

## The role of adenotonsillar tissues as a reservoir for *Helicobacter pylori* and *Helicobacter hepaticus*

Iraj Aliakbari<sup>1</sup>, Saeidollah Noohi<sup>1</sup>, Seyed Abbas Safavi<sup>1</sup>, Ali Goljanian Tabrizi<sup>1</sup>, Mehdi Bolfion<sup>1</sup>, Maryam Razaghi<sup>1</sup>, Hossein Goudarzi<sup>2</sup>, Hossein Dabiri<sup>2</sup>

<sup>1</sup>Department of Ear, Nose, Throat, Shahid Beheshti University of Medical Sciences, Tehran, Iran

<sup>2</sup>Departments of Medical Microbiology, Faculty of Medicine, Shahid Beheshti University of Medical Sciences, Tehran, Iran

### ABSTRACT

**Aim:** The aim of current study is to investigate whether tonsillar and/or adenoid tissue of patients with chronic adenotonsillitis plays a reservoir role for *Helicobacter pylori* (*H. pylori*) or *Helicobacter hepaticus* (*H. hepaticus*).

**Background:** Recently, there have been arguments regarding *Helicobacter pylori* (*H. pylori*) being reserved in adenotonsillar tissue.

**Patients and methods:** This study was performed with 90 patients with the diagnosis of chronic tonsillitis and adenoid hypertrophy, mean age  $36 \pm 22$ , 32 (36%) female and 58(64%) male. Presence of *H. pylori* and *H.hepaticus* were detected by *glmM* gene and 16S rRNA specific primers respectively.

**Results:** Of all patients 58 (65%) were found seropositive for *H. pylori* IgG while only 7(8%) patients had gentile gastrointestinal (GI) symptom, all gastritis. *H. pylori* and *H.hepaticus* was not detected in any of the patients by PCR.

**Conclusion:** There was no correlation between GI symptom and/or seropositivity of *H. pylori* with presence of *H. pylori* and *H. hepaticus* in adenotonsillar tissues. Our results did not support the role of adenotonsills as a reservoir for *H. pylori* or *H. hepaticus*.

**Keywords:** *Helicobacter pylori*, *Helicobacter hepaticus*, Adenotonsil, PCR.

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### Introduction

It is well documented that *Helicobacter pylori* (*H. pylori*) infection is worldwide, however, the precise transmission way have not been fully determined and remains vague. Transmission via the fecal-oral and oral – oral transmission is believed as the major mechanism of *H. pylori* distribution (1-3). *H. pylori* has been detected in the oral cavity samples from supragingival plaque, dental plaque and saliva by polymerase chain

reaction (3-7), but culture of the bacteria from oral cavity samples has been very rare. More recently, it has also been suggested that adenotonsillar tissues may act as a reservoir for *H. pylori*, thereby several studies evaluated the presence of *H. pylori* in adenoid and tonsil specimens (8, 9). There is a hypothesis that tonsillectomy may provide protection against *H. pylori* infection of the stomach since the tonsils may have a role as reservoirs for recurrent *H. pylori* infections (10). If infection of tonsillar tissue does indeed have an impact on *H. pylori* colonization of the gastric mucosa, clinicians would benefit from having

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**Reprint or Correspondence:** Hossein Dabiri, MSPH, PhD.  
Departments of Medical Microbiology, Faculty of Medicine,  
Shahid Beheshti University of Medical Sciences, Iran

**E-mail:** hdabiri@razi.tums.ac.ir

access to an easy, sensitive, and specific method of verifying the presence of *H. pylori* in tonsillar tissue. Eradicating known *H. pylori* from tonsillar tissue by adenotonsillectomy might make it easier to eradicate it in gastric mucosa.

In addition to *H. pylori*, several non-*pylori* helicobacter species such as *Helicobacter heilmannii*, *Helicobacter felis* and *Helicobacter bizozzeronii* have been detected in the human stomach while some others like *Helicobacter hepaticus* and *Helicobacter bilis*, have been shown in the human enterohepatic tracts (11-16). The original source of non-*pylori* *Helicobacter* species has been reported to be animals including dogs, cats, mice and pigs (13-16) in which they have in some cases been associated with human disease such as gastritis, gastric ulcers, MALT lymphomas and extra gastrointestinal diseases (13, 15-20). These non-*pylori* *Helicobacter* species can be transmitted to humans via close contact and have in some cases been associated with pathology. However the exact route of transmission and possible role of adenotonsillar area as a reservoir of these bacteria remains unknown.

