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Prevalence and Risk Factors of *Helicobacter pylori* Infection among Health Center Referrals in Khorramabad (West of Iran)

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ABSTRACT

Helicobacter pylori infects about one half of the world population. Since there was no information about the epidemiology of *H. pylori* infection in Khorramabad, west of Iran, this study was done to determine the prevalence and potential risk factors of *H. pylori* infection in this region. *Helicobacter pylori* specific ELISA for the presence of IgG antibodies was done on the sera of 381 cases. A questionnaire was used to gather the information related to demographic, hygienic and lifestyle variables. The overall prevalence of infection was 43%. A positive association was found with increasing age ($p = 0.002$), premastication of food by mother ($p = 0.019$) and education level ($p = 0.011$), but not with gender and family size. The prevalence of the infection was lower than that in other parts of the country and it is predicted that it will decrease in the future because of the increasing education rate and reduction of bad hygienic behaviors such as food premastication by mothers.

Key words: *H. pylori*, khorramabad, epidemiology, risk factors, prevalence

INTRODUCTION

Helicobacter pylori is a gram-negative spiral-shaped bacterium which infects the stomach of human beings (Frenck and Clemens, 2003; Mahmood and Hamid, 2010). Infection with this bacterium can lead to gastritis, peptic ulcer and sometimes gastric carcinoma (Schwarz *et al.*, 2008; Moghaddam *et al.*, 2009). The infection is usually treated with antibiotics, but recently, researchers are trying to develop a specific vaccine for preventing the infection. The effective use of vaccines requires some information on the prevalence of the infection in the target population (Moujaber *et al.*, 2008).

Helicobacter pylori prevalence differs greatly in different geographic regions and is usually higher in developing countries than in developed ones (Smith *et al.*, 2009). Moreover, the infection is acquired in early childhood and persists for the rest of lifespan if it is left untreated. The acquisition rate of the infection is related to socio-economic status, crowding and lifestyle (hygiene) factors (Queiroz and Luzza, 2006). About 50% of the world population is infected with this bacterium (Leal *et al.*, 2008).

The overall prevalence of the infection in Iran is not known, but some years ago several local studies have been done in some provinces (Alborzi *et al*., 2006). The studies show that the prevalence of this infection ranges from 59 to 70%, which is higher than the global prevalence which is 50%. Unfortunately, these studies have not investigated the risk factors associated with the infection. Moreover, no study has been done to determine *H. pylori* prevalence in the western part of this country with its specific climatic and cultural characteristics. The aim of this study was to determine the seroprevalence and associated risk factors of *H. pylori* infection among health center referrals of Khorramabad (west of Iran).

MATERIALS AND METHODS

This cross-sectional study was carried out in the first half of 2008. We enrolled 381 consecutive volunteers who attended different health care centers of Khorramabad in Lorestan province. Persons who had history of blood transfusion, immunosuppressive state or immunosuppressive drug consumption such as corticosteroids were excluded from the study. The group had a mean age of 28.5 years (range = 15-88 years) out of whom, 160 were male, 237 were female and the gender of the others was unknown. Ages were rounded to complete ages.

After signing a consent form, the subjects were interviewed in health centers by trained healthcare workers using a self-administered standardized questionnaire. The questionnaire contained two categories of questions; one category gathered demographic information (age, gender, job, education level [in years], mother education level, monthly income and the size of the family) and the other one included questions related to lifestyle (hygiene) behaviors (use of a common bowl for serving food during childhood, premastication of food by mother, past and current water source, frequency of raw vegetables consumption and consumption of unheated milk).

A blood sample was taken from 381 persons. Sera were isolated from all the samples. The sera were frozen at -20°C until the analysis was performed. We used Enzyme Linked Immunosorbent Assay (ELISA) test to verify *H. pylori* specific IgG antibody using a commercially available kit (Monobind, Inc., California, USA). To determine the infection status (positive or negative) of the subjects, we used a cut off point equal to 20 U mL^{-1} of anti-*H. pylori* antibody according to the manufacturer's instructions. Persons whose IgG levels were less than the cut off point were considered *H. pylori* negative.

