

*Research Article***Role of helicobacter pylori in cases of benign laryngeal lesions.**

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**Abstract**

**Introduction:** laryngeal tumors include malignant and a spectrum of benign lesions. Benign lesions of the larynx include mainly chronic laryngitis, vocal cord polyp, vocal cord nodule, Reinke's edema and many different benign lesions. Smoking and voice abuse are the most common cause and risk factor of laryngeal disorders. Helicobacter pylori (HP) were detected in some cases of chronic laryngitis. However, it has been confirmed by histology, tissue cultures or polymerase chain reaction in benign laryngeal lesions.

**Patient and methods:** 40 patients of both sexes, aged 6-75 years old, prepared for surgical excision of benign laryngeal lesions and histopathological examination to diagnose HP were involved in this prospective study. Follow up of laryngopharyngeal reflux (LPR), HP stool antigen test and histopathological evaluation of the biopsies to diagnose HP in the lesions.

**Results:** When we compared between positive cases which was 8 by Giemsa stain and negative cases (32 cases), there was no statistically significant difference according to age, sex and LPR. But there was statistically significant difference regarding the different types of laryngeal lesions with most positive cases found in patients with vocal fold polyp. There was also statistically significant difference as regard stool antigen test which showed significant agreement.

**Conclusion:** HP may be considered as a reliable cause and risk factor for benign laryngeal lesions especially VF polyp. Also stool antigen test could be used as a reliable test for diagnosis of HP such as histopathological examination.

**Key words:** Helicobacter pylori (HP), Laryngopharyngeal reflux (LPR), Vocal fold (VF) and Benign laryngeal lesions.

**Introduction**

laryngeal tumors include malignant and a spectrum of benign lesions. A benign lesion of the larynx includes noninfective and nontraumatic disorders; such lesions of the larynx involve mainly chronic laryngitis, VF polyp, VF nodule, Reinke's edema and various benign lesions. Smoking and voice are the most common causative and risk factors of laryngeal disorders (Soni et al., 2016).

Helicobacter pylori (HP) are one of the risk factors of adenocarcinoma. Thus, it was considered as a carcinogen in 1994 by the World Health Organization (WHO) (André et al., 2010). Recent studies have shown that HP can be responsible for the pathogenesis of upper gastro-esophageal tract lesions such as oral cavity. It

could be presented in dental plaques and secretions of the salivary glands, even when gastric involvement was not detected (Wichelhaus et al., 2011).

HP was found in some cases of chronic laryngitis by rapid urease test. However, it has been confirmed by histology, tissue cultures or polymerase chain reaction (PCR) in benign laryngeal lesions (Akbayir et al., 2005). Previous tests showed false positive or negative results, regardless of the behavior of the lesion (Megraud and Lehours, 2007).

The aim of this study is to evaluate the correlation between helicobacter pylori infection

and benign laryngeal lesions and its role as a risk factor in these lesions.

### Patients and methods

After approval of the local ethics and scientific committee of Minia University Hospital, all the patients assigned written informed consents after explanation of the procedure, 40 patients of both sex aged between 6-75 years old, prepared for endoscopic surgical excision of laryngeal lesions and histopathological examination to detect HP were enrolled in this prospective study. This study carried out at the department of Otorhinolaryngology in Minia University Hospital and was conducted between February 2018 and February 2019.

Patients were excluded from the study if they met the following criteria: patient refusal, uncooperative patients, patients with systemic disease making them unfit for surgery, patients with malignancy or inflammatory lesions of larynx, patients with speech defect due to CNS lesions and patients with positive history of using anti-HP drugs.

A careful assessment of medical history was taken. General systemic examination was carried out. Routine investigations were done to assess the general condition and surgery preparation. All patients were subjected to do HP stool antigen test to diagnose it in the GIT.

One day before the surgery, all the patients completed a specially designed questionnaire, which assessed LPR symptoms. Reflux symptom index (RSI): hoarseness, throat clearing, excess throat mucus, difficulty swallowing food, choking, troublesome cough, lump in the throat, heartburn, chest pain, regurgitation and indigestion. It was self-rated on 6-point scale from 0, no problem, to 5, severe problem.  $RSI \geq 13$  was considered abnormal (Belafsky et al., 2002).

Otorhinolaryngological examination was done by nasal and paranasal sinuses examination, examination of both ears and examination of the larynx by: External examination and Laryngoscopy to diagnose the different types of lesions.

