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Helicobacter pylori Colonization in Biopsies of the Adenotonsillectomy Specimens

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Abstract: Problem statement: *Helicobacter pylori* (*H. pylori*) is a microaerophilic, gram negative bacillus, which can cause peptic ulcer and gastric cancer. Recurrent infection with this agent is considered as one of the reasons for failure of peptic ulcer treatment. Some studies have reported colonization of *H. pylori* in dental plaques, tonsils and adenoid tissues. Therefore oral cavity could be the source of *H. pylori* and it might be the reason for unsuccessful eradication. **Approach:** In this cross sectional study, 95 patients with the average age of 12.1±7.5 years undergoing adenotonsillectomy were chosen consecutively. Intra-operatively a 2 mm section of the tonsils were removed and investigated for *H. pylori* by Rapid Urease Test (RUT). Post-operatively the removed tonsils were stained by hematoxylin-eosin (H and E) and Gimsa for direct investigation of *H. pylori* bacterium. Serum samples of the patients were also tested for the presence of *H. pylori* IgG antibody. **Results:** Overall 70 patients (73.7%) had positive anti- *H. pylori* IgG antibody in their sera. The results of RUT on adeno-tonsils showed that 42.1% of the specimens were positive for *H. pylori*. In histology examination, 9 patients (9.5%) were positive for the presence of bacterium. **Conclusion:** Based on our findings it seems that tonsils and adenoid tissues are the candidate places for the growth of *H. pylori*. Further studies about the role of tonsillar colonization of *H. pylori* in re-infection after treatment are recommended.

Key words: *Helicobacter pylori* colonization, adenotonsils, adenotonsillectomy

INTRODUCTION

Helicobacter pylori, is a worldwide bacterial infection that has a prevalence of 30% in developed to 90% in underdeveloped countries[1,2]. It is reported that the prevalence rate in Iran is between 50-90%[3]. This microaerophilic, gram negative rod is found mostly between epithelial surface and gastric mucus layer. Despite adherence of *H. pylori* to gastric epithelial cells, it can not enter into the epithelium. The enzymes urease, catalase and lipase produced by the bacterium may play some roles in pathogenesis of *H. pylori* induced gastrointestinal diseases[4,5]. Fecal-oral is the main route of transmission for H. *pylori*, however the bacteria can be transmitted through saliva as well[6]. Infection due to *H. pylori* causes gastritis, peptic ulcer, Mucosa Associated Lymphoid Tissue (MALT) lymphoma and gastric carcinoma[7]. Some studies have reported colonization of *H. pylori* in dental plaques, tonsils, saliva and adenoid tissues[8,9]. Considering the fecal-oral route transmission of *H. pylori* and its presence on the tonsils, it is possible that these organs have a potential role in colonization and transmission of the bacteria[9,10].

In treated patients the recurrence rate of peptic ulcer without eradication of *H. pylori* reaches to 70%, while with eradication of the infection this rate will reduce to 20%[11]. Re-infection with the new bacterium after eradication is unusual, however recrudescence of the infection with the same organism may occurs frequently[11,12]. In this respect another source of infection other than stomach might be involved[10]. Minocha has shown reduced colonization of *H. pylori* infection in coming years after surgical adenotonsillectomy[13]. Based on the reports of few studies with contradictory results, it has proposed that adeno- tonsils might be a source of *H. pylori* colonization. Accordingly this study was designed to detect colonization of *H. pylori* in the biopsy specimens of adenotonsil tissues in scheduled surgical patients.

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MATERIALS AND METHODS

Patients and methods: This cross-sectional study was performed on 95 patients who scheduled for adenotonsillectomy in Ear, Nose and Throat (ENT) ward of Shafa hospital (Kerman school of medical sciences) during the year 2007. All of these patients had common indications for adenotonsillectomy including recurrent tonsillitis, chronic tonsillitis and/or adenoiditis. None of the patients had received any antibiotic or gastric acid-lowering drugs during the last 2 weeks prior to surgery. All of the parents and/or patients agreed for surgery and the study proposal.

***H. pylori* diagnosis:** *H. pylori* tonsil colonization was diagnosed based on Rapid Urease Test (RUT) and histopathology on the adeno-tonsil tissues. Before removing the adeno-tonsils, a three millimeter biopsy specimen was taken from these tissues during general anesthesia with a sterile punch device washed with normal saline. The specimens were kept in rapid urease test solution tubes at room temperature. Results were recorded during 4 h after the test. Yellow and red color solutions were considered as negative and positive results respectively. After surgery the taken tonsils were put in 10% formalin and transferred to the pathology ward for histopathology examination. In this part samples were sectioned and stained with hematoxylin and eosin (H and E) and Gimsa. The processed specimens were examined by the pathologist. Serum samples of the patients were also tested for the presence of *H. pylori* IgG antibody by using quantitative enzyme immunoassay (EIA) (Monobind 1425-300 USA).

Statistical analysis: The analysis was based on SPSS 11.5 software with 95% confidence intervals (95% CI). Also Chi square and paired t-test were used for statistical analysis.

RESULTS

Ninety five patients including 50 females and 45 males were studied. Their average of age was 12.1±7.5 years with a range between 2-35 years. The average of the age in females was 3.5 years more than males ($p = 0.018$).

