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Original Article

Prevalence of Helicobacter Pylori Infection in Asymptomatic Children in Birjand, Eastern Iran

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Abstract

Introduction

Helicobacter pylori (*H. pylori)* is the cause of serious diseases including gastric cancer and gastric mucosa–associated lymphoid tissue lymphoma. About 50% of world population is infected by this microorganism and it based on epidemiologic studies, is mainly acquired during childhood . There is not enough evidence about prevalence of this infection in children and its risk factors so encourage us to study on it.

Methods and Materials

This cross-sectional study was conducted among 282 primary school students in Birjand, Iran. Samples were randomly selected. The stool assay was performed using the Helicobacter pylori Stool Antigen by Enzyme- immunoassay (EIA) test kit (ACON company). Data were analyzed at through SPSS version 21.

Results

91 boys and 191 girls, aged 9 to 12 years were evaluated. The prevalence of *H. pylori* colonization in 282 students was 13.1%. We found statistically significant relationship between *H. pylori* colonization and gender, duration of breast feeding, and family crowding ($P<0.05$); but there was not significant relationship with age , family history of dyspepsia, number of days in week consuming yogurt and economically stratified living region in present study ($P<0.05$).

Conclusion

Helicobacter Pylori is a big concern even in young asymptomatic children and it needs to be further studied about its potential risk factors and how to manage them for the goal of prevention.

Key words: Asymptomatic, Helicobacter Pylori, H. pylori Stool Antigen, Prevalence .

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Introduction

Helicobacter pylori, a Gram-negative bacterium that colonizes human gastric mucosa, was first introduced in 1984 in biopsy of chronic antral gastritis and peptic ulcer disease (1). It has been shown to be associated with serious diseases such as gastric cancer and gastric mucosa–associated lymphoid tissue lymphoma. Globally, it remains one of the most common infections, as an estimated 50% of the world's population are carriers of this organism (1,2). The mode of transmission for *H. pylori* is not absolutely known; however, epidemiologic studies strongly support person-to-person transmission and fecal-oral or oral-oral routes are the most likely (1).

It is known that the infection is mainly acquired during childhood, but the specific age of acquisition and the factors associated with its persistence are unknown (3). Infected mother and siblings is the most common familial source of this bacterium(4). Helicobacter pylori infection in children is usually acquired during the first 10 years of life in developing countries (3). In these countries, >80% of adults are colonized with *H. pylori*, and >50% of children are infected at 10 years of age in comparison with 30% of adults and 10% of children in developed countries (3,5). Comparison of infection prevalence of various age in developing countries shows that infection is minimal in children aged 7 to 8 years (36.84%) and reaches maximum levels in students aged 14 years (66.67%) (6). Screening result shows that there are 2 waves of *H. pylori* infection (the first peak was detected in 11 years, the second in 14 years). US data, as a developed nation, indicate that black children aged 5 to 9 years have an overall infection frequency of 30%. Around the world, infection among children ranges from approximately 35% in Russia, 20% in China and Poland, 12% in Korea and America to <10% in France, Belgium and Finland (7).

In our review of literature there are two studies about prevalence of *H. pylori* infection in pediatrics of Iran that showed high *H. pylori* infection prevalence (47% in center and 64.3% in west of Iran)(8,9).

There are invasive and noninvasive methods for diagnosis of infection. Invasive methods include endoscopy and biopsy for histology, culture and rapid urease test analysis. Noninvasive methods are serology, urea breath test and stool antigen analysis. The gold standard test for detection of *H. pylori* remains histology from gastric biopsy (1).

Serology-based evaluation of *H. pylori* status is limited because of a 30% false-positive rate, as immunoglobulin G testing reveals both previous (treated) and present infections (1). To avoid detection of previous *H. pylori* infection, the Urea breath test (UBT) (highly accurate for the diagnosis of *H. pylori* infection in children older than 6 years)(3) and stool antigen testing are useful(1). These tests are more accurate during childhood (4,10-12) but urea breath test has a relatively high cost and requires trained staff and well equipped laboratory instruments despite of its high sensitivity and specificity. The new, noninvasive, low-cost *H. pylori* antigen test in stool can replace UBT for detection of *H. pylori* infection in children with comparable reliability and accuracy (13).

The sensitivity, specificity, and positive and negative predictive values of Helicobacter pylori stool antigen test (HpSA) were found to be respectively 98%, 100%, 100%, and

96.5% (14). There are 2 methods for detecting antigen: based on monoclonal and polyclonal antibody; in polyclonal antibody method, it is possible that cross reactivity of the HpSA with nonviable or coccoid forms of the Helicobacter pylori (Hp*)* bacterium cause false-positive results (15).

