race, geographic area, methods of diagnosis, eradication therapy and socioeconomic status (16). In 2017, World Health Organization (WHO) has published lists of 16 bacteria that have a highest risk of human health. Therefore,

H. pylori is classified as one of the most important pathogens for the research and development of new and effective therapies (17). *Helicobacter pylori* stool antigen (HpSA) is simple, fast and inexpensive analytical process which could be easily achieved at home (just stool sample is needed) and no need to attend laboratory or fasting (18). The prevalence of *H. pylori* infections in the general population of Iran is very high (19). According to many studies have been done to evaluate the accuracy of rapid stool chromatographic immunoassays methods this assay was chosen in this study, as we wanted to diagnose patients in Qazvin, Iran with a simple, non-invasive technique. Accordingly, the aim of this retrospective cross-sectional survey was to show the high presence of *H. pylori* in patients referred to Mehr laboratory, during 20 months (April 2017- January 2019).

Materials and Methods

A sero-epidemiological study was carried out in Qazvin, Iran. *H. pylori* was diagnosed using a commercially available stool antigen test (HpSA). The stool samples were collected from referred patients to the laboratory. Then, stool samples were immediately tested for the presence of *H. pylori* antigen by chromatography method. Exclusion criteria of the stool samples were diarrhea, inadequate amount, and delayed delivery of the samples after collection. The

stool antigen test was done by using the stool antigen test kits (GENERIC ASSAYS, Germany) a direct immunoassay performed according to the manufacturing recommendations. The *H. pylori* CARD Test is a rapid immune chromatography assay (ICA) test that uses a monoclonal anti-*H. pylori* antibody on a strip for the detection of *H. pylori* infections in stool specimens. The test was performed according to the manufacturer's instructions by adding 1ML (approximately 20 drops) of diluent in a test tube. Small portion of stool specimen was added into the sample diluent and mixed well by shaking gently. The Tube test stayed at least 5 minutes for sedimentation. Top side of the liquid were extracted by a pipette and dispensed in a small tube or vial, enough to get a deepness 1cm or less and then the test tip was immersed to the liquid in the tube or vial. Finally, the test result was read 5 minutes after the immersion of the strip. A positive test result was indicated by the appearance of green band in the zone marked C (control line) and a red band in the zone marked T (result line). The sample was considered negative when only one green band appeared across the central window in the zone marked C. If no colored bands appeared or only one band appeared in the T zone, the result was regarded as invalid, and if an inconclusive result was obtained, the test was repeated with a new strip. The study period was April 2017- January 2019. The total study population was 434 patients that chosen from patient who were referred Mehr laboratory in Qazvin, Iran. Correlation between *H. pylori* and host characterizes were done by using chi-square. Data were analyzed and statistical comparisons were carried out through using SPSS 16.0xs.

(Table 3). Statistically significant difference was showed between *H. pylori* and age ($p<0.05$) (Table 2). Also, the highest rates of *H. pylori*

Results

HpSA was positive in 224 (51.6%) (Table 1). The infection was significantly associated with age (Table 2). Additionally, there was not a marginal significance in the relationship between *H. pylori* infection and sex (Table3).

From 434 patients referred to Mehr laboratory, 224 patients had been involved in H. pylori infection. The overall *H. pylori* infection was 51.6% (18.4% in males and 33.2% in females) were seen in two groups of 30 to 40 years old (63.9%) and 20 to 30 years old (63 %) (Table 2).

Table 1. Total Prevalence of *Helicobacter pylori* infection

		Frequency	Percent	Valid Percent	Cumulative Percent
Valid	Positive	224	51.6	51.6	51.6
	Negative	210	48.4	48.4	100.0
	Total	434	100.0	100.0	

Table 2. Prevalence of *Helicobacter pylori* infection according to the patients' age

			Age							Total
			higher than 60 years	1 to 10 years	10 to 20 years	20 to 30 years	30 to 40 years	40 to 50 years	50 to 60 years	
H.pylori	positive	Count	11	13	27	51	62	35	25	224
		% within *H.pylori*	4.9%	5.8%	12.1%	22.8%	27.7%	15.6%	11.2%	100.0%
		% within age	45.8%	20.0%	48.2%	63.0%	63.9%	53.8%	54.3%	51.6%
		% of Total	2.5%	3.0%	6.2%	11.8%	14.3%	8.1%	5.8%	51.6%
	negative	Count	13	52	29	30	35	30	21	210
		% within *H.pylori*	6.2%	24.8%	13.8%	14.3%	16.7%	14.3%	10.0%	100.0%
		% within age	54.2%	80.0%	51.8%	37.0%	36.1%	46.2%	45.7%	48.4%
		% of Total	3.0%	12.0%	6.7%	6.9%	8.1%	6.9%	4.8%	48.4%
Total		Count	24	65	56	81	97	65	46	434
		% within *H.pylori*	5.5%	15.0%	12.9%	18.7%	22.4%	15.0%	10.6%	100.0%
		% within age	100.0%	100.0%	100.0%	100.0%	100.0%	100.0%	100.0 %	100.0%
		% of Total	5.5%	15.0%	12.9%	18.7%	22.4%	15.0%	10.6%	100.0%

