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*.


**H**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 vacuolating 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 adeno- oronsillectomy procedure in the Department of Otorhinolaryngology of Ankara Research and Training Hospital, Ankara, Turkey, between May and...
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 hydrogen chloride [pH 8.3], 0.25M EDTA, and 1% sodium lauryl sarcosine) containing proteinase K (final concentration, 100 µg/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.*

**Figure 1.** Polymerase chain reaction amplification of *Helicobacter pylori,* performed with 16S rRNA primer pairs from tonsil and adenoid tissues. M indicates molecular size standard (100–base pair [bp] DNA ladder). Lanes 1 through 7, *H pylori*-positive tonsil and adenoid tissues (109 bp); lane 8, *H pylori* ATCC 43629 as positive control; lane 9, negative control.

**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 Mapstone et al. Three oligonucleotide primers were used with sequences (expressed 5’ to 3’) as follows: Hp1, CTG GAG AGA CTA AGC CCT CC (position 834-853); Hp2, ATT ACT GAC 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 2593-2612) and TTG TTG CCG 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 DNAmpl Ltd, Fareborough, 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

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**Table 1. Demographic Features of Patients**

<table>
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<th>Patient No./ Sex/Age, y</th>
<th>Type of Operation</th>
<th>Type of Anesthesia</th>
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</table>

Abbreviations: GA, general anesthesia; LA, local anesthesia.

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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 adeno-tonsillectomy, and 1 patient (4.5%) 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 adenotonsillectomy procedures (eg, recurrent tonsillitis, chronic tonsillitis, and mouth breathing). Subjects who had used antibiotics during the previous month and who had used bismuth-containing drugs or proton pump inhibitors during the previous 3 months were excluded. Patients were not questioned about dyspeptic complaints or gastroesophageal reflux.

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 about 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.
1-minute step at the specified annealing temperature and a 10-
minute elongation step at 72°C.11 The product of the PCR am-
plification reaction (expected size, 400 bp) was analyzed by elec-
trophoresis on agarose gels (Figure 2).

**RESULTS**

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. *Helico-
bacter pylori* was detected in only 1 of the 2 tonsil tis-
sues of each patient, and the results were evaluated ac-
cording to this detection rate. Of the 7 patients who were
PCR positive for *H pylori* DNA, the *CagA* gene was de-
tected 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 under-
gone 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 posi-
tive 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 posi-
tive for *H pylori* DNA. The PCR results for *H pylori* DNA
and *CagA* status are given in Table 2.

**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 stud-
ies have contradictory results. In a study of 19 patients, Un-
ver et al10 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 re-
sults could occur in patients who have achlorhydria. When
acid is absent, commensal organisms such as *Proteus spe-
cies* and *Klebsiella pneumoniae* may grow in the stomach
and produce urease.13 With the same approach, *Proteus spe-
cies* 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
al14 showed that there was no evidence of *H pylori* on ton-
sillar specimens by the CLO test and di Bonaventura et al15
also suggested that tonsils did not represent an extragas-
tric 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 stud-
ied previously on oral lesions,16 saliva,17 and especially den-
tal plaques, and the recovery rate of oral *H pylori* is
controversial, ranging from 0% to 100% according to au-
thors.18-23 Bickley et al18 showed no evidence of *H pylori*
in dental plaques using primers for urease C gene. In a study
by Riggio and Lennon,19 the rate of *H pylori* positivity in
subgingival dental plaques was found to be 33% by ampli-
fication of the 16S rRNA gene sequence of *H pylori*, and
subgingival plaque was suggested as a reservoir for *H pyl-
ori* infection.19 In a study by Song et al20 on 42 patients
with dyspepsia undergoing gastrointestinal endoscopy, the rate
of *H pylori* positivity in the oral cavity was 97%. In an-
other study on 40 randomly selected dental plaque samples,
the rate of *H pylori* positivity was found to be 100% by am-
plication of the 860-bp DNA sequence.21 In a study on

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

<table>
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<th>Patient No.</th>
<th>Tonsil Specimen</th>
<th>Adenoid Specimen</th>
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<td>23</td>
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Abbreviations: PCR, polymerase chain reaction; +, positive; −, negative.
*See Table 1 for operation type.

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329 patients with dyspeptic symptoms, the rate of *H. pylori* positivity in gingival pocket material was found to be 35% by PCR, and after eradication therapy, *H. pylori* DNA prevalence was determined to have fallen by 10%.\(^23\) In a study by Allaker et al.\(^23\) on 100 pediatric patients, the rate of *H. pylori* positivity in the mouth was 68%, and they suggest that oral-oral transmission may be the predominant mode of spread of *H. pylori* in children.

Our results are similar to these studies in demonstrating *H. pylori* presence in the oral cavity and oral-oral mode of transmission of the bacteria. However, to our knowledge, this is the first study investigating the CagA gene of the bacteria in tonsil and adenoid tissues. The CagA protein is a surface immunodominant antigen. It is present in 60% to 80% of *H. pylori* strains and was found to have a close association with the production of vacuolating cytotoxin.\(^4\) In our study, the CagA gene was detected in the 71% of *H. pylori*–positive tonsil and adenoid tissues. In a study by Lage et al,\(^1\) 40 (38.5%) of 104 patients with dyspepsia were positive for *H. pylori*, and the CagA gene was detected in 91.7% of the patients with duodenal ulcer and in 73% of those with chronic gastritis. The association between *H. pylori* and MALT has been shown by some reports demonstrating the regression of MALTomas of the stomach, rectum, and salivary glands after eradication of *H. pylori*.\(^24\)--\(^27\) The stimulatory effect of *H. pylori* on MALT would be expected on tonsillar tissue as well, since it is a component of MALT. Considering the high rate of CagA-carrying strains, it can be speculated that tonsillectomy and adenoidectomy may protect the host against *H. pylori* infestation of the stomach and a possible case of peptic ulcer or gastric cancer. However, it does not seem to be practical and logical for every patient when we consider the high prevalence of *H. pylori* infection throughout the world.

We think that PCR is a suitable tool for the diagnosis of this bacteria, which is difficult to culture. The application of nested PCR was carried out for the detection of *H. pylori* from various oral samples.\(^15\)--\(^24\) A recent study by our study group demonstrated the importance of PCR for detecting the *H. pylori* in mucosal tissue samples. In that study, *H. pylori* was detected in the sinus mucosa of some patients with chronic rhinosinusitis.\(^28\) In the present study, *H. pylori* DNA and its CagA gene were detected in tonsil and adenoid tissue samples.

The differences in the results of the studies using PCR for the detection of *H. pylori* are noticeable. We think that these differences are related to the primers, the conditions, and technical procedures of PCR assay used in the study. The accuracy of PCR can be affected by the choice of primers and the target DNA, the bacterial density of the sample, the preparation of the specimens, and variations in laboratory protocols. The primers used for the target DNA in the PCRs in our study were demonstrated to be highly sensitive and specific in previous studies.\(^9\)--\(^12\) But we have some doubts about the samples studied. We included only 1 core biopsy specimen from each tonsil and adenoid tissue in our study. Multiple biopsy samples from each tissue can be studied to detect the exact prevalence of the bacteria. However, our study is a preliminary report, and further studies can be performed by using PCR, which is a very useful diagnostic tool, especially in mucosal tissue samples.

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-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.

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### 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.