

## Investigation of *Helicobacter pylori* in the Middle Ear of the Patients with Chronic Otitis Media by CLO Test and <sup>14</sup>C Urea Breath Test

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**Objective:** The aim of this study was to investigate the presence of *Helicobacter pylori* by CLO test in the middle ear of the patients with chronic otitis media. We also investigated the relationship between *H. pylori* in the stomach and in the middle ear by <sup>14</sup>C urea breath test (<sup>14</sup>C UBT) for the possible source of this bacterium.

**Study Design:** A prospective controlled clinical study.

**Methods:** Tissue samples were obtained from the middle ear mucosa of 41 patients undergoing ear surgery for chronic suppurative otitis media and placed in the CLO test kit. <sup>14</sup>C UBT was performed in 24 patients and 20 normal subjects.

**Results:** The CLO test results were positive in 22 patients (53.6%). Results of <sup>14</sup>C UBT that was performed in 24 patients were positive in 19 patients (79.1%) and were positive in 6 control subjects (30%).

**Conclusion:** Fifty-three percent of the middle ear mucosae obtained from 41 patients with chronic otitis media were shown to be positive for *H. pylori*, and although our results suggest that the source of this bacterium is the stomach, further clinical studies are needed. **Key Words:** *Helicobacter pylori*—Chronic otitis media—CLO test—<sup>14</sup>C UBT.

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Since the detection of *Helicobacter pylori* in the gastric mucosa of patients with gastritis 20 years ago (1), much attention has been paid to this bacterium and its infections.

Colonization of *H. pylori* has been found in the oral cavity (saliva and dental plaque) (2), tonsils and adenoids (3), ethmoidal and maxillary sinuses (4,5), and, recently, in the middle ears of children with chronic otitis media with effusion (6).

*Helicobacter pylorus* is a spiral-shaped, gram-negative, microaerophilic bacterium first isolated from the gastric mucosa of patients with gastritis (1). It is not a commensal organism as it causes gastritis characterized by infiltration of both acute and chronic inflammatory cells (7,8). The bacteria can be found in approximately 50% of the world population (9).

*Helicobacter pylorus* is thought to be transmitted via oral route. Although many researchers think that *H. pylori* is transmitted by the orofecal route, it is also possible that *H. pylori* is transported from the stomach to the mouth in association with gastroesophageal

reflux (7). A significant percentage of patients with gastroesophageal reflux disease is actually infected with *H. pylori* (10), so transmission of *H. pylori* from the stomach to the middle ear by gastroesophageal reflux may occur.

The aim of this study was to investigate presence of *H. pylori* by CLO test in the middle ear of the patients with chronic otitis media. We also investigated the relationship between *H. pylori* in the stomach and in the middle ear by <sup>14</sup>C urea breath test (<sup>14</sup>C UBT) for possible source of this bacterium.

### MATERIALS AND METHODS

In the present study, 41 patients undergoing ear surgery for chronic suppurative otitis media and 20 healthy control subjects were included. Informed consent was obtained from the patients and controls. The patients who used antibiotics, bismuth compounds, H<sub>2</sub> receptor blockers and antacids until 4 weeks before the surgery and <sup>14</sup>C UBT procedure were excluded from the study. Tissue samples were obtained from the middle ear mucosa during surgery and then placed in the CLO test kit (Kimberly-Clark Corp., Roswell, GA). The CLO test kits were stored at room temperature. The tests were read first after half an hour and at 24 hours. The test results were recorded as positive if the indicator turned into red or orange, otherwise it was considered negative.

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$^{14}\text{C}$  urea breath test was performed in 24 patients and 20 normal subjects. The capsules of urea labeled with  $1\ \mu\text{Ci}$  of [ $^{14}\text{C}$ ] (HeliCap Urea Bulk Capsules, produced for Noster System AB, Stockholm, Sweden) for  $^{14}\text{C}$  UBT procedure were used. The patient swallowed the urea capsule with 20 mL of water. After 10 minutes, the patient was asked to inflate the test bag (Heliprobe BreathCard, Noster System AB), and then this BreathCard was inserted to Gieger-Müller counter (Heliprobe-analyser, Noster System AB), and activity was counted for 250 seconds. The results were assessed according to grading system suggested by the producer firm (0, negative; 1, uncertain; and 2, positive).

