H.pylori and Pepsin as a Putative Risk Factors in Laryngeal Cancer Patients

Original Article

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ABSTRACT

Background: Laryngeal cancer constitutes 26-30% of all head and neck malignant tumors. Chronic stimulation by acid reflux may lead to a malignant change in the laryngopharyngeal mucosa. Pepsin assay could be a rapid, sensitive, and specific diagnostic marker of extra-esophageal reflux (EES). Helicobacter pylori is involved in the pathogenesis of peptic ulcer, and gastric carcinoma with growing interest in the presence of Helicobacter pylori in the upper aerodigestive tract.

Aim: The aim of the study is to confirm the role of H.pylori and pepsin as risk factors in laryngeal cancer patients.

Patients and Methods: Seventy-five patients were enrolled in this study, and divided into 3 groups; Group (A) 32 patients with suspected laryngeal malignancy. Group (B) 20 patients with benign laryngeal lesions. Group (C) 22 patients free from laryngeal disease, scheduled for non-laryngeal surgery. All patients were subjected to laryngo-pharyngeal lavage (LPL) using sterile water, before starting the surgical procedure and was analyzed for detection of Pepsin an H. Pylori, also culture for H. Pylori was done.

Results: Strong positive association exist between H. pylori and Pepsin and occurrence of laryngeal carcinoma, while there was no statistically significant relationship with occurrence of a benign laryngeal lesions.

Conclusion: GER is a risk factor or co-carcinogen in the pathogenesis of laryngeal carcinoma and not considered as a risk factor for benign laryngeal lesions. H. pylori culture and Pepsin assay in LPL can be used as non-invasive tool for detection of GER.

Key Words: Bamford- Cancer larynx, H. pylori, pepsin.

INTRODUCTION

The 5-year survival of patients with head and neck squamous cell carcinoma (HNSCC) has not changed dramatically over the last decades despite the use of various diagnostic and therapeutic modalities[1]. Thus, new approaches remain necessary to prevent cancer, improve treatment and reduce recurrence. Laryngeal cancer constitutes 26-30% of all head and neck malignant tumors[2]. It is common in men and it is distributed between ages 40 and 70 years old. Smoking, alcohol, human papilloma virus and radiation are well-known etiologic factors of laryngeal cancer. Other reported risk factors include, genetics, carotene deficiency[3] and occupational exposure[4, 5].

The hypothesis that gastric reflux plays a role in the development of laryngeal squamous cell carcinoma, was first suggested by Gabriel and Jones[6] in 1960. Other study concluded that, chronic stimulation by acid reflux may lead to a malignant change in the laryngopharyngeal mucosa[7]. A high frequency of acid reflux has been reported Since the mid-80's in laryngeal cancer patients who do not drink alcohol or smoke cigarettes, this lead to an increased interest in the relationship between laryngeal cancer and acid reflux[8, 9, 10].

Despite the recognition that gastroesophageal reflux (GER) can provoke laryngeal cancer, the diagnosis of laryngopharyngeal reflux (LPR) remains a very difficult task. Initially, patients with laryngeal symptoms undergo laryngoscopy and chest X-ray to rule out malignancies. Once cancer is excluded, a diagnosis of LPR is suspected when the following laryngoscopic findings are seen: edema, erythema, ventricular obliteration, pseudosulcus and post-cricoid hyperplasia. However, these laryngoscopic findings

are also common in healthy volunteers, and this largely limits their diagnostic value[11]. Moreover, there are several controversies regarding how to confirm LPR. Upper gastrointestinal endoscopy has been demonstrated to have low sensitivity[12, 13], the proton pump inhibitor test has been shown to have low specificity[14], and radiologic studies have limited sensitivity and specificity[12]. Nowadays it was found that multichannel intraluminal impedance and pH monitoring (MII-pH) is able to detect not only acid but also non-acid reflux and proximal migration of the refluxate[15, 17]. However, there is still a great debate of controversy surrounding the significance of LPR role.

Pepsin exists in the form of pepsinogen, which is secreted mainly by stomach mucosa cells. Pepsinogen exerts its biological activity when converted to pepsin if the pH is decreased. Under normal conditions Pepsin is not detected in the laryngopharyngeal lavage (LPL). When reflux occurs into the throat, laryngeal mucosa cells may be damaged[18]. Recent studies have shown that pepsin can be used as a diagnostic marker of LPR[19, 20].

