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The Impact of *Helicobacter pylori* on Lesion Type and Matrix Metalloproteinase-9 Expression in Laryngeal Tumors

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Abstract

Background: *Helicobacter pylori* and matrix metalloproteinase-9 (MMP-9) play a significant part in the pathophysiological processes and expression of carcinogenesis, particularly in laryngeal Squamous Cell Carcinoma (LSCC).

Objective: Explore the link between the *H. pylori* bacterium, MMP-9 expression, and laryngeal squamous cell carcinoma development by examining the role of chronic inflammation and extracellular matrix remodeling in laryngeal carcinogenesis.

Patients and Methods: This cross-sectional study recruited 118 tissue samples, including 58 malignant LSCC and 60 benign laryngeal polyps (BLP). Histopathological and immunohistochemical methods were used to assess the interplay of *H. pylori*, MMP-9, and smoking status in laryngeal tumors.

Results: The prevalence of *H. pylori* was significantly higher in malignant lesions (63.79%) as compared to benign lesions (51.66%) (p=0.1). A significant correlation was found in patients with benign lesions between the level of MMP-9 and smoking and *H. pylori* status (r = 0.46, p = 0.001), and (r = 0.5, p = 0.001), respectively. While in patients with malignant lesions, the correlation test revealed a significant negative correlation between the level of MMP-9 and smoking status (r = -0.41, p 0.001), while *H. pylori* is a highest positively correlated with MMP-9 expression (r = 0.5, p = 0.001). The logistic regression analysis revealed that *H. pylori* infection could significantly predict high MMP-9 expression (OR =3.08, 95% CI: 1.15-8.28, p = 0.02).

Conclusion: These findings highlight the crucial role of *H. pylori* infection and smoking in tumor progression through MMP-9-mediated extracellular matrix remodeling, inflammation, and immune modulation. Suggesting the potential impact of targeting *H. pylori* infection and MMP-9 activity for managing carcinogenesis.

Keywords: *Helicobacter pylori*, MMP-9, Laryngeal squamous cell carcinoma, Benign laryngeal polyps.

Introduction

Laryngeal squamous cell carcinoma (LSCC) is the prevalent malignancy of the larynx, comprising a significant part of head and neck cancers. It is the second most common malignancy in the upper respiratory tract after cancer of the lung (1). LSCC accounts for over 95% of all laryngeal malignancies (2). This cancer arises from the epithelial cells of the larynx and is associated with various risk factors, including exposure to environmental toxins, smoking, and alcohol consumption

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(3). Smoking, in particular, is a major contributor to head and neck cancers, introducing carcinogens that lead to DNA damage, oxidative stress in respiratory epithelial tissues, and chronic inflammation. Additionally, lifestyle factors and chronic infections, notably *Helicobacter pylori* (*H. pylori*), have been involved in the development of LSCC (4). Smoking introduces a range of toxic substances that further irritate the mucosal lining, leading to chronic inflammation and increased susceptibility to infections. Synergistic effects of *H. pylori* infection, smoking, and chronic inflammation play an essential role in the progression of LSCC (5). *H. pylori* is a gram-negative organism, ordinarily known for its role in gastric diseases. Recent research suggests that *H. pylori* can also be found in the saliva, calculus, lymphoid tissue of the pharynx, nose, and sinus, oropharyngeal aphthous, discharge of the tympanic cavity, and larynx (6,7). It has been found in the upper aerodigestive tract and is supposed to play a role in inflammation-induced carcinogenesis in the larynx (8). Gastroesophageal reflux disease and laryngeal reflux cause an acidic environment for the larynx, where *H. pylori* can live. Due to its chronic inflammatory effect, *H. pylori* can lead to chronic illness and malignant tumor formation in the larynx (9). Thus, *H. pylori's* damage to the laryngeal protective layers, such as the mucosa and the epithelium, leads to inflammation, which later on could lead to chronic irritation also, abnormal proliferation, the result in laryngeal pathology that can range from a subtle inflammatory process to the most extreme malignant transformation of the cells (10). Matrix metalloproteinases (MMPs) are key regulators of metastatic processes. Their expression and activation have been implicated in various pathological events, including tumor invasion, inflammation, and metastasis. Among the MMP family, matrix metalloproteinase - 9 (MMP-9) is notably in relation to tumor development and progression (11). Matrix metalloproteinase (MMP) is a proteolytic metalloenzyme that is dependent on zinc. MMP-9 has a big role in pathophysiological processes and can break down extracellular matrix (ECM) components. Numerous illnesses are linked to MMP-9 overexpression and dysregulation. Therefore, controlling and inhibiting MMP-9 is a crucial therapy strategy of battling a number of illnesses involving cancer (12). The tumor in LSCC features excessive tissue and inflammation, processes strongly affected by the activity of protease enzymes like Matrix Metalloproteinase-9. MMP-9 is the enzyme responsible for the degradation of the extracellular matrix, especially IV-type collagen, a major component of basement membranes (13). In cancer, the unorganized MMP-9 expression is linked with tumor metastasis and angiogenesis, enabling cancer cells to breach the basement membrane and tissues (14). The study aims to explore the link between *H. pylori* infection, MMP-9 expression, and the initiation of laryngeal squamous cell carcinoma by examining the role of chronic inflammation and matrix remodeling in laryngeal carcinogenesis. Awareness of these interactions could provide worthy insight into the pathogenesis of laryngeal carcinoma and the way for new therapeutic strategies for managing this aggressive malignancy.

