Clinical Study

Chemotherapy

Chemotherapy 2006;52:80–82
DOI: 10.1159/000091727

Received: October 9, 2004
Accepted after revision: June 20, 2005
Published online: February 22, 2006

Histopathologic Examination for *Helicobacter pylori* as a Possible Etiopathogenic Factor in Laryngeal Carcinoma

Ahmet Kizilay[a] Levent Saydam[c] Abdullah Aydin[d] M. Tayyar Kalcioglu[a]
Orhan Ozturan[a] N. Engin Aydin[b]

Departments of [a]Otolaryngology and [b]Pathology, Inonu University Medical School, Malatya,
[c]Department of Otolaryngology, Bayindir Medical Center, Ankara, and
[d]Department of Pathology, Gaziantep University Medical School, Gaziantep, Turkey

Key Words
Helicobacter pylori · Head and neck cancer · Larynx cancer

Abstract
Objective: We investigated the presence of *Helicobacter pylori* in laryngeal cancer specimens to reveal whether or not this is a risk factor in the development of squamous cell carcinoma of the larynx. ***Methods:*** Sixty-nine total laryngectomy specimens with the pathologic diagnosis of squamous cell carcinoma and 30 laryngeal tissue samples that had been taken for the investigation of nonneoplastic (polyp, nodule) diseases were studied. Specimens of both tumor and control groups were stained with hematoxylin-eosin and modified Giemsa stains, and then they were examined under a light microscope. ***Results:*** In both groups, *H. pylori* could not be found in any of the cases. ***Conclusion:*** The histologic examination of our series did not reveal any clue related to the possible etiologic association between *H. pylori* and squamous cell carcinoma of the larynx.

Presented at the 1st World Conference on Magic Bullets – EHRLICH 2004, Nürnberg, Germany, September 9–11, 2004.

Introduction

Helicobacter pylori is a gram-negative microaerophilic spiral microorganism that has been identified and cultured in the past two decades [1]. There are numerous reports about its colonization in the stomach and oral cavity [2]. It can dwell in the gastric mucosa for years and is considered the main cause of chronic diffuse superficial gastritis of the fundus and antrum. *H. pylori* gastritis is a worldwide disease and may also be one of the most common chronic human infections. However, *H. pylori* can lead to the development of intestinal metaplasia in the stomach and gastric metaplasia in the duodenum. There is strong evidence that *H. pylori* infection is associated with the subsequent development of gastric carcinoma [3].

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 the oral cavity, dental plaque and saliva samples [2]. While the carcinogenic role of *H. pylori* in the stomach is well documented in the medical literature [3], little is known about the possible etiopathogenic role of *H. pylori* in the larynx and other upper aerodigestive tract cancers [2, 4, 5]. In this study we investigated the hypothesis that the development of squamous cell carcinoma of the larynx might be initiated or promoted by *H. pylori* infection.

KARGER

Fax +41 61 306 12 34
E-Mail karger@karger.ch
www.karger.com

0009–3157/06/0522–0080$23.50/0

Accessible online at:
www.karger.com/che

Ahmet Kizilay, MD
Department of Otolaryngology, Inonu University Medical School
Turgut Ozal Medical Center
TR–44300 Malatya (Turkey)
Tel. +90 422 341 0660, ext. 4605, Fax +90 422 341 0728, E-Mail akizilay@inonu.edu.tr

Materials and Methods

A total of 69 patients with histopathologically confirmed squamous cell cancer of the larynx were enrolled in the study between January 1997 and September 2003. We retrospectively reviewed charts of 66 male (95.7%) and 3 female (4.3%) patients who ranged in age from 35 to 78 years (median = 62). All the tumors were resected with total laryngectomy with or without partial pharyngectomy. We used the TNM classification system revised by the AJCC in 1997 for tumor staging. Eleven cases who received preoperative radiotherapy and/or chemotherapy were excluded and thus 58 patients remained for evaluation.

Preoperative hematologic values and nutritional status of the patients were all considered within normal limits. Unilateral or bilateral neck dissections were performed in all cases. The rules for biopsy taking and processing were established retrospectively. Larynx and neck specimens were removed en bloc. Immediately after resection the laryngectomy specimens were washed with phosphate-buffered saline (pH 7.3) and subsequently fixed in 4% neutral buffered formaldehyde for 24 h. Four-micrometer-thick cut sections of paraffin-embedded tissues were stained with hematoxylin and eosin for routine histopathologic examination. A modified Giemsa stain was used for *H. pylori* identification. Both tumor and neighboring normal-looking tissue of each specimen were examined under a light microscope with considerable mucosal surface evaluation. The presence of *H. pylori* was initially assessed in hematoxylin and eosin sections, then modified Giemsa-stained slides were examined for further confirmation.

Thirty patients who had undergone several laryngeal procedures for nononcologic diseases between January 1998 and September 2003 were randomly selected and served as a control group. They included patients with vocal nodule (17/30), vocal polyp (8/30) and chronic laryngitis (5/30). The same evaluation technique was used in the control group.

Results

Of the 58 patients, 26 had well-differentiated and 32 had moderately or poorly differentiated tumors. All of the patients had T3 or T4 tumors and squamous cell carcinoma was defined as primary squamous cell carcinoma arising in the larynx. Light-microscopic evaluation did not show any *H. pylori* microorganisms in the laryngeal cancer specimens or controls. There were no statistical differences related to age, gender or localization of the tumor.

We found a lack of histologic evidence for *H. pylori* in laryngeal cancer specimens.

Discussion

H. pylori has been estimated to infect almost half of the world's population [6]. The incidence in the Turkish population ranges from 60 to 84% [7]. Direct and indirect methods may be used to determine the presence of *H. pylori*. Epidemiological studies have generally used seropositivity as their diagnostic criteria such as serology and breath testing. Direct methods are both histologic and microbiologic studies such as urease test, polymerase chain reaction, culture and smears [2, 5].