Helicobacter pylori is a gram-negative bacterium involved in the pathogenesis of peptic ulcer, and gastric carcinoma. The ecological niche of H. pylori is the human stomach, where it establishes long-term colonization of the gastric mucosa[21]. There is growing interest in the presence of Helicobacter pylori in the upper aerodigestive tract[22]. The aim of this study is to investigate the role of H.pylori and pepsin as risk factors in laryngeal cancer patients.

PATIENTS AND METHODS:

This was a prospective study carried out between January 2018 and January 2020, after approval of the Institutional Review Board of Ain Shams University Hospitals. An informed consent was signed by all participants. Seventy five patients were enrolled in this study, and divided into 3 groups (A, B and C). All participants subjected to detailed history taking including history of reflux and full ENT examination. Group (A) 32 patients with suspected laryngeal malignancy. Group (B) 21 patients with benign laryngeal lesions. Group (C) 22 patients free from laryngeal disease, scheduled for non-laryngeal surgery (10 for septoplasty, 6 for tonsillectomy and 4 for myringoplasty) were included as control.

Prior to surgery routine laboratory investigations, chest X-ray and laryngeal photography were done. Contrast enhanced CT, only for laryngeal cancer patients. Group (A) underwent microlaryngosurgery for lesion mapping and biopsy taking. While excisional biopsy was done for Group (B). All biopsies were sent for histopathological examination. Group (A) patients with proven laryngeal malignancy underwent total, partial laryngectomy and neck dissection according to the staging of the lesions and extent of the pathology. Post-resection histopathology reports and disease staging were recorded.

Exclusion criteria were as follows: Previous surgery in the upper aerodigestive tract, eating disorders with vomiting, pregnancy and/or breastfeeding, use of aspirin, non-steroidal anti-inflammatory drugs, treatment for reflux either medical or surgical and underlying psychiatric illness.

All patients were subjected to laryngo-pharyngeal lavage (LPL) using sterile water, before starting the surgical procedure under general anesthesia. Samples were placed on ice and immediately transferred to the laboratory, where it was stored at −80°C until analyzed.

The stored samples were analyzed with:

1- Campylobacter-like organism "CLO" (rapid urease assay) kit (Ballard medical product, Draper, Utah, USA) which is based on the fact that the LPL can be inoculated into a gel (medium containing urea and phenol red, a dye that turns pink at pH 6.0 or higher). Thus the PH rises above 6.0 when H.pylori metabolizes urea to ammonia via urease activity. The sample that did not change in color and remained yellow was considered negative, while sample that turns orange or red recorded as H.pylori positive.

2- Enzyme-linked immunosorbent assay (ELISA) kit was used for detection of pepsin (Fujian Blueprint Technology Company, FuZhou, China). This assay was carried out according to the manufacturer's instructions. Absorbance values of the samples at 450 nm were determined using a microplate reader (BioTek Instruments, Inc., Winooski, VT). Results were classified as positive if pepsin was detected in LPL.

3- Culture of H. pylori on Columbia agar supplemented with lysed horse blood and Skirrow's supplement (containing vancomycin, trimethoprim, polymyxin B and amphotericin B) (Oxid-Unipath, limited, Pasingstoke, and Hampshire, England). The plates were incubated under microaerobic conditions (90% CO_2, 5% O_2, 5% N_2) at 37 °C for 4 to 7 days. Suspected colonies of H.pylori (small, circular, convex, translucent) were tested by gram stain, and catalase activities.

STATISTICAL METHODS:

Data were analyzed using IBM© SPSS© Statistics version 22 (IBM© Corp., Armonk, NY). Numerical variables were presented as mean and standard deviation and inter-group differences were compared using one-way analysis of variance (ANOVA) with application of the Tukey test for post hoc comparisons if needed.

Categorical variables were presented as ratio or as number and percentage and differences were compared using the Pearson chi-squared test. Associations between nominal variables on two levels were tested using the phi coefficient of association (φ). Multinomial logistic regression analysis was used to examine the relation between CLO test, H. pylori or positive pepsin test and occurrence of laryngeal lesions. Two-sided *p-values <0.05* are considered statistically significant.

