Detection of *Helicobacter pylori* and Its CagA Gene in Tonsil and Adenoid Tissues by PCR

Meltem Yalinay Cirak, MD; Ali Ozdek, MD; Dicle Yilmaz, MD; Unal Bayiz, MD; Erdal Samim, MD; Sevgi Turet, PhD

**Objective:** To determine the presence of *Helicobacter pylori* and, if detected, the prevalence of the CagA gene in adenotonsillectomy specimens by polymerase chain reaction (PCR).

**Design:** A prospective clinical trial.

**Setting:** Tertiary referral center.

**Patients and Methods:** The study population comprised 23 patients who had undergone adenoidectomy, tonsillectomy, or adenotonsillectomy under local or general anesthesia. *Helicobacter pylori* DNA was extracted from 3-mm-diameter tissue samples obtained from each tonsil and adenoid tissue specimens. The amplifications were performed for the 16S ribosomal RNA (rRNA) and CagA genes of *H pylori* in the samples of which *H pylori* DNA was detected.

**Results:** In examining all the samples, 7 (30%) of 23 patients were shown to be positive for *H pylori* DNA, 5 (71%) of whom also possessed the CagA gene.

**Conclusions:** Tonsil and adenoid tissues may be an ecological niche of the mouth without regard to transient or permanent colonization. Oral-oral transmission may be a possible mode of spread of *H pylori*.

*Arch Otolaryngol Head Neck Surg.* 2003;129:1225-1229

---

**ELICOBACTER PYLORI** is a gram-negative bacterium that colonizes the human stomach. Persistent *H pylori* infection is associated with chronic gastritis, peptic ulcer disease, and in some cases atrophic gastritis, mucosa-associated lymphoid tissue (MALT) lymphoma (MALToma), and gastric cancer. About 5% of gastric neoplasms are lymphoid tumors. Nearly all these lesions are now considered to be a consequence of long-term *H pylori* infection. The putative virulence gene (CagA) is an *H pylori* gene, and this cytotoxin-associated antigen codes for a 96- to 138-kDa protein that is associated with the vacuulating toxin. The presence of *H pylori* CagA-positive strains is associated with peptic ulcer disease, gastric cancer, and gastric MALTomas. Tonsil tissue is a component of MALT, and if *H pylori* has colonized this tissue, determining the prevalence of the CagA gene would also be important in determining its relation with gastric MALTomas.

Although *H pylori* is probably the most common chronic bacterial infection of humans and is present in almost half of the world’s population, the exact mode of transmission and natural reservoirs for *H pylori* are unknown. There are 3 proposed routes of transmission: oral-oral, gastric-oral, and fecal-oral. Gastritis, especially the acute stage, is often accompanied by increased episodes of intermittent gastroesophageal reflux or vomitus, and the tonsils might be colonized with *H pylori* and thus act as a reservoir. In fact, to our knowledge, there is only 1 study emphasizing the colonization of *H pylori* in the tonsils.

In the present study we aimed to determine the presence of *H pylori* and, if detected, the prevalence of the CagA gene in the adenotonsillectomy specimens by polymerase chain reaction (PCR). To our knowledge, this is the first study pursuing the detection of *H pylori* DNA and its CagA gene in tonsil and adenoid tissues by using molecular methods.

**METHODS**

**PATIENTS AND SAMPLE COLLECTION**

The study group comprised 23 consecutive patients (mean age, 17 [range, 4-42] years) who had undergone a tonsillectomy and/or adenoidectomy procedure in the Department of Otorhinolaryngology of Ankara Research and Training Hospital, Ankara, Turkey, between May and...
June 2002. There were 14 patients (61%) younger than 16 years, and 9 patients (39%) were older than 16 years. Eight (35%) of 23 patients were male. Thirteen (57%) of the patients had undergone tonsillectomy, 9 patients (39%) had undergone adenotonsillectomy, and 1 patient (4.3%) had undergone adenoidectomy. Patient demographics are given in Table 1. A total of 54 samples (44 samples from tonsil tissues and 10 samples from adenoid tissues) were collected from the surgical specimens.

