RESEARCH COMMUNICATION

Is there any Association between *Helicobacter Pylori* Infection and Laryngeal Carcinoma?

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Abstract

Objective: To investigate the possible role of *Helicobacter pylori* as a cause of squamous cell carcinoma of larynx in a case-control study in an otolaryngology ward at an academic university. Subjects and Methods: A total of 65 patients with laryngeal cancer and 65 matched cancer-free controls underwent esophagogastroduodenoscopy and biopsy of antral and body regions of the stomach for evaluation of *Helicobacter pylori* infection. Results: The proportion of subjects with a positive rapid urease test for gastric infection was similar between the two groups (49.2 % in cases vs. 40% in controls). However, a positive rapid urease test for body was less frequently seen in patients with laryngeal cancer whereas a positive rapid urease test for antrum was significantly higher (P=0.04). Conclusion: Our study failed to show *Helicobacter pylori* as one of the etiologies of laryngeal cancer. However, it supported the hypothesis that colonization of Helicobacter pylori only in the gastric body might have a protective effect against laryngeal cancer with decreasing gastric acid while antral *Helicobacter pylori*, increasing gastric acid due to G cell hyperplasia, may be a predisposing factor for laryngeal cancer, with acid reflux as a possible underlying etiology.

Keywords: *Helicobacter pylori* - laryngeal cancer - rapid urease test - gastric acid - esophagogastroduodenoscopy

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Introduction

Cancer of the larynx is the second most common malignancy of the upper aerodigestive tract, 85% to 95% of laryngeal malignancies are squamous cell carcinoma (SCC). Tobacco and alcohol use are the two primary risk factors for cancer of the larynx. Other risk factors include human papilloma virus (HPV), chemical carcinogens, positive family history for malignancy, previous radiotherapy and personal history of head and neck cancer (Flint et al., 2010).Although the relationship is not proven, acid pepsin diseases is regarded as a risk factor for laryngeal carcinoma (Doustmohammadian et al., 2010). HPV is epidemiologically considered as an etiologic factor for laryngeal cancer since it is shown to increase the proliferation of laryngeal epithelial cells (Jacob et al., 2002). Other infectious agents as *Helicobacter pylori* (HP) might also cause epithelial cell proliferation and cancer.

The prevalence of HP varies throughout the world and depends largely on the overall standard of living in the region. In developing parts of the world, 80% of the population may be infected by the age of 20, whereas the prevalence is 20-50% in industrialized countries (Fauci et al., 2008).

HP is a gram – negative spiral bacterium known to be one of the most virulent bacteria worldwide. It usually causes chronic bacterial infection in humans and has been associated with gastritis, peptic ulcer, gastric cancer and other gastrointestinal disorders (Wang et al., 2007; Fauci et al., 2008).

HP colonization induces a tissue response in the stomach termed chronic superficial gastritis(Fauci et al., 2008). Antral – predominant gastritis is most closely linked with duodenal ulceration,while gastric ulceration and adenocarcinoma arise in association with pan or corpus – predominant gastritis. The inflamed acid – producing gastric corpus produces less acid with consequent relative hypochlorhydria despite hypergastrinomia (Fauci et al.,2008).Much attention has been focused on the possible protective role of HP against Gastroesophageal Reflux Disease (GERD). The mechanism underlying this protective effect appears to include HP– induced hypochlorhydria (Fauci et al., 2008).Therefore, it could be postulated that HP of gastric corpus, decreasing gastric acid, is protective against GERD and laryngeal cancer. HP of antrum, increasing gastric acid, plays a role in the pathogenesis of laryngeal cancer.

HP infection is diagnosed by either invasive (endoscopy

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and biopsy)or non-invasive (urease breath test (UBT), serology, and stool antigen testing) techniques. invasive test, which require upper gastrointestinal endoscopy and are based on the analysis of gastric biopsy specimens. If endoscopy is performed, the most convenient biopsy – based test is the Urease Test in which biopsy specimens are placed into a gel containing urea and an indicator. The presence of HP, urease elicits a color change which often occurs within minutes but can require up to 24h (Fauci et al.,2008).

Several studies have been performed to evaluate the association between HP and laryngeal cancer. Some studies have shown that HP is as an etiologic factor for laryngeal carcinoma and some other studies have investigated the protective effect of HP against laryngeal cancer (Aygene et al., 2001; Zhuo et al., 2003; Akbayir et al., 2005; Nurgalieva et al., 2005; Fang et al., 2008;Titiz et al., 2008; Masoud et al.,2008).