After assessment of the patients, every patient was subjected to preoperative anesthetic evaluation and excisional biopsy was taken from the laryngeal lesions of all patients by micro-laryngeal surgery (MLS) under general anesthesia. Biopsies were sent for histopathology.

Each laryngeal specimen was divided into two slides: The first slides were stained with routine Hematoxylin and Eosin stain (H&E) and examined to prove the nature of the lesions and the second slides were undergone Giemsa staining for HP examination.

Evaluation was done according to the following: age, sex, LPR symptoms with RSI, HP stool antigen test, histopathological evaluation of the biopsies by H&E to know the nature of the lesions and Giemsa stain to diagnose HP in the lesions.

The collected data were tabulated, and statistically analyzed using SPSS program software version 25. Analyses were done for non-parametric quantitative data between two groups using Mann Whitney test and for qualitative data using Chi square and Fisher exact tests. Kappa test was done to predict the agreement between the results of the histopathological examination (slide test) and stool antigen test. Correlations between different variables were done using Nonparametric Spearman's rho correlation coefficient. The level of significance was taken at (P value < 0.05).

**Results:**

**Table (1): Patient's characteristic data as range, mean ± standard deviation, median, interquartile range or number and percentage.**

Variable		Descriptive statistics (n= 40)
<b>Age:</b>	-Range (years) -Mean ± SD -Median / IQR	(6-75) 39.6±17.4 38/(27.3-52.8)
<b>Sex:</b>	-Male -Female	26(65%) 14(35%)
<b>Lesion:</b>	-VF polyp -VF cyst -VF nodule -Reinke's edema -Leucoplekia -Papilloma	22 (55%) 8 (20%) 2 (5%) 4 (10%) 2 (5%) 2 (5%)
<b>Reflux:</b>	-Yes -No	21 (52.5%) 19 (47.5%)
<b>Stool test:</b>	-Positive -Negative	15 (37.5%) 25 (62.5%)
<b>Slide test:</b>	-Positive -Negative	8 (20%) 32 (80%)

**Positive versus negative cases of helicobacter pylori:**

When we compare between positive and negative cases of HP diagnosed by histopathological examination by Giemsa stain revealed the following:

**Table (2): Comparison according to age, sex and LPR. Data are presented as median, interquartile range or number and percentage. There was no significant difference.**

Variable		Slide test		P value
		Negative (n = 32)	Positive (n = 8)	
<b>Age:</b>	-Median -IQR	37.5 (27.3-53.8)	40 (27.8-50.8)	0.892
<b>Sex:</b>	-Male -Female	22(68.8%) 10(31.3%)	4(50%) 4(50%)	0.416
<b>Reflux:</b>	-Yes -No	17 (53.1%) 15 (46.9%)	4 (50%) 4 (50%)	1

Mann Whitney test for non-parametric quantitative data between the two groups -  
Fisher exact test for qualitative data between the two groups -  
Significant level at P value < 0.05.

**Table (3): Comparison according to nature of lesions. Data are presented as number and percentage. There was statistically significant difference between them except in VF nodule which was insignificant.**

Variable		Slide test		Total	P value
		Negative (n = 32)	Positive (n = 8)		
<b>Lesion:</b>	-VF polyp	17(77.3%)	5(22.7%)	22	<b>0.01*</b>
	-VF cyst	7(87.5%)	1(12.5%)	8	<b>0.0001*</b>
	-VF nodule	1(50%)	1(50%)	2	0.69
	-Reinke's edema	3(75%)	1(25%)	4	<b>0.02*</b>
	-Leucoplekia	2(100%)	0(0%)	2	< <b>0.001*</b>
	-Papilloma	2(100%)	0(0%)	2	< <b>0.001*</b>

- Chi square test for qualitative data between the two groups
- \*: Significant level at P value < 0.05

**Table (4): Spearman's correlation test between slide test and lesions. It shows weak insignificant correlation.**

Slide test vs lesions		Slide test	
		r	P value
<b>Lesion:</b>	-VF polyp	0.075	0.644
	-VF cyst	-0.094	0.565
	-VF nodule	0.172	0.288
	-Reinke's edema	0.042	0.799
	-Leucoplekia	-0.115	0.481
	-Papilloma	-0.115	0.481

- Non-parametric Spearman's rho correlation
- Significant level at P value < 0.05
- Degree of correlation: weak (r=0-0.24), fair (r=0.25-0.49), moderate (r=0.5-0.74), strong (r=0.75-1).

