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Abstract Clinical studies have shown that Helicobacter pylori can be found not only in the mucosa of the stomach, but in the pharyngeal and laryngeal regions as well. The aim of this prospective case-control study was to identify H. pylori infection in the biopsy material from the larynx of the patients suffering from benign laryngeal diseases (vocal fold polyps, laryngitis) and laryngeal cancer and to investigate the possible relationships between the laryngeal H. pylori and patients’ socio-demographic data and laryngopharyngeal reflux. The results of the biopsy material from 67 adult patients treated for benign laryngeal diseases and laryngeal cancer and 11 individuals of the control group revealed that H. pylori infection could be identified in more than one-third of the patients. In the majority of cases H. pylori was found in the patients with chronic laryngitis (45.5 %) and laryngeal cancer (46.2 %). The findings of these sub-groups significantly differed from those of the control group (9.1 %) (p < 0.05). No significant relationships between H. pylori infection found in the laryngeal region and patients’ demographic data, their unhealthy habits and reflux-related symptoms or signs were obtained. It could be concluded that H. pylori can colonize in the larynx of patients with benign laryngeal diseases and laryngeal cancer. To clarify the role of H. pylori as a risk factor for laryngeal diseases further research is needed.

Keywords Helicobacter pylori infection · Upper airway · Benign laryngeal diseases · Laryngeal cancer

Introduction

Helicobacter pylori infection caused by a gram-positive curved or spiral-shaped rod is one of the most common human bacterial infections in the world, which has affected more than half of the world’s population [1]. The infectivity in Lithuania is counted up to 51.7–78.5 % among adults and about 45 % among children [1, 2]. It has been proved that H. pylori has a significant impact on the pathogenesis of chronic gastritis, duodenal and gastric ulcers, adenocarcinoma, and gastric lymphoma [3]. Although, the primary localization of this bacterium is the stomach region, the recent studies suggest that H. pylori can also be found in calculus, saliva, pharyngeal lymphoid tissue, nasal and sinus mucosa, oropharyngeal aphthas, tympanic cavity secretion, and larynx [4]. The most recent study data have shown that H. pylori can cause the formation of vocal fold (VF) polyps, moreover, the evidence of this bacterium found significantly more often in the patients with laryngeal cancer than in those with benign laryngeal diseases can also be observed [5]. The recent...
5-year studies and meta-analysis have demonstrated that the bacterium can be an independent risk factor for the development of the hypopharyngeal–laryngeal cancer showing a two times higher risk of developing a laryngeal cancer for patients with identified H. pylori infection in the larynx than those for the control group [5–7].

Despite the fact that modern scientists have been performing researches on whether H. pylori may be an etiological factor for different laryngeal diseases, the data in this field are scanty. Furthermore, the routes of the bacteria entering the upper airway, as well as the relationship between gastroesophageal reflux and H. pylori in the upper airway also remains unclear.

The aim of this study was twofold: to identify H. pylori infection in the biopsy material from the larynx of the patients suffering from benign laryngeal diseases and laryngeal cancer and to investigate the possible relationships between the H. pylori in the larynx and patients’ demographic data, unhealthy habits and laryngopharyngeal reflux (LPR).

Materials and methods

Patients

The study was conducted at the University hospital from 2010 September to 2011 June. In 78 adult patients from 21 to 87 years of age, who had experienced endolaryngeal microsurgical operations on benign and malignant laryngeal diseases, agreed to participate in the study and did not take proton pump inhibitors (PPIs) for at least a week, were examined. The patients clinical group was divided into three sub-groups according to their diagnosis: the sub-group of VF polyps, which included 32 patients—16 males and 16 females (with an average age of 43.5 ± 13.1 years); the sub-group of chronic laryngitis—22 patients, 13 males, 9 females (with an average age of 53.3 ± 9.1 years); the sub-group of laryngeal cancer—13 patients, 12 males, and 1 female (with an average age of 63.5 ± 11.8 years). The control group consisted of 11 patients—1 male and 10 females (with an average age of 38.1 ± 12.2 years), who had been operated for “soft” VF nodules located in the mid-portion of the membranous VFs; however, a histological examination of the removed specimens revealed normal tissues of VFs.

