Screening for *Helicobacter pylori* Infection among Patients with Otorhinolaryngological Diseases May Spare Need for Surgical Interference: A PCR Confirmed Study

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Abstract: Objectives: To evaluate the coincidence of *Helicobacter pylori (H. pylori)* infection in patients presenting with varied otorhinolaryngological diseases. **Patients & Methods:** The current survey study included 292 patients; 173 males and 119 females with mean age of 25.9±15.4 years. All patients underwent complete otorhinolaryngological evaluation with special concern to the presenting complaint. Then, all enrolled patients underwent the urea breath test (UBT) using the Heliprobe 14C UBT and the obtained surgical specimens or effusion samples or swabs were examined by polymerase chain reaction (PCR) for detection of *H. pylori* DNA. **Results:** According to results of UBT, 41 patients were *H. pylori* infected, 107 patients had borderline infection and 144 patients were free of infection. PCR examination of *H. pylori* DNA detected 62 positive cases; 33 were positive and 29 were borderline UBT with a true positive rate of 80.5% for positive and 27.1% for borderline UBT. PCR assured *H. pylori* infection in 5 patients had CRS with polyposis, in 9 patients with CRS without polyposis, in 17 patients with tonsillitis and in 10 patients with pharyngitis without tonsillitis. PCR confirmed *H. pylori* infection in 15 patients with chronic otitis media (COM) with effusion and in 6 patients with laryngitis. **Conclusion:** Upper air passages could be considered as reservoir for *H. pylori* that must be considered as an underlying pathogenic mechanism for various otorhinolaryngological disorders and must be excluded prior to surgical decision making. Urea breath test could be considered as a good positive screening test for presence of *H. pylori* infection and must be applied as a routine test at otorhinolaryngological clinics.
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1. Introduction

Helicobacter pylori is a microaerophilic, gram-negative spiral organisms. Several factors have been associated with *H. pylori*'s epithelial damage and aggressiveness, including surface lipopolysaccharide, bacterial urease, flagella, surface adhesins, oxidizing radicals and cytokines produced by leukocytes. Like all gram-negative bacteria, cell wall lipopolysaccharide acts as a potent immunogen agent, stimulating an inflammatory reaction that could ultimately lead to the destruction of endothelial cells. *H. pylori* also possess the cytotoxin-associated gene A (cagA), which interferes with intracellular signaling pathways and mediates phenotypic alterations, strongly evoking neoplasic transformation **(Belair *et al.*, 2009; Parzecka *et al.*, 2009)**.

The unique reservoir of *H. pylori* is the stomach of humans and the potential sources of contamination are vomit, saliva and feces. In infected subjects, *H. pylori* is always present in vomit and can survive a few hours in the environment. Saliva is sometimes positive because of regurgitations, but feces only contain viable organisms when there is a short transit **(Mégraud, 2003; Kindermann and Lopes, 2009)**.

However, there are many articles on the association of *H. pylori* infection with disorders elsewhere in the body; a possible association between *H. pylori* infection and eye diseases, including Sjögren syndrome, blepharitis, central serous chorioretinopathy and uveitis, has been proposed. Moreover, systemic *H. pylori*-induced oxidative damage may be the mechanism which links oxidative stress, *H. pylori* infection and the damage to the trabecular meshwork and optical nerve head that result in glaucoma **(Izzotti *et al.*, 2009)**. **Abdou *et al.* *(2009)*** reported that *H. pylori* may have a role in the exacerbation of urticarial symptoms, even though it is not involved directly in its etiology, and the severity of symptoms is dependent on the density of bacterial infection, and so its eradication may lead to symptom improvement.

