

Helicobacter pylori in benign versus malignant laryngeal lesions

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Objective

The aim was to investigate the existence of immunoglobulin (Ig)G antibody against *Helicobacter pylori* (*H. pylori*) in blood sample from patients having laryngeal lesions and comparing its level in benign versus malignant lesions.

Patients and methods

Under general anesthesia, direct laryngoscopy was performed for patients having laryngeal lesions, and biopsy was taken and sent for histopathology. Anti-*H. pylori* IgG antibodies were measured by enzyme-linked immunosorbent assay from venous blood samples from each patient.

Results

Within the included 56 patients, 30 had benign lesion and 26 (46%) had squamous cell carcinoma (SCC). Overall, 73.3% of patient with benign lesions were seropositive and 92.3% of patient with laryngeal SCC were seropositive. The mean anti-*H. pylori* IgG antibody level was significantly ($P=0.0041$) higher in patients who had SCC (23.93 ± 19.6) than patients who had benign laryngeal lesions (38.9 ± 27.5).

Conclusion

Laryngeal lesions are commonly associated with *H. Pylori* infection and showed significantly higher level in laryngeal SCC than benign lesions, reflecting more association of larynx cancer with *H. pylori* infection.

Keywords:

benign, *H. pylori*, larynx, malignant

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Introduction

Helicobacter pylori (*H. pylori*) are gram-negative microaerophilic microorganisms. It is estimated that more than 50% of the population worldwide carry this bacterium in their gastrointestinal tract [1]. It has been verified that *H. pylori* plays an important role in the pathogenesis of peptic ulcers, chronic gastritis, gastric lymphoma, and adenocarcinoma [2]. Immigration of *H. pylori* in the upper aerodigestive zone was proven [3], and the association between *H. pylori* and laryngeal malignancy was investigated; however, it is still a matter of controversy [4].

Laryngeal squamous cell carcinoma (SCC) is one of the most common malignant tumors of the head and neck, representing approximately 25% of the cases [5]. The potential for epithelial and mucosal inflammation and damage could result in chronic harm and epithelial cell proliferation, leading to laryngeal pathology [6,7].

The aim of this study was to investigate the existence of immunoglobulin (Ig)G antibodies against *H. pylori* in blood sample from patients who had laryngeal lesions and to compare *H. pylori* level in benign versus malignant lesions.

Patients and methods

After approval by the institutional review board and obtaining a written informed consent from the patients, patients having laryngeal lesions who were scheduled for elective direct laryngoscopy for either biopsy or excisional biopsy in Otorhinolaryngology Department, Zagazig University Hospitals, and Benha teaching hospitals, during the period from February 2015 to April 2017 were chosen to participate in the study. Patients who had known hypersensitivity to used anesthetic drugs, unfit patients for general anesthesia, congenital laryngeal lesions, and syndromic patients were excluded from the study. Nonsteroidal anti-inflammatory drugs were avoided for 2 weeks before surgery. All patients were subjected to full history taking, general and local clinical examination, and routine preoperative laboratory testing.

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Under general anesthesia, direct laryngoscopy was performed, and a biopsy was taken if the lesion was suspected to be malignant or total surgical excision for clinically suspected benign lesion, and all biopsies were sent for histopathology.

Laboratory investigation

Quantitative determination of IgG antibody against *H. pylori* was done by enzyme-linked immunosorbent assay (ELISA).

Specimen collection and storage

Five ml of venous blood sample was taken from each patient and left to clot at 4°C in a sterile, clean, dry tube. After clotting, the samples were centrifuged for 10 min at 5000 rpm. Serum was separated and stored immediately at -20°C till the time of analysis.

Anti-*H. pylori* IgG antibodies were measured by ELISA using commercially available kit from RD Radio Diagnostics (catalog no. E HLG KO8, www.RDlab.com; Germany) Principle of the test: calibrators and unknowns are incubated in microtitration wells coated with purified and inactivated *H. pylori* antigen. After incubation and washing, the wells are treated with the conjugate, composed of anti-human IgG antibodies labeled with peroxidase. After a second incubation and washing step, the wells are incubated with the substrate tetramethylbenzidine. An acidic stopping solution is then added, and the degree of enzymatic turnover of the substrate is determined by wavelength absorbance measurement at 450 nm. The absorbance measured is directly proportional to the concentration of anti-*H. pylori* IgG antibodies present. Results: the cutoff control corresponds to calibrator 1. If the absorbance of the sample is higher than that of the cutoff, the sample was positive for the presence of

specific IgG. The ratio between the average optical density of the sample and that of the cutoff was calculated. The sample was considered positive, if the ratio was more than 1.1; doubtful, if $\pm 10\%$ of the cutoff; and negative, if the ratio was less than 0.9. Results were expressed in u by interpolating the optical density values of the 5 calibrators and comparing the value of the sample with this curve.

