State of the Globe: Diagnostic Tests to Detect *Helicobacter Pylori* Tonsillitis

Infection with *Helicobacter pylori* is a worldwide public health problem which chronically affects the gastric system. Asymptomatic infection with this bacterial organism has been widely reported; however, its association with gastritis, peptic ulcers, and gastric cancer[1] has been frequently recorded in the literature. The presence of *H. pylori* in the upper aerodigestive tracts and locations including oral cavity[2] and saliva,[3,4] tonsils, and adenoid glands,[5,6] nose and sinuses mucus,[7-10] and even in middle ear[11] has been documented.

Some researchers believe that the tonsil and the adenoid tissue may constitute a reservoir of the *H. pylori* colonization and consecutive oropharyngeal and gastric infections and clinical outcomes;[12-15] whereas, others reported controversial findings.[16-18] To resolve this debate, also to determine the significance of *H. pylori* tonsillitis, diagnosis of the infection in these tissues should be improved. This editorial writing is to summarize the state of the globe in diagnostic tests applied for the detection of *H. pylori* tonsillitis.

A variety of laboratory methods, mostly rapid urease test (RUT), conventional PCR and real-time PCR, have been used for the detection of *H. pylori* in the clinical samples of suspected patients.

The RUT is easy to use and has been broadly used as one of the most conventional methods for the diagnosis of *H. pylori* infections in clinical samples. However, the specificity of RUT was found insufficient when used in the detection of throat and tonsil specimens.[19,20] Urease production by other bacterial species colonized in tonsil, throat, and mouth is documented; this can interfere with the RUT, leading to low specificity for the detection of *H. pylori*.[21] Yilmaz *et al.* used the CLO-test on the adenotonsillectomy specimens obtained from 50 children; they found no infection in the specimens, though the *H. pylori* Ag was detected in the stools of 50% of the children.[16]

Access this article online	
Quick Response Code:	Website: www.jgid.org
	DOI: 10.4103/0974-777X.96765

In another study, Eyigor and colleagues examined 47 patients with chronic tonsillitis and adenoid hypertrophy for the detection of *H. pylori* using RUT and found only 5.5% positivity.[20] Due to the lack of gold standard, it is difficult to discuss about the accurate validity of this test in this study. Less accuracy of RUT compared to PCR in detection of *H. pylori* in adenotonsillectomy specimens was observed in a study sample of 20 children with the specificity of only 56%;[15] in this study, samples with consistent *H. pylori* positive with both RUT and PCR were considered as a "gold standard". There is not yet a single method capable of serving as a "gold standard" for detection of the infection in upper digestive tract samples, but a combination of two or three methods can confirm actual positive samples to establish a gold standard for the methodology surveys.

Immunoflourescence, immunoelectron microscopy, and application of cytotoxin-associated antigen A are also of important and useful methods for investigation of *H. pylori* in the clinical materials. These have been used by Kusano *et al.* in a survey of 55 tonsillectomy specimens from recurrent pharyngotonsillitis or immunoglobulin A (IgA) nephropathy and detected the organism in 78.2% of the samples.[14] They found a significant association between tonsillar and gastric *H. pylori* infections, also concluded that tonsillar *H. pylori* may be one of the antigens causative of IgA nephropathy. In an earlier study on palatine tonsil of pharyngotonsillitis and IgA nephropathy patients, using immuno-tissue based techniques and *in situ* hybridization, Kusano *et al.* found similar results.[22] Light microscopy seems to be an inaccurate technique for *H. pylori* diagnosis. Aslan and colleagues could not detect it in pronto dry test positive tonsil tissues stained with hematoxylin–eosin, Giemsa, or Warthin–Starry silver stains.[23]

Culture and isolation of the organism from clinical samples is difficult. Kusano *et al.* used conventional cultures for *H. pylori* with different circumstances,[14] but the culture failed to detect and isolate the organism in the same specimens that were positive at 78.2% by the other methods described earlier. In contrast with this study, isolation of *H. pylori* from culture media inoculated by tonsillar specimens was successful in a few studies.[24,25] In a recent study, Wibawa *et al.*[26] utilized the culture method in combination with other methods, including immunohistochemistry and

modified Giemsa stained histological survey; they could isolate *H. pylori* and found bacterial colonization in 15.7% of tonsillar tissues of chronic tonsillitis cases.

