Arch Iran Med. October 2020;23(10):665-671

ARCHIVES OF IRANIAN MEDICINE

doi 10.34172/aim.2020.84

http://www.aimjournal.ir

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Original Article

An Update of *Helicobacter pylori* Prevalence and Associated Risk Factors in Southern Iran: A Population-Based Study

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Abstract

Background: The childhood period is considered to be the primary period for acquisition of the *Helicobacter pylori*. The high prevalence rates from developing countries are associated with gastric cancer. A decreasing trend of its prevalence has been reported from different parts of the world. Determining the prevalence rate could be important in choosing preventive strategies. This study aimed to determine the prevalence of *H. pylori* among a group of children from southern Iran to provide an update on the current status of the disease.

Methods: This is a cross-sectional population-based study conducted in Shiraz, southern Iran, from January 2014 to December 2015. Four groups including neonates, children aged 6 months to 3 years, 10- and 15-year-old children were included. Multi-monoclonal stool antibody test was used for diagnosis.

Results: Among 436 participants, 24.8% (95% CI: 20.8–29.1) had a positive test for *H. pylori*: 25% in neonates (95% CI: 16.2–36.1), 22% in children aged 6 months to 3 years (95% CI: 15.2–30.2), 19.5% in the 10-year-old (95% CI: 12.3–29.4), and 29.2% in 15-year-old children (95% CI: 21–39). Sex, age, number of siblings, owning a pet, parents' smoking status, parental education, residential area, birth weight, and feeding status were not found to be statistically significant predictors of *H. pylori* antigen positivity ($P>0.05$).

Conclusion: The prevalence of *H. pylori* was estimated to be low in southern Iran in comparison with previous reports or other developing countries. Preventive strategies with respect to low prevalence rates may be considered in the childhood period.

Keywords: *Helicobacter pylori*, Iran, Monoclonal antibodies, Prevalence

Cite this article as: Ziyaee F, Alborzi A, Pouladfar G, Pourabbas B, Asaee S, Roosta S. An update of *Helicobacter pylori* prevalence and associated risk factors in southern Iran: a population-based study. Arch Iran Med. 2020;23(10):665–671. doi: 10.34172/aim.2020.84.

Received: March 2, 2019, Accepted: April 13, 2020, ePublished: October 1, 2020

Introduction

It is estimated that more than 50% of the global population is infected with *Helicobacter pylori*.[1] The prevalence is higher in developing compared with developed countries.[2] The highest prevalence rate is in South America and Asia.[3] Also, high prevalence rates of *H. pylori* infection have been reported from different parts of Iran. In Shiraz, southern Iran, in 2006, the prevalence rate ranged 57% to 98% in children from different age groups according to the stool antigen test (SAT).[4] In another study in Rasht, northern Iran, in 2007, the prevalence of infection in high school children was reported to be 40% using the SAT.[5]

The bacterium, although mostly asymptomatic, is associated with some upper gastrointestinal and extra-intestinal diseases including lymphoma of the mucosa, chronic gastritis, peptic ulcers, iron deficiency anemia, vitamin B12 deficiency and most importantly, gastric carcinoma.[6,7] Among risk factors of the coronary artery diseases, hyperlipidemia is associated with *H. pylori*.[8] Specifically in the Iranian population, gastric cancer has been strongly associated with diet and *H. pylori* infection.[9]

The childhood period is considered to be the primary period for acquisition of *H. pylori* infection.[9,10] It has been suggested that screening for *H. pylori* infection and precancerous lesions may serve as a preventive strategy against the development of gastric adenocarcinoma.[11] A few guidelines on *H. pylori* infection from around the world indicate that eradication of *H. pylori* would result in reduction of the incidence of gastroduodenal diseases, including gastric cancer, and would decrease new infections in future generations.[12-14] Based on these guidelines, Japan health insurance systems approved the coverage of the diagnosis and eradication of *H. pylori* in all infected patients.[12,15] In order to develop a preventive strategy, evaluation of *H. pylori* prevalence is important.