The study was conducted after the reception of the permission from Kaunas Regional Ethics Commission for Biomedical Research. All the patients signed a consent form to participate in the study.

Methods

Questionnaires

Before the surgery, all the patients filled a specially designed questionnaire, which assessed demographics, unhealthy habits, and LPR symptoms and signs (history, upper gastrointestinal endoscopy findings, reflux-related symptoms and videolaryngoscopic data). Reflux-related symptoms were assessed with a standardized, validated questionnaire Reflux symptom index (RSI) of nine symptoms common in laryngopharyngeal and gastroesophageal reflux: hoarseness, throat clearing, excess throat mucus, difficult swallowing food, coughing after ate, choking, troublesome cough, lump in the throat, heartburn, chest pain, regurgitation and indigestion. Symptoms were self-rated on 6-point scale from 0, no problem, to 5, severe problem. RSI ≥13 was considered abnormal [8].

Clinical examination of the patients’ ear, nose, throat, and larynx condition were registered in specially designed form. Reflux-related findings were assessed applying videolaryngoscopy. Videolaryngoscopy was performed using the Kay Elemetrics 70° endoscope with evaluation for subglottic edema, ventricular obliteration, erythema, VF edema, diffuse edema, posterior commissure hypertrophy, granuloma and thick endolaryngeal mucus with validated Reflux finding score (RFS) scale. RFS ≥7 was considered abnormal [9].

Helicobacter pylori identification methods

The infection was identified by two methods: (1) histochemical evaluation of the biopsy material from the larynx: the 2–4 μm thickness paraffin sections were prepared and stained by modified Giemsa method (H. pylori acquires a dark blue color [10]) (Fig. 1); and (2) rapid urease test: the sterilely taken biopsy (min 2 × 2 mm pieces) was placed in a medium with urea and pH indicator—if the material contained H. pylori, urease enzyme resolved the endogenous urea into ammonia and carbon dioxide, pH was changed by the released ammonia, and the indicator changed its color from yellow to orange, red, or purple. CLO (Kimberly-Clark, USA) test was used. The color changes according recommendations were evaluated after 20 min, as well as 1, 3, and 24 h [11] (Fig. 2). All the specimens from the larynx of clinical and control group patients were obtained from the same region—a membranous part of the VFs. If at least one result of the two studies was positive, H. pylori was identified in the biopsy material of the larynx [H. pylori (+)].
Statistical analysis

Statistical analysis was performed using the statistical package for SPSS 17 (SPSS Corp., Chicago, IL, USA). Differences in non-parametric data were calculated using Chi-squared or Fisher’s exact tests. The unpaired Student’s t test (two-tailed) was used to compare the differences between two groups for quantitative data. * level of significance of 0.05 was used. The correlation coefficient (r) was calculated using Pearson’s and Spearman’s tests.

Results

Our study showed that *H. pylori* infection in the biopsy material from the larynx was found in 38.8% (26 of 67) of all the patients from the clinical group and these results significantly differ from those of control group where *H. pylori* infection was found in 9.1% (1 of 11) of investigated individuals (*p* < 0.05).

The analysis of the results of the clinical sub-groups demonstrated the highest rate of *H. pylori* infection in the biopsy material of the patients with chronic laryngitis and laryngeal cancer: 45.5% (11 of 32) and 46.2% (6 of 13) of cases, respectively (Fig. 3). The data of these two subgroups were significantly higher from the data of the control group (*p* < 0.05). No significant difference between the prevalence of *H. pylori* among the patients with benign laryngeal diseases and laryngeal cancer was obtained (*p* > 0.05).