In this study variables included: sex, patients' level of education, income of the family, residence (place of stay) and level of education of the fathers (Table 1). No relationship was found between these variables and colonization of *H. pylori* in adeno- tonsil tissues.

Duration of pharyngeal symptoms in these patients was 1 month to 27 years. Most of them (54.7%) had their symptoms for 1-5 years. The common surgical findings were inflamed adeno-tonsils with or without blockage. In biopsy specimens, RUT was positive in 40 cases (42.1%). In histology examination, bacteria were observed in adeno-tonsil tissues in 9 patients (9.5%). The serology test for *H. pylori* IgG antibody was positive in 70 (73.7%) of the patients. Seventeen patients (17.9%) had history of stomach upset (Table 2). *H. pylori* serology was positive in 15 of 17 (88.2%) symptomatic patients, while it was positive in 56 of 78 (71.8%) asymptomatic cases ($p = 0.007$).

Table 1: Demographic features in patients undergone adenotonsillectomy (N= 95)

Variable	Group	Number	Percent
Sex	Female	50	52.6
	Male	45	47.4
Education	Uneducated	1	1.1
	Preliminary	29	30.5
	Primary school	31	32.6
	High school	25	26.3
	Diploma	4	4.2
	University student	2	2.1
	Bachelor (of degree)	2	2.1
	Master of Science	1	1.1
Monthly income	Low	56	59.0
	Medium	35	36.8
	High	4	4.2
Residence	City (urban)	46	48.4
	Village (rural)	49	51.6
Father's education	Uneducated	22	23.2
	Primary school	14	14.7
	High school	31	32.6
	Diploma	20	21.0
	Tow years after diploma	1	1.1
	Bachelor (of degree)	6	6.3
	Ph.D.	1	1.1

Table 2: The frequency of symptoms and findings in patients undergone adenotonsillectomy (N = 95)

Variable	Group	Female frequency	Percent	Male frequency	Percent	p-value
Digestive sign	Negative	36	46.2	42	53.8	0.007
	Positive	14	82.4	3	17.6	
Duration of pharynges symptoms	<1 year	10	47.6	11	52.4	0.030
	1-5 years	23	44.2	29	55.8	
	>5 years	17	77.3	5	22.7	
Surgery (indication) finding	Inflammation	12	60.0	8	40.0	0.700
	Blocking	24	49.0	25	51.0	
	Both	14	53.8	12	46.2	
Rapid urease test	Negative	29	52.5	26	47.5	0.98
	Positive	21	52.5	19	47.5	
Serology for *H. pylori*	Negative	13	52.0	12	48.0	0.941
	Positive	37	52.9	33	47.1	
Histology for *H. pylori*	Negative	45	52.3	41	47.7	0.854
	Positive	5	55.6	4	44.4	

DISCUSSION

H. pylori is a world wide distributed chronic infection which is associated with gastritis, peptic ulcer, gastric carcinoma and lymphoma. In developing countries most of the children are affected by this bacterium up to the age of 10 as the infection rate will reach 80% at the age of 50. A significant number of these subjects do not show any obvious clinical symptoms[14]. Stomach is the main place of *H. pylori* colonization and the eradication of infection from the stomach is the mainstay of therapy for prevention of peptic ulcer recurrence. It has been shown that by eradication of *H. pylori*, peptic ulcer recurrence will be reduced to less than10% annually or even it may result in MALT regression[15]. Re-infection or bacterial reactivation is an important factor in recurrence of peptic ulcer. The re-infection rate in different studies has been reported from 1% to 20% annually[16]. Among the factors related to infection recurrence or colonization, mouth cavity is the issue which has been doubted and argued by the researchers. This hypothesis was propounded when some studies have shown the colonization of *H. pylori* in dental plaques, tonsils and adenoid tissues[8,9]. In our study, serology test was positive in 73.7% of patients revealing high rate of infection in the first and second decades of life. RUT was positive in 42% of patients and histology showed bacterium in 9.5% of the samples. In Yilmaz et al study from Turkey on 50 samples of adenotonsillectomy in children aged 2-10 years, the results of *H. pylori* serology was positive in 50% of cases but none of the surgical samples were positive for rapid urease test[17]. The results of another study from Turkey on 19 patients by Unver *et al* showed that rapid urease test was positive in 57.8% of adenotonsillectomy samples[18]. In addition, in another study carried out by Aslan on 52 patients, pronto dry test, which shows *H. pylori* infection, was positive in 42%; however, no *H. pylori* was found in H and E staining[19]. The difference of results in various studies might be due to different laboratory methods but the positive results of urease test or histology in some of these studies reflect the existence of *H. pylori* on adeno-tonsil tissues. In our study, RUT was positive in 42.1% of cases while histology was positive just in 9.5% of samples. This difference might be explained by the possible existence of other urease producing bacteria in the mouth or adeno- tonsil tissues. Also it's possible that *H. pylori* may contaminate the oral cavity temporarily or invade the adeno-tonsil tissues superficially, in contrast of its permanent and deep replacement in gastric mucosa.

CONCLUSION

Existence of *H. pylori* shown by RUT and in some histology findings potentiate the hypothesis that this bacterium might be able to live in oral cavity, where it can be regarded as one of the infection resources and may play a role in oral transmission of this common bacterium.

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