

# Determination of *Helicobacter pylori* in Patients With Chronic Nonspecific Pharyngitis

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**Objectives/Hypothesis:** To determine if there is a relationship between *Helicobacter pylori* colonization in the pharynx mucous membrane and chronic nonspecific pharyngitis.

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

**Methods:** Seventy patients with chronic pharyngitis and 20 healthy control subjects were examined with polymerase chain reaction (PCR) and culture for *H. pylori* colonization in the pharynx mucous membrane between March 2008 and October 2008. Patients with pharyngitis were separated into two groups (35 patients in each) by using C-14 urea breath test, according to the presence of gastric *H. pylori* infection.

**Results:** In the control group, none of the patients had *H. pylori* in the pharynx. In the chronic pharyngitis group, in 12 patients (34.3%) with gastric *H. pylori* infection and in seven patients (20%) without gastric infection, *H. pylori* colonization in pharynx mucosa was determined with the PCR method. In only two of chronic pharyngitis patients (5.8%), *H. pylori* infection was detected with culture. In the pharynx mucosa, the *H. pylori* infection rate was significantly higher in the chronic pharyngitis groups than in the control group ( $P = .002$  between C-14 positive and control groups,  $P = .040$  between C-14 negative and control groups). There was not a significant difference in *H. pylori* colonization in the pharynx of patients who had chronic pharyngitis with or without gastric ailments and *H. pylori* infection ( $P = .179$ ).

**Conclusions:** Chronic nonspecific pharyngitis without gastric *H. pylori* infection is significantly related to *H. pylori* colonization in the pharynx, and gastric involvement increases the rate of this spread.

The gold standard for detection of *H. pylori* infection is the PCR method.

**Key Words:** Chronic nonspecific pharyngitis, *Helicobacter pylori*, polymerase chain reaction.

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## INTRODUCTION

In all otorhinolaryngology outpatient clinics the most common complaint among patients is sore throat, and the most frequent diagnosis is chronic nonspecific pharyngitis. Chronic pharyngitis, a chronic inflammation of the pharyngeal mucosa and underlying etiopathogenesis, is still controversial. In general, chronic nonspecific pharyngitis is related to different processes, such as nasal obstruction and mouth breathing, laryngopharyngeal reflux, and acute or chronic upper respiratory tract infection.<sup>1</sup> These patients present with symptoms such as chronic throat irritation, sore throat, chronic cough, foreign-body and globus sensation in the throat, cervical dysphagia, and intermittent hoarseness persisting for three months or more.<sup>1,2</sup> Treatment is usually difficult and based on reducing the symptoms by medical or behavioral methods.

*Helicobacter pylori* is a well-known microaerophilic, Gram-negative pathogenic micro-organism responsible for chronic inflammation of gastric mucosa, gastric and duodenal ulceration, atrophic gastritis, mucosa associated lymphoid tissue lymphoma, and gastric carcinoma.<sup>3–5</sup> In the gastrointestinal system, *H. pylori* is able to colonize in the human stomach, saliva, gastric juice, and feces of patients.<sup>6,7</sup> However, there are also some studies that report extragastric *H. pylori* presence in salivary secretions,<sup>8,9</sup> tonsil and adenoids,<sup>10,11</sup> oral cavity,<sup>12</sup> nasal and sinus mucosa,<sup>13,14</sup> middle ear,<sup>15</sup> and dental plaques.<sup>8,9</sup>

In this study, because extragastric localizations of *H. pylori* are very close to each other, we aimed to determine if there is a relationship between *H. pylori* infection and chronic nonspecific pharyngitis by using polymerase chain reaction (PCR) and *H. pylori* culture methods. With this study, we also tried to find out if gastric *H. pylori* infection contributes to chronic pharyngitis.