Statistical analysis was performed using SPSS 14.0 statistical software for Windows (SPSS Inc., Chicago, Illinois, USA). The significance level was set at *P* less than or equal to 0.05.

Results

Within the included 56 patients, 30 (53.5%) patients were histopathologically proved to have benign lesion, including 24 (80%) males and six (20%) female. 20/30 (66.6%) cases were polyp, 6/30 (20%) were nodules, 2/30 (6.6%) was cyst and 2/30 (6.6%) was Reinke's edema. On the contrary, malignant lesions were proved in 26 (46%) patients, and all were males and had SCC (Table 1).

The mean age of all patients was 47.57 \pm 11.9 (age range: 28–70) years. The mean age for patients with benign lesions was 40.8 \pm 9.88 years and in malignant lesions was 40.3 \pm 8.34 years. A total of 46 (82.1%) patients were seropositive, with 22/30 (73.3%) benign and 24/26 (92.3%) malignant, whereas 10 (17.85%) patients were seronegative, with eight (26.7%) benign and two (7.7%) malignant. Anti-*H. pylori* IgG antibodies level in patients who had benign lesions ranged from 3.2302 to 85.4 (mean=38.9 \pm 27.5) whereas anti-*H. pylori* IgG antibodies level in malignant lesions ranged from 8.247 to 87.275

Table 1 Epidemiological and anti-*H. pylori* immunoglobulin G antibody results in benign and malignant laryngeal lesions

Pathology	Mean age	Male [n (%)]	Female [n (%)]	Seropositive [n (%)]	Seronegative [n (%)]	Anti- <i>H. pylori</i> IgG antibodies
Benign lesion [30 (53.5%)]						
Polyp	20/30 (66.6%)					
Nodules	6/30 (20%)	24 (80)	6 (20)	22 (73.3)	8 (92.3)	38.9 \pm 27.5
Cyst	2/30 (6.6%)					
Reinke's edema	2/30 (6.6%)					
Malignant lesions [26 (46%)]						
Squamous cell carcinoma	26/26 (46%)	13 (100)	0 (0)	24 (26.7)	2 (7.7)	23.93 \pm 19.6
<i>P</i> value	0.84 (NS) (<i>t</i> =0.2029)	0.0158 (S) ($\chi^2=5.824$)		0.06445 (NS) ($\chi^2=3.419$)		0.0041 (S) (<i>t</i> =2.9967)

S, significant.

(mean: 23.93±19.6). The mean anti-*H. pylori* IgG antibodies level was significantly ($t=2.9967$, $P=0.0041$) higher in patients who had malignant laryngeal lesions (23.93±19.6) than patients who had benign laryngeal lesions (38.9±27.5) (Table 1), reflecting significance association between *H. pylori* and cancer larynx, which could be attributed to the importance of *H. pylori* level in pathogenesis of cancer larynx. We took into consideration that both groups of patients were matched for age; all patients were smokers and nonalcoholics.

Discussion

H. pylori infection, resulting from a gram-positive spiral-shaped or curved rod, is one of the commonest bacterial infections in human [8], with a prevalence reaching 90% in underdeveloped countries and 30% in developed countries [9].

Although the stomach is the primary site of this bacterium, *H. pylori* could also be detected in calculus, pharyngeal lymphoid tissue, saliva, nasal and sinus mucosa, oropharynx, tympanic cavity secretion, and larynx [10].

The mean age of our patients was 47.57±11.9 years near to the mean age reported by Barakat *et al.* [11] and Siupsinskiene *et al.* [12].

Malignant lesions were proved in 46% of included patients, which is near to the percent registered by Barakat *et al.* [11] However, Siupsinskiene *et al.* [12] found that 16.67% patients had malignant lesion. This is mostly because we included only cases scheduled for surgery, so all malignant lesions were included, whereas many patients with benign lesions do not prefer to undergo surgery.