SATs are noninvasive and inexpensive diagnostic tests

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for *H. pylori* infection.[12] Early SATs used an enzyme immunoassay (EIA), based on polyclonal antibodies. While they provided reliable results for the diagnosis of *H. pylori* infection, controversial results were sometimes observed in post-eradication assessment due to false positives.[16,17]

Monoclonal antibody-based techniques generally have higher specificity. SATs based on monoclonal antibodies have been developed and found to be more accurate than those using polyclonal antibodies.[18] A meta-analysis also showed that the specificity of SATs based on monoclonal antibodies was 0.97 (95% CI: 0.96-0.98).[19] Monoclonal antibody-based enzyme-linked immunosorbent assays (ELISA) have provided a non-invasive tool for the evaluation of bacterial colonization in the pediatric population with sensitivity and specificity of more than 85% and 90%, respectively, compared with UBT, culture or histology.[20-22]

In this study, we evaluated the prevalence of *H. pylori* and its associated factors among a group of children from southern Iran, in order to determine changes in the infection trend and to provide an update on the current condition of the disease. Our study also included neonates which provided a more comprehensive assessment of the pediatric population. In addition, we used multiple monoclonal assays, which have high accuracy.

Regarding the worldwide epidemiology of *H. pylori* in the pediatric population, infection rates vary from 4.9%[23,24] to 73.3% in a group of children from Kenya.[25]

A study in 2013 found that among 516 children and young adults (12–25 years old) in Belgium, the prevalence of *H. pylori* using UBT was 11% (3%–60%) and the most important factor affecting the prevalence was geographical distribution or place of residence.[26]

In 2015, Krueger et al used the augmented data from the National Health and Nutrition Examination Survey (NHANES) from the United States to evaluate the association between a positive serum for *H. pylori* (positive IgG) and different factors. In their multivariate analysis, they found that among participants aged 3 to 19 years, increased age, race, crowded housing (persons per room) and lower income were associated with higher rates of *H. pylori*.[27]

Materials and Methods

Study Protocol

This is a cross-sectional population-based study conducted in Shiraz, Iran, during a 2-year period from January 2014 to December 2015. Data was collected from four different age groups including neonates (younger than 28 days) who visited a phototherapy center and Well Baby unit, children aged 6 months to 3 years who attended kindergartens, 10- and 15-year-old children attending primary schools and high schools, respectively. Children with any gastrointestinal symptoms including diarrhea, abdominal pain, nausea, vomiting and those who used any antibiotics during the two weeks prior to sampling were excluded from the study.

Data Collection

Shiraz is one of the five largest cities in southern Iran with a population of about 1.5 million. The city is divided in 10 regions. For the first age group (neonates), samples were collected from Hafez hospital affiliated to the Shiraz University of Medical Sciences. In Hafez hospital, women of different socioeconomic status are admitted for delivery. The neonates were selected using simple random sampling from apparently healthy ones. For the rest of the study population, data was collected from all regions to provide heterogeneity in terms of the participants' socio-economic status. Samples were obtained from all ten regions of Shiraz using simple random sampling. A questionnaire was used to obtain demographic data from each individual.

Questions were classified into three parts, namely those related to the child, parents, and region of residence. In the section related to the child, data was gathered on age, sex, birth weight, type of feeding in the neonatal group (breast feeding or formula feeding), route of delivery in the neonatal group (natural vaginal delivery or caesarean section), present or past gastrointestinal symptoms, and antibiotic use in the previous two weeks. In the section related to the parents, data was gathered on parental education, number of children, smoking habits (number of cigarettes per day or using water-pipe), and owning a pet.

Parents were asked to collect stool samples from their children at home and keep it at 2–8 degrees centigrade for a maximum of 72 hours and to bring it to the preselected coordinated centers as soon as possible. Stool samples were transferred daily to the referral lab at Professor Alborzi Clinical Microbiology Research Center, Shiraz, Iran. The samples were then frozen at -70°C.

