Association of cagA⁺ Helicobacter pylori with Adenotonsillar Hypertrophy

YASEMIN BULUT, AHMET AGACAYAK, TURGUT KARLIDAG,⁠ ¹ ZULAL ASCI TORAMAN and MUSTAFA YILMAZ

Department of Microbiology, and ¹Department of Otorhinolaryngology, College of Medicine, Firat University, Elazig, Turkey

BULUT, Y., AGACAYAK, A., KARLIDAG, T., TORAMAN, Z.A. and YILMAZ, M. Association of cagA⁺ Helicobacter pylori with Adenotonsillar Hypertrophy. Tohoku J. Exp. Med., 2006, 209 (3), 229-233 —— Cytotoxin-associated gene A (cagA) of Helicobacter pylori (H. pylori) encodes a highly immunogenic and virulence-associated protein. The presence of cagA⁺ H. pylori strains in tonsil and adenoid tissues may affect clinical outcome. The aim of the present study was to determine the presence of H. pylori cagA gene in tonsil and adenoid tissues and to establish the potential association of cagA⁺ H. pylori in recurrent adenotonsillitis (RAT) and adenotonsillar hypertrophy (ATH). For this aim, a total of 118 tissue samples (71 tonsil and 47 adenoid tissues) were collected from a total of 71 children: 28 cases with RAT and 43 cases with ATH. The samples were analyzed for glmM gene to detect the infection with H. pylori by polymerase chain reaction (PCR). H. pylori-positive samples were further analyzed for the presence of the cagA gene. The PCR analysis showed that 29 samples (24.6%) were positive for H. pylori. Seventeen out of these 29 samples (58.6%) were found positive for cagA; the cagA gene was detected in 12 samples of ATH and 5 samples of RAT. The presence rate of cagA gene was significantly higher (p < 0.05) in ATH patients than that found in RAT patients. These results suggest that presence of cagA⁺ H. pylori may be associated with development of ATH. ———

Helicobacter pylori (H. pylori) is a microaerophilic, gram-negative spiral bacterium. This pathogen causes one of the most common chronic bacterial infection in humans and it is accepted to have a role in development of chronic gastritis and gastric ulcer (Forman et al. 1991; Suerbaum and Michetti 2002). In addition to gastric specimens, detection of H. pylori from different regions such as oral region suggests that the bacterium may lead to a broad spectrum of clinical manifestations (Li et al. 1995; Cammarota et al. 1996; Mravak-Stipetic et al. 1998; Jun et al. 2005). The presence of H. pylori in oral samples may be a cause of the high incidence of H. pylori infection in the world (Unver et al. 2001; Ozdek et al. 2003; Morinaka et al. 2003).

Despite some published reports on epidemiology of the bacterium (Li et al. 1995; Mravak-Stipetic et al. 1998; di Banaventura et al. 2001; Cirak et al. 2003; Morinaka et al. 2003), mode of transmission and role of H. pylori detected from tonsil and adenoid tissues on its epidemiology...
have not been completely understood. There are contradictory data on the presence of *H. pylori* in these tissues. For instance, Unver et al. (2001) reported the *H. pylori* was present in the adenoid and tonsil tissues of 57% of the 19 patients with tonsillitis and adenoiditis, whereas Yilmaz et al. (2004) reported that *H. pylori* was not detected in the tonsil and adenoid tissues of the 50 children with chronic or recurrent tonsillitis and adenoiditis.

Cytotoxin-associated gene A (*cagA*) encodes a highly immunogenic and virulence-associated protein with molecular mass of 120-140 kDa (Covacci and Rappuoli 2003). Recent studies have shown that *cagA* gene-positive (*cagA*+) *H. pylori* strains cause more severe gastric infections (Aydin et al. 2004; Yamazaki et al. 2005). However, to the best of our knowledge, there is only one study about *cagA*+ in tonsil and adenoid tissues. Cirak et al. (2003) reported that presence rate of *cagA* in *H. pylori*-positive tonsil and adenoid tissues was 71%.

Detection of *H. pylori* by culturing is difficult due to the fastidious growth requirements of the pathogen. Polymerase chain reaction (PCR) is considered as a rapid, highly sensitive and specific method for detection of *H. pylori* from a variety clinical samples (Lu et al. 1999). The Campylobacter-like organism (CLO)-assay is a rapid urease test used for detection of Campylobacter-like-organisms including *H. pylori*. The surface of *H. pylori* is covered with urease enzyme. Therefore, the diagnosis of *H. pylori* can usually be established by testing for urease activity using CLO-assay (Yilmaz et al. 2004).

