



Survey on the prevalence of active infection caused by *Helicobacter pylori* in the patients carried out endoscopy in Tonekabon

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ABSTRACT

H.pylori is one of the common infectious agent that involves in acute and chronic gastritis, peptic scar and gastric cancer. The infection episode in children ranges between 10 to 80 percent and the high frequency in developing countries. The goal of this study was to determine the incident of *H.pylori* in the patients carried out to endoscopy and the effective factors. In this study, 50 patients of 3 to 15 years old range that were admitted in endoscopy ward were studied. After endoscopy, the specimen of gastric mucus was studied using rapid urease Test (RUT). The specimen with urease positive test were taken as the case and specimen with negative urease test were taken as control group and the factors involves in *H.pylori* were studied. The Odds ratio was calculated in the specimen and confidence interval was performed in 95% probability and the data was analyzed by using common statistical tests. The results showed that of 50 children, 9 patients (18%) were diagnosed with Pelvic ulcer disease (PUD) and positive *H.pylori*, 5 patients (10%) had gastritis and free from contamination to concerned organism. The 6-15 years old age group showed the highest number of bacteria (30%). Of all the patients subject of study, 7 cases were male (14%) and 8 (16%) were females. 25 patients (54%) had less than high school diploma education and 23 cases (46%) had university education. In this study, age, sex, water consumption, number of family members and the economic-social status were the criteria of study and it was shown that the infection essentially happens in young ages and the chance of infection through contaminated water is high. It is concluded that *H.pylori* infection is an important factor involving the gastritis and duodenum inflammation.

1. Introduction

H.pylori infection is one of the most frequent chronic bacterial infections in world; especially in developing countries (Matysiak et al. 1997). The epidemiological pattern of this infection differs in industrial countries from the developing countries; i.e., in industrial countries,

as the patient gets older, the infection frequency grows but in developing countries, most children are exposed to the bacteria in young age and almost a large number of young adolescents that reach adulthood show the sign of infection and there is a 60 to 90 percent frequency in all age groups (Matysiak et al., 1997; Atherton et al.,

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1998). Although it has been claimed that the risk of getting this infection in childhood is high (Bahremand et al., 2006; Malaty et al., 2002). The most common age of getting this infection is still unknown and it differs in the developing and developed countries (Mitchell et al., 1992). This bacterium is the main cause of chronic gastric type B and is a secondary factor in developing diseases such as ulcer peptic, gastric ednocarcinoma and mucosa associated lymphoid tissue (MALT) lymphoma (Hunt et al., 2010). Different studies have shown that treating and uprooting *H.pylori* infection has been effective in decreasing and omitting peptic ulcers and controlling MALT lymphoma (Malaty et al., 2002). In addition, it has been shown that this bacterium is one of the environmental factors and an initiator of gastric mucosa damages (Cornelius et al., 1989). Following the symptoms of chronic gastritis (atrophy), the intestine metaplasia and dysplasia grows to change into gastric adnocarsinom. Different studies show that there is a direct relationship between socio-economic level of people and the frequency of the infection. Graham and colleagues in their studies in Heydarabad, India, reported that 80 percent of <20 year old people with low socio-economic level had positive *H.pylori* antibody (Graham et al., 1991; Atherton et al., 1998; Ramakrishnan et al., 2007), while in the developed countries with high socio-economic levels, the infection prevalence of 3.1 to 19.4 percent has been reported (Pateo et al., 1994; Hornemann et al., 1997). The relative frequency of *H.pylori* in <20 years old people of Ardebil city was 47 percent (Cornelius et al., 1989), and in the city of Yazd, it has been reported to be 43.8 percent in 2-14 years old children (Valizadeh Tosi et al., 1994). Based on studies, the prevalence of microbial contamination in Iran is 30 to 40 percent and in children suffering from chronic abdomen pain in peptic diseases is around 80 percent. The prevalence of the infection increases as the person grows older. Based on this, the frequency of infection in the children without symptoms in Iran has been reported to be 12 to 40 percent and around 40.9 percent of the patients were 7 to 18 years old and young adults (Bahremand et al., 2006). Researchers believe that man is the only

definite source of this infection. In sum, 50 percent of people in the world have contamination (Wylie, 1999; Bchrman et al., 1996). The transmission of this infection is still unknown, although the existence of *H.pylori* in dental plaques, saliva and feces show the oral-oral and oral-fecal transmission (William et al., 2007). The aim of this study was to determine the amount of *H.pylori* existence and assess its amount with the histological findings, the age-group and study the existence of organism in chronic gastric and intestine metaplasia (Lennita et al., 2011).

2. Material and Methods

Fifty children with 3 to 15 years old in Tonekabon in 2010 were studied. The patients reported different complaints such as abdomen pain and hemorrhage from upper and lower digestive system, maldigestion, nausea, and frequent vomiting and chronic diarrhea and underwent gastric and duodenum endoscopy at the endoscopy ward of Tonekabon hospital. After analysis of the specimens, the questionnaire form containing questions on age, gender, records of digestive problems in the individual and parents as well as the socio-economic status [questions on parent's education and jobs, house ownership, substructure level per individual, type of consumption water-piping, and non-piping- accommodation place, number of family members and methods of school management (public, private)] was completed.