To diagnose *H. pylori* in the stool samples, we used a laboratory-based monoclonal stool antibody test, the Premier Platinum HpSA Plus (HpSA ELISA II; Meridian Diagnostics, Inc., Cincinnati, OH, United States), which uses multiple murine monoclonal antibodies. Testing was done according to the manufacturer's guidelines. The cut-off values for the HpSA EIA defined by optical density at 450 nm and 450/630 nm, were 0.140, and 0.1, respectively. This test has a sensitivity of 96.1% and specificity of 95.7%. Positive and negative predictive values for this test are 96%.[28]

Definition of Variables

The regions from which the samples were randomly selected included ten regions from Shiraz. Each region differed from the others in terms of socio-economic conditions.

Educational status was classified as illiterate, high school education (diploma) and lower, and university education. Low birth weight in the neonatal group was defined as birth weight under 2500 g. Exclusive breast feeding in the neonatal group was defined as no episode of formula feeding.

Statistical Analysis

A minimum sample size of 96 children was calculated considering 60% prevalence, 10% precision and 95% confidence interval. Data analysis was done using the SPSS® for Windows®, version 20 (SPSS Inc., Chicago, IL, USA).

The association between each qualitative variable and *H. pylori* antigen status was determined using the chi-square and the Fisher's exact tests. A multiple logistic regression analysis was used to evaluate the simultaneous association of variables with *H. pylori* antigen status. Due to the large number of variables for the regression analysis, only those variables with a P value of less than 0.2 in the bivariate comparison between the antigen positive and the antigen negative groups were included in the logistic model with enter method. In the enter method, all the input variables are entered simultaneously. Data are presented as frequencies and percentages, where appropriate. A P value < 0.05 was considered as statistically significant.

Results

Overall, 436 participants entered the study, of whom 24.8% (95% CI: 20.8–29.1) tested positive for *H. pylori* in their stool samples. Eighty children were included in the neonatal group, 128 children in the 6 month–3 year old group, 92 in the 10-year-old group and 106 in the 15-year-old group. Evaluating each age group separately showed that 25% of the neonates (95% CI: 16.2–36.1), 22% of the children aged 6 months to 3 years (95% CI: 15.2–30.2), 19.5% of those aged 10 years (95% CI: 12.3–29.4) and 29.2% of those aged 15 years (95% CI: 21–39), had a positive antigen for *H. pylori*.

None of the variables including sex (P = 0.980), age (P = 0.105), region of residence (P = 0.153), number of siblings (P = 0.275), parental education (neither mother's education (P = 0.135) nor father's education (P = 0.513)), owning a pet (P = 0.189), parents' smoking status (P = 0.843), birth weight (P = 0.443) and feeding status (P = 0.981) were statistically different between the antigen positive and the antigen negative groups (Table 1).

Multiple logistic regression analysis demonstrated that age (P = 0.226), region of residence (P = 0.353), owning a pet (P = 0.839) and mother's education (P = 0.292) were not significant predictors of *H. pylori* antigen positivity (Table 2). The total sample size and the number of cases (people with a positive test) included in analysis for the model presented in Table 2 were 319 and 77, respectively. According to Concato et al[29] and Peduzzi et al,[30] the minimum sample size based on the rule of event-per-variable of 10 is acceptable for logistic regression. In this study, there were 16 parameters (including the intercept) to be estimated in the logistic regression model presented in Table 2; so, at least 160 cases were required but there were only 107 cases with many missing values for variables according to Table 1. This lack of data may lead to selection bias and hence, reduced statistical power and precision for modeling. Also, sparse data were evident for some variables in Table 1 including region included in the model (Table 2) and some confidence limits were unrealistically large suggesting "sparse-data bias".[31]

Discussion

Here, we have studied the prevalence and associated factors of *H. pylori* using multiple monoclonal stool antibodies among different age groups of pediatric participants in southern Iran. We found that the overall prevalence of *H. pylori* was 24.8%, which was the highest among 15-year-old children (29.2%). The most interesting findings in our study is probably the significant decrease in *H. pylori* prevalence compared with that reported from Shiraz 10 years ago according to a population-based study which showed an overall prevalence of 82%.[4] This shows improvements in diagnostic modalities (decreasing false positive rates), treatment and socio-economic status, especially among the pediatric population.