None of the published studies compared the existence of HP in two separate parts of the stomach - corpus and antrum - in patients with laryngeal carcinoma and in cancer-free controls.

Materials and Methods

This study was approved by ethics committee of Liver and Gastrointestinal Disease Research Center, Tabriz university of Medical Sciences. Study procedures were explained by a designated research assistant and written information was also given. Informed written consent was obtained from each patient who wished to participate. Totally, 65 patients who had undergone laryngoscopy and biopsy with the definite diagnosis of squamous cell carcinoma as well as those who had undergone total or partial laryngectomy between May 2006 and February 2008 at academic hospitals affiliated to Tabriz University of Medical Sciences were evaluated. These patients underwent esophagogastroduodenoscopy (EGD) and biopsy of the antrum and body of the stomach. Tissue specimens from 2-3 cm above the pylorus and 2-3 cm below the cardia with separate two forceps were placed on a gel containing urea and an indicator and then color change was evaluated during the first hour and after 24 hours. After 24 hours, a yellow color indicated a negative and an orange to red color indicated a positive result.

Control group (65 patients) were hospitalized patients with anemia or other illnesses not associated with HP. These patients did not have signs of laryngeal cancer in their medical history and laryngoscopy. These controls, similar to the case group, underwent EGD and biopsy of the stomach. A complete medical history including demographic data such as age and sex, medical history, history of cigarette smoking and alcohol was obtained from each patient.

Patients who had used antibiotics, antiacid drugs or proton – pump inhibitors for peptic ulcer during the previous 2-4 weeks were excluded from this study. Tissue specimens from laryngeal cancer (65 patients) and benign laryngeal lesions (40 patients) were also investigated for the existence of HP with histopathologic evaluation and rapid urease test.

Table 1. Characteristics of Patients with and without Laryngeal Cancer

Characteristic	with	without	p-value
Mean age (Yr ± SD)	61.8 ± 7.70	61.7 ± 7.62	
Tobacco use	51 (78.5%)	48 (73.8%)	
Alcohol use	19 (29.2%)	17 (26.2%)	
RUT Body HP	5/32 (15.6%)	10/26 (38.5%)	
RUT Antral HP	27/32 (84.3%)	16/26 (61.5%)	

RUT, rapid urease test positive

Clinical characteristics and laboratory variables were compared by Independent sample T-test, $\chi 2$ test and Fisher exact test. A p-value less than 0.05 was considered statistically significant. All analyses were performed using the Statistical Package for the Social Science Software (SPSS).

Results

Our case group included 63 males and 2 females with a mean age of 61.8 ± 7.7 years. The control group consisted of 63 males and 2 females with a mean age of 61.7 ± 7.6 years. In the population with laryngeal cancer, the prevalence of smoking and alcohol consumption was 78.5 % and 29.2%, respectively. In the control group, they were 73.8 % and 26.2 %, respectively. The characteristics of the case and the control group are presented in Table 1. The cases and controls were matched in terms of age, sex, amount and duration of tobacco and alcohol consumption and did not have any significant differences. The proportion of subjects with a positive rapid urease test (RUT) for gastric HP was similar between the two groups [32 (49.2 %) in cases vs. 26 (40 %) in controls, p=0.290]. However, a positive RUT for body HP was less frequently seen in patients with laryngeal cancer than in controls without laryngeal cancer and difference was statistically significant. (5/32 in cases vs. 10/26 in controls, p = 0.04). A positive RUT for antrum HP was significantly higher in patients with laryngeal cancer than in controls and difference was statistically significant. (27/32 in cases vs. 16/26 in controls, p = 0.04). Tissue specimens from laryngeal cancer (65 patients) and benign laryngeal lesions (40 patients)were also investigated for the existence of HP with histopathologic evaluation and RUT. During rapid urease testing, none of the subjects (in either of the groups) showed color change during the first hour. While 65 slides of laryngeal cancer (squamous cell carcinoma) and 40 slides of benign laryngeal lesions (polyp, nodule) were stained with haematoxylin-eosin, none revealed HP existence.

Discussion

Upper aerodigestive tracts have many common etiologies such as smoking and alcohol use. Larynx is a part of the upper aerodigestive tract. Therefore, since HP is a known etiologic factor for gastric cancer, it could be postulated that HP plays a role in the pathogenesis of laryngeal cancer. The present study found no evidence in support of this hypothesis.