RESULTS:

Patients were divided into three groups. Group (A) 32 patients with suspected laryngeal malignancy. Their mean age was 57.0±10, and there were 30 (93.75%) males and 2 (6.25%) females. 24 (75.0%) of them were smoker. Group (B) 21 patients with benign laryngeal lesions, their mean age was 46 ± 10, and there were 11 (52.4%) males and 10 (47.6%) females. 11 (52.4%) of them were smoker. Group (C) 22 patients free from laryngeal disease, scheduled for non-laryngeal surgery (10 for septoplasty, 6 for tonsillectomy and 4 for myringoplasty) were included as control. Their mean age was 47 ± 9, and there were 16 (72.7%) males and 6 (27.3%) females. 14 (63.4%) of them were smoker.

Clinical and histopathological characteristics of group (A) And (B) are reveled in (Table 1, 2). Results of CLO test, H. pylori culture and pepsin test in the three studied groups are presented in (Table 3) and (Figure 1). There was a relatively strong positive association between CLO, H. pylori or Pepsin and occurrence of laryngeal carcinoma (phi coefficient, φ = 0.61, 95% CI = 0.44 to 0.79, *p-value <0.001*) (Table 4).

The sensitivity, specificity, efficiency, and prevalence of CLO, H.pylori or pepsin for prediction of laryngeal malignancy in patients presenting with a laryngeal lesions were (88%, 71%, 81% and 60% respectively). After adjustment for patient' age, sex and smoking status, there was no statistically significant relation between CLO (or H. pylori or pepsin) and occurrence of a benign laryngeal lesion (odds ratio = 1.3, 96% CI = 0.3 to 5.3, *p-value = 0.753*). However, the relation between CLO (or H. pylori or pepsin) and malignant laryngeal lesion was statistically significant (odds ratio = 31.0, 96% CI = 5.4 to 178.4.4, *p-value <0.001*). (Table 5).

Table 1: Clinical and histopathological characteristics of malignant laryngeal lesions

TNM stage, histopathological type	Number of patients (%)
T stage	
T1	6 (18.8%)
T2	1 (3.1%)
T3	14 (43.8%)
T4	11 (34.4%)
N stage	
N0	16 (50.0%)
N1	9 (28.1%)
N2	6 (18.8%)
N3	1 (3.1%)
M stage	
M0	32 (100.0%)
M1	0 (0.0%)
Clinical stage	
Stage I	6 (18.8%)
Stage II	0 (0.0%)
Stage III	10 (31.3%)
Stage IV	16 (50.0%)
Histopathological type	
Carcinoma in-situ	1 (3.1%)
Well differentiated carcinoma	11 (34.4%)
Moderately differentiated carcinoma	16 (50.0%)
Poorly differentiated carcinoma	4 (12.5%)

Table 2: Clinical and histopathological characteristics of benign laryngeal lesions

Site and histopathological type	Number of patients (%)
Site	
Supraglottic	0 (0.0%)
Glottic	21 (100%)
Transglottic	0 (0.0%)
Histopathological type	
Vocal fold polyp	8 (38.1%)
Postglottic granuloma	4 (19.0%)
Reinks edema	3 (14.3%)
Laryngeal papillomatosis	4 (19.0%)
Vocal fold keratosis	2 (9.5%)

Table 3: Results of CLO culture, H. pylori culture and pepsin test in the three study groups

Test	Result	Cancer larynx (n=32)	Benign laryngeal lesions (n=21)	No laryngeal lesion (n=22)	*p-Value†*
CLO	Negative	4 (12.5%)	15 (71.4%)	17 (77.3%)	*<0.001*
	Positive	28 (87.5%)	6 (28.6%)	5 (22.7%)	
H. pylori culture	Negative	4 (12.5%)	15 (71.4%)	17 (77.3%)	*<0.001*
	Positive	28 (87.5%)	6 (28.6%)	5 (22.7%)	
Pepsin	Negative	4 (12.5%)	15 (71.4%)	17 (77.3%)	*<0.001*
	Positive	28 (87.5%)	6 (28.6%)	5 (22.7%)	