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## MATERIALS AND METHODS

### Patients and Sample Collection

In our clinic, between March 2008 and October 2008, we prospectively evaluated a total of 70 consecutive cases with one or more symptoms of chronic nonspecific pharyngitis, such as sore throat, chronic cough, chronic throat irritation, globus or foreign-body sensation, angina in the pharynx, nausea, cervical dysphagia, and intermittent hoarseness persisting for 3 months or more, and 20 cases with no pharyngeal complaints. The people in the control group had no specific pharyngitis or stomach ailment history, such as gastric ulcer or chronic active gastritis. The shortest disease history in pharyngitis group was 3 months and the longest was 4 years.

A comprehensive questionnaire was completed and otorhinolaryngological examinations were performed including nasal, pharyngeal, and laryngeal endoscopic evaluations. Patients who had nasal obstruction and mouth breathing, chronic upper respiratory infections, chronic periodontal infections, chronic exposure to irritating inhaled substances, such as tobacco or industrial fumes, who generally ate hot or cold foods, drank alcohol, had pharyngeal neurosis, had to use angiotensin-converting enzyme inhibitors or long term antiseptic lozenges, or had neoplasm and vasculitis were excluded from the study.<sup>1</sup> All patients were asked about the presence of classic symptoms of gastroesophageal reflux (heartburn, regurgitation, and acid taste), and these data were recorded for further evaluation. A C-14 urea breath test was used to detect *H. pylori* infection of the gastric mucosa, and 70 patients with chronic nonspecific pharyngitis were divided into two groups consisting of 35 patients in each, according to the positive or negative results of this test.

*H. pylori* infections in the pharynx of the people in the control group and the patients suffering from chronic pharyngitis were examined with PCR and *H. pylori* culture. Three groups were studied:

- Group 1 consisted of 35 cases with chronic pharyngitis and negative C-14 urea breath test.
- Group 2 consisted of 35 cases with chronic pharyngitis and positive C-14 urea breath test.
- Group 3 consisted of 20 control cases with no pharyngitis and negative C-14 urea breath test.

In group 1, 14 cases (40.0%) were males and 21 cases (60.0%) were females, with mean age of  $41.91 \pm 8.226$  (ranging from 27 years to 59 years old). In group 2, six cases (17.1%) were males and 29 cases (82.9%) were females, with mean age of  $43.46 \pm 15.816$  (ranging from 18 years to 74 years old). In group 3, 12 cases (60.0%) were males and eight cases (40.0%) were females, and mean age of these patients were  $45.15 \pm 12.770$  (ranging from 19 years to 68 years old).

In the present study, all procedures performed before extraction of DNA were carried out under aseptic conditions. Consequently, the bacterial species detected probably represented the bacteria present in the pharynx. To collect tissue from the pharynx of each patient, the surface of the pharynx mucous membrane was sprayed with 20 g/L amethocaine for anesthesia. The secretion at the pharynx was washed out by water from the epithelial tissue so that the results were not affected by gastric juice and saliva. Epithelial tissue in the pharynx was then collected by punch biopsy. Our research was approved by the ethical and human rights committee of the Ministry of Health, Ankara Tarining and Research Hospital (registration no: 0269/1980), and informed consents were obtained from control patients before starting the procedure.

### C-14 Urea Breath Test

A C-14 urea breath test (Helicap, Noster System AB, Stocholm, Sweden) was used in order to detect *H. pylori* infection of the gastric mucosa. None of the patients had undergone upper gastrointestinal operations or had used nonsteroidal anti-inflammatory drugs, antibiotics, bismuth, H<sub>2</sub> receptor antagonists, or proton pump inhibitors during the previous 4 weeks. Capsules of urea (Breath CARD, Kibion, Stocholm, Sweden) labeled with 1  $\mu$ Ci of <sup>14</sup>C were used. The patient swallowed the urea capsule with 20 mL of water. After 10 minutes, the patient was asked to inflate a balloon to provide a breath sample. The results were given in counts per minute (cpm) by a Heliprobe analyzer (Kibion AB, Uppsala, Sweden). Samples with values <25 cpm were considered negative for *H. pylori*, samples with values between 25 cpm and 50 cpm were considered doubtful, and >50 cpm were positive.