The correlation analysis showed no significant relationships between *H. pylori* infection in the laryngeal region and patients’ demographic data (*r* = 0.04–0.09, *p* > 0.05), as well as their unhealthy habits (*r* = 0.10–0.04, *p* > 0.05) and LPR symptoms or signs (*r* = 0.12–0.18, *p* > 0.05). A comparative data between the patients in whom *H. pylori* was identified in the biopsy material from the larynx [*H. pylori* (+)] and the patients in whom *H. pylori* was not found [*H. pylori* (−)] are shown in Table 1.

Discussion

The prevalence of *H. pylori* in the patients with laryngeal diseases

Literature data reveal that *H. pylori* is not considered a normal laryngeal flora [12]. Recent studies are intended to determine whether the bacterium could be one of the etiological factors for laryngeal diseases and whether bacterial eradication might be effective in the treatment of
laryngeal diseases. Thus, one of the aims of this study was to determine the prevalence of *H. pylori* in the patients with benign laryngeal diseases and laryngeal cancer. The results of this study indicate that *H. pylori* has been identified in the biopsy material from the larynx of more than one-third (38.8 %) of all the clinical group patients, equally suffering from benign laryngeal disease and laryngeal cancer. The highest rate of *H. pylori* infection has been identified in the biopsy material of the patients with chronic laryngitis (45.5 %) and laryngeal cancer (46.2 %) which significantly differed from the control group (9.1 %).

The results of our study correspond to the scarce literature data. Similarly to the results of this study, the investigations performed over the past decade have shown that *H. pylori* is identified in almost half of the investigated patients with benign laryngeal diseases—in 40.6–47.3 % of cases [13–14]. The most recent studies of *H. pylori* determined by an accurate method of polymerase chain reaction (PCR), while identifying *H. pylori* DNA and the most important antigen of the bacterium in the investigated material, support the hypothesis of a possible relationship between the etiological *H. pylori* and benign laryngeal diseases: in the investigated material from the laryngeal region *H. pylori* was found in 9.1–71.4 % of the cases [15–19]. In 2008, Fang et al. [20] hypothesized that *H. pylori* may be an etiological factor of VF polyps. Using the rapid urease test, the authors investigated a laryngeal histologic material taken from 53 hoarse patients whose complaints had been caused by VF polyps and nodules; in 39.9 % of cases *H. pylori* was found only in the biopsy material from VF polyps, while no *H. pylori* was found in any of the material samples from VF nodules. In this study, *H. pylori* was identified in the polyps of a similar percentage of the patients—34.4 %, but no statistically significant difference between the sub-group of VF polyps and the control group that consisted of the persons treated for VF nodules, was obtained, possibly due to a small number of subjects in the control group.

The majority of current research is being directed towards the investigation of *H. pylori* infection and laryngeal cancer risk. Literature data confirm the importance of *H. pylori* infection in the development of laryngeal cancer. The data of meta-analysis indicate that the risk of developing laryngeal cancer is two times higher for the subjects infected with *H. pylori*, than for those in the control group [7]. Using both serological studies data that show immune response to *H. pylori*, a marker of prior infection with this organism, and the research data of the biopsy material from the laryngeal region, *H. pylori* is found in 46–70 % of cases of the patients with laryngeal cancer, i.e., significantly more often than in the patients operated for benign laryngeal lesions, which suggests that *H. pylori* may be an independent factor of squamous cell carcinoma of the larynx [5, 6, 14, 21]. Although, no significant difference between the prevalence of *H. pylori* among the patients with laryngeal cancer and benign laryngeal diseases was obtained in this study, the patients with laryngeal cancer were registered as the most often infected.

The relationship between *H. pylori* infection found in the larynx and laryngopharyngeal reflux

One of the aims of this study was to determine the relationships between *H. pylori* infection in the laryngeal region and patients’ demographic data, as well as their unhealthy habits and LPR. According to the findings of this study and based on the examination of the data from 78 patients’, no significant relationships between *H. pylori* infection in the laryngeal region and patients’ demographic data, their unhealthy habits and LPR symptoms or signs,

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**Table 1** Comparison of the demographics, unhealthy habits and reflux-related symptoms and signs between the patients positive and negative for *Helicobacter pylori* in the biopsy material from the larynx