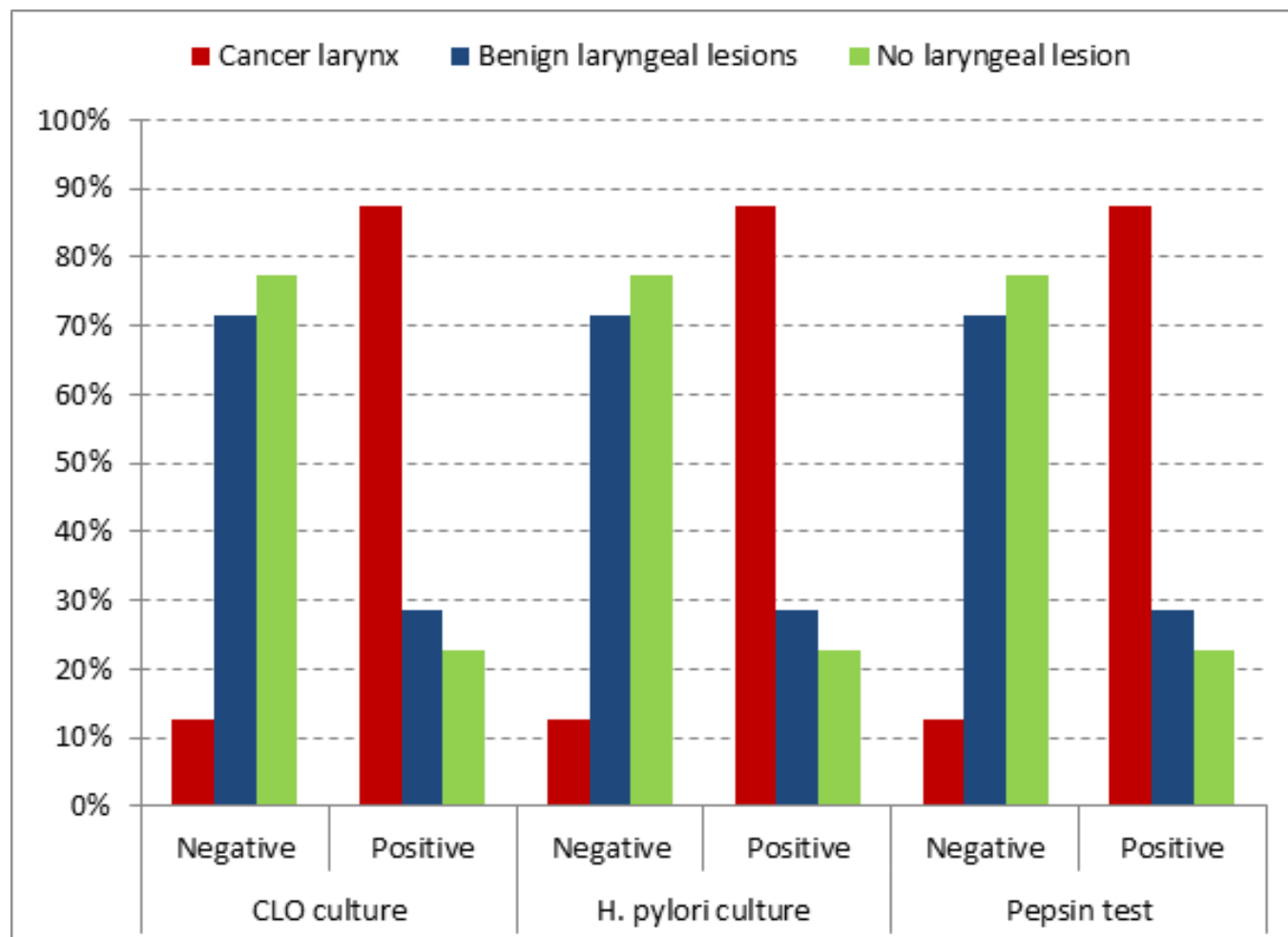Data are number (percentage).
†. Pearson chi-squared test.

Fig. 1: Results of CLO culture, H. pylori culture and pepsin test in the three study groups.

Table 4: Association between CLO, H. pylori or pepsin and the occurrence of laryngeal cancer

		Test (CLO, H. pylori or Pepsin)				
		Positive	Negative	Columns total	Measure of association	
Criterion	Malignant laryngeal lesion	28	4	32	Phi (φ)	0.61
	No or benign laryngeal lesion	11	32	43	95% CI	0.44 to 0.79
	Rows total	39	36	75	*p-Value*	*<0.001*

Data are counts.