We used Pearson chi-square and Fisher's exact test to assess the association between *H. pylori* infection and the possible risk factors. To measure the strength of association of some variables (i.e., age) with the infection prevalence, we used linear by linear association test and the corresponding 95% Confidence Intervals (CI) were calculated. p-values of <0.05 were required for statistical significance.

RESULTS

The mean age of the participants was 28.5 years. The overall prevalence in the study sample was 43%. Seroprevalence of infection in the men (42.5%) and the women (43.6%) was not statistically significant ($p = 0.46$). Out of demographic variables; age, job and education level of the participants were associated with the prevalence of infection. There was a robust association between age and prevalence ($p = 0.01$). Out of the four age groups, the lowest (22.9%) and the highest (52.3%) prevalence rates were found in <20 year and 30-39 year age groups, respectively. This association had a linear status ($p = 0.002$).

The prevalence differed significantly (p = 0.036) in different job groups. The highest rate (53.5%) was found in the housewives and the lowest (31%) was found in the students. There was an inverse significant (p = 0.032) relationship between the participant's education level and the infection rate. Those with higher education (academic level) had the lowest infection rate (35%). The illiterate persons had the maximum (62.5%) infection rate. This inverse linear relationship (p = 0.047) was also found between mother's education level and infection rate, but it was not statistically significant (p = 0.137). There was not any significant relationship between monthly income or family size and the infection prevalence (Table 1).

Among lifestyle or hygiene variables, only premastication of food by mother had a significant relationship (p = 0.019) with *H. pylori* infection rate. The seroprevalence rate in the group with

Table 1: The relationship between seroprevalence of *H. pylori* infection and demographic variables. The rate of infection in persons with unknown variables such as gender has not been stated

	Frequency		95%		
	----------------------------------		confidence		Linear by linear
Variables	Total	Positive*	Interval**	p-value	association test
Age (year)					
<20	35	8 (22.9)	9.0 -36.8	0.01	0.002
20-29	215	86 (40)	33.5-46.5		
30-39	71	38 (53.5)	41.9-65.1		
>=40	44	23 (52.3)	37.6-67.0		
Gender					
Male	153	65 (42.5)	34.7-50.3	0.46	
Female	225	98 (43.6)	37.2-50.0		
Occupation					
Housewife	99	53 (53.5)	43.7-63.3	0.036	
Unemployed	58	22 (37.9)	25.4-50.4		
Employee	48	24 (50)	35.9-64.1		
Self-employed	100	41 (41)	31.4-50.6		
Student	42	13 (31)	17.0-45.0		
Others	24	6 (25)	7.7 -42.3		
Education level (year)					
0	40	25 (62.5)	47.5-77.5	0.032	0.011
1-8	96	41 (42.7)	32.8-52.6		
9-12	143	62 (43.4)	35.3-51.5		
>12	100	35 (35)	25.7-44.3		
Mother education level (year)					
0	201	96 (47.8)	40.9-54.7	0.137	0.047
1-8	126	51 (40.5)	31.9-49.1		
>=9	48	16 (33.3)	19.9-46.7		
Monthly income (USD)* **					
<200	40	19 (47.5)	33.6-61.4	0.811	0.698
200-399	153	64 (41.8)	34.7-48.9		
>=400	75	32 (42.7)	31.5-53.9		
Family size					
<6	201	79 (39.3)	32.6-46.0	0.086	0.141
>=7	155	73 (47.1)	39.3-54.9		

*Numbers in parentheses indicate the percentages of positive cases among each category, **Shows confidence interval of frequency percentages of seropositivity in each category, ***United States Dollar

Table 2: The relationship between Seroprevalence of *H. pylori* infection and lifestyle variables