### PCR

Biopsy specimens collected for culture were placed in 1 mL of sterile saline 0.85%, and those collected for PCR were immediately placed in 1 mL of Tris ethylenediamine tetraacetic acid (EDTA) buffer. Both specimens were transported to the laboratory without delay. Samples for culture were processed within 20 minutes and homogenized. Afterwards they were cultured on Brain Heart infusion agar (Oxoid, Cambridge, UK) containing 7% horse blood and *H. pylori* selective supplement (Oxoid). Plates were incubated at 37°C for 7 days in microaerophilic conditions that were provided using CampGen packs (Oxoid). For suspected colonies Gram's stain, catalase, oxidase, and urease tests were performed. Catalase, oxidase, and urease tests that were positive, and curved Gram-negative rods were defined as *H. pylori*.

For PCR examination samples were preserved in Tris EDTA buffer at -20°C. DNA was extracted from samples using Heliosis DNA extraction kit (Metis Biotechnology, Ankara, Turkey) according to manufacturer's instructions. *H. pylori* specific 16S rRNA gene nested PCR amplifications were done by using Heliosis *H. pylori* PCR kit (Metis Biotechnology). Amplification reactions were performed with an automated thermal cycler (Techne-Comm Ltd., Hampshire, UK). Reaction mixtures without DNA and *H. pylori* NCTC 11637 were used as negative and positive controls, respectively. The specific PCR amplification products of 110 base pairs were analyzed using gel electrophoresis with 2% agarose and ethidium bromide staining.

### Statistical Analysis

The data were analyzed with SPSS for Windows 11.5 (SPSS Inc., Chicago, IL). Whereas age was expressed as mean  $\pm$  standard deviation, nominal data were presented as number of cases and (%). Mean ages were compared by one-way analysis of variation. Pearson chi-square test was applied for evaluation of sex distributions and PCR results among groups. A *P* value less than .05 was considered statistically significant.

## RESULTS

In the present study, *H. pylori* infections in the pharynx of patients who had chronic nonspecific pharyngitis and control group were examined with PCR and culture techniques. There was not a significant difference between the age distribution of three groups ( $41.91 \pm 8.226$  in group 1,  $43.46 \pm 15.816$  in group 2, and  $45.15 \pm 12.770$  in group 3, *P* = .655). When gender was evaluated, the rate of female patients was

significantly higher in group 2 than groups 1 and 3 (rate of female cases was 60.0% in group 1 and 40.0% in group 3, whereas 82.9% in group 2;  $P = .034$  between groups 1 and 2;  $P < .001$  between groups 2 and 3) but there was not a significant difference between groups 1 and 3 ( $P = .153$ ).

Table I, summarizes the results of *H. pylori* analysis by PCR and culture methods in the pharynx mucosa of patients with chronic nonspecific pharyngitis and in control subjects. There was a statistically significant difference in rates of *H. pylori* presence in the pharynx mucosa with PCR method among the three groups ( $P = .011$ ). When the groups were compared to each other in pairs, a significant difference was also found between pharyngitis and the control groups ( $P = .002$  between groups 1 and 3;  $P = .040$  between groups 2 and 3); however a significant difference was not found between groups 1 and 2 ( $P = .179$ ).

When all patients who had chronic nonspecific pharyngitis were evaluated, 27.1% (19 of 70 patients) of these patients had *H. pylori* infection in the pharynx. When the patients were evaluated in detail according to the presence of *H. pylori* infection in gastric mucosa (positive or negative C-14 urea breath test), 34.3% (12 of 35 patients) had gastric *H. pylori* infection, and 20% (7 of 35 patients), who did not have infection in gastric mucosa, had *H. pylori* infection in pharynx mucosa with the PCR technique. In the control group (20 patients), none of the patients had gastric ailment *H. pylori* infection in the gastric and pharynx mucosa. With culture, *H. pylori* infection was positive in only two cases in the chronic pharyngitis groups, one patient in group 1, and one patient in group 2. There was no statistical correlation between the results of the PCR technique and the results of the culture.