Table 5: Multinomial logistic regression for the relation between CLO, H. pylori or pepsin and occurrence of benign or malignant laryngeal lesion as adjusted for patients' age and sex

| | | | | | | | 95% CI for Exp(B) | |
Outcome*	Independent variable	B	SE	Wald (df1)	*p-Value*	Exp(B)	Lower Bound	Upper Bound
Benign laryngeal lesion	Intercept	0.674	1.552	0.189	0.664			
	Age (years)	-0.006	0.033	0.029	0.865	0.994	0.931	1.062
	Male sex †	-0.962	0.891	1.166	0.280	0.382	0.067	2.190
	Smoking ‡	0.165	0.872	0.036	0.850	1.180	0.213	6.524
	Positive CLO (or H. pylori or pepsin) §	0.207	0.740	0.078	0.780	1.230	0.288	5.251
Malignant laryngeal lesion	Intercept	-9.051	3.138	8.318	0.004			
	Age (years)	0.110	0.050	4.915	0.027	1.117	1.013	1.231
	Male sex †	3.028	1.322	5.249	0.022	20.654	1.549	275.430
	Smoking ‡	-1.250	0.985	1.611	0.204	0.287	0.042	1.975
	Positive CLO (or H. pylori or pepsin) §	3.434	0.893	14.797	*<0.001*	31.002	5.389	178.359

95% CI = 95% confidence interval, B = regression coefficient, df = degree of freedom, Exp(B) = odds ratio, SE = standard error, Wald = Wald chi-squared statistic.
*. The reference category is: No laryngeal lesion.
†. The reference category is: Female sex.
‡. The reference category is: No smoking.
§. The reference category is: Negative CLO (Negative H. pylori or Negative pepsin, respectively)

DISCUSSION

An important emerging risk factor for laryngeal cancer is GER. Many studies evaluating the incidence of laryngeal cancer, found that the age-adjusted incidence increased during the last decades[23, 24, 25], despite decreasing numbers of people who smoke[26]. Such observations, along with the high reported prevalence of GER in patients with laryngeal carcinoma[10, 27, 37] suggested that GER may be an important risk factor for laryngeal carcinoma. In this study, we evaluated the role of H. pylori and pepsin as risk factors in laryngeal cancer patients.

In the present study, 75 patients were included and divided into 3 groups. Group (A) 32 patients with laryngeal malignancy. Group (B) 21 patients with benign laryngeal lesions. Group (C) 22 patients free from laryngeal disease, scheduled for non-laryngeal surgery.

Results of CLO test in group A,B,C were (positive results in 87.5%,28.6% and 22.7% respectively) and were negative in (12.5%, 71.4% and 77.3% respectively).H. pylori culture results were positive in (87.5%, 28.6% and 22.7% respectively), and were negative in (12.5%, 71.4% and 77.3% respectively).

Pepsin assay results were (positive results in 87.5%, 28.6% and 22.7% respectively) and were negative in (12.5%, 71.4% and 77.3% respectively).

There was a relatively strong positive association between CLO, H. pylori and Pepsin with occurrence of laryngeal carcinoma (phi coefficient, φ = 0.61, 95% CI = 0.44 to 0.79, *p-value <0.001*) (Table 4). The sensitivity, specificity, efficiency, and prevalence of CLO, H.pylori and pepsin for prediction of laryngeal malignancy in patients presenting with a laryngeal lesions were (88%, 71%, 81% and 60% respectively). After adjustment for patient' age, sex and smoking status, there was no statistically significant relation between CLO, H. pylori and pepsin and occurrence of a benign laryngeal lesion (odds ratio = 1.3, 96% CI = 0.3 to 5.3, *p-value = 0.753*). However, the relation between CLO, H. pylori and pepsin with malignant laryngeal lesion was statistically significant (odds ratio = 31.0, 96% CI = 5.4 to 178.4.4, *p-value <0.001*).

These results showed that GER may play a role in the development of laryngeal cancer by increasing the risk of cancer irrespective of other established risk factors as smoking, the phenomenon of independent causation or through enhances the carcinogenic potential of this factor by synergistic mechanisms, the phenomenon of co-carcinogenesis.

Many studies suggested an independent role for GER in laryngeal carcinogenesis[29, 39, 32, 37]. Mercante *et al*., compared the incidence of GER in 274 patients with malignant neoplasm of the oral cavity, of the pharynx and of the larynx, examined by esophago-gastro-duodenoscopy (EGD) with 636 controls in a retrospective study. The GER prevalence in this nonsmoking cancer subgroup was 21%, significantly higher than the 5% found in the nonsmoking controls.