The study was approved by the local ethics committee, and informed consent was obtained from all patients. All patients were operated on following routine surgical indications for adenoidectomy. Tonsillectomy procedures were performed using the dissection and snare technique, and adenoid tissues were removed using adenoid curettes.

Fourteen patients were operated on while under general anesthesia, and 9 patients were operated on while under local anesthesia. Tonsillectomy procedures were performed using the dissection and snare technique, and adenoid tissues were removed using adenoid curettes.

After the completion of the operation, a core biopsy specimen (containing a 3-mm-diameter sample) was taken from each tonsil and adenoid tissue. A different blade was used to collect each specimen, and gloves were changed after the procedure. Collected samples were placed immediately into Tris EDTA buffer and transported to the laboratory of the Gazi University Faculty of Medicine, Department of Microbiology and Clinical Microbiology, Ankara.

**PREPARATION OF DNA FOR PCR**

As previously described, DNA was extracted from tonsil and adenoid tissues. Briefly, the sample tissues (containing about 10 mg of the specimen) were homogenized and suspended in 100 µL of digestion buffer (0.1M sodium chloride, 0.01M Tris hydrochloride [pH 8.3], 0.25M EDTA, and 1% sodium lauryl sarcosine) containing proteinase K (final concentration, 100 mg/mL) and incubated at 55°C for 3 hours. Afterwards, proteinase K was inactivated by heating the sample for 10 minutes at 95°C. During all sample collection and preparation steps, great care was taken to avoid contamination. The supernatant was used for the amplification of the 16S rRNA gene of *H pylori*.

**PCR PRIMERS AND AMPLIFICATION**

The DNA amplification for the 16S rRNA gene was performed according to the method used by Saiki et al., with primer sequences previously described and tested by Ho et al. and Mappstone et al. Three oligonucleotide primers were used with sequences (expressed 5’ to 3’) as follows: Hp1, CTA AGC CCT CC (position 834-853); Hp2, ATT ACG GCT GAT TGT GC (position 744-763); and Hp3, AGG ATG AAG GTT TAA GGA TT (position 407-426). The DNA amplification for the *CagA* gene was performed according to the method used by Lage et al., with the previously described primer sequences 93089 and 93261: AAT ACA CCA AGC CCT CCA AG (position 2593-2621) and TTG TTG CCG CCT TTG CTC TC (position 2992-2973).

The first amplification was performed with the Hp1 and Hp3 primers (Metis Biotechnology Ltd, Ankara) in a 30-µL reaction mixture containing 3 µL of ×10 PCR buffer, 2 µL of magnesium chloride; 3 µL of deoxynucleotide triphosphate mixture (final concentration, 1 mM [each] dATP, dCTP, dGTP, and dTTP); 3 µL of both Hp1 and Hp3; 1 µL of template DNA; 2 µL of dimethyl sulfoxide; and 1 U of *Taq* DNA polymerase. All reagents were purchased from DNAmp Ltd, Farmborough, England. For the second amplification, 1 µL of the primary amplification product was used in a 30-µL reaction mixture with primers Hp1 and Hp2 (Metis Biotechnology Ltd). The first and second rounds of amplification were performed as previously described. The product of the nested PCR amplification reaction (expected size, 109 base pairs [bp]) was analyzed by electrophoresis on agarose gels (Figure 1). As a positive control, DNA extracted from *H pylori* ATCC 43629 (American Type Culture Collection, Manassas, Va) was used. As a negative control, a reaction mixture without DNA was included and subjected to the same steps.