Comparisons between these two methods in children have shown that the monoclonal antibody has higher sensitivity than the polyclonal antibody(98% *vs.* 93.8%) and replace it (4,16). This test seems suitable to monitor the success of anti- *H. pylori* therapy and screening of asymptomatic subjects (17,18); also the test was approved by the United States Food and Drug Administration (FDA) as a pre-endoscopic diagnostic test for *H. pylori* infection in adults. Newly there is a new generation of rapid monoclonal antibody based HpSA test that work with lateral flow immunochromatography technique in 5 minutes. It is a convenient office-based method for detection of *H. pylori* antigen in stool specimens. The diagnostic accuracy of this test was as high as that of HpSA immunoassay (HpSA ELISA) in children (19). Some important risk factors for transmission of *H. pylori* include: age , race , living in rural area , overcrowding , Socioeconomic status(SES) , poor sanitary conditions , mothers with lower educational level , poor diet and poor water supply (1-3,7,10,20). Even in populations in the same country, low SES is associated with infection acquisition(2); there is no association between intestinal parasitosis and *H. pylori* infection but Giardia was closely associated with it (21). Some other associations are iron deficiency anemia, diarrheic disease, and impairment of growth, weight, and cognitive functions (4). In attention of little evidence about prevalence of *H. pylori* in children and importance of this issue due to its potential risk for severe gastrointestinal complications, this study was designed. The aim of this study was to evaluate the prevalence of *H. pylori* infection and its associated risk factors in school aged children in East of Iran, Birjand.

Methods and Materials

This was a population based cross sectional study in 9 to12 year- old children lived in Birjand, the capital of Southern Khorasan Province. Just being at 9 to12 year- old child who study in Birjand was the inclusion criteria, and exclusion criterias for this study were: dissatisfaction in cooperation , significant gastrointestinal sign and symptom, antibiotic or antacid usage Proton pump inhibitors (PPI) or H2 blocker and diarrhea at the time of sampling. The study was reviewed and authorized by the Ethics and Investigation Committees of the Birjand University of Medical Science. The parents signed an informed consent form authorizing their children's participation.

Sample size of this study was 282 persons that calculate with comparison of proportions formula according to the prevalence that achieve from Dr. Soltani et al. study (9). Sampling was done in a randomized cluster manner from 12 primary schools (4 male and 8 female primary schools). Schools were selected from 4 economic regions in Birjand . After explaining of study, completion of a written consent by student`s parents and verbal consent by students, and dismissing of exclusions, the participants fill a questionnaire of some demographic data and stool samples was obtained and analysed with *H. pylori* antigen EIA test kits (ACON company). This was a solid phase enzyme qualitative and quantitative detection of *H. pylori* antigen in human stool. The

microwell plate is coated with anti-*H. pylori* antibodies. During testing, the antigens are extracted out with extraction solution and added to the antibodies coated microwell plate with the enzyme conjugated antibodies to *H. pylori* and then incubated. If the specimens contain *H. pylori* antigens, it will bind to the antibodies coated on the microwell plate and simultaneously bind to the conjugate to form immobilized antibody-*H. pylori* antigen-conjugate complexes.

If the specimens do not contain *H. pylori* antigens, the complexes will not be formed. After initial incubation, the microwell plate is washed to remove unbound materials. Substrate A and substrate B are added and then incubated to produce a blue color indicating the amount of *H. pylori* antigens present in the specimens. Sulfuric acid solution is added to the microwell plate to stop the reaction producing a color change from blue to yellow. The color intensity, which corresponds to the amount of *H. pylori* antigens present in the specimens, is measured with a microplate reader at 450/630-700 nm or 450 nm. The sensitivity and specificity of this kit have been obtained 98.6% and 95.4% in a previous survey(according to the data in brochure of kit). In each run of testing 65-70 sample, 2 control and 4 calibrator was analysed .

In this study for increase of test accuracy results in each run 3 samples was tested twice and the results compare with each other. Values below 0.045 µg/ml, between 0.045-0.055µg/ml, and above 0.055µg/ml considered as negative, equivocal and positive, respectively.

Finally data was analysed with IBM SPSS 21.0 and statistical 10.0 and the level of significance considered below 5%.

Results

Results shown 191 (67.7%) of participants were girls and 91(32.2%) were boys. The mean age of the participants for girls was (10.54$\pm$ 0.398) years and for boys (10.42 $\pm$ 0.735) years. The overall prevalence of *Helicobacter pylori* colonization in 282 students was 13.1% (Table.1). The prevalence of infection in girls was 16.2% and in boys was 6.6%, so the relationship between *H. pylori* infection and gender was statistically significant (P=0.025) (Table.1). The prevalence of infection among 9, 10, 11 and 12 year- old-children was respectively 12.9%, 13.4%, 16.4% and 10.5%, and there was no significant relationship between age of children and *H. pylori* infection (Table.1)(P>0.05) . Results shown that there was strong negative relationship between duration of breast feeding and prevalence of *H. pylori* infection (P=0.002) (Table.1). Also there was a significant positive relationship between the number of siblings (family crowding) and *H. pylori* infection (P=0.004) (Table.1). Although there were some evidence that advocate potential anti *H. pylori* activity of probiotics, we didn`t find any significant association or trend between the number of days that family consume yogurt (as a probable source of probiotics) and *H. pylori* infection (P>0.05). Presence of gastric complaint (such as flatulence, heart burn, regurgitation, as a general term: dyspepsia) in households has a visual positive trend with *H. pylori* infection but the relationship was not statistically significant (P>0.05). There was not any relationship between *H. pylori* infection and living regions that economically stratified across the city (P>0.05).