Audiological examinations and otoscopy were performed in all patients before the surgery.

Statistical analyses were performed using SPSS 11.0.0 for Windows (SPSS, Inc., Chicago, IL). A  $p$  value of less than 0.05 was considered significant. For overall comparisons of the groups (patients with chronic suppurative otitis media and controls), Mann-Whitney  $U$  test was performed. Pearson correlation analysis was used to investigate relationship between the quantitative variables.

## RESULTS

The patient group consisted of 41 patients with chronic suppurative otitis media with a mean age of 29.5 years (range, 7–65 years). Eleven were female and 30 were male patients. Of 41 patients, 15 had chronic suppurative otitis media with cholesteatoma, and 26 had chronic suppurative otitis media without cholesteatoma. Tympanoplasty in 9 patients, tympanoplasty with mastoidectomy canal wall up in 16 patients, and radical mastoidectomy procedures in 16 patients were performed. The control group consisted of 20 healthy subjects with a mean age of 27.6 years (range, 26–29 years). There were 4 female and 16 male subjects. There was no statistically significant difference between the ages and sexes of the patients and controls ( $p > 0.05$ ).

The CLO test results were positive in 22 patients (53.6%) (Table 1). Results of  $^{14}\text{C}$  UBT that was performed in 24 patients were positive in 19 patients (79.1%).  $^{14}\text{C}$  UBT was performed in 14 of 22 patients who had positive CLO test result. The  $^{14}\text{C}$  UBT results of these 14 patients were positive (100%).  $^{14}\text{C}$  UBT was performed in 10 of 19 patients who had negative CLO test result. Five (50%) of 10 patients had a positive  $^{14}\text{C}$  UBT result. There was a significant positive correlation between CLO test results and  $^{14}\text{C}$  UBT results in the patient group ( $r = 0.607$ ,  $p = 0.002$ ).

The  $^{14}\text{C}$  UBT results were positive in 6 control subjects (30%). The  $^{14}\text{C}$  UBT results of the patients and controls were significantly different ( $p = 0.001$ ) (Table 2).

In the patient group, preoperative mean hearing level of air conduction was 54 dB, and bone conduction was 19 dB. There was no correlation between the positive

**TABLE 1.** Patient group CLO test results

	Positive	Negative	Total
No. of patients (%)	22 (53.6%)	19 (46.4%)	41

**TABLE 2.** Patient and control group  $^{14}\text{C}$  UBT results

	Patient Group (n = 24)		Control Group (n = 20)	
	Positive	Negative	Positive	Negative
No. of patients (%)	19 (79.2%)	5 (20.8%)	6 (30%)	14 (70%)

CLO test results and the hearing levels of air and bone conduction ( $p > 0.05$ ).

Of 41 patients, 31 had a history of ear discharge, and 10 had dry ear. The CLO test was positive in 18 (58.6%) of 31 patients with wet ears and in 4 (40%) of 10 patients with dry ears. Positive CLO test results of the patients with wet and dry ears were not significantly different ( $p > 0.05$ ).

## DISCUSSION

*Helicobacter pylori* could be found in approximately 50% of the world population (9). Therefore, many diagnostic methods have been developed for detecting the bacterium and its infections. Diagnostic methods are categorized into two groups as invasive and noninvasive tests. Invasive tests include histological diagnosis, CLO (rapid urease) test, culture, and polymerase chain reaction. Noninvasive tests include  $^{14,13}\text{C}$  UBT and antibody tests (7).  $^{14}\text{C}$  UBT is a very accurate test for detecting *H. pylori* infection with a sensitivity and specificity better than many other tests (11,12). The organism produces a large amount of urease an enzyme that breaks down urea to form ammonia and soluble carbon dioxide, which is expired in the exhaled breath. Labeling of urea with isotope allows the  $^{14}\text{C}$  to be detected in the expired breath. The CLO test is also a very accurate test for detecting *H. pylori* with 90% sensitivity and 100% specificity rate (12).