**Table (5): Comparison according to stool antigen test. Data are presented as number and percentage. There was statistically significant difference between them and this is reevaluated by Kappa test for agreement.**

Variable		Slide test		P value
		Negative (n = 32)	Positive (n = 8)	
<b>Stool test:</b>	-Positive	9 (28.1%)	6 (75%)	<b>0.036*</b>
	-Negative	23 (71.9%)	2 (25%)	

- Fisher exact test for qualitative data between the two groups - \*: Significant level at P value < 0.05

**Table (6): Kappa test for agreement between slide test and stool antigen test. Data are presented as number and percentage. It shows fair significant agreement.**

Variable		Slide test		Kappa test	
		Negative (n = 32)	Positive (n = 8)	K	P value
Stool test:	-Positive	9 (28.1%)	6 (75%)	<b>0.353</b>	<b>0.014*</b>
	-Negative	23 (71.9%)	2 (25%)		

- Kappa test for agreement
- \*: Significant level at P value < 0.05
- Degree of Agreement: slight (K=0-0.20), fair (K=0.21-0.40), moderate (K=0.41-0.60), substantial (K=0.61-0.80), near perfect or perfect (K=0.81-1)

## Discussion

*Helicobacter pylori* are mobile, microaerophilic gram-negative bacteria. It is the most common etiological factor of chronic infection and is widespread throughout the world (Özcan et al., 2009) (Kim et al., 2007).

The precise mode of transmission for extra gastric involvement are still under study. The possible routes of HP colonization are person-to-person, oral-oral, gastric-oral, fecal-oral or through contaminated water (Özcan et al., 2009) (Morinaka et al., 2003).

There was also regarding the nature of the lesions 22 (55%) patients with VF polyp, 8 (20%) with VF cyst, 2 (5%) with VF nodule, 4 (10%) with Reinke's edema, 2 (5%) with leucoplekia and 2 (5%) with papilloma. When we compared between positive and negative cases, there was no statistically significant difference between them according to age, sex of the patients and LPR.

But there was statistically significant difference between positive and negative cases regarding the different types of laryngeal lesions with the majority of positive cases found in patients with VF polyp and there was insignificant correlation between them.

Our results indicate association between HP and VF polyp, VF nodule, while in cases of leukoplakia and single papilloma lesions are not. The previous association indicates that, the voice abuse is not the only risk factor for these 2 lesions but LPR play a role.

The study done by Burduk et al., 2011 investigating the association of HP colonization in chronic rhinosinusitis and benign laryngeal diseases. This controlled study included a series of 30 patients with nasal polyps and normal nasal mucosa and 30 patients with benign laryngeal diseases. For benign larynx diseases (polyps and Reinke's edema) 30 patients (12 males, 18 females, mean age 48.7 years, range: 26-71).

Samples obtained from fresh tissues were subjected to HP ureA detection by the polymerase chain reaction (PCR) HP diagnostic test. Samples which were positive for ureA HP gene were evaluated for cagA HP gene. HP ureA gene was detected in all patients with nasal polyps, concha bullosa and laryngeal diseases. Presence of HP cagA gene was identified in 7(23.3%) of 30 patients of HP-positive larynx samples and no positive result was detected in nasal polyps and concha bullosa.

The results of Burduk et al., 2011 were nearly similar to the results of the present study regarding the number and percentage of HP positive cases which was 8 (20%). In our present study, we enrolled larger number of total cases

which was 40 patients of all types of benign laryngeal lesions (26 males, 14 females, mean age 39.6 years, range: 6-75 years).

Burduk et al., 2011 did their study on 30 cases of VF polyp and Reinke's edema only when they compared to the same cases of our study which was 26 cases. We found 6 (23.1%) positive cases from the total 26 patients with VF polyp and Reinke's edema with the majority of positive cases was observed in patients with VF polyp.

Furthermore, Izadi et al., 2012 had investigated the presence of HP in benign laryngeal lesions by PCR. This observational cross-sectional study involved 55 patients (33 or 60% males, 22 or 40% females, mean age 22 years, range: 5-55) with confirmed benign laryngeal lesions (21 or 38.18% cases of VF polyp, 14 or 25.45% cases of VF nodule and 20 or 36.36% cases of papillomatosis). The samples were taken from all patients with benign laryngeal lesions and prepared for PCR analysis to detect HP. They revealed 5 (9.09%) positive results among 55 patients with benign laryngeal lesions. Of them, 3 (14.28%) were polyp among 21 patients, 1 (7.14%) was nodule among 14 patients and 1 (5%) was papilloma among 20 patients.