Prior investigators have studied HP as an etiologic

factor for laryngeal carcinoma. Kizilay et al (2006) investigated 69 total laryngectomy specimens with squamous cell carcinoma and 30 laryngeal tissue samples with non-neoplastic diseases (polyp, nodule) but none demonstrated HP infection. However, diagnosis was solely on the basis of histology. Akbayir et al(2005) examined 50 patients with laryngeal cancer and 50 benign laryngeal biopsy specimens by histopathological and immunohistochemical techniques, but again none demonstrated HP infection .In our study, HP was not detected in malignant and benign specimens of larynx when both rapid urease testing and pathology were applied, therefore, it is unlikely for HP to be colonized in larynx. Nevertheless, we suppose that larynx is not a permanent reservoir for HP. More exact researches with Polymerase Chain Reaction study for HP are recommended. In our study, a positive RUT for gastric HP was similar between the two groups but a positive RUT for antral HP was significantly different between the two groups.

Several serologic studies have been performed to evaluate the association between HP and laryngeal cancer. Aygene et al investigated the presence of IgG antibodies against HP antigens by ELISA technique in 26 patients with squamous cell carcinoma of larynx and 32 matched controls. They found that 73.1% of the patients with squamous cell carcinoma and 40.6 % of the controls were seropositive. ($P < 0.05$) (Aygene et al,2001).

Rubin et al reported that the presence of HP antibodies was significantly higher in patients with laryngeal dysplasia or frank carcinoma of the head and neck in comparison with their associated controls (Rubin et al., 2003).

It should be noted that the prevalence of HP varies throughout the world. In developing parts of the world such as Iran, 80 % of the population may be infected by the age of 20 and therefore, serologic studies are not suitable for such societies. In a study from Iran, seropositivity of HP (ELISA) in the age range of 41-50 and 51-60 was 73.46% and 75.75%, respectively (Jafarzadeh et al.,2007).

Jaspersen et al(1998) investigated the association between HP and chronic laryngitis. Thirty six patients with chronic laryngitis underwent gastroscopy. Biopsies were taken from the gastric antrum and body and lower, middle and upper esophagus. HP was diagnosed through RUT and histology. They concluded that there was no evidence for the existence of HP – associated laryngitis, suggesting that acid reflux is the underlying etiology. Absence of a control group and few cases of the study were limitations of this study.

In our study, a positive RUT for body HP was less frequently seen among patients with laryngeal cancer than in controls and the difference was statistically significant. It could support the hypothesis that body HP may be protective against laryngeal cancer through decreasing gastric acid.

The only Study that has investigated the protective role of body HP against laryngeal cancer was performed by Nurgalieva et al(2005).The aim of this study was to investigate whether HP infection also protects against laryngopharyngeal carcinoma. One hundred and ninety case subjects and 111 control subject were included in the study. The proportion of subjects with anti – HP IgG was similar between the two groups (32.8 % in cases vs. 27.0 % in controls, $p = 0.342$) but the difference was not statistically significant. This study had some limitations. First, HP of the body and antrum were not separately evaluated and second, the study was based on serologic findings.

In our study, a positive RUT for Antrum HP was higher in patients with laryngeal cancer than in controls and the difference was statistically significant. Antral HP may be a predisposing factor for laryngeal cancer, probably via increasing gastric acid, suggesting that acid reflux is the underlying etiology .

In conclusion, our study failed to show *Helicobacter pylori* as one of the etiologies of laryngeal cancer. However, a positive RUT for body HP was less frequently seen among patients with laryngeal cancer than in controls and the difference was statistically significant. It could support the hypothesis that body HP may be protective against laryngeal cancer with decreasing gastric acid. A positive RUT for antrum HP was higher in patients with laryngeal cancer than in controls and the difference was statistically significant. Antral HP may be a predisposing factor for laryngeal cancer, probably via increasing gastric acid due to G cell hyperplasia, suggesting that acid reflux may be the underlying etiology and acid suppression postlaryngeal cancer therapies may have protective effects on laryngeal cancer recurrence rates.

In our study, a certain association between gastric acid with laryngeal cancer was not found, since no PH study was carried out. But HP in two separate areas of stomach (antrum and body)in two groups was different, probably HP in these two areas had different effect on gastric acid and laryngeal cancer. More research with PH study is recommended.

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