Table 3. Prevalence of *Helicobacter pylori* infection according to the patients' sex

Crosstab					
			gender		Total
			Male	female	
H.pylori	positive	Count	80	144	224
		% within *H.pylori*	35.7%	64.3%	100.0%
		% within gender	54.4%	50.2%	51.6%
		% of Total	18.4%	33.2%	51.6%
	negative	Count	67	143	210
		% within *H.pylori*	31.9%	68.1%	100.0%
		% within gender	45.6%	49.8%	48.4%
		% of Total	15.4%	32.9%	48.4%
Total		Count	147	287	434
		% within *H.pylori*	33.9%	66.1%	100.0%
		% within gender	100.0%	100.0%	100.0%
		% of Total	33.9%	66.1%	100.0%

Discussion

The incidence of *H. pylori* has decreased rapidly in Asia. This has been shown in both seroprevalence-based and endoscopy-based surveys. In the present study we found that 51.6%(table 1) of the individuals were positive for *H. pylori* by using the stool antigen test kit (Rapid immune chromatography assay, GENERIC ASSAYS, Germany) among all population, which is parallel with Lankarani survey (20) a Meta analysis study of the Prevalence of *H. Pylori* Infection in Iran. Seroprevalence of *H. pylori* infection in Rural areas in Qazvin was 83.3% higher than urban areas 75.8% (21) and 58.8% by normal endoscopic in Qazvin (22). The rate of infection with *H. pylori* reported in other surveys are very high in the entire population of Iran (23). In Tehran, Iran the rate of *H. pylori* infection was 78% and 82% for men and women, respectively (24). In Rasht, 83.6% (19), and 66.8% in Golestan province. Most of the epidemiological studies in Qazvin province have been conducted by other methods, also there has not yet been a study on the prevalence of *H. pylori* in a private laboratory in Qazvin. *H. pylori* infection is one of the most common health problems in Iran. This is the first report in Qazvin, Iran showing the prevalence of the *H. pylori* by using the stool antigen to evaluate prevalence of this organism. The findings of the current study were comparable to the performance of other methods. In some studies, there is a meaningful relationship between H. pylori with male gender (25). in both Spain and Japan, the prevalence was Higher in boys ($p < 0.01$) (25). It should be noted, in parallel with many other surveys in our country which is implicated that there is no significant difference between the outbreak of *H. pylori* infection and gender (26, 27) also, In the current study there was not a marginal significance in the relationship between *H. pylori* infection and sex(Table 3). On the other side, we observed a significant association between age and *H. pylori* infection prevalence, with the highest infection rate in the age groups of 30-40 and 20-30 (63.9% and 63%, respectively) which decreased to 20% and 43.8% in 1-10 and >60 years, respectively (Table 2). This finding was lower than Nouraie et al study in which *H. pylori* was found in 79.2% and 74.7% of 46-55 and over 56 years individuals, respectively(28). The present study reveals a significant decline in the prevalence of *H. pylori* infection in the studied population. It seems that in parallel with better therapeutic approaches and limitation of bacteria, an improvement in the personal hygiene and living conditions of the Iranian population contribute to lower prevalence of *H. pylori*. The immune chromatographic test is much more cost-effective, cheaper, faster (less than 20minutes), and it does not require any special equipment. in the small laboratories that cannot afford the urea breath test and the ELISA test It is useful method. However, the collection of the stool samples during diarrhea should be avoided, as diarrhea may dilute the *H. pylori* antigen in the fecal, and as a result lowering the sensitivity of the test (29)