Patients and Methods

Specimens' collection: This cross-sectional study involved tissue samples acquired from 118 paraffin-embedded archived blocks from July 2024 to October 2024 at Educational Laboratories of Medical City, Al-Yarmouk Teaching Hospital, and private laboratories. These samples were divided into two groups. The first group consisted of 58 patients (47 males and 11 females) suffering from laryngeal squamous cell carcinoma (LSCC) (mean age 56.32 years) and was divided into two groups: 24

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smokers and 34 non-smokers. The second group consisted of 60 patients (40 males and 20 females) suffering from benign laryngeal polyps (BLP) (mean age of 50.32 years), who included 23 smokers and 37 nonsmokers. The study focused on patients complaining of laryngeal squamous cell carcinoma and benign laryngeal polyps, and those who had received antibiotics or treatment to eradicate *H. pylori* before tissue collections were excluded. Any comorbid condition, such as diabetes or immunocompromised, is also excluded. Archival specimens of lousy quality or insufficient for analysis, such as small biopsy material, damaged tissues, or incomplete data, were excluded. The diagnosis was reaffirmed histopathologically by reviewing newly prepared hematoxylin and eosin-stained slides held in the research laboratory of the College of Medicine / Mustansiriyah University and the Educational Laboratories of Medical City.

Histological evaluation for detection of *H. pylori* infection: To determine whether or not *H. pylori* was found in the larynx tissue specimens, a histopathological examination was performed using standard methods, including the Giemsa stain, to diagnose the specimen and to identify the *H. pylori*, as well as immunohistochemical analysis for *H. pylori* and MMP-9. The diagnostic criteria for malignant and benign biopsies depend on histopathological examination, which includes examining the tissue under a microscope to evaluate cellular structure, morphology, and other criteria. Each block of paraffin was sectioned into a thickness of 4 micrometers. Three sections were taken from blocks: one was stained with hematoxylin and eosin for histopathology review, and the other two were placed on positively charged slides (Fisher brand) and stained with anti- *H. pylori* and anti-MMP-9 monoclonal antibodies using immunohistochemical techniques. Two separate pathologists re-evaluated the hematoxylin and eosin (H and E-stained sections) to determine the morphological kinds of the tumor, the grade, and other criteria.

Immunostaining for the detection of *H. pylori* infection: For the immunohistochemical examination of *H. pylori*, polyclonal antibodies against *H. pylori* obtained from rabbits were used (Abcam/UK code (ab20459) 1:100). On the other hand, antibodies against human MMP-9 and polyclonal antibodies [Abcam/UK code, anti-MMP-9 antibody (ab73734)] were used. The detection of gene expression by utilizing monoclonal antibodies specific to a particular gene is regarded as the fundamental premise of the immunostaining method, which is used to identify a particular protein in normal, benign, and cancerous cells. These antibodies could bind to the nuclear targets found in the cytoplasm. The detection of bounded primary antibodies, which is achieved by conjugating secondary antibodies with a particular chromogen (3, 3-diaminobenzidine, DAB), results in the formation of brown-color sediment at the antigen position in tissue that indicates a positive response. In the peroxidase secondary detection technique, a positive response is shown when brown products react at the location of the target antigen. This reaction is connected with the appearance of the brown products. After that, the cell was stained with a counterstain to produce a blue color.