In a study carried out by Bacciu *et al*. (38) based on laryngeal cancer patients with no history of smoking or drinking and a normal control group consisting of individuals with no abnormal laryngeal findings, GER was noticed in 27.7% in the laryngeal cancer group and 4.8% in the control group.

Morrison *et al*[29] found that 48% of patients in the nonsmoking cancer group in his study had experienced reflux suggesting that GER may be a risk factor for laryngeal cancer. Similarly, Ward and Hanson[39], published a retrospective series of 138 cancer patients. 12% of patients in their series were nonsmokers and nonalcoholic with moderate to severe GER, again suggesting that GER is an independent risk factor in development of laryngeal carcinoma.

Moreover, GER may act as co-carcinogen by enhancing the carcinogenic effects of smoking and alcohol. The cocarcinogenic potential of GER seems plausible given the injurious nature of refluxate to the larynx[28, 31, 40]. El-Serag *et al*[10] showed that the GER patients were 2.31 times more likely to develop cancer; smoking increased the risk by 2.60 times, but in the presence of both smoking and GER, the risk increased by 2.79 times. Silent GER was evaluated in many studies using dual-probe pH monitoring in patients with laryngopharyngeal squamous cell carcinoma. GERD was evident in cancer patients compared to control groups[27, 33, 34, 35, 36]. From these studies, it might be easy to conclude that GERD has causal role in carcinogenesis given its high prevalence in laryngeal cancer patients.

Reflux has been documented by detection of pepsin in the trachea, lung, sinus, middle ear, combined sputum and saliva, and breath condensate[41]. In this regard, two review articles have highlighted that pepsin assay is a rapid, sensitive, and specific tool for correlation of reflux with airway disease and is a reliable diagnostic marker of extra-esophageal reflux (EES) which means that EER can now be detected by the non-invasive identification of pepsin in saliva[24, 43].

Another study by Bardhan *et al*[43] also showed that higher concentrations of pepsin and frequency of exposure through an increased number of reflux events were more likely to result in injury of the susceptible laryngopharynx. Furthermore, the severity of reflux was related to salivary pepsin, with sever laryngopharyngeal symptoms in subjects who had salivary pepsin compared to those who did not[44].

On the other hand, some studies found that the strength of association between GER and laryngeal cancer is uncertain. Geterude *et al*[45] compared the occurrence and frequency of reflux in 40 consecutive cancer patients and 40 healthy volunteers. The authors reported that the number of reflux episodes and fraction of time of the pH less than 4 at both the proximal and distal esophageal probes were not significantly different between the patients and controls. Chen *et al*[46] conclude in their study that, the prevalence of GER in cancer and noncancer groups were not statistically significant. Ozlugedik *et al*.[47] reported a 62% occurrence rate of LPR and a 45% occurrence rate of pathologic GER in laryngeal cancer patients, although there was no significant difference when compared to the control group.

Lundell *et al*.[48] showed that acid is an uncommon cause of laryngopharyngeal symptoms. A similar finding was demonstrated in study by Ang *et al*.[49] suggesting that acid and non-acid refluxes do not play different roles in the genesis of extra-esophageal symptoms.

Our study showed that no statistically significant relation between CLO, H. pylori and pepsin and occurrence of a benign laryngeal lesion (odds ratio = 1.3, 96% CI = 0.3 to 5.3, *p-value = 0.753*). This was in accordance with, Yadlapati *et al*[50] who found that oropharyngeal pH testing and salivary pepsin analysis could not distinguish between healthy volunteers and individuals with a combination of laryngeal benign lesion. However Beltesis *et al*, in their study showed that 75% of patients with a VFP had laryngopharyngeal reflux (LPR), and this value is greater than that among patients with normal laryngoscopy findings[51]. Koufman *et al*., concluded in their study that, the incidence of LPR was significantly higher in the VFP group than in the control group[27].

CONCLUSION

There was a relatively strong positive association between H. pylori and pepsin with occurrence of laryngeal carcinoma which suggest the role of GER as a risk factor or co-carcinogen in the pathogenesis of laryngeal carcinoma. On the other hand GER is not a risk factor for benign laryngeal lesions. H. pylori culture and Pepsin assay in LPL can be used as non-invasive tool for detection of GER.

CONFLICT OF INTEREST

There are no conflicts of interest.

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