In contrast to the results of the present study, Izadi et al., 2012 found smaller number and percentage of HP positive cases than our study which was 8 (20%) positive cases in spite of, we conducted our study on smaller number of patients (40 patients). This may be due to the difference of the technique of HP detection. But this study was nearly similar with our present study according to number and percentage of sex and mean and range of age (26 or 65% males, 14 or 35% females, mean age 39.6 years, range: 6-75).

Izadi et al., 2012 did their study on only 3 types of benign laryngeal lesions which was VF polyp, VF nodule and papilloma when they compared to the same cases of our study which was 26 cases. We found 6 (23.1%) positive cases from the total 26 patients with VF polyp, VF nodule and papilloma with the majority of positive cases was observed in patients with VF polyp. The results of positive cases of their study

was approximately similar to our present study which was 5 (22.7%) were polyp among 22 patients, but differ according to the other lesions which was 1 (50%) was nodule among 2 patients and no positive cases from 2 cases of papilloma.

The study conducted by Titiz et al., 2008 compared between the presence of HP in laryngeal tissues of patients with squamous cell carcinoma and benign laryngeal lesions. This study was carried out over 40 patients (all patients were males, mean age 52.4 years, range: 33-72) and PCR investigations were carried out in biopsy samples of 21 patients with squamous cell carcinoma and of 19 patients with benign laryngeal pathologies like VF polyp (12 patients) and nodules (7 patients).

They concluded that the PCR results of 17 out of the 21 samples (80.9%) of the patients with squamous cell carcinoma were positive for HP. Moreover, any genomic material of HP was not found in the PCR results of the 19 patients with benign laryngeal pathology.

The results of Titiz et al., 2008 are not in agreement with our results regarding age, sex and the positive cases of HP which were 8 (20%) patients.

The difference between the results of this study and the results of the current study may be related to; the difference in the study design and protocol in the form of number of total patients of benign laryngeal lesions which was 19 patients this is less than our study which was 40 patients (lower number of patients), age of the patients (mean age 52.4 years, range: 33-72) while our study was (mean age 39.6 years, range: 6-75), sex of the patients which was all males while our study was 26 (65%) male patients and 14 (35%) female patients, and finally the difference in the method of diagnosis or detection of HP between this study (PCR) and our study (Giemsa staining).

Another study created by Siupsinskiene et al., 2013 evaluating HP infection in the biopsy material from the larynx of the patients with benign laryngeal diseases (VF polyps, laryngitis) and laryngeal cancer and to explore the possible

relationships between the laryngeal HP and patients' socio-demographic data and LPR. This prospective case-control study enrolled 67 adult patients investigated for benign laryngeal diseases and laryngeal cancer and 11 individuals of the control group (mean age 21-87 years) were evaluated for HP diagnosis by Giemsa staining and rapid urease test. The patients were divided into: the sub-group of VF polyp was 32 patients—16 males, 16 females (with an average age of 43.5 years); the subgroup of chronic laryngitis were 22 patients—13 males, 9 females (with an average age of 53.3 years); the sub-group of laryngeal cancer were 13 patients—12 males, 1 female (with an average age of 63.5 years). The control group consisted of 11 patients—1 male and 10 females (with an average age of 38.1 years), who had been operated for “soft” VF nodules. They found 11 positive cases (32%) from total 32 patients with VF polyp, 9 positive cases (45.5%) from total 22 patients with chronic laryngitis, 6 positive cases (46.2%) from total 13 patients with laryngeal cancer and 1 positive cases (9.1%) from total 11 patients with VF nodule (control group). No significant relationships between HP infection found in the larynx and patients' demographic data, their unhealthy habits and LPR were obtained.

The results of Siupsinskiene et al., 2013 agree with the results of the current study regarding age of the patients which was 43.5 years in cases of VF polyp and 38.1 in cases of VF nodule and 39.6 years in our study. Also, the results of positive cases in VF polyp patients (11 or 32% positive cases from total 32 patients) were approximately like the results of our study which was 5 or 22.7% positive cases from total 22 patients. And also, in the insignificant relationships between HP and patients' demographic data, their unhealthy habits and LPR.

In contrast to the results of the present study, Siupsinskiene et al., 2013 found 1 (9.1%) positive case in the control group (VF nodule) among 11 patients which differ from our study which was 1 (50%) positive case among 2 cases. This is may be due to smaller number of cases in our study.

**Conclusion:** HP may be considered as a reliable cause and risk factor for benign laryngeal lesions especially VF polyp. Also stool antigen test could be used as a reliable test for diagnosis of HP such as histopathological examination

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