The number of siblings, parental education and region of residence, as parameters of socioeconomic status, were not associated with a positive antigen test. The prevalence rate in different parts of Shiraz varied from 13.5% in region 1 to 52.6% in region 8, which correlates with socioeconomic condition of these regions. For example, region 1 has moderate to high socioeconomic status but region 8 has low socioeconomic status. Although region 8, with the highest population density among all the ten regions, also had the highest rate of *H. pylori* prevalence compared with the other nine regions in our study, the impact of region of residence was not statistically significant. Our study included a wide range of geographical areas in Shiraz which provided strong heterogeneity in terms of socio-economic status and distribution of baseline characteristics across the comparison groups.

In the neonatal group, we also studied the association of birth weight, feeding status (breast-fed or formula-fed), and route of delivery (natural vaginal delivery or caesarean section) but they were not related to higher prevalence of *H. pylori*. Only few studies have reported the prevalence of *H. pylori* in neonates. In an Italian study from 2002 to 2005, formula feeding and admission to the NICU was associated with positive SAT in neonates.[32] In another study in Norway in 2006, route of delivery was an important predictor of *H. pylori* antigen positivity and 59% of the neonates born through natural vaginal delivery had positive SAT compared with 10% of the neonates who were born through caesarean section.[33]

Table 1. Baseline Characteristics of Participants and Comparison of Antigen-positive and Negative Individuals

Variables	Antigen				P value
	Positive		Negative	Total	
	No. (%)	95% CI*	No. (%)		
Sex					
Male	55 (51.4)	42.0–60.6	167 (51.5)	222	0.980
Female	52 (48.6)	39.3–57.9	157 (48.5)	209	
Total	107	—	324	431	
Age					
1-28 day	20 (20.6)	13.8–29.7	60 (19.4)	80	0.105
6 m/o-3 y/o	28 (28.9)	20.8–38.6	100(32.4)	128	
10 y/o	18 (18.5)	12.1–27.4	74 (23.9)	92	
15 y/o	31 (32.0)	23.5–41.8	75 (24.3)	106	
Total	97	—	309	406	
Region# (80 samples collected from neonates of Hafez hospital are not included)					
1	5 (6.5)	2.8–14.3	32 (13.2)	37	0.153
2	4 (5.2)	2.0–12.6	16 (6.6)	20	
3	8 (10.4)	5.4–19.2	27 (11.2)	35	
4	8 (10.4)	5.4–19.2	23 (9.5)	31	
5	12 (15.6)	9.1–25.3	38 (15.7)	50	
6	11 (14.3)	8.2–23.8	34 (14.0)	45	
7	4 (5.2)	2.0–12.6	22 (9.1)	26	
8	10 (13.0)	7.2–22.3	9 (3.7)	19	
9	4 (5.2)	2.0–12.6	16 (6.6)	20	
10	11 (14.3)	8.2–23.8	25 (10.3)	36	
Total	77	—	242	319	
Number of siblings					
None	31 (32.3)	23.8–42.2	109 (35.5)	140	0.275
One	34 (35.4)	26.6–45.4	128 (41.7)	162	
More than one	31 (32.3)	23.8–41.2	70 (22.8)	101	
Total	96	—	307	403	
Mother's education					
Illiterate	11(11.4)	6.5–19.4	17(5.5)	28	0.135
High school and lower	57 (59.4)	49.4–68.7	195 (63.3)	252	
University education	28(29.2)	21.0–38.9	96 (31.2)	124	
Total	96	—	308	404	
Father's education					
Illiterate	5 (5.2)	2.2–11.6	11(3.6)	16	0.513
High school and lower	65(67.7)	57.8–76.2	197 (64.0)	262	
University education	26(27.1)	19.2–36.7	100 (32.5)	126	
Total	96	—	308	404	
Owning a pet					
Yes	17 (17.5)	11.2–26.3	38 (12.3)	55	0.189
No	80 (82.5)	73.7–88.8	271 (87.7)	351	
Total	97	—	309	406	
Smoking Parent					
Yes	21 (21.6)	14.6–30.8	64 (20.7)	85	0.843
No	76 (78.4)	69.2–85.4	245 (79.3)	321	
Total	97	—	309	406	
Low birth weight					
Yes	2 (12.5)	3.5–36.0	12 (21.1)	14	0.443
No	14 (87.5)	64.0–96.5	45 (78.9)	59	
Total	16	—	57	73	
Exclusive breast-feeding					
Yes	14 (87.5)	51.2–88.2	50 (87.7)	64	0.981
No	2 (12.5)	2.9–31.4	7 (12.3)	9	
Total	16	—	57	73	