PCR-based methods have been also considered by several workers to detect *H. pylori*; satisfactory specificity has been more frequently reported with these techniques in comparison with the RUT. However, insufficient specificity with the conventional PCR, targeting *ureC* gene, is also reported.[27] Studies on different clinical samples, including gastric juice[28] and peptic ulcer,[29] demonstrated a high sensitivity with the PCR assay. PCR was also used for *H. pylori* detection in adenotonsillar samples and presented good results.[13,15] However, no PCR test was found to be positive when used for investigation of 47 samples of chronic tonsillitis using *glmM* gene.[20,30] It should be noted that only three samples were positive with the RUT test in this study and no other confirmatory method has been used here, therefore the PCR sensitivity cannot be discussed based on these study data.

Both the real time PCR and the scorpion real time PCR have been reported to show high specificity and sensitivity for the detection of *H. pylori* in various clinical samples.[31] The scorpion real-time PCR has been used by a number of researchers.[12,13,21,32,33] Utilization of the conventional PCR and the scorpion real time PCR in comparison with URT showed a strong agreement between PCR-based methods in detection of the bacterium DNA in the specimens of chronic tonsillitis and found the scorpion real-time PCR as a preferred method for diagnosis of such specimens.[34]

The PCR-based methods, in particular the real-time PCR and the scorpion real-time PCR, proved to be more valid comparing to the other methods, i.e. RUT, immunohistochemistry, light microscopy, and *in vitro* cultures. However, the precise validity of the available diagnostic methods for the detection of tonsillitis *H. pylori* in the relevant clinical samples require further evaluation with characterization and usage of a gold standard.

The PCR technology provided the tools for genotype characterization of the *H. pylori* isolates with concern to a variety of resolutions, i.e. pathogenic relevance, common or different colonization sources, drug resistance, etc. There are many studies focused on gastric isolates using *cagA* and *vacA* genes, but this sort of studies on upper digestive tract isolates and the pertaining data are limited. A report by Cirak *et al.* showed 71% cagA in the *H. pylori*-positive adenotonsillar specimens.[12] In another genotyping, Bulut *et al.* found association of cagA(+) *H. pylori* with adenotonsillar hypertrophy.[13] In addition, Pavlik *et al.* found isolates from otorhinolaryngology patients having different genotypes from those of other patients.[32] Genotype characterization of *H. pylori* isolates from tonsil in comparison with those of peptic isolates may be of important requirements to examine possible connection between the two sources of the bacterial colonization and their contribution to the relevant diseases.

In addition to the suitability for bacterial detection, the molecular methods showed to be an appropriate alternative to the traditional phenotypic methods in distinguishing drug (clarithromycin)-resistant isolates. Elviss *et al.* used 3'-mismatched reverse primer PCR in comparison with real-time PCR and PCR-RFLP developed earlier.[35] The DNA-based techniques completely correlated with the traditional methods.[36] A single-step PCR process based on the scorpion primer technology was used to discriminate between resistant and/or sensitive-associated specific alleles.[36] As one of the important diagnostic dimensions, methods for identification of drug-resistant isolates of *H. pylori* require further development.

In overall oropharyngeal *H. pylori* investigations, the attempts resulted in valuable findings that provided insight into the issue; however, small size samples in some studies may interfere with the appropriate interpretation of methodological diagnostic investigations of tonsil-related *H. pylori* infections. It worth noting that, to date, a single method may not be exclusively relied for detection of oropharyngeal *H. pylori* and a combination of diagnostic methods can be recommended.

Asghar Fazaeli

Department of Medical Parasitology, School of Medicine, Zanjan University of Medical Sciences and Health Services, Zanjan, Iran

Address for correspondence:
Dr. Asghar Fazaeli, E-mail: afparm1@yahoo.co.uk

REFERENCES

1. Dunn BE, Cohen H, Blaser MJ. *Helicobacter pylori*. Clin Microbiol Rev 1997;10:720-41.
2. Skinner LJ, Winter DC, Curran AJ, Barnes C, Kennedy S, Maguire1 AJ, *et al. Helicobacter pylori* and tonsillectomy. Clin Otolaryngol Allied Sci 2001;26:505-9.
3. Zhang C, Yamada N, Wu YL, Wen M, Matsuhisa T, Matsukura N. *Helicobacter pylori* infection, glandular atrophy and intestinal metaplasia in superficial gastric, gastric erosion, erosive gastritis, gastric ulcer and early gastric cancer. World J Gastroenterol 2005;11:791-6.
4. Zhang JP, Peng ZH, Zhang J, Zhang XH, Zhang XH, Zheng QY. *Helicobacter pylori* infection in the pharynx of patients with chronic pharyngitis detected with TDI-FP and modified Giemsa stain. World J Gastroenterol 2006;12:468-72.