The *CagA* reactions were performed in 50 µL of reaction mixture containing 0.4µM concentration of each primer (primers 93089 and 93261); 0.2mM concentration of each deoxynucleotide triphosphate (dATP, dCTP, dGTP, and dTTP); and reaction buffer (100mM Tris hydrochloride [pH 8.3], 500mM potassium chloride, and 15mM magnesium chloride). The amplifications consisted of 35 cycles of denaturation at 94°C for 1 minute, annealing at 59°C for 1 minute, and elongation at 72°C for 1 minute, followed by a final cycle comprising a
In examining all the tissue samples of the 23 patients, 7 patients (30%) were found to be positive for *H pylori* DNA (in tonsil tissues of 4 patients and in adenoid tissues of 3 patients) and 16 patients (70%) were negative. *Helicobacter pylori* was detected in only 1 of the 2 tonsil tissues of each patient, and the results were evaluated according to this detection rate. Of the 7 patients who were PCR positive for *H pylori* DNA, the *CagA* gene was detected in 5 (71%) in 3 [75%] of the 4 patients who were positive in their tonsil tissues and in 2 (67%) of the 3 who were positive in their adenoid tissues.

Two (15%) of the 13 patients who had undergone only tonsillectomies were PCR positive for *H pylori* DNA in only 1 tonsil. The *CagA* gene was detected in 1 (50%) of these 2 patients. Two (22%) of the 9 patients who had undergone adenotonsillectomies were PCR positive for *H pylori* DNA in tonsil tissues, and the *CagA* gene was detected in both of these patients (100%). Two of the 9 patients (22%) who had undergone adenotonsillectomy were PCR positive for *H pylori* DNA in adenoid tissues, and the *CagA* gene was detected in both of these patients (100%) as well. One patient undergoing only adenoidectomy was PCR positive for *H pylori* DNA. The PCR results for *H pylori* DNA and *CagA* status are given in Table 2.

**RESULTS**

**COMMENT**

*Helicobacter pylori* DNA was detected in 7 patients (30%) in our study group, 5 (71%) of whom were *CagA* positive. To our knowledge, no other published study seems to have examined the prevalence of *H pylori* DNA and its putative virulence gene *CagA* by PCR. There are only a few studies regarding the presence of *H pylori* on tonsils, and these studies have contradictory results. In a study of 19 patients, Unver et al[^6] found a high *H pylori* colonization rate (57.89%) by the *Campylobacter*-like organism (CLO) test. This high positive rate can be related to the false positivity of the CLO test, since it was indicated that false-positive CLO test results could occur in patients who have achlorhydria. When acid is absent, commensal organisms such as *Proteus species* and *Klebsiella pneumoniae* may grow in the stomach and produce urease. With the same approach, *Proteus species* and *K pneumoniae* can be found on tonsil and adenoid tissues. Moreover, since tonsil and adenoid tissues do not have an acidic environment, this situation can also affect the results of the CLO test. On the other hand, Skinner et al[^12] showed that there was no evidence of *H pylori* on tonsillar specimens by the CLO test and di Bonaventura et al[^13] also suggested that tonsils did not represent an extragastric reservoir for *H pylori* infection by using PCR.

The presence of the organism in the mouth supports the potential spread of the organism via a person-to-person route. Oral colonization of *H pylori* has been studied previously on oral lesions[^14], saliva[^15] and especially dental plaques, and the recovery rate of oral *H pylori* is controversial, ranging from 0% to 100% according to authors[^16]. Bickley et al[^17] showed no evidence of *H pylori* in dental plaques using primers for urease C gene. In a study by Riggio and Lennon[^18], the rate of *H pylori* positivity in subgingival dental plaques was found to be 33% by amplification of the 16S rRNA gene sequence of *H pylori*, and subgingival plaque was suggested as a reservoir for *H pylori* infection[^19]. In a study by Song et al[^20] on 42 patients with dyspepsia undergoing gastrointestinal endoscopy, the rate of *H pylori* positivity in the oral cavity was 97%. In another study on 40 randomly selected dental plaque samples, the rate of *H pylori* positivity was found to be 100% by amplification of the 860-bp DNA sequence[^21]. In a study on