Oral cavity as a reservoir for *H. pylori* bacteria was a point of debate, **Dowsett & Kowolik (2003)** documented that oral cavity has been proposed as a reservoir for gastric *H. pylori*, which has been detected by culture and PCR in both dental plaque and saliva, while **Loster *et al.* (2006)** denied such data and documented that oral cavity does not serve as bacterial reservoir to infect gastric mucosa. Later, **Liu *et al.* (2008)** reported that oral cavity may be a reservoir for *H. pylori* infection in children and *H. pylori* in dental plaque may play a role in the occurrence of dental caries, and poor oral hygiene may represent a risk

factor for *H. pylori* in the oral cavity. **Chen *et al.* (2011)** reported that *H. pylori*-positive rate from saliva is closely related to the types of gastrointestinal disease in patients, and it is correlated with the periodontal diseases as well and oral cavity with periodontal diseases is an ecological niche of *H. pylori* which might be an important cause for occurrence and re-occurrence of gastrointestinal disease.

Considering the oral cavity is a common entrance channel for both gastrointestinal and respiratory systems, the current study aimed to evaluate the coincidence of *H. pylori* infection in patients presenting with varied otorhinolaryngological diseases.

2. Patients and Methods

The current survey study was assigned to include all patients presenting to the otorhinolaryngological outpatient clinic of Benha University Hospital since Oct 2010 till Jan 2012, irrespective of age or gender or complaints. All patients underwent complete otorhinolaryngological evaluation with special concern to the presenting complaint.

Then, all enrolled patients underwent the urea breath test (UBT) using the Heliprobe 14C UBT (Kibion Heliprobe System, Stockholm, Sweden). The test validity character for screening of *H. pylori* infection was documented by **Peura *et al.* (1996) and Allardyce *et al.* (1997)** to have sensitivity exceeds 97%, with a specificity of 95%. Heliprobe 1 µCurie ^{14}C capsule was given, after 10 min, patients were asked to blow (only exhaled) into the Heliprobe breath-Card until the card indicator of the breath card changed from orange to a yellow color. Then, the Breath-Card was inserted into the small desktop GM-counter (Heliprobe Analyser). The results were graded as Grade 0: not infected; Grade 1: borderline; and Grade 2: infected.

All patients received the appropriate management according to the diagnosis of their complaints after completion of respective investigations. The obtained surgical specimens or effusion samples or swabs were examined by polymerase chain reaction (PCR) for detection of *H. pylori* DNA.

PCR assay:

The chromosomal DNA of *H. pylori* was prepared by physical rupture using a Disruptor Genie cell disrupter with zirconium and tungsten carbide beads. Briefly, the homogenized material was mixed with an equal amount of PBS and centrifuged at 12,000 g for 10 min. Subsequently, 300 ml lysis buffer (50 mM Tris/HCl, pH 8.0; 1 mM EDTA; and 1% SDS), 0.3 g zirconium beads and a tungsten carbide bead (3 mm diameter) were added to the precipitate, which was then boiled at 100^{o}C for 10 min. Then, bacteria were ruptured using a cell disrupter at room temperature for 3 min. The DNA was then purified by repeated phenol/chloroform extraction, precipitated with 100% ethanol and re-suspended in 100 ml TE (10 mM Tris/HCl, pH 8.0; and 1 mM EDTA). Each 10 ml volume of the PCR mixture contained 0.25 mM deoxynucleotide triphosphates, 10 mM Tris/HCl (pH 8.3), 50 mM KCl, 2 mM $MgCl_2$, 5 U Taq polymerase, 2 mM primer and 2 ml template DNA. Amplification was conducted using a PCR thermal cycler with the following temperature profile: 94 uC for 2 min, followed by 30 cycles of 94 uC for 10 s, 55 uC for 15 s and 72 uC for 1 min, with a final extension at 72 uC for 5 min. The amplified products were loaded onto 1.5% (w/v) agarose gels, separated by electrophoresis, stained with ethidium bromide (0.5 mg ml21) and photographed under UV light. The following primer with 5' to 3' sequence was used after **Nyan *et al.* (2004)**:

Forward: CTATGACGGGTATCCGGC
Reverse: ATTCCACCTACCTCTCCCA

3. Results

The study included 292 patients; 173 males (59.2%) and 119 females (40.8%) with mean age of 25.9±15.4; range: 4-64 years. Age strata and gender distribution of enrolled patients were shown in table 1. Patients' distribution according to definite otorhinolaryngological diagnosis was shown in table 2.