Scoring: After seeing 10 different fields with a high-powered microscope, the immunohistochemistry (IHC) signaling data were analyzed (100x). By dividing the number of stained cells in each field by the total number of cells in each field across all 10 fields, we were able to determine the percentage of positively stained cells in each of these fields using the formula below: mean percentage of the stained cells across all 10 fields.

Evaluation of Immunostaining: The immunostaining was performed by an

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independent histopathologist who was blinded to the clinical diagnosis of the tissues at the time of evaluation and expression of *H. pylori* (Negative, meaning no expression) and (Positive, meaning there was expression) (15, 16). When the tissue's IHC signaling values were more than 10% of total tumor cells, it was determined that the tissue contained MMP-9 (17, 18).

Statistical analysis

Data were analyzed using an accessible statistical program, SPSS-28 (Statistical Packages for Social Sciences- version 28). Data analysis was carried out, and the results were presented as the mean and standard deviation, while categorical variables were presented as percentages. For comparing two independent groups, T-tests were used, or the ANOVA was employed to observe the statistical differences between more than two independent variables. Pearson's Chi-square statistic was used to analyze the percentage differences (qualitative data). To investigate the nature of the connection between the variables, qualitative correlation coefficients (r) were calculated. The prediction of lesion type (benign vs. malignant) and high MMP-9 was performed through Logistic regression analysis. The results are presented as beta coefficients and odds ratios with a 95% confidence interval. The P-value ≤ 0.5 was considered statistically significant.

Results

***H. pylori* Expression in Benign and Malignant Lesions:** Presence of *H pylori* protein expression, which was measured and confirmed by immunohistochemistry, was heterogeneous dark brown staining in the tissue that was revealed in Figure 1A and Figure 2A in 37 (63.79%) of the malignant lesions and 31 (51.66%) of the benign polyps, as shown in Table 1. The mean age group of patients with malignant lesions was 56.32 ± 9.8 SD, while those with benign lesions were 50.32 ± 8.3 SD, with significant differences.

Regarding sex distribution, a higher prevalence rate of males was evident in both groups, 47 (81.03%) in patients with malignant lesions and 40 (66.66%) in benign polyps, as illustrated in Table 1. Table 2 demonstrates a combined analysis using chi-square and Pearson correlation, which was done to evaluate the association between demographic features (independent variables: age, sex, and smoking status) and *H. pylori* infection (dependent variable) in patients who have malignant squamous cell carcinoma. The Age didn't show any significant association with *H. pylori* infection regarding ≤ 50 years and ages > 50 years in terms of *H. pylori* positivity (p = 0.46), also a weak non-significant positive correlation was observed (r = 0.15, p = 0.25). No significant correlation was seen between sex and *H. pylori* infection (p = 0.85, r = -0.09, p = 0.49). Smoking status was significantly correlated with *H. pylori* infection (p = 0.008, r = -0.46, p =0.001). Table 3 evaluates the association between demographic features (independent variables: age, sex, and smoking status) and *H. pylori* infection (dependent variable) in patients who have benign lesions. There was no statistically significant association between age and *H. pylori* infection (p = 0.404, r = 0.04, p = 0.76) and sex (p = 0.428, r = -0.07, p = 0.6). A significant positive correlation was illustrated between smoking status and *H. pylori* infection (p = 0.036, r = 0.36, p = 0.004).

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Table 1. Comparative analysis of *H. pylori* infection in benign and malignant lesions. N.S.: not significant ($p > 0.05$).

Variable	No. (118)	Mean age (year) ±SD	Male	Female	*H. pylori* positive	*H. pylori* Negative
Malignant lesion	58	56.32 ± 9.8	47 (81.03%)	11 (18.96%)	37 (63.79%)	21 (36.20 %)
Benign lesion	60	50.32 ± 8.3	40 (66.66%)	20 (33.33%)	31 (51.66%)	29 (48.33%)
P value		p < 0.05	0.07 NS	NS	0.1 NS	N.S

Table 2. Chi-Square and Pearson correlation analysis of demographic features with *Helicobacter pylori* in malignant squamous cell carcinoma lesions. *Chi-square for independence, the p-value is less than 0.05, indicating a significant correlation. # Pearson Correlation Coefficient Measures linear relationship strength and significance at ($p < 0.05$).