The aim of this study was to determine the prevalence of *H. pylori* in the patients with benign and malignant laryngeal diseases.

ELISA is noninvasive serology method for detection of *H. pylori*. It is rapid test, and even though it is less sensitive, it is specific. This serological test is relatively widely available and cheap; therefore, it may be helpful in screening or in confirming the presence *H. pylori* infection [13]. Although serology is not appropriate for determining the underlying gastrointestinal disease [13], this is not our concern in the current study. Thus, we used ELISA serology test for measuring anti-*H. pylori* IgG antibodies, which is available in our institute and could quantitatively measure antibody

level. ELISA was used similar to Urita *et al.* [14] and Rezaii *et al.* [15].

Overall, 82.1% of our patients who had laryngeal pathology scheduled for surgery were seropositive. Thus, laryngeal lesions are commonly associated with *H. pylori* infection. In addition, 73.3% of patient with benign lesions were seropositive and 92.3% of patient with laryngeal SCC were seropositive, reflecting the more association of cancer larynx with *H. pylori* infection.

On quantitative measure of anti-*H. pylori* IgG antibodies level, the view becomes clearer as the mean anti-*H. pylori* IgG antibodies level was significantly ($P=0.0041$) higher in patients who had malignant laryngeal lesions than patients who had benign lesions, reflecting significance association between *H. pylori* and cancer larynx, which could be attributed to the importance of *H. pylori* level in etiopathogenesis of cancer larynx. We took into consideration that benign and malignant groups of patients were matched for age and sex. Importance of detection of anti-*H. pylori* IgG antibodies level with laryngeal lesions might direct attention to treatment of *H. pylori* infection in the postoperative regime for laryngeal pathology.

Barakat *et al.* [11] similarly commonly detected positive anti-*H. pylori* IgG antibody with laryngeal lesions but did not detect significant differences between benign and malignant lesions. Similarly, in 2008, Fang *et al.* [16] hypothesized that *H. pylori* may be an etiological factor of VF polyps.

The important association between *H. pylori* infection and the development of laryngeal cancer was proved by a meta-analysis by Tominaga [5], which indicated that the risk of developing laryngeal cancer is twice as high for the patients infected with *H. pylori* [7]. Using serological studies, *H. pylori* infection was significantly more often exist in patients operated for malignant than benign laryngeal lesions, which suggests that *H. pylori* may be an independent factor of laryngeal SCC [4,17–19]. Similarly, Rezaii *et al.* [15], using ELISA for detection of anti-*H. pylori* IgG antibodies, concluded that *H. pylori* is an independent risk factor for laryngohypopharyngeal carcinoma. Similar results were also reported by Titiz *et al.* [20], using PCR to detect genetic material from *H. pylori* in the larynx, and recorded significantly more *H. pylori* infection with cancer larynx than benign laryngeal diseases. In agreement with our results, some studies detected high prevalence of *H. pylori* in patients with

laryngeal SCC by serology [18–21]. In contrast, Nurgalieva *et al.* [22], using ELISA to detect antibodies to *H. pylori*, found that *H. pylori* infections do not either protect against or promote laryngopharyngeal carcinoma.

Compared with ELISA, PCR is more accurate with strong sensitivity and specificity for the detection of *H. pylori* in laryngeal mucosa [13], but it is less available and of high cost, limiting its use.

H. pylori infection was considered to cause epithelial cell proliferation in the laryngeal mucosa as its effect in gastric mucosa, which eventually could progress to laryngeal carcinoma [13].

Early treatment of *H. pylori* is important to decrease the incidence of occurrence of laryngeal pathology especially malignant lesions because it could be considered as a risk factor of cancer larynx according to this study, which reflects significant association between *H. pylori* and cancer larynx. Accordingly, the treatment of *H. pylori* should be continued after surgery to help avoid recurrence. Other studies are needed to determine the effect of treatment for *H. pylori* on recurrence of laryngeal lesions. Moreover, another study is needed to examine this organism within tissue samples of recurrent cases.

Conclusion

Laryngeal lesions are commonly associated with *H. pylori* infection, which showed significantly higher level in laryngeal SCC than benign lesions, reflecting more association of larynx cancer with *H. pylori* infection.

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Conflicts of interest

There are no conflicts of interest.

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