In the current study, we aimed to investigate whether adenoid and/or tonsillar tissue from patients diagnosed with chronic adenotonsillitis was a reservoir for *H. pylori* and/or *H. hepaticus* using molecular techniques. Comparison of the surface versus the core of adenotonsillar tissue was investigated to determine contamination with *H. pylori* and *H. hepaticus*. Informed consent was obtained from all patients, and the protocol was approved by the ethical committee of Research Center for Gastroenterology and Liver Diseases in Shaheed Beheshti University of Medical Science.

## Patients and Methods

Out of 120 patients who underwent adenotonsillectomy in Taleghani General Hospital between April 2009 to February 2010, 90 patients

were included in the study. The criteria for undergoing tonsillectomy was six or more tonsillitis attacks in a year, while the criteria for adenoidectomy was defined as 2/3 blockage of the choana on endoscopy which resulted in obstruction symptoms. All patients underwent adenoidectomy and/or tonsillectomy operations under general anesthesia. In the operating theatre, following excision of the tonsils or adenoids all surgical specimens were washed with sterile normal saline and were cut into the desired sections using a newly sterilized blade under sterile conditions. Three core and three surface samples (overall six with 2-3 mm wide) were obtained from each adenotonsillectomy patients (one from each tonsil and one from the adenoid). Following the preparation of the samples they were immediately sent to microbiology laboratory in sterile screwcap tubes containing normal saline or TE buffer.

The *H. pylori* seroprevalence in selected patients was measured by enzyme-linked immunosorbent assay (ELISA) for the immunoglobulin G (IgG) antibody against *H. pylori* (Pishtaz Teb, Tehran, Iran).

DNA from each *H. pylori* isolate was extracted using a commercially available kit (Qiagen, Hilden, Germany). The *glmM* (*ureC*) gene was used for molecular detection of *H. pylori* strains. The primer sequence of *glmM* used in current study was as follows: forward 5'-GCT TAC TTT CTA ACA CTA ACG CGC-3' and reverse, 5'-GGA TAA GCT TTT AGG GGT GTT AGG GG-3'. *Helicobacter hepaticus* specific primers; forward: 5'-GAAACTGTTACTCTG -3'; reverse: 5'-TCAAGCTCCCCGAAGGG -3' were used to amplify a 405 bp fragment of the 16S rRNA gene (19, 21). Primers PC04 (5'-CAA CTT CAT CCA CGT TCA CC-3') and GH20 (5'-GAA GAG CCA AGG ACA GGT AC-3') were used to amplify a 268-bp fragment of the human  $\beta$ -globin gene as a control gene to monitor specimen processing and

DNA extraction (22). All PCR mixtures were prepared in a volume of 25  $\mu$ L containing 1  $\times$  PCR buffer, 500 nM of each primer, 1.5 mM MgCl<sub>2</sub>; 200  $\mu$ M each dNTP, 1.5U Taq DNA polymerase, and 300 ng DNA sample. The mixtures were placed in a thermocycler (Eppendorf AG 22331, Hamburg, Germany), PCR products were visualized by electrophoresis in 1.5% agarose gel, stained with ethidium bromide, and examined under UV illumination. The primers and methods for *H.pylori* detection were controlled by using same primers, material and methods from gastric biopsies.

The Fisher's exact test and the Chi-square test were used for analysis of categorical data. Analysis was done using Sigma Stat for Windows V2.03 (SPSS, Chicago, IL). A p-value of <0.05 was considered statistically significant.

## Results

All 90 patients underwent adenotonsillectomy included in current study. The age of the patients were  $36 \pm 22$  varied from 6 to 80. Of patients 32 were female (36%) while 58 were male (64%). All patients have not received any antibiotic at least one month prior to adenotonsillectomy. None of the symptoms and physical examination findings supported the existence of any preoperative gastroesophageal reflux disease (GERD). Serology for *H. pylori* IgG showed that 58 (65%) of 90 patients were positive in ELISA test regardless to clinical manifestation. Although by increasing age, we detected an increased prevalence of *H. pylori*, however there was no statically significant correlation between patients' age and seopositivity of *H. pylori* ( $p > 0.05$ ) in our study. Overall 7(8%) patients had gastrointestinal disorder; all gastritis with no sign of ulcer or cancer, mean age  $45 \pm 20$ . We could not find any correlation between GI symptom and other variable such as age and sex. Successful DNA extraction was confirmed by positive PCR

product for the human  $\beta$ -globin gene as an internal control in all of the specimens. However, no *H. pylori* gene was detected in any of the specimens, both in the core and surface of samples. Also, once we performed PCR for *Helicobacter hepaticus* as a member of extra gastrointestinal *Helicobacter spp.* No samples were found positive for DNA in all studied specimens.