5. Oshowo A, Gillam D, Botha A, Tunio M, Holton J, Boulos P, *et al. Helicobacter pylori*; the mouth, stomach and gut axis. Ann Periodontol 1998;3:276-80.
6. Lin HC, Wu PY, Friedman M, Chang HW, Wilson M. Difference of *Helicobacter pylori* colonization in recurrent inflammatory and simple hyperplastic tonsil tissues. Arch Otolaryngol Head Neck Surg 2010;136:468-70.
7. Morinaka S, Ichimiya M, Nakamura H. Detection of *Helicobacter pylori* in nasal and maxillary sinus specimens from patients with chronic sinusitis. Laryngoscope 2003;113:1557-63.
8. Ozdek A, Cirak MY, Samima E, Bayiz U, Safak MA, Turet S. A possible role of *Helicobacter pylori* in chronic rhino sinusitis: A preliminary report. Laryngoscope 2003;113:679-82.
9. Schwartz K, Monsur J, Northrup J, West P, Neale AV. Pharyngitis clinical prediction rules: Effects of inter observer agreement: A Metro Net study. J Clin Epidemiol 2004;57:142-6.
10. Aladag I, Bulut Y, Guven M, Eyibilen A, Yelken K. Seroprevalence of *Helicobacter pylori* infection in patients with chronic non-specific pharyngitis: Preliminary study. J Laryngol Otol 2008;122:61-4.
11. Karlidag T, Bulut Y, Keles E, Kaygusuz I, Yalcin S, Ozdarendeli A, *et al.* Detection of *Helicobacter pylori* in children with otitis media with effusion: A preliminary report. Laryngoscope 2005;115:1262-5.
12. 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;129:1225-9.
13. Bulut Y, Agacayak A, Karlidag C, Toraman ZA, Yilmaz M. Association of cag A+ *Helicobacter pylori* with adenotonsilar hypertrophy. Tohoku J Exp Med 2006;209:229-33.
14. Kusano K, Inokuchi A, Fujimoto K, Miyamoto H, Tokunaga O, Kuratomi Y, *et al.* Coccoid *Helicobacter pylori* exists in the palatine tonsils of patients with IgA nephropathy. J Gastroenterol 2010;45:406-12.
15. Abdel-Monem MH, Magdy EA, Nour YA, Harfoush RA, Ibreak A. Detection of *Helicobacter pylori* in adenotonsillar tissue of children with chronic adenotonsillitis using rapid urease test, PCR and blood serology: A prospective study. Int J Pediatr Otorhinolaryngol 2011;75:568-72.
16. Yilmaz M, Kara CO, Kaleli I, Demir M, Tümkaya F, Büke AS, *et al.* Are tonsils a reservoir for *Helicobacter pylori* infection in children? Int J Pediatr Otorhinolaryngol 2004;68:307-10.
17. Jelavic B, Bevanda M, Ostojic M, Leventic M, Vasilj M, Knezevic E. Tonsillar colonization is unlikely to play important role in *Helicobacter pylori* infection in children. Int J Pediatr Otorhinolaryngol 2007;71:585-90.
18. Vayisoglu Y, Ozcan C, Polat A, Delialioglu N, Gorur K. Does *Helicobacter pylori* play a role in the development of chronic adenotonsillitis? Int J Pediatr Otorhinolaryngol 2008;72:1497-501.
19. De Korwin JD. Advantages and limitations of diagnostic methods for H. pylori infection. Gastroenterol Clin Biol 2003;27:380-90.
20. Eyigor M, Eyigor H, Gultekin B, Neriman A. Detection of *Helicobacter pylori* in adenotonsiller tissue specimens by rapid urease test and polymerase chain reaction. Eur Arch Otorhinolaryngol 2009;266:1611-13.
21. Jabbari-Moghaddam Y, Rafeey M, Radfar R. Comparative assessment of *Helicobacter pylori* colonization in children tonsillar tissues. Int J Pediatr Otorhinolaryngol 2009;73:1199-201.