Demographic features	No. 58	*H. pylori* Positive (37)	*H. pylori* Negative (21)	*P* value (Chi-square)	Pearson correlation coefficient P-value
Age. (Years)					
≤ 50	22 (37.93%)	12 (32.43%)	10 (47.61%)	0.46	r=0.15
> 50	36 (62.06%)	25 (67.56%)	11 (52.38%)		p=0.25
Sex					
Male	47 (81.03%)	31 (83.78%)	16 (76.19%)	0.85	r=-0.09
Female	11 (18.96%)	6 (16.21%)	5 (23.80%)		P=0.49
Smoking status					
Smoker	24 (41.37)	9 (24.32%)	15 (71.42%)	0.008*	r=-0.46
Non-smoker	34 (58.62%)	28 (75.67%)	6 (28.57%)		p=0.001#

Table 3. Chi-Square and Pearson correlation analysis of demographic features with *helicobacter pylori* infection in benign lesions. *In the Chi-square test for independence, the p-value was less than 0.05, indicating a significant correlation. # Pearson Correlation Coefficient Measures linear relationship strength and significance at ($p < 0.05$).

Demographic features	No. 60	*H. pylori* Positive (31)	*H. pylori* Negative (29)	*P* value (Chi-square)	Pearson correlation coefficient P-value
Age. (Years)					
≤ 50	26 (43.33%)	12(38.70%)	14 (48.27%)	0.404	r = 0.04
> 50	34 (56.66%)	19(61.29%)	15 (51.72%)		p = 0.76 NS
Sex					
Male	40 (66.66%)	22 (70.96%)	18 (62.06%)	0.428	r = -0.07
Female	20 (33.33%)	9 (29.03%)	11 (37.93%)		p = 0.6 NS
Smoking status					
Smoker	23 (38.33%)	15 (65.21%)	8 (34.78%)	0.036*	r = 0.36
Non-smoker	37 (61.66%)	16 (43.24%)	21 (56.75%)		p = 0.004#

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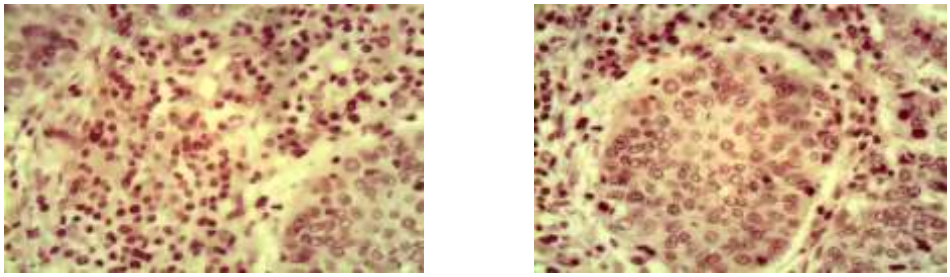
MMP-9 Expression in Benign and Malignant Lesions: An analysis assessed the link between MMP-9 expression as the dependent variable and independent variables as age, sex, smoking, and *H. pylori* status in patients with malignant lesions. No significant differences in MMP-9 expression with weak, non-significant negative correlation were observed between aged ≤ 50 years and aged > 50 years ($p > 0.05$, $r = -0.2$, $p > 0.05$, respectively). The expression of the mean percentage of MMP-9 did not significantly differ between males and females, with weak and no significant correlation ($p > 0.05$, $r = -0.2$, $p > 0.05$, respectively). Smoking status and *H. pylori* infection significantly affect the level of MMP-9 expression, imposing a higher significant increase in MMP-9 expression in the smokers' group and patients with *H. pylori* compared to non-smokers and patients with no *H. pylori* ($p = 0.02$ and $p = 0.001$, respectively). Pearson correlation test revealed a significant negative correlation between the level of MMP-9 and smoking status ($r = -0.41$, $p < 0.001$), while *H. pylori* is a highest positively correlated with MMP-9 expression ($r = 0.5$, $p = 0.001$) as seen in Table 4. MMP-9 protein expression was measured by immunohistochemistry. The tissue has heterogeneous dark brown staining, as shown in Figure 1 B.

Table 4. Association between MMP-9 expression (% positivity) and clinical variables in the malignant lesions. ^t-test for independence, the p-value was less than 0.05, indicating a significant correlation. # Pearson Correlation Coefficient Measures linear relationship strength and significance at ($p < 0.05$).