The purpose of the present study was to determine *H. pylori* *cagA* gene in tonsil and adenoid tissues and to establish the potential association of *cagA*+ *H. pylori* in recurrent adenotonsillitis (RAT) and adenotonsillar hypertrophy (ATH).

**Subjects and Methods**

**Patients and samples**

Biopsy samples were collected from 71 children aged from 4 to 10 years who were admitted to the Ear, Nose, and Throat Clinic of Firat Medical Center, Firat University, Elazig, Turkey from April 2002 to July 2004. Twenty-eight of the children who had at least five episodes of inflammation caused by group A β-hemolytic streptococci within the preceding year assigned as RAT group. The remaining 43 patients who were experiencing dysphagia, mouth breathing, snoring and hyponasal voice without RAT and/or symptoms of obstructive sleep apnea secondary to ATH were assigned as ATH group. Prior to sampling, families of the patients were informed about the study and their consents were obtained. The present study was approved by the Ethical Committee of Firat University Medical School. The samples were taken under general anesthesia from all of the patients. Twenty-four patients had tonsillectomy, whereas 47 had adenotonsillectomy (Table 1). Only right tonsil samples were used in this study for analysis. Thus, this study was carried out with 71 tonsil and 47 adenoid samples totaling 118 samples. About 3 mm diameters from the core and surface of the samples were used for CLO-assay. The remaining tissue was stored at −80°C for PCR analysis.

**CLO-assay**

CLO-assay was performed with each biopsy sample using commercial CLO-assay kit by following the procedures recommended by the manufacturers (Trimed Inc.,

<table>
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<th>Type of operation and sex</th>
<th>Tonsillectomy (n = 24)</th>
<th>Adenotonsillectomy (n = 47)</th>
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<tbody>
<tr>
<td>F</td>
<td>M</td>
<td>F</td>
</tr>
<tr>
<td>RAT (n = 28)</td>
<td>4</td>
<td>6</td>
</tr>
<tr>
<td>6.4 ± 1.5</td>
<td></td>
<td>8</td>
</tr>
<tr>
<td>ATH (n = 43)</td>
<td>6</td>
<td>8</td>
</tr>
<tr>
<td>5.6 ± 1.2</td>
<td></td>
<td>10</td>
</tr>
<tr>
<td>Total (n = 71)</td>
<td>10</td>
<td>14</td>
</tr>
</tbody>
</table>

RAT, recurrent adenotonsillitis; ATH, adenotonsillar hypertrophy; F, female; M, male.
Osborne Park, Australia). The test results were evaluated according to the definitions recommended by the manufacturer.

**DNA extraction and PCR**

DNA was obtained from the biopsy samples using a commercial DNA extraction kit (Wizard Genomic DNA Purification System, Promega Co., Madison, WI, USA). The DNA was dissolved in sterile dH2O and stored at −80°C until analyzed by PCR.

The PCR protocol used was previously described (Lu et al. 1999). In this study, initially, the amplifications of glmM gene regions were carried out for the detection of presence of *H. pylori* from samples. If detected, another different primer set was used for detection of *cagA* gene (Cirak et al. 2003). PCR reactions were performed in a total reaction volume of 100 μl containing 10 μl of 10X PCR buffer (100 mM Tris-HCl, pH 8.0, 500 mM potassium chloride, 15 mM magnesium chloride), 250 μM of each of the four deoxynucleotide trifosphates, 2 U *Taq* DNA polymerase (Promega Co.), 10 μM of each of primers (Table 2) and 10 μl of template sample DNA. The samples were obtained at 94°C for 5 min and then 34 cycles at 94°C for 1 min, at 56°C for 1 min and at 72°C for 2 min. The PCR products were run on a 2% agarose gel and the products were visualized by ethidium bromide staining.

During DNA extraction and PCR method, *H. pylori* ATCC reference strain (ATCC 43504) was used as a positive control. The sensitivity of PCR was determined with 10-fold serial dilution, from 50 ng to 5 fg, of the purified DNA extracted from the reference strain as described previously (Gok et al. 2001). Each dilution was analyzed by PCR. The detection limit of PCR assay carried out the purified DNA extracted from the *H. pylori* ATCC reference strain was found as about 7 bacteria (0.05 pg) per reaction. To eliminate false positivity and false negativity, negative and positive controls were included in each experiment.

**Statistical analysis**

The relationship between the presence of *cagA* in the samples and groups was analyzed using the Chi-square test.