Although most studies have shown that stool antigen test is also a precise method for verifying eradication of *H. pylori* 4-8 weeks after treatment. these favorable results in the post treatment setting have not been confirmed in other studies, and further researches are required to describe these discrepancies. The novel monoclonal fecal antigen test is more precise than the polyclonal test both in the pre- and in the post treatment setting, and Allowing a clearer and more reliable distinction between positive and negative outcomes. Finally, the stool antigen test seems to be a cost-effective method in order to diagnosis of *H. pylori* infection in patients(30). One of the most important disadvantage of the serological tests that detect antibodies against *H. pylori* is that this methods are not able to distinguish between active infection and previous exposure to *H. Pylori* (31). For these reasons, the UBT and HpSA tests are the only noninvasive suitable methods for *H. pylori* infection detection, eradication and control (32). One of the most important benefits of HpSA tests are ease of use, quick result times, and reduced cost in comparison with UBT (33). therefore, HpSA tests may be the only noninvasive assay to

detect *H. pylori*. The technical specifications of HpSA methods should be addressed. results would be detectable in few minutes and do not require any complicated laboratory equipment. Lower sensitivities for HpSA assays have occurred in certain circumstances, such as those for patients undergoing proton pump inhibitors or bismuth therapy and for patients with liver cirrhosis or gastrointestinal bleeding (34). Although we eliminated samples related to patients who were undergoing proton pump inhibitor or bismuth therapy, a few patients with hidden gastrointestinal bleeding may have been included in the study, which may have resulted in the false-negative HpSA tests. nevertheless, Low bacterial colonization in the stomach and therefore low concentrations of *H. pylori* antigens in the stool can be sufficient to result in false negative results (35). Positive false reactions may result from other *Helicobacter* species, and this may result in false positive tests(36). In conclusion, The GENERIC ASSAYS *H. pylori* stool antigen test is rapid, easy to use, and does not require expensive equipment, but when only rapid HpSA diagnostic tests are used in the laboratory, it is so important to know the diagnostic accuracies of rapid tests and evaluate the results according to the sensitivities and specificities of these tests. Reliable HpSA tests with high quality results may be useful for small laboratories and for primary care physicians that need to test for *H. pylori* infection. The findings from this study point to the fact that, by spending a lot of expenses on the production of various antibiotics to counteract pathogenic bacteria, it can be easily seen that the level of pathogen bacteria is still high. Evidences and the results of this study in a private and non-hospital laboratory indicate the ineffectiveness of the treatment by antibiotics, and using of promising methods such as microbiota, modern molecular techniques such as CRISPR and using of the beneficial aspects of phages should be considered and put them into practice.

Treatment against *H. pylori* is based on the use of antibiotics, but the treatment failure can account for more than 20% and is essentially due to an increase in the prevalence of antibiotic-resistant bacteria, which has led to the search for alternative therapies. phytotherapy and probiotics are new suggestions for treatment. Probiotics are live organisms or produced substances that are orally used, usually in addition to conventional antibiotic therapy. They may modulate the human microbiota and promote health, stimulate the immune response, prevent antibiotic side effects, and directly compete with pathogenic bacteria. Phytomedicine Includes the use of herbal extracts as drugs or health enhancing agents, but in most cases the molecular mode of action of the active ingredients of these herbal extracts is unknown (37).

Recent research suggests that probiotics modulate *H. pylori* colonization of the gastric epithelial cells. It is increasingly recognized that changes in the microflora of the intestine play an important role in development of complication and patient's intolerance during anti-*H. pylori* treatment. Probiotic supplement can reduce the adverse effects of antibiotics and as a result, maximize the success of the treatment (38). combining first line anti *H. pylori* therapy with probiotic species, composed of *Bacillus subtilis* and *streptococcus faecium* reduced side effects, improved patient's tolerance and enhanced the eradication rate of *H. pylori* (39). A vaccine against *H. pylori* would solve the problem of pathogenic strains and work as a prophylactic (40). There is no available vaccine yet, but several are being developed (41). study after study has shown that Phage therapy may be able to treat and eradicate *H. pylori*. Bacteriophage therapy shows potential as an alternative to antibiotics but it will take some time to verify whether it is feasible as it requires phage isolation and characterization (37). many studies have shown that Honey and propolis, garlic, red win, Cranberry and Antioxidants also successfully have anti-*helicobacter pylori* activities (42).

Conclusion

In conclusion, The GENERIC ASSAYS *H. pylori* stool antigen test is rapid, easy to use, and does not require expensive equipment, but when only rapid HpSA diagnostic tests are used in the laboratory, it is so important to know the diagnostic accuracies of rapid tests and evaluate the results according to the sensitivities and specificities of these tests.

Funding information

This research did not receive any specific grant from funding agencies in the public, commercial, or not-for-profit sectors**.**

Ethics approval and consent to participate

Not needed.

Conflict of interest

The authors declare no competing financial interest.

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