y/o, years old; m/o, months old.

*95% confidence interval for *H. pylori* antigen positivity. #Shiraz (one of the five largest cities in Iran) is divided in 10 regions (in terms of socio-economic conditions) and samples were obtained from all 10 regions using simple random sampling. * Chi-square test. ** Fisher›s exact test.

Table 2. Multiple Logistic Regression Analysis for Acquiring a Positive Antigen Test for *H. pylori*

Variables	OR	95% CI for OR	*P* Value
Age			0.505
1–28 day	0.93	0.51–1.82	0.671
6 m/o–3 y/o	0.86	0.42–1.76	0.684
10 y/o	0.56	0.26–1.19	0.135
15 y/o	Reference	—	—
Region*			0.337
1	0.32	0.09–1.10	0.072
2	0.56	0.14–2.17	0.410
3	0.70	0.23–2.15	0.538
4	0.82	0.27–2.45	0.723
5	0.72	0.26–1.96	0.528
6	0.67	0.24–1.88	0.453
7	0.45	0.12–1.66	0.233
8	2.69	0.68–10.68	0.157
9	0.69	0.18–2.66	0.597
10	Reference	—	—
Owning a pet			
Yes	1.15	0.54–2.46	0.701
No	Reference	—	—
Mother's education			0.625
Illiterate	1.34	0.26–6.75	0.718
High school and lower	0.79	0.40–1.52	0.484
University education	Reference	—	—

y/o, years old; m/o, months old.
*Shiraz (one of the five largest cities in Iran) is divided in 10 regions (in terms of socio-economic conditions) and samples were obtained from all 10 regions using simple random sampling.

However, in our study, 23% of the neonates born through natural vaginal delivery had positive SAT compared with 26% of the neonates who were born through caesarean section. *Candida albicans* is likely a carrier for *H. pylori* and colonization of the vagina with *Candida albicans* is one of the transmission routes of *H. pylori* from mothers to neonates.[34]

In one of the most recent studies in 2007 in northern Iran, 961 children between the ages of 7 to 11 years were evaluated for *H. pylori* SAT using a polyclonal assay. The prevalence of *H. pylori* was found to be 40% and 43.6% in the total population and children aged 10–11 years, respectively.[5] Similar to our study, the number of family members and parents' education were found to be insignificantly associated with *H. pylori* prevalence. We found the prevalence rate of *H. pylori* to be lower among 10-year-old children (19.5% compared with 43.6% in the former study). This could be attributed to multiple factors including the diagnostic kits which were used in the studies, as we used multiple monoclonal based kits which are more accurate than the polyclonal kit which were used in the mentioned study.

In another study which used a polyclonal antibody-based assay in the same city where the current study has been conducted, 593 children from five different age groups were studied during 2006. They found that none of the age groups, including those aged 8–10 months, 2–3 years, 5–6 years and 9–10 years, showed a statistically significant difference regarding *H. pylori* prevalence (83%, 98%, 88% and 89%, respectively); however, they did find the group aged 14–15 years to have a significantly lower prevalence of *H. pylori* (58%).[4] Dissimilarly in this study, none of the age groups showed a significant difference regarding *H. pylori* status.