## DISCUSSION

Chronic pharyngitis is a chronic inflammation of the pharyngeal mucosa due to a wide variety of specific active micro-organisms, gastroesophageal reflux disease, functional dyspepsia, and other physical processes that

cause mouth breathing.<sup>1,6,16</sup> When these patients visit a clinic, the routine procedure is to eliminate the causative factors and to treat the patient with nonspecific antibiotics, anti-inflammatory agents, or behavioral methods in order to rehabilitate chronic nonspecific pharyngitis symptomatically without looking for an active pathogenic micro-organism.<sup>1</sup> As there are only a few studies about antibiotic therapy in chronic pharyngeal complaints in which it is reported that up to 20% of the patients benefit from such treatment, the general thought is that bacteria may be responsible for this condition.<sup>1</sup>

*H. pylori* is an accepted cause of chronic persistent gastritis and has a major causative role in peptic ulceration, intestinal metaplasia in the stomach, and gastric metaplasia in the duodenum, gastric adenocarcinoma, and mucosa associated lymphoid tissue lymphoma.<sup>17,18</sup> The mucosa of the upper aerodigestive tract is in continuity with the gastric mucosa, and as a consequence there are several studies confirming *H. pylori* colonization in localizations besides the gastrointestinal cavity with or without having the bacteria in the stomach, such as the oral cavity,<sup>10,12</sup> dental plaque,<sup>8,9</sup> saliva samples,<sup>8,9</sup> adenotonsillar tissues,<sup>10,11</sup> nasal and sinus mucosa of some patients with chronic rhinosinusitis,<sup>13,14</sup> and tracheo-bronchial secretions.<sup>19</sup> *H. pylori* can settle in the most proximal part of the digestive tract by gastroesophageal reflux or contamination by an exogenous route without any gastric regurgitation.<sup>20</sup> As this bacteria can exist in the oral cavity and other extragastric regions independent from stomach presentation, it can be theorized that *H. pylori* can also colonize in the larynx and pharynx. Pharyngeal reflux in the presence of *H. pylori* infection of the stomach would expose the pharynx to the *H. pylori* bacterium or retrograde flow of gastric contents containing acid, bile, and pepsin, and could therefore conceivably act as a cofactor for the development of inflammatory or malignant conditions.<sup>21-23</sup> In the present study, *H. pylori* was studied as a potential cause for nonspecific pharyngeal symptoms and pharyngeal inflammation in patients with chronic nonspecific pharyngitis and in healthy subjects. In humans, *H. pylori* is probably the most common chronic bacterial infection, especially in the gastrointestinal system, and is present in almost half of the world's population, asymptomatic throughout their lives.<sup>24,25</sup> It was reported in several studies that *H. pylori* positivity increased as a factor of age, and gender has no effect on *H. pylori* infection.<sup>21</sup> In our study, the mean age of patients who had gastric *H. pylori* infection was  $41.91 \pm 8.226$ , and there was no significant difference when compared to the patients who did not have gastric infection. In the group of chronic pharyngitis patients without gastric infection, the female gender rate was significantly higher.

In the study of Zhang et al.<sup>7</sup> it was reported that in patients with chronic nonspecific pharyngitis who had gastric ailments, 68.8% of the cases had infection with *H. pylori* in the pharynx, whereas only 23.5% of the cases without gastric complaints had infection, and this was statistically significant. In the study of Aladag et al.<sup>1</sup> they found a high rate of *H. pylori* seroprevalence

TABLE I.

