

Clinical Study

The Role of *H. pylori* in the Development of Laryngeal Squamous Cell Carcinoma

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Aim. This study aims to investigate the possible role of *H. pylori* as a cause of laryngeal squamous cell carcinoma. **Method.** This controlled study was performed with 31 consecutive laryngeal cancer and 28 cancer-free patients who underwent direct laryngoscopy and biopsy of laryngeal lesions. To document the previous *H. pylori* infection, serological analysis of the antibody titers was done. Immunohistochemical analyses were applied to the tissue samples. **Results.** Serology was found positive at the 90.3% of the laryngeal cancer patients and 96.4% of the benign group. There were no statistically significant differences between the two groups ($P > 0.05$). Immunohistochemical analysis results were determined as negative at all of the specimens of laryngeal cancer patients and patients with benign lesions. **Conclusion.** There were no signs of colonization of *H. pylori* in laryngeal tissues of both groups' patients. It is thought that no relationship exists between the *H. pylori* infection and laryngeal squamous cell carcinoma.

1. Introduction

Laryngeal squamous cell carcinoma (LSCC) is one of the common cancers of the upper aerodigestive system. It accounts for 25% of all the cancers of head and neck and 2-3% of the cancers of the whole body [1]. Tobacco is the most important risk factor for the LSCC. The other risk factors might be some viruses, bacteria, diet type, radiation exposure, gastroesophageal reflux, occupation, and genetical inheritance [2].

H. pylori, a helical shaped gram-negative microaerobic bacterium which was described as a type 1 carcinogen by the International Agency for Research on Cancer Working Group (IARC-1994), is related to gastric cancer and MALT lymphoma [3, 4]. *H. pylori* has also been shown in the oral cavity, dental plaques, and saliva [5]. The existence of *H. pylori* in the oral cavity and stomach can indicate the colonization of the bacteria in the laryngeal mucosa. It is suggested that *H. pylori* might play a role in the development of laryngeal

cancer by forming chronic inflammation and raising the exposure to the carcinogens by destroying mucosal and immune barriers.

There are several studies investigating the relationship between *H. pylori* and laryngeal cancer, the results of which still show conflict about the subject [6–12]. The aim of the present study is to investigate the existence of *H. pylori* in the laryngeal specimens of the patients with the diagnosis of LSCC and to make a comparison with the patients with benign larynx pathologies.

2. Patients and Method

A total of 59 patients with laryngeal pathologies were enrolled in the study. The laryngoscopic biopsies of 31 patients revealed the result of LSCC. Twenty eight patients had benign larynx pathologies such as polyps or nodules. Patients with a history of using medical treatment in terms of the eradication of *H. pylori* were excluded from the study.

TABLE 1: The comparison of the variables between the malignant and benign groups.

	Laryngeal pathology		P
	Malignant (n = 31) n (%)	Benign (n = 28) n (%)	
Serology, n (%)			
Positive	28 (90.3)	27 (96.4)	0.614
Negative	3 (9.7)	1 (3.6)	
Immunohistochemistry		0	

H. pylori IgG antibody titers were measured from the serum samples of the patients via Enzyme-Linked Immunosorbent Assay (ELISA) test in order to display a previous infection. Euroimmun (Luebeck/Germany) study kit was used, and levels more than 20 U/mL were accepted as seropositive.

The existence of *H. pylori* antigens in the specimens of all patients was investigated with immunohistochemical staining, which was applied with streptavidin biotin peroxidase (Str. ABC/HRP) method. As a control, biopsy specimens taken from the antral mucosa of a patient who had *H. pylori* gastritis were stained with the same method and observed in light microscope, and the existence of *H. pylori* was detected.

The results were analyzed with SPSS 15.0. Pearson chi-square analyses were made for the comparison of the categorical variables and Student's *t*-test for the numerical variables. The normal distribution of the data was analyzed with Shapiro-Wilk test. To determine the risk factors for malignancy single and multiple regression analysis were made. $P < 0.05$ value was accepted as statistically significant.

3. Findings

The mean age of the patients enrolled was 50.6 ± 10.4 (27–70). Thirty one (52.5%) patients had malignant, and 28 (47.5%) had benign larynx pathologies. Forty five (76.3%) of the patients with LSCC had a history of smoking. IgG antibody was positive in 90.3% of the patients with LSCC and 96.4% of the ones with benign pathologies. There were no statistically significant differences between the two groups ($P > 0.05$) in terms of the results of serology. None of the slides of both laryngeal cancer and benign laryngeal lesions revealed *H. pylori* existence histochemically (Table 1).