The diversity in *H. pylori* prevalence recorded in our age groups could be due to the auto-curability and the re-infection phenomena, as some studies have documented annual auto-curability rates to be as high as 5.5% among the pediatric population.[35]

Similar to previous studies from Iran, we did not find an association between *H. pylori* and sex, age, parents' smoking status and some socio-economic factors including: number of siblings and parental education. Birth weight and type of feeding in the neonatal group were also insignificant predictors of *H. pylori* stool antigen positivity. Compared with other countries, especially those with higher socio-economic status, we did have higher prevalence rates of *H. pylori*.

A similar decrease has been recorded in other studies, as well. Bures et al found a significant decrease in *H. pylori* prevalence during a ten-year period from 2001 to 2011 (41.7% vs. 23.5%) in the Czech Republic.[36] After an appropriate socio-economic indexing score is introduced, a more comprehensive understanding of the association between *H. pylori* and socio-economic status will be understood. We propose larger studies in the context of cohort studies that consider multiple socio-economic factors to evaluate the association between these factors and the prevalence of *H. pylori*.

Our study had some strong points and some limitations. One issue with different studies relates to the screening method used for the detection of *H. pylori*. Unlike our study in which we used a multiple monoclonal antibody-based stool ELISA test for the diagnosis of *H. pylori*, many of the previous studies in the region, as stated before, have used polyclonal antibody-based stool EIA tests. Polyclonal antibody-based stool EIA's have lower specificity than monoclonal antibody-based stool EIA tests[13] which have a diagnostic accuracy equivalent to that of the UBT which is considered the best test for the diagnosis of *H. pylori* among non-invasive diagnostic tests.[37] We did not have a standard index for the evaluation of socio-economic status and different factors related to socio-economic status were separately assessed. Perhaps after a suitable indexing score has been introduced, evaluation of socio-economic status may show a more significant impact on *H. pylori* prevalence. Also, considering the fact that we excluded children who had any gastrointestinal symptoms and those who used any antibiotics during the two weeks prior to sampling, the prevalence of *H. pylori* infection may be underestimated because of incidence-prevalence bias. In addition, we were not able to determine the time of acquisition of infection. It may blur etiologic associations (for example, between *H. pylori* infection and low birth weight) because of reverse causality bias. Also, missing data in variables is another limitation of the study which may result in selection bias.

Our study showed that the prevalence of *H. pylori* among the pediatric population in southern Iran had a significant decrease from a decade ago and in some regions, the prevalence is even similar to developed countries. Regarding the difference in prevalence, perhaps the effect of different variables such as age that have been shown to be associated with *H. pylori* in other regions of the world, is highly dependent on geographical area and plays an insignificant role in the prevalence of *H. pylori* among Iranian children.

In developing countries, the preferred method for *H. pylori* management would be prophylactic vaccination.[38] In 2016, an oral vaccine against the bacterium was used in China for children; after one year of follow-up, it was 71% effective.[39] The decrease in the rate of *H. pylori* prevalence in Iran shows that from now on, it would be cost-effective to use a vaccination protocol to prevent the disease and especially its side effects, including gastric cancer.

Authors' Contribution

PG, PB and MR: Study concept and design. ZF: Data collection. AS: Material preparation. ZF, PG, PB and RS: Acquisition, analysis, or interpretation of data. RS: Statistical analysis. ZF and PG: Drafting of the manuscript. AA and MR: Critical revision of the manuscript for important intellectual content. All authors commented on previous versions of the manuscript. All authors read and approved the final manuscript.

Conflict of Interest Disclosures

The authors have no conflict of interest to declare regarding the manuscript.

Ethical Statement

The study protocol was approved by the institutional review board of both Shiraz University of Medical Sciences and the Professor Alborzi Clinical Microbiology Research Center. The study was in accordance with the declaration of Helsinki guidelines. All parents gave their written consent to enter the study.

Acknowledgements

The authors would like to thank Shiraz University of Medical Sciences for their financial support of the study (grant #8675), Dr. Nasrin Shokrpour for editorial assistance at the Center for Development of Clinical Research of Nemazee Hospital. This study was extracted from the thesis presented for obtaining the specialty of pediatrics degree by Fateme Ziyaee, MD.

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