Variables	No. 58	MMP-9 Expression Mean ± SE %	T-test *P* value	Pearson Correlation Coefficient P-value
Age (year)				
≤ 50	22 (37.93%)	43.29 ± 6.3	P= 0.438	r = -0.2 P = 0.13
> 50	36 (62.06%)	53.75 ± 2.4		
Sex				
Male	47 (81.03%)	52.44 ± 1.8	P= 0.91	r = -0.2 P = 0.12
Female	11 (18.96%)	53.18 ± 6.54		
Smoking status				
Smoker	24 (41.37%)	57.91 ± 3.06	P= 0.02^	r = -0.41 P = 0.001#
Non-smoker	34 (58.62%)	48.82 ± 2.21		
H. pylori status				
H. pylori Positive	37 (63.79%)	58.92± 1.95	P = 0.001^	r = 0.59 P = 0.001#
H. pylori Negative	21(36.20%)	41.43± 2.54		



A **B**

Figure 1. Immunohistochemical staining (IHC) of anti-*H. pylori* and MMP-9 proteins in the tissue of LSCC. Staining by DAB chromogen (dark brown) counterstained with H&E. (A) positive anti-*H. pylori* immunostaining (X400). (B) Positive MMP-9 immunostaining. (X400).

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Table 5 demonstrates the results that assessed the link of MMP-9 expression as the dependent variable with the other characteristics that represent independent variables: age, sex, smoking, and *H. pylori* status in patients with benign lesions. No statistically significant differences in MMP-9 expression with weak, non-significant positive correlation were observed between aged ≤ 50 and aged > 50 years ($p > 0.05$, $r = 0.14$, $p = 0.2$, respectively). The mean expression of MMP-9 did not significantly differ between males and females, and no significant correlation ($p > 0.05$, $r = 0.02$, $p = 0.83$, respectively). Smoking and *H. pylori* status significantly affect the level of MMP-9 expression, imposing a higher significant increase in MMP-9 expression in smokers and patients with *H. pylori* compared to non-smokers and patients with no *H. pylori* ($p = 0.001$). Pearson correlation test revealed a significant correlation between the level of MMP-9 and smoking and *H. pylori* status ($r = 0.46$, $p= 0.001$), and ($r = 0.5$, $p = 0.001$), respectively. Figure 2B demonstrates MMP-9 protein expression in dark brown staining in the tissue.

Table 5. Association of MMP-9 expression and clinical variables in the benign lesion. ^t-test for independence, the p-value was less than 0.05, indicating a significant correlation. # Pearson Correlation Coefficient Measures linear relationship strength and significance at ($p < 0.05$).

Variables	No. 60	MMP-9 Expression Mean ± SE %	P value	Pearson Correlation Coefficient P-value
Age (year)				
≤ 50	26 (43.33%)	43.07 ± 2.6	P = 0.2	r=0.14 P= 0.2
> 50	34 (56.66%)	46.91 ± 2.2		
Sex				
Male	40 (66.66%)	45.5 ± 2.0	P= 0.84	r= 0.027 P= 0.83
Female	20 (33.33%)	44.75 ± 3.0		
Smoking status				
Smoker	23(38.33%)	53.04 ± 1.9	P= 0.001^	r= 0.46 P= 0.001#
Non-smoker	37 (61.66%)	40.41 ± 2.1		
H. pylori status				
H. pylori Positive	31 (51.66%)	52.58 ± 1.9	P=0.001^	r= 0.57 P= 0.001#
H. pylori Negative	29 (48.33%)	37.41 ± 2.0		

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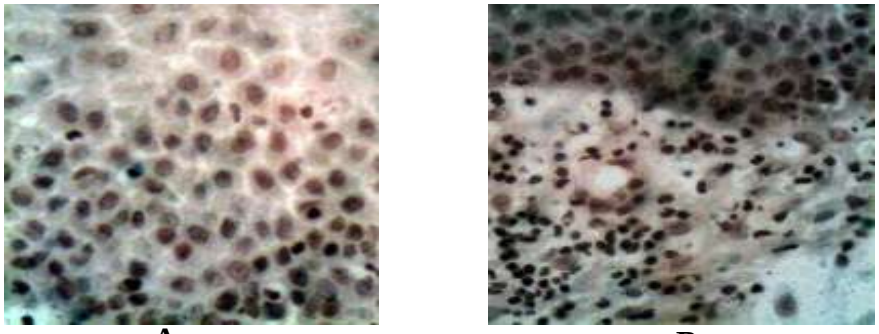

A B

Figure 2: Immunohistochemical staining (IHC) of anti - *H. pylori* and MMP-9 proteins in the tissue of BLP. Staining by DAB chromogen (dark brown) counterstained with H&E. (A) Positive anti-*H. pylori* immunostaining (X400). (B) Positive MMP-9 immunostaining. (X400).