## Discussion

*Helicobacter pylori* is a major human pathogen that is estimated to infect half of the worlds' population and more than 90% of the Iranian people (5). *H. pylori* infection is a major cause of chronic gastritis, peptic ulcer disease (PUD), gastric carcinoma (GC) and mucosa associated lymphoid tissue (MALT) lymphoma (23, 24). Although the stomach is believed to be the primary reservoir for *H. pylori*, it has been detected from dental plaque, the oral cavity and saliva (4). Thus, it is possible that the upper aerodigestive tract including tonsil and adenoid tissue might act as a reservoir for *H. pylori* especially for oral-to-oral mode of transmission. This idea is based on the results of previous studies, in which the bacteria were detected in dental plaques, oral lesions and saliva (4, 25, 26). Determination of *H. pylori* colonization can be made either invasively using culture of biopsy specimens collected at endoscopy or noninvasively using serologic analysis, the rapid urease test (RUT), CLO-test (commercially prepared RUT) or molecular techniques such as PCR (polymerase chain reaction). Of advantage of PCR, that it is able to detect very low numbers of microorganisms, including the non- cultivable coccoid form of *H. pylori*. Also if highly specific primers are used, PCR does not cross react with other urease producing organisms as has been observed using RUT. Thereby PCR is one of the best choices for detection of *H. pylori* and other microorganisms of

interest based on their specific genes targets. The results of the current study would suggest that adenotonsillar tissue is not a reservoir for *H. pylori*. This finding is consistent with previous studies which failed to detect *H. pylori* colonization of adenotonsillar samples (27-30). In contrast, a number of studies have reported the detection of a high prevalence of *H. pylori* in adenotonsillar tissues (9, 31, 32). Of reasons may account for the divergent results in detection of *H. pylori* from adenotonsillar specimen in different studies, might be the type of *H. pylori* detection method as main issue for such diversity. Examination of previous studies shows that in the majority of those in which *H. pylori* was detected in in adenotonsillar tissues, the RUT (rapid urease test) was employed (9, 32). Given the abundance of bacteria in the non- acidic oral cavity the UBT is likely to lead to a high rate of false-positive results due to the known presence of other urease producing bacteria (30, 33, 34). Thus the high rates of positivity in the studies conducted using only RUT test are likely to be unreliable. In the current study, the primers, materials and methods were controlled as by using same material and methods we were able to detect *H. pylori* from gastric biopsy sources (35-37), Therefore, we think that the negative results for *H. pylori*, observed in this study, are not caused by the used material or techniques.

Recently increasing evidence that non-pylori helicobacters may play a role in human gastrointestinal and extra gastrointestinal disease has been reported. To date, several studies have investigated the adenotonsillar tract as reservoir of *H. pylori*, while none have examined these tissues as possible reservoirs for *non-pylori helicobacter* isolates. Thereby we considered *H. hepaticus* in our study program. Similar to *H.pylori* results, all samples were negative for *H. hepaticus* too, which shows that this bacteria like to *H.pylori* do not colonize on adenotonsillar tissue surface or core.

As our knowledge, this is the first report regard to study of *non-pylori helicobacter* strain in adenoid and tonsils tissues. In conclusion, the fail of current study to detect any positive PCR result for *H. pylori* DNA is in agreement with majority of studies which have excluded the possible role of tonsils and adenoid as reservoir of *H.pylori* infection. Also we could not detect any *H. hepaticus* colonized on adenotonsillar tissues which result out the possible role of adeoid and tonsils as a reservoir for this bacterium.