According to results of UBT, 41 patients were infected by *H. pylori* (grade 2), 107 patients had borderline *H. pylori* infection and 144 patients were free of *H. pylori* infection (grade 0). PCR examination of *H. pylori* DNA, (Fig. 1) in obtained specimens, biopsies, effusion or discharge detected 62 positive cases; 33 of those had positive UBT and 29 of those had borderline UBT with a true positive rate of 80.5% for positive UBT and 27.1% for borderline UBT.

There was variant distribution of patients with different professional diagnoses among grades of *H. pylori* infection. About 30% of patients had chronic rhinosinusitis with or without polyposis gave positive UBT; about 26% of patients had pharyngitis and/or tonsillitis gave positive UBT; about 19% of patients had otitis media with effusion gave positive UBT and about 10% of patients had laryngitis gave positive UBT (Table 3).

Among patients had CRS with polyposis, PCR assured *H. pylori* infection in 5 patients; 2 had UBT grade I and 3 had UBT grade II and in 9 patients with CRS without polyposis; 5 had UBT grade I and 4 had UBT grade II with a collective positive PCR rate of 77.8% and about 30% of patients had CRS with or without polyposis. Among patients had pharyngitis with tonsillitis, PCR assured *H. pylori* infection in 17 patients; 7 had UBT grade I and 10 had UBT grade II and in 10 patients with pharyngitis without tonsillitis; 4 had UBT grade I and 6 had UBT grade II with a collective positive PCR rate of 48.6% and 35.7% of patients had pharyngitis with or without tonsillitis.

PCR confirmed *H. pylori* infection in 15 patients with COM with effusion; 10 had UBT positive and 5 had borderline UBT result. PCR confirmed *H. pylori* infection in 6 patients with laryngitis; 2 had UBT positive and 4 had borderline UBT result with a collective positive PCR rate of 37.5% and 40% of patients had chronic otitis media with effusion and laryngitis, respectively (Table 4).

Table (1): Patients' enrollment data

			Number (%)	Mean±SD
Age (years)	Strata	<10 years	51 (17.5%)	7.4±1.4 (4-9)
		>10-15 years	52 (17.8%)	13.1±1.3 (11-15)
		>15-25 years	55 (18.8%)	20.5±3 (16-25)
		>25-35 years	60 (20.5%)	31.3±2.9 (26-35)
		>35-45 years	41 (14.1%)	40.3±3.3 (36-45)
		>45-55 years	16 (5.5%)	50.8±2.7 (46-54)
		>55 years	17 (5.8%)	59.9±2.7 (56-64)
	Total		292 (100%)	25.9±15.4 (4-64)
Gender	Males		173 (59.2%)	
	Females		119 (40.8%)	

Data are presented as mean±SD & numbers; ranges & percentages are in parenthesis

Table (2): Patients' distribution according to professional diagnosis

Diagnosis	Number (%)
Chronic otitis media with effusion	69 (23.6%)
Tonsillitis	63 (21.6%)
Pharyngitis	57 (19.5%)
Chronic rhinosinusitis	52 (17.8%)
Laryngitis	31 (10.6%)
Chronic rhinosinusitis with polyposis	20 (6.8%)
Total	292 (100%)

Data are presented as numbers; percentages are in parenthesis

Table (3): Patients' distribution according to professional diagnosis

Diagnosis		Grade 0	Grade I	Grade II	Total
Chronic rhinosinusitis	with polyposis	11 (55%)	5 (25%)	4 (20%)	20 (100%)
	without polyposis	31 (57.7%)	16 (32.7%)	5 (9.6%)	52 (100%)
Pharyngitis	With tonsillitis	28 (36.5%)	24 (46%)	11 (17.5%)	63 (100%)
	Without tonsillitis	29 (43.8%)	23 (47.4%)	5 (8.8%)	57 (100%)
Chronic otitis media with effusion		29 (34.8%)	27 (46.4%)	13 (18.8%)	69 (100%)
Laryngitis		16 (41.9%)	12 (48.4%)	3 (9.7%)	31 (100%)
Total		144 (49.4%)	107 (36.6%)	41 (14%)	292 (100%)