22. Kusano K, Tokunaga O, Ando T, Inokuchi A. *Helicobacter pylori* in the palatine tonsils of patients with IgA nephropathy compared with those of patients with recurrent pharyngotonsillitis. Hum Pathol 2007;38:1788-97.
23. Aslan S, Yilmaz I, Bal N, Sener M, Butros R, Demirhan B, *et al.* Investigation of *Helicobacter pylori* in tonsillary tissue with Pronto Dry test and pathologic examination. Auris Nasus Larynx 2007;34:339-42.
24. Fonseca TL, Moraes EP, Juliano CR, Silva AM, Scaini CJ, Mendoza-Sassi RA, *et al.* Detection of *Helicobacter pylori* by phenotypic and genotypic methods. Dig Dis Sci 2010;55:1643-8.
25. Cho AR, Lee MK. A comparison analysis on the diagnosis of *Helicobacter pylori* infection and the detection of clarithromycin resistance according to biopsy sites. Korean J Lab Med 2010;30:381-7.
26. Wibawa T, Surono A, Widodo I. Isolation of viable *Helicobacter pylori* in the tonsillar tissues of chronic tonsillitis patients. J Infect Dev Ctries 2011;5:561-4.
27 Megraud F, Lehours P. *Helicobacter pylori* detection and anti-microbial susceptibility testing. Clin Microbiol Rev 2007;20:280-322.
28. Yakoob J, Rasool S, Abbas Z, Jafri W, Abid S, Islam M, *et al.* Gastric juice for the diagnosis of *H. pylori* infection in patients on proton pump inhibitors. World J Gastroenterol 2008;14:1539-43.
29. Lo CC, Lai KH, Peng NJ, Lo GH, Tseng HH, Lin CK, *et al.* Polymerase chain reaction: A sensitive method for detecting *Helicobacter pylori* infection in bleeding peptic ulcers. World J Gastroenterol 2005;11:3909-14.
30. Dibonaventura G, Neri M, Nerietal G. Do tonsils represent an extra gastric reservoir for *Helicobacter pylori* infection. J Infect 2001;42:221-2.
31. Liu H, Rahman A, Semino-Mora C, Doi SQ, Dubois A. Specific and Sensitive Detection of *H. pylori* in Biological Specimens by Real-Time RT-PCR and *In Situ* Hybridization. PLoS One 2008;3:e2689.
32. Pavlík E, Lukes P, Potuzníková B, Astl J, Hrdá P, Soucek A, *et al. Helicobacter pylori* isolated from patients with tonsillar cancer or tonsillitis chronica could be of different genotype compared to isolates from gastrointestinal tract. Folia Microbiol (Praha) 2007;52:91-4.
33. Naserpour Farivar T, Pahlevan A, Johari P, Safdarian F, Aslani Mehr M, Ahmadpour F, *et al.* Assessment of *Helicobacter pylori* prevalence by scorpion Real-time PCR in chronic tonsillitis patients. J Global Infect Dis 2012;4:38-42.
34. Najafipour R, Naserpour Farivar T, Pahlevan AA, Johari P, Safdarian F, Asefzadeh M. Agreement rate of rapid urease test, conventional PCR, and Scorpion real-time PCR in detecting *Helicobacter pylori* from tonsillar samples of patients with chronic tonsillitis. J Global Infect Dis 2012;4:106-9.
35. Elviss NC, Lawson AJ, Owen RJ. Application of 3'-mismatched reverse primer PCR compared with real-time PCR and PCR-RFLP for the rapid detection of 23S rDNA mutations associated with clarithromycin resistance in *Helicobacter pylori*. Int J Antimicrob Agents 2004;23:349-55.
36. Burucoa C, Garnier M, Silvain C, Fauchère JL. Quadruplex Real-Time PCR Assay Using Allele-Specific Scorpion Primers for Detection of Mutations Conferring Clarithromycin Resistance to *Helicobacter pylori*. J Clin Microbiol 2008;46:2320-6.

How to cite this article: Fazaeli A. State of the globe: Diagnostic tests to detect *Helicobacter pylori* tonsillitis. J Global Infect Dis 2012;4:99-101.