Helicobacter pylori Colonization in Biopsies of the Adenotonsillectomy Specimens

1M.J. Zahedi, 1S. Darvish Moghadam, 2M. Ahmadi Mosavi, 3T. Mirshekari and 1M. Hayatbakhsh
1Department of Gastroenterology, 2Department of Ear, Nose and Throat, 3Department of Pathology, Medical School and Physiology Research Center, Kerman, Iran

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 adenotonsils 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.
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 pharyngeal 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               | 52.6    | 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[8,9]. 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 adeno-tonsil tissues[8,9]. In our study, serology test was positive in 50% 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 adenoto-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.

ACKNOWLEDGEMENT

Kindly cooperation of Research Deputy of Kerman University of Medical Sciences and Physiology Research Center in conducting this study is appreciated.

REFERENCES

1. Baldwin, D.N., B. Shepherd, P. Kraemer, M.K. Hall and L.K. Sycurso et al., 2007. Identification of *Helicobacter pylori* genes that contribute to stomach colonization. Infect. Immunol., 75: 1005-1016. DOI: 10.1128/IAI.01176-06
2. Magalhaes Queiroz, D.M. and F. Luzza, 2006. Epidemiology of *Helicobacter pylori* infection. Helicobacter, 11: 1-5. http://direct.bl.uk/bld/PlaceOrder.do?UIN=193702725&ETOC=RN&from=searchengine
3. Hashemi, M.R., M. Rahnavardi, B. Bikdeli and M. Dehghani Zahedani, 2006. *H. pylori* infection among 1000 southern Iranian dyspeptic patients. World J. Gastroenterol., 12: 5479-5482. http://www.ncbi.nlm.nih.gov/pubmed/17006984
4. Beswick, E.J., I.V. Pinchuk, G. Suarez, J.C. Sierra and V.E. Reyes, 2006. *Helicobacter pylori* CagA-dependent macrophage migration inhibitory factor produced by gastric epithelial cells binds to CD74 and stimulates procarcinogenic events. J. Immunol., 176: 6794-6801. http://www.jimmunol.org/cgi/content/abstract/176/11/6794
5. Lin, Y.F., C.Y. Chen, M.H. Tsai, M.S. Wu, Y.C. Wang, E.Y. Chuang et al., 2007. Duodenal ulcer-related antigens from *Helicobacter pylori*: Immunoproteome and protein microarray approaches. Mol. Cell Proteom., 6: 1018-1026. http://www.mcponline.org/cgi/content/abstract/6/6/1018
6. Mladenova, I., M. Durazzo and R. Pellicano, 2006. Transmission of *Helicobacter pylori*: Are there evidences for a fecal-oral route? Minerva Med., 97: 15-18. http://www.ncbi.nlm.nih.gov/pubmed/16565693
7. Sagaert, X., C. de Wolf-Peeters, H. Noels and M. Baens, 2007. The pathogenesis of MALT lymphomas: Where do we stand? Leukemia, 21: 389-396. http://www.ncbi.nlm.nih.gov/pubmed/17230229
8. Gebara, E.C., C.M. Faria, C. Pannuti, L. Chehter, M.P. Mayer and L.A. Lima, 2006. Persistence of Helicobacter pylori in the oral cavity after systemic eradication therapy. J. Clin. Periodontol., 33: 329-333. http://www.ncbi.nlm.nih.gov/pubmed/16634953
9. Bulut, Y., A. Agacayak, T. Karlidag, Z.A. Toraman and M. Yilmaz, 2006. Association of cagA+ Helicobacter pylori with adenotonsillar hypertrophy. Tohoku J. Exp. Med., 209: 229-233. http://www.ncbi.nlm.nih.gov/pubmed/16778369
10. Jelavic, B., M. Bevanda, M. Ostojic, M. Leventic, M. Vasilj and E. Knezevic, 2007. Tonsillar colonization is unlikely to play important role in Helicobacter pylori infection in children. Int. J. Pediatr. Otorhinolaryngol., 71: 585-590. http://www.ncbi.nlm.nih.gov/pubmed/17239446
11. Bose, A.C., V. Kate, N. Ananthakrishnan and S.C. Parija, 2007. Helicobacter pylori eradication prevents recurrence after simple closure of perforated duodenal ulcer. J. Gastroenterol. Hepatol., 22: 345-348. http://www.ncbi.nlm.nih.gov/pubmed/17295765
12. Saad, R.A., 2006. Re-infection following successful eradication of Helicobacter pylori infection. Aliment Pharmacol. Ther., 24: 695-696. http://www.ncbi.nlm.nih.gov/pubmed/16907902
13. Minocha, A., C.A. Raczkowski and R.J. Richards, 1997. Is a history of tonsillectomy associated with a decreased risk of Helicobacter pylori infection? J. Clin. Gastroenterol., 25: 580-582. http://www.ncbi.nlm.nih.gov/pubmed/9451666
14. Das, J.C. and N. Paul, 2007. Epidemiology and pathophysiology of Helicobacter pylori infection in children. Indian J. Pediatr., 74: 287-290. http://www.ncbi.nlm.nih.gov/pubmed/17401270
15. Komoto, M., K. Tominaga, B. Nakata, T. Takashima, T. Inoue and K. Hirakawa, 2006. Complete regression of low-grade Mucosa-Associated Lymphoid Tissue (MALT) lymphoma in the gastric stump after eradication of Helicobacter pylori. J. Exp. Clin. Cancer Res., 25: 283-285. http://www.ncbi.nlm.nih.gov/pubmed/16918142
16. Cameron, E.A., G.D. Bell, L. Baldwin, K.U. Powell and S.G. Williams, 2006. Long-term study of re-infection following successful eradication of Helicobacter pylori infection. Aliment Pharmacol. Ther., 23: 1355-1358. http://www.ncbi.nlm.nih.gov/pubmed/16629941
17. Yilmaz, M., C.O. Kara, I. Kaleli, M. Demir, F. Tumkaya and A.S. Buke et al., 2004. Are tonsils a reservoir for Helicobacter pylori infection in children? Int. J. Pediatr. Otorhinolaryngol., 68: 307-310. http://www.ncbi.nlm.nih.gov/pubmed/15129940
18. Unver, S., U. Kubilay, O.S. Sezen and T. Coskuner, 2001. Investigation of Helicobacter pylori colonization in adenotonsillectomy specimens by means of the CLO test. Laryngoscope, 111: 2183-2186. http://www.ncbi.nlm.nih.gov/pubmed/11802022
19. Aslan, S., I. Yilmaz, N. Bal, M. Sener, R. Butros and B. Demirhan et al., 2007. Investigation of Helicobacter pylori in tonsillary tissue with pronto dry test and pathologic examination. Auris Nasus Larynx, 34: 339-342. http://www.ncbi.nlm.nih.gov/pubmed/17196780