## References

1. Zali MR. Facing resistance of H. pylori infection. Gastroenterol Hepatol Bed Bench 2011; 4: 3-11.
2. Azevedo NF, Guimaraes N, Figueiredo C, Keevil CW, Vieira MJ. A new model for the transmission of Helicobacter pylori: role of environmental reservoirs as gene pools to increase strain diversity. Crit Rev Microbiol 2007; 33: 157-69.
3. Azevedo NF, Huntington J, Goodman KJ. The epidemiology of *Helicobacter pylori* and public health implications. Helicobacter 2009; 14: 1-7.
4. Momtaz H, Souod N, Dabiri H. Comparison of the virulence factors of *Helicobacter pylori* isolated in stomach and saliva in Iran. Am J Med Sci 2010; 340: 345-49.
5. Alborzi A, Soltani J, Pourabbas B, Oboodi B, Haghghat M, Hayati M, et al. Prevalence of *Helicobacter pylori* infection in children (south of Iran). Diagn Microbiol Infect Dis 2006; 54: 259-61.
6. Czesnikiewicz-Guzik M, Bielanski W, Guzik TJ, Loster B, Konturek SJ. *Helicobacter pylori* in the oral cavity and its implications for gastric infection, periodontal health, immunology and dyspepsia. J Physiol Pharmacol 2005; 56: 77-89.
7. Liu Y, Yue H, Li A, Wang J, Jiang B, Zhang Y, et al. An epidemiologic study on the correlation between oral *Helicobacter pylori* and gastric *H. pylori*. Curr Microbiol 2009; 58: 449-53.
8. Agirdir BW, Bozova S, Derin AT, Turhan M. Chronic otitis media with effusion and *Helicobacter pylori*. Int J Pediatr Otorhinolaryngol 2006; 70: 829-34.
9. Unver S, Kubilay U, Sezen OS, Coskuner T. Investigation of *Helicobacter pylori* colonization in