4. Discussion

Laryngeal squamous cell carcinoma is one of the most common cancers of the upper aerodigestive system. Tobacco appears as the major risk factor. The other risk factors are, alcohol, chemical carcinogens, vocal abuse, positive family history of cancer, previous radiation exposure of the head and neck, and human papilloma virus (HPV). HPV is considered as a causative agent which is shown to increase proliferation in laryngeal epithelial cells. Epithelial cell proliferation could also be caused by other infectious agents. *H. pylori*, a gram negative spiral, flagellated bacillus,

has been reported as infectious bacteria of various areas of the stomach and duodenum and major causative agent of various gastric diseases [13, 14]. This bacterial infection has prevalence of about 30% in developed countries and up to 90% in underdeveloped countries [15]. *H. pylori* infection is identified as an important risk factor for gastric cancer [16, 17]. In 1994, the International Agency for Research on Cancer and the World Health Organization classified the *H. pylori* infection as a group I carcinogen [18].

Although its role is well documented in gastric cancer, little is known about the possible association of *H. pylori* infection with other carcinomas. Nowadays dental plaques, oral lesions, saliva, and adenotonsillar tissue are considered as reservoir of *H. pylori* as well as stomach. Besides, the colonization of the *H. pylori* in the upper aerodigestive tract may be facilitated by oral route or gastric oral route (Gastroesophageal reflux) and there might be positive association between *H. pylori* infection and head and neck cancer.

H. pylori is considered to cause epithelial cell proliferation in the laryngeal mucosa just like it does in gastric mucosa, which eventually leads to laryngeal carcinoma [6]. Several studies have been attempted to investigate the relationship between *H. pylori* infection and laryngeal cancer. Whereas some of these studies suggested an increased susceptibility to laryngeal cancer, the others failed to show a positive association. Serological studies performed to investigate the relationship between *H. pylori* infection and laryngeal cancer show conflicting results [7, 9, 11]. Essentially, these studies reflect the previous *H. pylori* infection, and serological positivity may be caused by the colonization of the gastric mucosa.

Tests to confirm the presence of *H. pylori* can be divided into two categories: those that rely on indirect methods to detect infection such as serology and breath urease testing and those that are named as direct methods require invasive interventions such as endoscopy and biopsy. Direct methods include histopathology, culture, and rapid urease test. Each test has its own strengths and weaknesses.

Serological tests can accurately reflect the presence or absence of *H. pylori* infection, and they do not indicate colonization of the bacteria in laryngeal tissues. The best way to know whether the laryngeal tissues are involved by *H. pylori* is to show bacterial presence in tissue. However there are few studies that have run histological investigation of *H. pylori* in laryngeal tissues [8, 10].

The first study investigating the presence of *H. pylori* in laryngeal tissue was performed by Borkowski et al. [8]. In this study, laryngeal biopsy specimens of 35 patients with chronic laryngitis were analyzed to investigate the presence of *H. pylori* by using a rapid urease test. The test was positive in six of 35 patients (17.1%). Rapid urease test positivity in laryngeal tissue without other evidence of *H. pylori* infection is not meaningful from the standpoint of colonization. Laryngeal tissue can be colonized by other microbiological agents containing urease enzyme [6].

Kizilay et al. [10] refused the presence of *H. pylori* in specimens from both cases with LSCC and patients with non-neoplastic diseases of the larynx by using light microscope. Specimens of both tumor and control groups were stained with hematoxylin-eosin and modified Giemsa stains.

Several attempts have been made for an accurate detection of *H. pylori* in histological sections of various tissues. Immunostaining with monoclonal and polyclonal antibodies has been reported as a sensitive and specific method for identifying *H. pylori* [19, 20]. Akbayir et al. [6] investigated *H. pylori* in specimens from 50 patients with LSCC and in 50 patients with benign laryngeal pathologies using histopathological and immunohistochemical methods. *H. pylori* was identified in 28 LSCC patients under light microscopy. However, immunohistochemical evaluation of the samples failed to provide *H. pylori*-specific staining. They stated that the absence of *H. pylori* in laryngeal carcinoma tissue suggests lack of its role in the development of laryngeal squamous cell carcinoma and absence in benign laryngeal tissue suggests lack of *H. pylori* colonization in the larynx.

In the present study, it was found that patients with LSCC were no more likely than patients with benign lesions to have *H. pylori* infection. Although there is a high incidence of serological evidence of *H. pylori* infection in both groups of patients, we could not identify *H. pylori* in any of biopsy samples immunohistochemically. Taken together, the current data suggest that the larynx is not a reservoir for colonization of *H. pylori*, and *H. pylori* does not play a role in the pathogenesis of laryngeal neoplasia.

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