Data are presented as numbers; percentages are in parenthesis

Table (4): PCR positive results among patients had borderline (grade I) and positive (grade II) UBT

Diagnosis	UBT Grade I	PCR	UBT Grade II	PCR	Total PCR +ve
CRS with polyposis	5 (25%)	2 (40%)	4 (20%)	3 (75%)	5 (55.6%)
CRS	16 (32.7%)	5 (31.3%)	5 (9.6%)	4 (80%)	9 (40.9%)
Pharyngitis with tonsillitis	24 (46%)	7 (29.2%)	11 (17.5%)	10 (90.9%)	17 (48.6%)
Pharyngitis	23 (47.4%)	6 (26.1%)	5 (8.8%)	4 (80%)	10 (35.7%)
COM with effusion	27 (46.4%)	5 (22.2%)	13 (18.8%)	10 (84.6%)	15 (37.5%)
Laryngitis	12 (48.4%)	4 (30%)	3 (9.7%)	2 (66.7%)	6 (40%)
Total	107 (36.6%)	29 (27.1%)	41 (14%)	33 (80.5%)	62 (41.9%)

Data are presented as numbers; percentages are in parenthesis

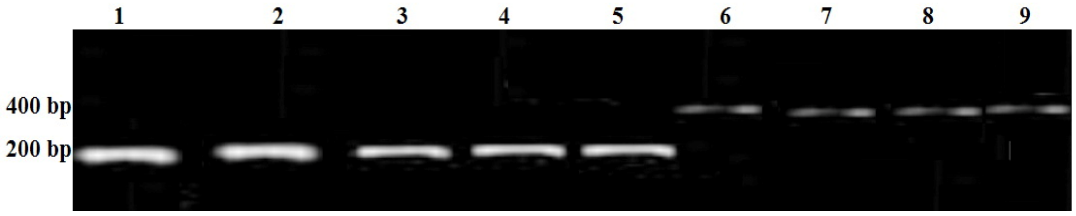

Fig (1): Agarose gel electrophoresis stained with ethidium bromide showing *H. pylori* DNA after digestion by restriction endonucleases. Lanes 1, 2: MM homozygous genotype, Lane 3: TT homozygous genotype, Lanes 4-6: MT heterozygous genotype, M: Molecular DNA marker

4. Discussion

Among studied with variant otorhinolaryngeal complaint and were free of GERD; 41 patients were UBT positive and 107 patients were UBT borderline with a detection rate of 14% for infected cases and 36.7% for cases with query infection. PCR examination of *H. pylori* DNA in obtained specimens detected 62 positive cases; 33 had positive and 29 had borderline UBT. These data point to a fact that upper air passages could be considered as reservoir for *H. pylori* and that there is a coincidence between *H. pylori* infection and presence of otorhinolaryngological diseases.

PCR assured *H. pylori* infection in 77.8% and about 30% of patients had CRS with or without polyposis who gave positive and borderline UBT, respectively. In line with these figures; **Jelavic *et al.* (2012)** analyzed nasal polyps of 40 patients with CRS, undergoing functional endoscopic sinus surgery for the presence of *H. pylori* using immunohistochemistry and found that nasal polyps in 28 (70%) patients were positive for *H. pylori*.

Among patients had tonsillitis or pharyngitis who gave positive or borderline UBT, PCR detected *H. pylori* infection in 48.6% and 35.7% of patients, respectively; such result go in hand with **Dağtekin-Ergür *et al.* (2008)** who detected the presence of *H. pylori* in palatine and pharyngeal tonsil tissues of children using rapid urease test and immunohistochemical analysis and suggested that these tissues can be a reservoir for *H. pylori*. **Aladag *et al.* (2008)** found patients with chronic nonspecific pharyngitis have a significantly higher rate of *H. pylori* seropositivity than control subjects. **Kaptan *et al.* (2009)** detected *H. pylori* colonization in mucosa of 34.3% of chronic pharyngitis patients with gastric *H. pylori* infection and in 20% of patients without gastric infection. **Lin *et al.* (2010)** found *H. pylori* can colonize in human palatine tonsil tissues and a significantly higher positive *H. pylori* rate was present in patients who underwent tonsillectomy for chronic recurrent tonsillitis (48%) compared with those had tonsillectomy for sleep-related breathing disorders (24%). **Abdel-Monem *et al.* (2011)** also, reported that adenotonsillar tissue may constitute an extra-gastric reservoir for *H. pylori* in symptomatic children with chronic adenotonsillitis.