The results of current study demonstrated that the rate of presence of *H. pylori* in the middle ear of patients with chronic otitis media was 53.6%, and positivity rate of  $^{14}\text{C}$  UBT result was 79.1% in the patient group and 30% in the control group. The differences between the positivity rates of  $^{14}\text{C}$  UBT result of the patient and control groups were statistically significant ( $p = 0.001$ ), and there was a significant positive correlation between CLO test and  $^{14}\text{C}$  UBT results. Thus, there is a colonization of *H. pylori* in the middle ear of patients with chronic otitis media, and the source for the bacteria may be the stomach because the positivity rate of  $^{14}\text{C}$  UBT in the patient group is considerably higher than in the control group.

We found an indirect relationship between the results of the CLO test and  $^{14}\text{C}$  UBT in patients and controls. There was a statistically significant positive correlation between the CLO test and  $^{14}\text{C}$  UBT results in the patient group. Based on this correlation,  $^{14}\text{C}$  UBT findings were analyzed to compare the patient and control results, and we found a statistically significant result. Therefore, we

established an indirect relation between patients' CLO test and controls'  $^{14}\text{C}$  UBT results.

In 1963, Wittenborg and Neuhauser (13) demonstrated that fluid in the nasopharynx entered the nasopharyngeal orifice of the normal physiologic state. This study demonstrates the mechanism by which the eustachian tube may be exposed to gastric juice refluxed into the nasopharynx. Exposure of ciliated respiratory epithelium, found in the eustachian tube, to a pH of less than 4 results in ciliostasis. Ciliostasis of the eustachian tube mucosa impairs mucociliary clearance, potentiating exposure to gastric juice to cause colonization of *H. pylori* in the middle ear.

The relationship between gastroesophageal reflux and chronic otitis media with effusion has been well established, but studies about the relationship between gastroesophageal reflux and chronic suppurative otitis media have been limited. Some studies demonstrated that gastroesophageal reflux might cause middle ear disease in adult patients with chronic otitis media (14,15).

The middle ear with tympanic membrane perforation is much more influenced by gastroesophageal reflux than the middle ear with intact tympanic membrane because of lack of positive middle ear air pad. This positive middle ear air pad, present in a normal middle ear, prevents the entering of reflux fluid into the middle ear. However, further clinical studies are needed to understand whether this hypothesis is correct.

Chronic suppurative otitis media develops from a chronic bacterial infection, and the mechanism of infection of the middle ear is postulated to be translocation of bacteria from the external auditory canal through a tympanic membrane perforation into the middle ear, but some authors suggest that the pathogenic organisms may enter through reflux of the eustachian tube (16–18).

In the pathophysiology of chronic suppurative otitis media, the inflammatory response creates mucosal edema; ongoing inflammation eventually leads to mucosal ulceration and consequent breakdown of the epithelial lining. *Pseudomonas aeruginosa* is the most commonly recovered organism in the chronic suppurative otitis media, and *P. aeruginosa* produces protease, lipopolysaccharide, and other enzymes. The tissue damage from bacterial and inflammatory enzymes creates further damage, necrosis, and, eventually, bony erosion (19).

In the studies addressing how *H. pylori* forms peptic ulcer disease, it has been stated that *H. pylori* produces urease, catalase, oxidase, protease, and other enzymes such as VacA and CagA cytotoxins, and these enzymes and cytotoxins lead to local mucosal inflammation and, consequently, ulceration (7,8,20). We speculate that *H. pylori* can create an inflammation in the middle ear mucosa by a similar way.

## CONCLUSION

In the present study, colonization of *H. pylori* in the middle ear of patients with chronic suppurative otitis media was investigated. Fifty-three percent of the middle ear mucosae obtained from 41 patients were shown to be

positive for *H. pylori*, and positivity rate of  $^{14}\text{C}$  UBT result was 79.1% in patient group. The relationship between *H. pylori* in the stomach and in the middle ear was also investigated for possible source of this bacterium, and our results suggest that the source of this bacterium is the stomach. The current study demonstrated an association between *H. pylori* and chronic suppurative otitis media, but it could not show a causal relationship. Further clinical studies are needed for demonstrating the pathogenesis.

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