Variables	Frequency Total	Positive*	95% confidence interval**	p-value
Common bowl usage in childhood				
Yes	224	102 (45.5)	39.0-52.0	0.255
No	140	58 (41.5)	33.3-49.7	
Premastication of food by mother				
Yes	108	57 (52.8)	43.4-62.2	0.019
No	253	102 (40.2)	34.2-46.2	
Past water source				
Well/spring	100	44 (44)	34.3-53.7	0.478
Municipal/Running	279	120 (43)	37.2-48.8	
Current water source				
Well/spring	15	5 (33.3)	9.5 -57.1	0.324
Municipal/Running	359	154 (42.9)	37.8-48.0	
Frequency of raw vegetable consumption				
Rarely	59	23 (39)	26.6-51.4	0.769
Daily	92	41 (44.6)	34.5-54.7	
Weekly	224	98 (43.8)	37.4-50.2	
Unheated milk consumption				
Yes	103	43 (41.7)	32.2-51.2	0.358
No	265	118 (44.5)	38.5-50.5	

*Numbers in parentheses indicate the percentages of positive cases among each category, **Shows confidence interval of frequency percentages of seropositivity in each category

mothers having this behavior was 52.8% (vs., 40.2% in the other group). The seroprevalence rate in the subjects who had used a common bowl for serving food in childhood were 45.5% (compared to 41.4% in the other group who did not use a common bowl), but this difference was not statistically significant. Other lifestyle variables were not related to the seroprevalence rate (Table 2).

DISCUSSION

Prevalence of *H. pylori* differs remarkably among underdeveloped and developed countries (Perez-Perez *et al*., 2004). There is a lack of information about the prevalence of infection in the west of Iran. Besides, other studies in this country have not assessed risk factors associated with *H. pylori* prevalence. In this study we determined the prevalence and its associated risk factors of *H. pylori*. The overall prevalence rate of the infection was 43%. Previous local studies (not published internationally) have found higher prevalence rates (59-70%) in other regions of the country (Alborzi *et al*., 2006). These studies have been done on samples which differ from our sample, so the results are simply not comparable. The educational and socioeconomic status of Khorramabad region may be different from those in other parts of Iran. Lower prevalence rate in a sample study may be due to auto-curability of the infection in higher age groups (Perri *et al*., 1998; Rothenbacher *et al*., 2002) and differences in types of *H. pylori* in adults in comparison with children. In the United States, auto-curability was 0.3 per year in the black race and 5.5% per year in white young persons (7-21 years old) (Torres *et al*., 2000).

We found a direct correlation between age and the infection rate, so seroprevalence was found to be higher in older age groups. This finding is in accordance with the results of other studies

(Mbulaiteye *et al.*, 2006; Santos *et al.*, 2005; Yim *et al.*, 2007). Infection is usually acquired in earlier ages (Frenck and Clemens, 2003) and persists frequently for lifetime (Go, 2002). Prevalence is higher in adults than in earlier age groups. This phenomenon is called cohort effect (Replogle *et al.*, 1996).

There was no difference in prevalence among the men and the women. The results of many previous studies (Alborzi *et al.*, 2006; Sasidharan and Uyub, 2009; Tam *et al.*, 2008) are in agreement with this finding, but there is a conflict with other studies (Dube *et al.*, 2009; Yim *et al.*, 2007) which state that the infection rate is higher in males than in females. There is no rationale to justify the male-predominance of the infection.

Seroprevalence decreased with increasing education level of the participants and their mothers. The result is compatible with the results of other studies (Sykora *et al.*, 2009; Yucel *et al.*, 2009) and can be justified by better economic and hygienic status in more educated persons. Illiteracy of mother has been stated as a potential risk factor for *H. pylori* infection (Frenck and Clemens, 2003). In a study in Brazil, it has been shown that higher education level of parents could act as a protective factor against *H. pylori* infection (Santos *et al.*, 2005). The implication of this finding is that the prevalence of the infection will decrease in the future, since the education level is increasing in the region.