Table1: Relationship between some associated factors and H. pylori colonization

Variables	Stratification				Significance	P value
Gender	male		female		yes	*0.025
	6.6%		16.2%			
Age	9	10	11	12	no	0.789
	12.9%	13.4%	16.4%	10.5%		
Breast feeding	4m-1y	1-2y	2y		yes	*0.002
	28.0%	18.9%	8.8%			
Children number	=<3		>3		yes	*0.011
	10.5%		89.5%			
Yogurt consumption(day)	=<3		>3		no	0.33
	11.3%		15.2%			
FHx of dyspepsia	Mother &father	Mother &father	Sibling	Other	no	0.096
	37%	14.8%	9.1%	20.0%		
Economic status	Low level		High level		no	0.287
	15.3%		11.0%			

Discussion

The prevalence of infection in our study was surprisingly lower than in other similar studies. Many predisposing and inhibitory factors involves in prevalence of this infection in different populations, some of these factors are socio-economic level, crowding, level of sanitation, care taker`s literature, and positive family history of gastro-intestinal complaint.

In attention to the difference of prevalence between two related studies in west and center of Iran (8,9) and our study it seems that may be such regional factors like climate and food habits influence it but it needs to be further studied.

Moreover in Falsafi *et al*. study in Tehran (8), the study population was both symptomatic and asymptomatic children and adolescent that explain the larger prevalence. Our study was designed in asymptomatic primary school student population, most of families were young and the mean number of siblings was 2.5.

We try to sample homogeneously in socio-economic status from students of Birjand population, for this reason we subgroup the population into four socio-economic level by living region and randomly select the primary schools in each region because we want to reach the total prevalence of infection in Birjand. These reasons may be

some causes for lower prevalence in our study. There was statistically significant relationship between gender and infection in our study (16.2% in girls and 6.6% in boys), Queiroz et al. and Hestvik et al. (4,30) have significant relationship between gender and infection, but in those infection in boys were higher; so it seems that this was an accidental relationship.

Our population age range was narrow (9 to12 year), may be this is the reason for that there was not a significant relationship between increasing age and prevalence of infection in present study, despite studies of Hestvik (3), Falsafi (8), Soltani (9), Mirada (10), Escobar-Pardo (21) and Rasheed (24) had significant relationship.

We found that number of siblings had a significant relationship with prevalence of infection, it can be explained by the root of transmission of *H. pylori* infection (person to person by fecal-oral and oral-oral), because of poor sanitation that usually is in larger families; results of Falsafi et al. (3) and Soltani et al. (9) are agree with this current study and Miranda et al. (10) and Queiroz et al. (32) are disagree with us in this issue.

In order of family members dyspepsia and infection in the case we find just a positive trend but it was not statistically significant, but at Li et al. and Falsafi et al. results, the relationship surveyed was significant (7,8).

There was very significant negative relationship between duration of breast feeding and *H. pylori* infection; these finding support the protective effect of breast feeding against this infection like a lot of other protective and immunologic effect of breast milk. finding of Hestvik et al. and Zhang et al. advocate this result (30,33) but Soltani et al., Miranda et al. and Carter et al. are in opposition of it (9,10,31).

There were some evidence that probiotics-new generation of therapy in a wide range of infectious and non-infectious diseases, have good effects in treatment of *H. pylori* infection (36) and some that assert dairy product based probiotics have a better effect in this issue (37). We consider the number of days that family consume yogurt as a variable and its relationship with prevalence of infection but we did not find a significant relationship between these two factors, however we could not assert about role of probiotics in control of infection, because we did not sure that the dairy products inevitably have probiotic strains; this issue is not studied in any other research.

The last factor that we investigate was relationship between economic region of living and infection acquiring risk. We divide the areas of city into four economic regions by the aid of office of education in Birjand and from each region we select one male and one female primary school, after completion of this stage because the sample size was insufficient, we have to continue sampling from four randomly selected female primary school from each region again, so finally we have samples from two female and one male primary school from each region.

We did not find any relation between these variables, Miranda *et al.* did not find such relationship too (8), but Carter et al. and Zhang et al. find positive relationship between these two variables (31,33), this positive relationship seems that provide by logic but it is difficult to determine absolute factors that indicate the economic state of family; it seems that living region in the city may be not enough indicator of economic level of family in our study and should be modify simultaneously by other indicators.

Conclusion

The prevalence of Helicobacter Pylori is different between areas of a country and health care providers should plan strategies for exploring and modifying its risk factors. determine absolute factors that indicate the economic state of family; it seems that living region in the city may be not enough indicator of economic level of family in our study and should be modify simultaneously by other indicators.

Conflict of Interest

The authors declare that they have no competing interests.

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