Grandis et al. [4] reported a lack of serologic evidence for *H. pylori* infection in head and neck cancer. The results of the present study confirmed no correlation between *H. pylori* infection and larynx cancer. But, a high seropositivity for *H. pylori* in the patients with squamous cell carcinoma of the larynx was reported in a recent study [5]. A few studies reported in the literature investigating the relation between *H. pylori* infection and head and neck cancers are shown in table 1.

Several studies have reported the isolation of these bacteria from the oral cavity, dental plaque and saliva [2]. A strong association of *H. pylori* infection with peptic ulcer disease, gastric carcinoma and gastric mucosa-associated lymphoid tissue (MALT) lymphoma made us search for larynx cancer. *H. pylori* infection may be an initiator or a promoter of gastric malignancies, because many arise in sites of chronic inflammation [6]. In both ulcer disease and MALT lymphoma, evidence suggests that *H. pylori* is a causal agent, because following the eradication of the microorganism, these proliferation-associated findings were significantly reduced [4]. Chronic infection may play an etiologic role in squamous cell carcinoma of the larynx; however, the results of present study did not confirm *H. pylori* infection.

On the other hand, the role of gastroesophageal reflux in the pathogenesis of several disorders has been of interest to otolaryngologists. Involvement of cardia mucosa in *H. pylori* infection results in insufficiency of the lower esophageal sphincter and this can lead to an increased gastroesophageal reflux. There is an increasing awareness and concern that gastroesophageal reflux disease may be a contributing factor to the development of laryngeal dysplasia and a possible risk factor for laryngeal and hypopharyngeal carcinoma [8, 9]. Besides, *H. pylori* has not been found to be a reason for chronic laryngitis [10].

Histologic examination is a direct and specific method for investigating the presence of *H. pylori* but one disadvantage of the present study is the fact that patients' specimens were collected from the routine files retrospective-

Table 1. Reports on the relationship between *H. pylori* and squamous cell carcinomas of the head and neck

Study	Region	Study design	*H. pylori* detection methods	Results
Grandis et al. [4]	Head and neck cancer	Case-control	Serology	No difference
Okuda et al. [2]	Oral cancer	Case-control	Reverse transcription polymerase chain reaction and culture	Present in the oral cancer surface only as a transient organism
Aygenc et al. [5]	Larynx cancer	Case-control	Serology	Significant seropositivity in larynx cancer
Rubin et al. [11]	Severe laryngeal dysplasia and carcinoma of the head and neck	Case-control	Serology	A higher level of seropositivity particularly in the age group of 41–61 years
Kizilay et al. (current study)	Larynx cancer	Case-control	Histopathology	No evidence

ly, sera were not available and there was a lack of urease tests. Before this point can be finally clarified, however, further studies will have to be done prospectively; a greater number of cases should be examined by serologic, histologic and microbiologic methods as well as other potentially associated factors such as peptic ulcer disease and gastroesophageal reflux should be investigated.

Conclusion

The histologic examination of the laryngeal cancer specimens and nonneoplastic laryngeal tissue samples revealed no clue regarding *H. pylori* as an etiologic association. The use of more than one diagnostic method may enhance the detection of the role of *H. pylori* in larynx and other aerodigestive tract cancers.

References

1 Marshall BJ, Warren JR: Unidentified curved bacilli in the stomach of patients with gastritis and peptic ulceration. Lancet 1984;i:1311–1315.
2 Okuda K, Ishihara K, Miura T, Katakura A, Noma H, Ebihara Y: *Helicobacter pylori* may have only a transient presence in the oral cavity and on the surface of oral cancer. Microbiol Immunol 2000;44:385–388.
3 Eidt S, Stolte M: The significance of *Helicobacter pylori* in relation to gastric cancer and lymphoma. Eur J Gastroenterol Hepatol 1995;7:318–321.
4 Grandis JR, Perez-Perez GI, Yu VL, Johnson JT, Blaser MJ: Lack of serologic evidence for *Helicobacter pylori* infection in head and neck cancer. Head Neck 1997;19:216–218.
5 Aygenc E, Selcuk A, Celikkanat S, Ozbek C, Ozdem C: The role of *Helicobacter pylori* infection in the cause of squamous cell carcinoma of the larynx. Otolaryngol Head Neck Surg 2001;125:520–521.
6 Parsonnet J, Hansen S, Rodriguez L, Gelb AB, Warnke RA, Jellum E, Orentreich N, Vogelman JH, Freidman GD: *Helicobacter pylori* infection and gastric lymphoma. N Engl J Med 1994;330:1267–1271.
7 Us D, Hascelik G: Seroprevalence of *Helicobacter pylori* infection in an asymptomatic Turkish population. J Infect 1998;37:148–150.
8 Ulualp SO, Toohill RJ: Laryngopharyngeal reflux: state of art diagnosis and treatment. Otolaryngol Clin North Am 2000;33:785–802.
9 Harrill WC, Stasney CR, Donovan DT: Laryngopharyngeal reflux: a possible risk factor in laryngeal and hypopharyngeal carcinoma. Otolaryngol Head Neck Surg 1999;120:598–601.
10 Jaspersen D, Weber R, Diehl KL, Kind M, Arps H, Draf W: Ist die chronische Laryngitis *Helicobacter-pylori*-assoziiert? Ergebnisse einer prospektiven Studie. Z Gastroenterol 1998;36:369–372.
11 Rubin JS, Benjamin E, Prior A, Lavy J: The prevalence of *Helicobacter pylori* infection in malignant and premalignant conditions of the head and neck. J Laryngol Otol 2003;117:118–121.