The Prevalence of *Helicobacter pylori* Infection in the Pharynx Mucous Membrane of Patients With Chronic Nonspecific Pharyngitis and Healthy Subjects.

	Chronic Nonspecific Pharyngitis		Control Group (n=20)
	C-14 Urea Breath Test (+) (n=35)	C-14 Urea Breath Test (-) (n=35)	
<b>PCR</b>			
<i>Helicobacter pylori</i> Infection in Pharynx			
Negative	23 (65.7%)	28 (80.0%)	20 (100.0%)
Positive	12 (34.3%)*	7 (20.0%)†	0 (0.0%)‡
<b><i>H. pylori</i> culture</b>			
Negative	34 (97.1%)	34 (97.1%)	20 (100.0%)
Positive	1 (2.9%)	1 (2.9%)	0 (0.0%)

\*Comparison between group 1 and 2 ( $P = .179$ ).

†Comparison between group 2 and 3 ( $P = .040$ ).

‡Comparison between group 1 and 3 ( $P = .002$ ).

(78%) in patients with chronic nonspecific pharyngitis who had no gastric ailments. In the control groups, although none of the cases was found to be infected with *H. pylori* in the pharynx with PCR method,<sup>7</sup> the rate of *H. pylori* infection with serological methods was 46.7%.<sup>1</sup> The patients with chronic nonspecific pharyngitis in our study were divided into two groups in order to find out the relationship between gastric ailments and gastric *H. pylori* infection and the presence of *H. pylori* in the pharynx mucosa. Our results were correlated with these authors' results. As in the pharyngitis cases with gastric ailments, 34.3% were determined to be infected with *H. pylori* in the pharynx, which was higher than in the cases without gastric ailments (20%), but was not significant. This means that in the etiology of chronic nonspecific pharyngitis, *H. pylori* infection is significantly determined to be one of the bacterial agents not only in the patients with gastric ailments and *H. pylori* infections, but also in the patients who did not have these complaints. If the patients had chronic nonspecific pharyngitis, they are associated with higher rates of *H. pylori* in the pharynx more than healthy patients.

In the diagnosis of *H. pylori* infections, direct and indirect methods may be used to determine the presence of *H. pylori*. Direct methods are both histologic and microbiologic studies, such as urease test, PCR, culture, and smears.<sup>26,27</sup> Biopsy-based methods, such as culture, PCR, and histopathology, have been accepted as the gold standard by many authors, but they are technically difficult and expensive.<sup>15</sup> Studies concerning culture methods may have underestimated the prevalence of *H. pylori* in the oral cavity, but PCR-based assays may be promising for detecting *H. pylori* because of their high sensitivity and specificity.<sup>28</sup> PCR has been considered a rapid, sensitive, and accurate method for detection of *H. pylori* in various clinical specimens.<sup>13,29,30</sup> We preferred to use culture and PCR methods. However, with culture it was difficult for us to detect *H. pylori* in the pharynx because in only two cases (2.9%) culture was positive for *H. pylori* infection. The results of the present study suggest that PCR is an important tool for the detection of *H. pylori* from mucosal tissue samples. With PCR technique, 7% of the patients were tested positively for *H. pylori* in dental plaque, but only two cases were positive with culture.

## CONCLUSION

From this study, it can be determined whether there is a relationship between *H. pylori* infection of the pharynx and chronic pharyngitis. Moreover, it can be determined if a history of certain gastric diseases may contribute to this infection. As a result it can be said that *H. pylori* is not detected in the pharynx of healthy people, chronic nonspecific pharyngitis is significantly related to *H. pylori* infection, and a stomach ailment history is associated with a higher rate of *H. pylori* infection of the pharynx. Thereafter, in the medical treatment of chronic pharyngitis, antibiotics that are efficacious on eradication of *H. pylori* infection can also be used. PCR is a suitable tool for the diagnosis of this

bacteria at the mucous membranes of various organs, which is difficult to culture.

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