We could not find a significant relationship between current economic status and the prevalence rate of the infection. Santos *et al.* (2005) have shown that significant association of present socio-economic variables (including current family income) will be lost after controlling past socio-economic indicators. Moreover, in another study in Greenland, researchers have not found any significant relationship between economic status and infection rate (Koch *et al.*, 2005). The correlation between past economic status and the infection rate has been emphasized by many researchers (Peach *et al.*, 1997; Rothenbacher *et al.*, 1997). Our unexpected result can be explained by the point that we assessed current economic status which can be different in comparison to the past.

In contrast to other studies (Goodman *et al.*, 1996; Peach *et al.*, 1997; Rothenbacher *et al.*, 1997), we could not find a significant relationship between family size and *H. pylori* seroprevalence. This unusual finding can be justified by more socialization between Iranian families in comparison to other countries. This cultural habit exposes the members of low-sized families to the high risk of getting infection from the members of high-sized families.

Among the lifestyle variables, only premastication of food by mother was associated significantly with the infection. This finding confirms that a mother could act as a potential source of infection for her children. Konno *et al.* (2005) in a study which was done in Japan have shown that DNA fingerprinting of *H. pylori* isolates is identical in mothers and their children. In another study in Germany, it was shown that the odds ratio for *H. pylori* infection of a child was 12.9 in the case of mother seropositivity for *H. pylori* (Weyermann *et al.*, 2006). The results of other studies which were done in Ethiopia and Bangladesh are not compatible with our results (Lindkvist *et al.*, 1998).

The main route of the infection transmission is oral-oral or fecal-oral (Schwarz *et al.*, 2008). We hypothesized that the use of a common bowl for serving food in this region-especially in the past- could be associated with the infection rate. But, our results did not confirm this hypothesis unexpectedly, because prevalence of infection in those who had been used to eating food in a common bowl in childhood and those without this behavior did not differ. This result can be explained by the homogeneity of the study population with regard to this susceptibility factor.

Although researchers have isolated *H. pylori* from water (Hulten *et al.*, 1996) and the bacterium could survive in water for about one week (Frenck and Clemens, 2003), we were not able to find any relationship between past and current water sources (running vs. well or spring water) and the infection rate. Two other research groups (Lin *et al.*, 2005; Nguyen *et al.*, 2006) have also shown that there is no relationship between water source and the infection. But, the main body of the evidence contradicts this finding (Brown, 2000; Glynn *et al.*, 2002; Queralt *et al.*, 2005). This discrepancy could be explained by the presence of many springs in the study region and the usual behavior of the habitants to frequently drink water from both sources.

In contrast to the results of Goodman *et al.* (1996), our study could not show a relationship between the infection rate and frequency of raw vegetables consumption. This could be due to the homogeneity of the study population because of widespread contamination of vegetables with *H. pylori*. Using untreated waste water for irrigating vegetables is common in this region.

Consumption of unheated milk also was not related to the infection rate, although *H. pylori* DNA has been isolated from 60% of cases of sheep milk in one study (Dore *et al.*, 2001). The intact bacterium itself has also been isolated from sheep milk (Go, 2002). Since, there are no reliable data about the contamination of "unsafe milk" and "raw vegetables" in this geographic area, the role of these factors as the sources of *H. pylori* infection would be neither clear nor conclusive.

One limitation of our study was the method of sampling. Convenient sampling from health center referrals could create a potential source of bias. The sample size was inadequate for assessing some risk factors. It was better to select the sample in a way that it could be more representative of the study population. The history of "pre-mastication of food by mother" belongs to many years ago, so some participants may not be a reliable source of data in this respect.

The findings of this study show that seroprevalence of *H. pylori* in this geographic region is lower than that reported as a mean percentage for the world population. Increasing education level in the region could promise a more decrease in the infection rate in the future. Finally, we suggest doing a population based study with a larger sample size in the region or even on a national scale to get better knowledge on the prevalence of *H. pylori*.

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