---

**Table 2. Helicobacter pylori DNA and CagA Status by PCR**

<table>
<thead>
<tr>
<th>Patient No.</th>
<th><em>H pylori</em> DNA</th>
<th>CagA</th>
<th><em>H pylori</em> DNA</th>
<th>CagA</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>−</td>
<td>+</td>
<td>−</td>
<td>+</td>
</tr>
<tr>
<td>2</td>
<td>−</td>
<td>+</td>
<td>−</td>
<td>+</td>
</tr>
<tr>
<td>3</td>
<td>−</td>
<td>+</td>
<td>−</td>
<td>+</td>
</tr>
<tr>
<td>4</td>
<td>+</td>
<td>−</td>
<td>+</td>
<td>−</td>
</tr>
<tr>
<td>5</td>
<td>−</td>
<td>+</td>
<td>−</td>
<td>+</td>
</tr>
<tr>
<td>6</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>7</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>8</td>
<td>−</td>
<td>+</td>
<td>−</td>
<td>+</td>
</tr>
<tr>
<td>9</td>
<td>−</td>
<td>+</td>
<td>−</td>
<td>+</td>
</tr>
<tr>
<td>10</td>
<td>−</td>
<td>+</td>
<td>−</td>
<td>+</td>
</tr>
<tr>
<td>11</td>
<td>−</td>
<td>+</td>
<td>−</td>
<td>+</td>
</tr>
<tr>
<td>12</td>
<td>−</td>
<td>+</td>
<td>−</td>
<td>+</td>
</tr>
<tr>
<td>13</td>
<td>−</td>
<td>+</td>
<td>−</td>
<td>+</td>
</tr>
<tr>
<td>14</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>15</td>
<td>−</td>
<td>+</td>
<td>−</td>
<td>+</td>
</tr>
<tr>
<td>16</td>
<td>−</td>
<td>+</td>
<td>−</td>
<td>+</td>
</tr>
<tr>
<td>17</td>
<td>−</td>
<td>+</td>
<td>−</td>
<td>+</td>
</tr>
<tr>
<td>18</td>
<td>−</td>
<td>+</td>
<td>−</td>
<td>+</td>
</tr>
<tr>
<td>19</td>
<td>−</td>
<td>+</td>
<td>−</td>
<td>+</td>
</tr>
<tr>
<td>20</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>21</td>
<td>−</td>
<td>+</td>
<td>−</td>
<td>+</td>
</tr>
<tr>
<td>22</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>23</td>
<td>−</td>
<td>+</td>
<td>−</td>
<td>+</td>
</tr>
</tbody>
</table>

*Abbreviations: PCR, polymerase chain reaction; +, positive; −, negative. *See Table 1 for operation type.*
In our study, the colonization rate of *H pylori* in tonsil and adenoid tissues was demonstrated to be 30% by using molecular techniques. Moreover, most of the strains (71%) were shown to possess the CagA gene. According to our findings, we postulate that the tonsil and adenoid tissue may be an ecological niche of the mouth without regard to transient or permanent colonization, and oral-transmission may be a possible mode of spread of *H pylori*. The risk of peptic ulcer disease, gastric cancer, and MALTomas of the stomach can be decreased with therapies for eradicating the bacteria. This study emphasizes the mode of transmission and colonization of *H pylori*, but further studies must be performed for more specific results.

Submitted for publication December 19, 2002; final revision received March 7, 2003; accepted March 12, 2003.

Corresponding author and reprints: Meltem Yalnay Cirak, MD, Noktali (Seyit Omer Haluk Sipahioglu) sok No: 7/18, Gaziosmanpasa, 06700 Ankara, Turkey (e-mail: meltemyc@gazi.edu.tr).

REFERENCES


**Correction**

**Error in Figure.** In the article by Kosuda et al titled “Feasibility and Cost-effectiveness of Sentinel Lymph Node Radiolocalization in Stage N0 Head and Neck Cancer,” published in the October issue of the Archives (2003;129:1105-1109), in Figure 1 on page 1107, the labels for the bottom 2 arms of the upper tree should have read “True Negative” and “False Positive.” This correction was made previously to online versions of this article.