After completing the questionnaire forms and information collection, the data was analyzed by using SPSS statistical software. In this study, two specimens were prepared from gastric tissues and were stored in 0.2 ml sterile distilled water. The biopsy specimen was placed in a sterile plate and was ground by a sterile scapple. The specimen was then inoculated in a BHI environment containing 7% horse blood and brucella blood agar with 5 percent sheep blood, tri-metoprim, vancomycin and polymyxin B. The plates were then incubated for 7 to 10 days in 37°C in micro-aerophilic and were studied daily. In order to accelerate in low growth culture development from concerned micro-organism, a subculture was prepared and

was then incubated for three to five days. Those conditions continued for 10 days to obtain results. In this condition, the colonies suspicious to *H.pylori* were tested to check the activities of urease catalysis and oxidize and a corrected warm coloring method was used. The presence of organism was confirmed by exhibition of negative gram curved bacilli and a positive test for urease, oxidase and catalase.

3. Results

Of 50 children subject of study, 9 cases (18%) were diagnosed with gastric ulcer PUD and infection with *Helicobacter* positive, and 5 patients (10%) had gastric ulcer free from organism contamination. 30 percent of patients with ulcer were in 6 to 15 years old age group while no positive cases in the age group 0 to 5 years were observed. Regarding gender, 7 patients (38.8%) were male and 8(25%) were females (table 1). The frequency distribution of disease in urban regions (62%) was more than rural areas (38%) (Table 2). The disease transmission in urban region was reported to be in 6-person urban families and 6 person families in rural areas. For showing the *H.pylori* histopathology, the distribution of this microorganism in tissue cuts which were stained in the two Giemsa and hematoxylin-eosine methods. The changes observed in the gastric mucous included atrophy, inflammation, and intestine metaplasia. The degree of inflammation changes in the gastric biopsy specimen of patients with ulcer was studied. The results showed that the most common changes were inactive chronic gastric, followed by active chronic gastric and atrophy changes. In the histological findings, malignant and ednocarcinoma metaplasia were observed. In addition, only in 20 percent of cases, the gastric mucous was normal. The results obtained from culture by using the classic methods in 80 percent of cases showed low growth of microorganism on the culture environment, low number of colonies, low transparency and very small size. At the same time, the amount of bacterial contamination in culture medium was high and the contaminating bacteria included *Pseudomonas*, *Proteus*, and *Klebsiella*. The

growth speed of *H.pylori* in non-selective classic Colombia agar was very slow as much as in most cases, 5 to 7 days were needed for observing colonies. In addition, when the selective modified colombia urea agar (MCUA) medium was used. Most isolated bacterial colonies were larger in terms of size. In addition, the amount of contamination significantly decreased and microorganism separation was performed in 24 hours. The frequency distribution of infections with emphasize on endoscopy and histology findings in the subjects of study showed that 14 percent of patients had gastric, 6 percent had inactive chronic gastric, 4 percent with normal endoscopy, 2 percent with duodenum ulcer, 2 percent with gastric ulcer and 2 percent showed atrophic changes.

4. Discussion

This study is descriptive-interval type. Factors such as age, socio-economic level of parents, gender, using urban or rural water and number of family members were studied. With respect to the results of this study, contamination to *H.pylori* was relatively high (22%), which agreed with the results of researches conducted other researcher in the city of Rasht, Iran, showing 40% frequency (Mansour et al., 2009). Although assessment of frequency of infection with *H.pylori* reveals important information on the range of infection in the population subject of study, it needs studies on infection pathogenesis to specify the process of optimized load of infection as well. The clinical consequences of the infection depend on the counter relations between the bacterial, host and environment factors (Lennita et al., 2011).

Study the sources and ways of infection transmission show that they are among factors that contribute to the amount of infection prevalence to that organism. In this study, it was shown that infection is common in young ages too, and the prevalence is in 6 to 15 years old age group (Peter et al., 2005). Ghanai and his colleagues in their research studied the 7 to 11 years old age groups and it was shown that most of the subjects were positive in feces test. They were showed that high frequency of infection

Table 1. The frequency of *H. pylori* in different age and gender

Age group	Gender								
	Male			Female			Total		
	Number	+	%	Number	+	%	Number	+	%
0-5	1	0	0	2	0	0	3	0	0
6-10	8	4	50	11	3	27.2	19	7	36.8
11-15	9	6	66.6	19	5	26.3	28	11	39.2
Total	18	10	55.5	32	8	25	50	18	36

Table 2. Comparing the abundance of *H. pylori* as per number of family members and the location

Number of family members	Location	
	Urban	Rural
Less than four	12	4
4-6	13	7
Equal or bigger than 7	6	8
Total	31	19

Table 3. The distribution of patients with *H. pylori* in patients subjects of study as per gender and education