Logistic Regression Analysis: Table 6 presents the results of two logistic models, one for predicting lesion types and the second for predicting high MMP-9 expression. These models were used to estimate the odds ratio (OR) and coefficients using age, sex, smoking, and *H. pylori* status as independent variables and both lesion type and MMP-9 level as dependent variables. To predict malignant lesions, none of the predictors reached the significant level, although the male sex approached the effect (p=0.07, OR=3.63, 95% CI: 0.9-14.3). The first model had a strong R2 of 0.56, which can explain more than half of the variance in the lesion types by the predictors. While the second model assesses the prediction of high MMP-9 level expression, only *H. pylori* significantly predicts the increase in MMP-9 expression (p=0.02, OR=3.08, 95% CI: 1.15-8.28). The model had a limited explanatory power of R2 of 0.09.

Table 6. Logistic regression for predicting lesion type and high MMP-9 expression. p-value < 0.05 is statistically significant. odds ratio (OR) > 1 shows a positive association, and < 1 shows a negative association. Confidence interval (CI) at 95% and nagelkerke R-squared values.

Variables	Lesion Type			High MMP-9		
	β (SE)	P value	OR 95% CI	β (SE)	P value	OR 95% CI
Age (> 50)	0.64 (0.49)	0.19	1.89 (0.71-4.96)	-0.34 (0.5)	0.5	0.71 (0.26-1.95)
Sex (Male)	1.29 (0.72)	0.07	3.63 (0.9-14.3)	-0.08 (0.6)	0.89	0.9 (0.72-3.16)
Smoking (yes)	-0.43 (0.67)	0.5	0.65 (0.18-2.3)	-0.5 (0.5)	0.3	0.58 (0.2-1.7)
H. pylori (Positive)	0.84 (0.55)	0.12	2.3 (0.8-6.61)	1.13 (0.51)	0.02*	3.08 (1.15-8.28)
Model Fit (R^2)	0.558			0.093		

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Discussion

It is important to explore the associated risk of laryngeal carcinoma and find novel methods and approaches to its prevention and treatment. For many years, there has been debate on the potential link with *H. pylori* and laryngeal and pharyngeal carcinomas. Several investigations have been conducted to illustrate this cause-and-effect relationship. This work studied the relationship between benign and malignant larynx conditions with *H. pylori* and smoking, in addition to the age and sex of the sample. The cases of malignant laryngeal tumors show more association with *H. pylori* infection than benign lesions, which suggests that this is statistically significant. This means *H. pylori* might be associated with malignant tumor formation. *H. pylori*, a colonizing bacterium that is found in the gastric in addition to the laryngeal mucosa, tonsil, and saliva, is linked to specific diseases and can survive in acidic environments (5, 6). This has been supported by many researchers (8). However, it was against a study that postulated no differences among the cases of *H. pylori* and malignancy (9). Gastroesophageal reflux disease and laryngeal reflux cause an acidic environment for the larynx where *H. pylori* can live and lead to chronic illness and malignant tumor formation in the larynx due to its chronic inflammatory effect (19). Thus, the effect of *H. pylori* to damage the laryngeal protective layers, such as the mucosa and the epithelium, leads to inflammation and later on could progress to chronic irritation with abnormal proliferation, in laryngeal pathology that can be a subtle inflammatory process to the most extreme malignant transformation of the cells (20). There is a significant relationship between smoking status and *H. pylori* in cases of squamous cell carcinoma of the larynx, where smokers tend to have an increased proportion of *H. pylori*-negative results, and the Pearson correlation shows a moderate negative relationship between smoking and *H. pylori* infection. That suggests the smoking may reduce the likelihood of *H. pylori* in patients with malignant tumors of the larynx. This is supported by the study of Ferro et al. (21). Smoking leads to reduced *H. pylori* infection through several mechanisms: nicotine can lead to reduced gastric acid secretion, which creates an unfavorable environment for *H. pylori*, another mechanism is changing in the microbiota in the stomach that is useful for *H. pylori* in addition to its main effect on the immune system where it leads to immune suppression, but also smoking results in the organized of proinflammatory cytokines for example IL-6, TNF-α and IL-1β. Chronic inflammation can promote gastric mucosal damage and repair processes that may make the stomach less conducive to *H. pylori* survival, especially in gastric ulceration or carcinoma, where significant damage to the stomach lining may occur (11, 22). In benign lesions of the larynx, there is a significant positive role for smoking on H. pylori; smoking was associated with higher *H. pylori*-positive cases in comparison to non-smokers. This finding is supported by Bateson (23). Relating to the results as mentioned earlier, the bimodal smoking effect on benign and malignant lesions might be explained by a complex interplay between smoking, the immune system, the gastric environment, the laryngeal environment, and the type of lesion. Nicotine's effect on cellular immunity, especially T cell response, can be summarized in the impairment of the immunity and shift of Th1-IFNy response (necessary in controlling bacterial infection) to Th2, which may reduce the effectiveness in eradicating *H. pylori*. The immune system's inability to clear the infection due to nicotine's immunosuppressive effects could increase the risk of prolonged inflammation. This persistent inflammation caused by smoking may encourage *H. pylori* infection in benign conditions (24).