<table>
<thead>
<tr>
<th>Variables</th>
<th>Helicobacter pylori (+)</th>
<th>Helicobacter pylori (--)</th>
<th>p value*</th>
</tr>
</thead>
<tbody>
<tr>
<td>Gender: males/females (%)</td>
<td>40.5/27.7</td>
<td>59.5/72.2</td>
<td>0.36</td>
</tr>
<tr>
<td>Age (years)</td>
<td>50.6</td>
<td>47.7</td>
<td>0.23</td>
</tr>
<tr>
<td>Body mass index (kg/m²)</td>
<td>25.7</td>
<td>24.4</td>
<td>0.12</td>
</tr>
<tr>
<td>Smokers (%)</td>
<td>37.0</td>
<td>25.5</td>
<td>0.64</td>
</tr>
<tr>
<td>Drinkers (%)</td>
<td>63.0</td>
<td>60.8</td>
<td>0.48</td>
</tr>
<tr>
<td>History of GERD (%)</td>
<td>31.3</td>
<td>11.1</td>
<td>0.12</td>
</tr>
<tr>
<td>Patients with reflux symptoms (%)</td>
<td>53.6</td>
<td>46.0</td>
<td>0.52</td>
</tr>
<tr>
<td>Reflux finding score (average ± SD)</td>
<td>10.0 ± 3.0</td>
<td>9.2 ± 3.4</td>
<td>0.56</td>
</tr>
</tbody>
</table>

* Patients with identified laryngeal *H. pylori*

b Patients with unidentified laryngeal *H. pylori*

c Patients with pathologic (≥13) reflux symptom index

d Standard deviation

* Difference is significant at the significance level *p* < 0.05
including heartburn, were obtained. These findings possibly suggest that *H. pylori* is not a transient infection, which invades the larynx from the stomach. Literature data dealing with this problem are scarce and contradictory. In the majority of such studies, the relationships between the identified *H. pylori* infection found in the stomach and the LPR were examined, which were not confirmed [22]. In 2012, according to the data of the study by Cekin et al. [16], which included 43 patients with benign laryngeal disease and laryngeal cancer and after the analysis of the identified *H. pylori* in the laryngeal region with the help of PCR method, no significant relationships between the bacterium and LPR symptoms were also found. Meanwhile, in 2006, the results of the study published by Oridate et al. [23] showed that *H. pylori* infection in the patients with LPR predict a better positive response to the anti-reflux treatment. Having examined the serological IgG titer of *H. pylori* for 42 patients, it was found that the response of the *H. pylori*-infected group to the 2-month PPIs treatment was clinically superior to that of the uninfected individuals. On the other hand, some studies suggest that while treating the patients with LPR and prescribing both PPIs and *H. pylori* eradication therapy, the laryngological symptoms and findings disappear in up to 80% of patients [24].

Thus, having summarized the data revealing the relationship between the laryngeal pathology and *H. pylori* infection, it can be assumed that *H. pylori* infection may be a risk factor for the development of laryngeal diseases, but its relevance in otolaryngology should be confirmed by larger scale, well designed, multicentre research studies that would examine the relationship between gastrointestinal reflux and *H. pylori* infection in the upper airway, and would assess the effect of *H. pylori* eradication on the course of *H. pylori*-associated laryngeal diseases.

**Conclusions**

*Helicobacter pylori* in the biopsy material from the larynx was identified in more than one-third of the patients, equally suffering from benign laryngeal disease and laryngeal cancer, but significantly more often than in the control group subjects. Patients with chronic laryngitis and laryngeal cancer showed the highest rate of *H. pylori* infection in the larynx.

No significant relationships between laryngeal *H. pylori* and patients’ demographic data, their unhealthy habits and reflux-related symptoms and signs, were obtained.

Further research studies are needed that would justify or deny the importance of *H. pylori* infection for the development of different laryngeal diseases, as well as the effect of *H. pylori* eradication on the course of laryngeal diseases.

**Conflict of interest** The authors have no conflict of interest.

**References**