Education	Gender								
	Male			Female			Total		
	Number	+	%	Number	+	%	Number	+	%
Illiterate	13	1	15.3	3	0	0	16	4	18.7
Less than high school Diploma	11	5	45.4	10	4	40	21	5	23.8
+High school diploma	6	4	66.6	7	4	57.1	13	10	79.9
Total	30	10	33.3	20	8	40	50	18	36

Table 4. The distribution of patients with *H. pylori* in the subjects of study as per gender and lesions

Lesions	Gender								
	Male			Female			Total		
	Number	+	%	Number	+	%	Number	+	%
PUD	5	2	40	8	3	37.5	13	5	38.4
NUD	7	3	42.8	6	4	66.6	13	7	53.8
Tumor	0	0	0	0	0	0	0	0	0
Duodenum ulcer	4	1	25	7	3	42.8	11	4	38.3
Normal mucous	2	0	0	11	3	27.2	13	3	23
Total	18	7	38.8	31	13	40.6	50	19	38

Table 5. The distribution of patients with *H. pylori* in patients subjects of study in different gender and findings of endoscopy and histology

Lesions	Male			Female			Total		
	Number	+	%	Number	+	%	Number	+	%
Normal endoscopy	3	1	33.3	4	1	25	7	2	28.5
Diagnosis of gastric based on endoscopy	6	4	66.6	5	3	60	11	7	63.6
Diagnosis of Duodenum ulcer	1	0	0	3	1	33.3	4	1	35
Gastric ulcer Diagnosis	1	0	0	3	1	33.3	4	1	25
Normal mucous in Histology	4	0	0	4	1	25	8	1	12.5
Inactive chronic Gastric	3	1	33.3	5	2	45	8	3	37.5
Active chronic Gastric	0	0	0	3	1	33.3	3	1	33.3
Atrophic changes	0	0	0	3	1	33.3	3	1	33.3
Metaplasia	0	0	0	0	0	0	0	0	0
Dysplasia	0	0	0	2	0	0	2	0	0
Total	18	6	33.3	32	34.3	34.3	50	16	32

among children who consumed urban water, compared to the subjects that used boiled water.

That study showed no significant differences between the amounts of infection and age, gender or socio-economy levels of people (Mansour et al., 2009; Hunt et al., 2010). The high frequency of infection in children indicates the infection development in the developing countries (Selvi et al., 2012). Another study revealed that the prevalence of *H.pylori* in <10 years old children in developing countries is 80% and in developing countries is less than 10% and up to 50 percent of children in low economic-social status in those countries are affected by the infection (Rowland et al., 2000). Ertem in his study based on urease respiration test, as conducted in turkey, showed that as the age grows, the prevalence of infection increases (Ertem et al., 2003). These studies show that the risk of repeated infection in the children in developing countries is high and the infection is mostly through contaminated water. Childhood is a critical period in acquiring infection and transmission could be through contaminated water, person to person, oral-oral or oral-fecal.

Consuming vegetables and foods from street stands are among the effective factors (Lennita et al., 2011). In present study, it was shown that the highest positive results were found in families with less than four members and most positive cases were for parents with university education. In a study on 224 female and male children showed no statistically significant

relationship between infection to *H.pylori* and gender (Malaty et al., 2002). Study on the frequency of *H.pylori* and its relationship with number of family members, was shown that as the number of family members increases, the contamination with *H.pylori* increases (Mokhtari et al., 2002). In a similar research, it was shown that there is a significant relationship between the variable of family members and the amount of infection; i.e., by increasing the number of family members, there will be an increase in *H.pylori* infection frequency (Pirouz et al., 2000).

Therefore, with respect to the studies, one could conclude there is a significant relationship between infection to that infection and increase in the number of family members, as accommodation of more people in a shared place increases the chance of transmission and microbial contamination. In another study in Brazil in 2006 on studying the relationship between *H.pylori* infections among family members showed close relationship in *H.pylori* infection transmission from mother to child through oral contents of mother's mouth (Ito et al., 2006). The findings of the research in *H.pylori* culture with similar work as performed in Iraq showed the chance of bacterial isolation in shorter time with less contamination in using selective culture media than classic media. The histopathological results showed antrum specimen in 20% of normal mucous cases while atrophic changes were lower. In fewer cases,

gastric and duodenum ulcers were found while the endoscopy results was agreed with the histopathology results in gastritis terms (24 cases in endoscopy compared to 20 cases of histopathology of antrum biopsy).

The gastritis cases showed considerable percentage in 6 to 15 years old study. In this study, the biopsy specimen of all patients with gastritis showed positive in urease tests. The necessity of conducting more studies in Iran from the point of molecular diagnostic methods such as Restriction Fragment Length Polymorphisms (RFLP) could make significant contribution in detecting the infection transmission ways. In addition, recommending performing controlled studies could be a factor in specifying the relationship between the risk factors and infection acquire. It is concluded that

high frequency of infection in the population subject of study and infection may develop gastritis and duodenum ulcers. It is also suggested that *H.pylori* infection is an important factor in the gastritis and duodenum inflammation.

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