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However, in malignant lesions, smoking causes damage to the tissue. It reduces the immune system due to the effect of cancer, making the environment less favorable for the organism's life (25). Targeting prevention and treatment strategies for gastric diseases linked with smoking and *H. pylori* can be made easier with an understanding of these dynamics. This is one of the areas that needs to be studied by other researchers to explain in more detail the exact way smoking affects *H. pylori* prevalence. For both the benign and malignant laryngeal lesions, gender does not have a significant effect on *H. pylori*. However, the aging significantly affect results, it is clear that immune system behavior different at aging and a decline with two types of immunity (the adaptive and the native), which makes a person more vulnerable to infection by many organisms, and one of them is *H. pylori* and the infection, inflammation consequences of having tissue changes and finally progression to cancer (26-28). Moreover, a significant positive correlation exists between smoking habit and *H. pylori* with MMP-9 expression in laryngeal malignancy, where smoking and *H. pylori* infection are associated with a higher chance of having MMP-9 expression. These results agree with the study described by Montiel-Jarquin et al. (12), who also found an increase in MMP-9 expression with smoking. The finding of increased *H. pylori* with MMP-9 expression in cancer is confirmed by the study of Liu et al. (22). Gelatinases, particularly MMP-9, play important functions in several inflammatory processes associated with malignancies. MMP-9 affects the tumor environment that favors tumor overrun and metastasis through the effect on the inflammatory cells and their mediators by the cleaving of interleukin-2α (IL-2α), shed intercellular adhesion molecule-1 (ICAM-1), and activate the transforming growth factor (TGF-β), all of them are linked to negative regulation of the immunological response to cancer which all cause tumor growth (28). In addition, MMP-9 influences macrophage polarization to M2 (tumor-activating pathway). The M2 phenotype is crucial for tissue healing, remodeling, and immune tolerance, but its dysregulation can contribute to tumor progression, chronic inflammation, and fibrosis (29). M2 macrophages, which are enriched in tumors, secrete a variety of cytokines (IL-10, TGF-β) that can inhibit immune responses (anti-tumor) and promote cancer cell proliferation (12). This immune suppression, coupled with ECM degradation and tissue remodeling, creates a microenvironment that supports tumor survival, growth, and metastasis. Also, it facilitates the interaction between tumor cells and fibroblasts by breaking down ECM proteins, allowing cancer cells to access growth factors, like vascular endothelial growth factors (VEGF), and fibroblast growth factors (FGFs), that are often sequestered within the ECM. This enhances tumor cell survival, proliferation, and motility; besides these, MMP-9 can affect processing of a novel vessel formation (angiogenesis), which has a significant role in tumorigenesis by degrading the ECM and allowing new vessel formation. As a result, more oxygen and nutrients are delivered to the tumor (29). Helicobacter pylori's effect on the immune system and immune mediator release, like IL-1β, IL-8, TNF-α, and IL-6, can progress to chronic irritation of the tissue and dysplasia of the mucosal lining cells. At the same time, it can evade the immune cells through the modulation of both regulatory and helper 17-T cells, which allows it to persist in the mucosal lining and causes chronic inflammation and tumor formation. Also, reactive oxygen species cause mutagenic effects on the DNA, with carcinogenic effects. The most virulent factor of *H. pylori* is the CagA protein that causes cell evasion of apoptosis and loss of epithelial-mesenchymal transition and cell survival

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through the release of EGF. All of these factors favor cell growth and cancer formation. Tumor invasion and metastasis with more control over the malignant lesion and better survival (19).