- adenotonsillectomy specimens by means of the CLO test. *Laryngoscope* 2001; 111: 2183–86.
10. Minocha A, Raczkowski CA, Richards RJ. Is a history of tonsillectomy associated with a decreased risk of *Helicobacter pylori* infection? *J Clin Gastroenterol* 1997; 25: 580–82.
  11. Owen RJ. *Helicobacter*- species classification and identification. *Br Med Bull* 1998; 54: 17–30.
  12. Trebesius K, Adler K, Vieth M, Stolte M, Haas R. Specific detection and prevalence of *Helicobacter heilmannii*-like organisms in the human gastric mucosa by fluorescent in situ hybridization and partial 16s ribosomal DNA sequencing. *JCM* 2001; 39: 1510–16.
  13. Fox JG. The non- *H. pylori* helicobacters: their expanding role in gastrointestinal and systemic diseases. *Gut* 2002; 50: 273–83.
  14. Neiger R, Dieterich C, Burnens A, Waldvogel A, Corthésy-Theulaz I, Halter F, et al. Detection and prevalence of *Helicobacter* infection in pet cats. *J Clin Microbiol* 1996; 36: 634–37.
  15. Nilsson HO, Pietroiusti A, Gabrielli M, Zocco MA, Gasbarrini G, Gasbarrini A. *Helicobacter pylori* and extragastric diseases— other helicobacters. *Helicobacter* 2005; 10: 54–65.
  16. Goldman CG, Mitchell HM. *Helicobacter* spp. other than *Helicobacter pylori*. *Helicobacter* 2010; 15: 69–75.
  17. O'Rourke JL, Grehan M, Lee A. Non-*pylori* *Helicobacter* species in humans. *Gut* 2001; 49: 601–606.
  18. Okiyama Y, Matsuzawa K, Hidaka E, Sano K, Akamatsu T, Ota H. *Helicobacter heilmannii* infection: clinical, endoscopic and histopathological features in Japanese patients. *Pathol Int* 2005; 55: 398–404.
  19. Hamada T, Yokota K, Ayada K, Hirai K, Kamada T, Haruma K, et al. Detection of *Helicobacter hepaticus* in human bile samples of patients with biliary disease. *Helicobacter* 2009; 14: 545–51.
  20. Takayama S, Takahashi H, Matsuo Y, Okada Y, Takeyama H. Effect of *Helicobacter bilis* infection on human bile duct cancer cells, *Dig Dis Sci* 2010; 55: 1905–10.
  21. Fox JG, Dewhirst FE, Shen Z, Feng Y, Taylor NS, Paster BJ, et al. Hepatic *Helicobacter* species identified in bile and gallbladder tissue from Chileans with chronic cholecystitis. *Gastroenterology* 1998; 114: 755–63.
  22. Saiki RK, Scharf S, Faloona F, Mullis KB, Horn GT, Erlich HA. Enzymatic amplification of beta-globin genomic sequences and restriction site analysis for diagnosis of sickle cell anemia. *Science*. 1985; 230: 1350–54.
  23. Wotherspoon AC, Ortiz-Hidalgo C, Flazon MR, Isaacson PG. *Helicobacter pylori*-associated gastritis and primary B-cell gastric lymphoma. *Lancet* 1991; 338: 1175–76.
  24. Marshall BJ, Warren JR. Unidentified curved bacilli in the stomach of patients with gastritis and peptic ulceration. *Lancet* 1984; 1: 1311–15.
  25. Carr MM, Poje CP, Ehrig D, Brodsky LS. Incidence of reflux in young children undergoing adenoidectomy. *Laryngoscope* 2001; 111: 2170–72.
  26. Birek C, Grandhi R, McNeill K, Singer D, Ficarra G, Bowden G. Detection of *Helicobacter pylori* in oral aphthous ulcers. *J Oral Pathol Med* 1999; 28: 197–203.
  27. Skinner LJ, Winter DC, Curran AJ, Barnes C, Kennedy S, Maguire AJ, et al. *Helicobacter pylori* and tonsillectomy. *Clin Otolaryngol* 2001; 26: 505–509.
  28. Yilmaz MD, Aktepe O, Cetinkol Y, Altuntaş A. Does *Helicobacter pylori* have role in development of otitis media with effusion? *Int Pediatr Otorhinolaryngol* 2005; 69: 745–49
  29. Vilarinho S, Guimarães NM, Ferreira RM, Gomes B, Wen X, Vieira MJ, et al. *Helicobacter pylori* colonization of the adenotonsillar tissue: fact or fiction? *Int J Pediatr Otorhinolaryngol* 2010; 74: 807–11.
  30. Eyigor M, Eyigor H, Gultekin B, Aydin N. Detection of *Helicobacter pylori* in adenotonsillar tissue specimens by rapid urease test and polymerase chain reaction. *Eur Arch Otorhinolaryngol* 2009; 266: 1611–13.
  31. Cirak MY, Ozdek A, Yilmaz D, Bayiz U, Samim E, Turet S. Detection of *Helicobacter pylori* and its *CagA* gene in tonsil and adenoid tissues by PCR. *Arch Otolaryngol Head Neck Surg* 2003; 129: 1225–29.
  32. Khademi B, Imanieh MH, Gandomi B, Yeganeh F, Niknejad N. Investigation of *H. pylori* colonization in adenotonsillectomy specimens by means of rapid urease (CLO) Test. *Iran J Med Sci* 2005; 30: 138–40.
  33. Bitar M, Mahfouz R, Soweid A, Racoubian E, Ghasham M, Zaatari G, et al. Does *Helicobacter pylori* colonize the nasopharynx of children and contribute to their middle ear disease? *Acta Otolaryngol* 2006; 126: 154–59.
  34. Brandi G, Biavati B, Calabrese C, Granata M, Nannetti A, Mattarelli P, et al. Urease-positive bacteria other than *Helicobacter pylori* in human gastric juice and mucosa. *Am J Gastroenterol* 2006; 101: 1756–61.

35. Dabiri H, Bolfion M, Mirsalehian A, Rezadehbashi M, Jafari F, Shokrzadeh L, et al. Analysis of *Helicobacter pylori* genotypes in Afghani and Iranian isolates. *Pol J Microbiol* 2010; 59: 61-66.

36. Dabiri H, Maleknejad P, Yamaoka Y, Feizabadi MM, Jafari F, Rezadehbashi M, et al. Distribution of *Helicobacter pylori* cagA, cagE, oipA and vacA in

different major ethnic groups in Tehran, Iran. *J Gastroenterol Hepatol* 2009; 24: 1380-86.

37. Rostami Nejad M, Rostami K, Yamaoka Y, Mashayekhi R, Molaei M, Al Dulaimi D, et al. Clinical and histological presentation of *Helicobacter Pylori* and gluten related Gastroenteropathy. *Arch Iran Med* 2011; 14: 115-19.