PCR confirmed *H. pylori* infection in 6 patients with laryngitis; 2 had UBT positive and 4 had borderline UBT result. Such outcome goes in hand with **Gong *et al.* (2010)** who reported that *H. pylori* infection as confirmed by PCR exists in the laryngeal mucosa, but with the higher rate of infection in the patients with laryngeal squameous cell carcinoma than that in the patients with benign laryngeal lesions and suggested that *H. pylori* may be one of pathogenic factors of laryngeal squameous cell carcinoma. Moreover, **Tiba *et al.* (2010)** detected 23S ribosomal RNA gene of *H. pylori* by RT-PCR in 10 of 14 patients (71.4%) with vocal fold minimal lesions which are mostly benign.

The data obtained through the current study indicated the possibility of *H. pylori* infection of multiple sites of upper respiratory passages. In support of such assumption, **Ozyurt *et al.* (2009)** detected *H. pylori* DNA by real-time PCR in 59.4% of nasal polyps, 70.4% of nasal mucosa samples, and 58.6% of larynx samples and identified cagA in 78.9, 89.5, and 82.4% of positive polyp, nasal mucosa, and larynx samples, respectively. Recently, **Burduk *et al.* (2012)** reported the presence of *H. pylori* DNA in nasal polyps, concha bullosa and benign larynx diseases and cagA-positive *H. pylori* was observed only in laryngeal tissues and concluded that these results may have implications for a possible role of *H. pylori* in laryngeal diseases.

On controversy of the obtained results and these literature documents; **Vayisoglu *et al.* (2008)** suggested that *H. pylori* would not colonize in tonsil tissue of patients with chronic tonsillitis. **Cvorovic *et al.* (2008)** documented that a possible role of *H. pylori* in CRS with polyposis may be suggested and if a patient has *H. pylori* in his gastric mucosa and is positive for GER symptoms, *H. pylori* may be found in polyps as well. **Ozcan *et al.* (2009)** using Campylobacter-like organism test, immunohistochemical examination on nasal polyp tissue biopsy specimens and serological analysis reported that the suggested role of *H. pylori* in the previous reports regarding nasal polypi may

demonstrate transient occurrence of *H. pylori* and it may not be treated as a possible etiological factor in nasal polypi.

These discrepant results could be attributed to the methodology used for diagnosis of infection; the current study relied on surveying enrolled patients using breath test and the result was confirmed using PCR determination of *H. pylori* DNA considering PCR as the gold standard diagnostic modality. In hand with such reliance on PCR; **Bak-Romaniszyn *et al.* (2007)** compared the detection of *H. pylori* in gastric mucosa in children by conventional method and PCR and reported that PCR technique in mucosa enables to diagnose *H. pylori* infection even in patients with the infection not detected with standard methods. Also, **Weiss *et al.* (2008)** documented that PCR assay identified a significant number of *H. pylori* infections that would not be detected by immunohistochemical analysis and/or breath tests. **Sukhanov *et al.* (2011)** reported that the coefficients of correlation for histologic, cytologic methods and PCR for diagnosis of *H. pylori* was 0.79, 0.34 and 0.53 and with indices of gastritis activity were 0.49, 0.37 and 0.52, respectively.

The obtained results and review of literature allowed concluding that upper air passages could be considered as reservoir for *H. pylori* that must be considered as an underlying pathogenic mechanism for various otorhinolaryngological disorders and must be excluded prior to surgical decision making. Urea breath test could be considered as a good positive screening test for presence of *H. pylori* infection and must be applied as a routine test at otorhinolaryngological clinics.

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