**RESULTS**

Results of CLO-assay and PCR analysis for *glmM* and *cagA* genes are presented in Table 3. Results of CLO-assay indicated that 16 out of 118 samples were positive (13.55%). In contrast to the results of CLO-assay, 29 of 118 samples (24.57%) were found positive for *glmM* gene when analyzed by PCR. Further analysis of these 29

<table>
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<th>Table 2. Primers used for polymerase chain reaction analyses of the tonsil and adenoid samples.</th>
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<td><strong>Target regions</strong></td>
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</table>
| glmM gene region | Primer 1; 5′-AAGCTTTTAGGGGTGTTAGGGGTTT-3′  
|                   | Primer 2; 5′-AAGCTTACTTTCTAACACTAAACGC-3′ |
| cagA gene region  | Primer 1; 5′-AATACACCAACGCCTCCA-3′  
|                   | Primer 2; 5′-TTGTTGCGCTTTGTGCTA-3′ |

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<th>Table 3. Results of polymerase chain reaction analyses and CLO-assay carried out from tonsil and adenoid tissues.</th>
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<tr>
<td><strong>Markers</strong></td>
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<tr>
<td>CLO*</td>
</tr>
<tr>
<td>glmM*</td>
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<tr>
<td>cagA*</td>
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RAT, recurrent adenotonsillitis; ATH, adenotonsillar hypertrophy; a, total numbers of samples from RAT patients; b, total numbers of samples from ATH patients.
positive samples revealed that the cagA gene was present in 17 samples (58.62%). When the cagA positive samples were distributed based on the source of tissues, 12 cases (70.58%) were in the ATH group and 5 cases were in the RAT group. Furthermore, the cagA gene was detected in 12 (80%) of 15 H. pylori positive samples in ATH group while it was detected in only 5 (35.71%) of 14 H. pylori positive samples in RAT group (Table 3). Chi-square analysis of the data revealed that presence rate of cagA gene was significantly higher ($p < 0.05$) in ATH patients that were positive for H. pylori than that found in RAT patients.

**DISCUSSION**

In the present study, presence of H. pylori was determined in the tonsil and adenoid tissue samples of patients with ATH and those with RAT followed by determination of cagA gene in H. pylori positive samples. Detection of H. pylori from tonsil or adenoid tissues has been studied by a number of other investigators using CLO or PCR. Some researchers did not detect any H. pylori (di Bonaventura et al. 2001; Skinner et al. 2001; Yilmaz 2004), whereas the other reported various percentages for the presence of the pathogen, even higher than reported in the present study (Unver et al. 2001; Cirak et al. 2003). The difference observed between those studies may be due to particular characteristics of population or diversity of the methods used.

PCR assay relies on the detection of genetic material regardless of the viability of the agents. It might be argued that bacterial DNA, H. pylori DNA in our case, was left from a previous acute attack. Thus, the CLO test was used to confirm the presence of live bacterial species in this study. According to CLO-assay results, 16 (13.55%) of 118 biopsy samples were positive. The results suggest that adenoid and tonsil tissues may be an extra-gastric reservoir for H. pylori. Also, the tissues may play a role in the epidemiology of the pathogen. However, to date, there is no evidence about persistence of H. pylori in these tissues.

For presence of H. pylori from the tissues in the present study, the idea of gastroesophageal reflux may be speculative, because H. pylori may come to the tonsil and adenoid tissues from the stomach through gastroesophageal reflux (GER), as reported by Phipps et al. (2000). However, GER was not investigated objectively in this study, and there was no information about the presence of H. pylori in the gastric mucosa of the patients.

Preliminary reports have suggested that cagA+ affect development of duodenal ulcer or gastricadenocarcinoma (Aydin et al. 2004; Yamazaki et al. 2005). Moreover, cagA protein is also known to contribute to gastric inflammation. However, to date there is only one study about cagA+ in tonsil and adenoid tissues. Cirak et al. (2003) reported cagA in 71% of the H. pylori-positive tonsil and adenoid tissues. In our study, cagA+ was found in 17 (58.62%) of the 29 samples detected as H. pylori positive by PCR analysis. In addition to the detection of presence of cagA+ from the tissues, relationship between cagA+ and clinical outcome was investigated. In PCR results, 12 (70.58%) of 17 cagA+ tissues were detected from ATH group, others were RAT group. Furthermore, cagA gene was detected in 12 (80%) of 15 H. pylori positive samples in ATH group while it was detected in only 5 (35.71%) of 14 H. pylori positive samples in RAT group. In other words, presence rate of cagA gene was significantly higher ($p < 0.05$) in ATH patients that were positive for H. pylori than that found in RAT patients. These results suggest that H. pylori carrying cagA gene may influence development of ATH.

**Acknowledgments**

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**References**


Cirak, M.Y., Ozdek, A., Yilmaz, D., Bayiz, U., Samim, E. &