Even in benign larynx lesions, smoking and *H. pylori* show a positive correlation with MMP-9 expression. These findings were in agreement with the study of Koyama (30), and there was no effect of age and sex on the level of expressivity. The logistic regression analysis further supported these findings, showing that only *H. pylori* infection emerged as a significant predictor of having high MMP-9 expression with an odds ratio of 3.08 (95% CI: 1.15-8.28), suggesting that this bacterial infection could increase the risk of having high MMP-9 over three times than non-infected individuals. While age, sex, and smoking did not predict the increase in MMP-9, indicating they are less likely to confound this. In the other model that predicts lesion type (malignant vs. benign), none of the predictors achieves a significant level. Still, the male sex (p=0.07) and *H. pylori* (p=0.1) approach support the impact of this bacterium in laryngeal carcinogenesis (6, 31-33). Although the model was fit for the prediction of lesion type (R2=0.558), its clinical utility is limited because of the lack of significant prediction for the variables. However, it still offers a view of the analytic process, suggesting a validation through a larger, more powerful study. By focusing on these factors, clinicians can better identify at-risk individuals and tailor preventive or therapeutic interventions to improve patient outcomes.

Conclusions

This study highlights the aggravating effect of smoking and *H. pylori* infection on MMP-9 expression, particularly in malignant cases, suggesting their involvement in tumor progression and highlighting the importance of these factors in preventing and management strategies.

Recommendations

Targeting MMP-9 through immunomodulatory therapies or its natural inhibitors shows a promising strategy for controlling tumor invasion and metastasis. Preventing measures, including avoiding smoking and eradicating *H. pylori*, might decrease the chance of this overexpression of MMP-9 and reduce the danger of laryngeal cancer progression.

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Ethical clearance: This study was conducted per the rules outlined in the Declaration of Helsinki. Verbal consent and analytical approval were obtained from all participants before specimen collection. The protocol of the study, along with the subject information and consent form, was reviewed and approved by the local ethics committee of the Microbiology Department at the Medical College of Mustansiriyah University, numbered (13) for the academic year 2024.

Conflict of interest: None.

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تأثير بكتيريا الملوية البوابية على نوع الافة وتعبير مصفوفة ميتالوبروتينايز-٩ في اورام الحنجرة

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الملخص

الخلفية: تلعب بكتيريا الملوية البوابية ومصفوفة الميتالوبروتينايز-٩ دوراً مهماً في العمليات المرضية الفسيولوجية والتعبير عن التسرطن، وخاصة سرطان الخلايا الحرشفية الحنجرية.

الأهداف: استكشاف الارتباط المحتمل بين عدوى الملوية البوابية و التعبير عن مصفوفة ميتالوبروتينايز-٩ ، و تطور سرطان الخلايا الحرشفية الحنجرية من خلال دراسة دور الالتهاب المزمن و التغيرات في تركيب المادة الخلالية خارج الخلية في سرطان الحنجرة.

المرضى والطرق: شملت الدراسة المقطعية ١١٨ عينة نسيجية منها ٥٨ حالة كانت سرطان الخلايا الحرشفية الحنجرية، و ٦٠ حالة كانت سلائل حنجرية حميدة. تم استخدام الفحص النسيجي المرضي الكيميائي المناعي لتقييم تأثير بكتيريا الملوية البوابية وتعبير مصفوفة ميتالوبروتينايز-٩ وحالة التدخين في اورام الحنجرة.

النتائج: كان انتشار بكتيريا الملوية البوابية ملحوظاً الافة الخبيثة (٦٣,٧٩٪) مقارنة بالآفات الحميدة (٥١,٦٦٪) (P=0.01). وجد ارتباط ذو دلالة احصائية لدى مرضى الآفات الحميدة بين مستوى التعبير عن مصفوفة ميتالوبروتينايز-٩ والتدخين وحالة الاصابة ببكتيريا H. pylori ((P= 0.46, p=0.001 و (r=0.5, p= 0.001) (على التوالي. اما مرضى الآفات الخبيثة, فقد كشف اختبار الارتباط عن ارتباط سلبي ذي دلالة احصائية بين مستوى مصفوفة ميتالوبروتينايز-٩ وحالة التدخين (p=-0.41, p=0.001) بينما كانت بكتيريا H. pylori اعلى ارتباط ايجابي مع تعبير مصفوفة ميتالوبروتينايز -٩(r=0.5, p=0.001) . كشف تحليل الانحدار اللوجستي ان عدوى بكتيريا H. pylori يمكن ان تتنبأ بشكل كبير بارتفاع تعبير مصفوفة ميتالوبروتينايز-٩ .(OR=3.08, 95%CI: 1.15.28, p=0.02).

الاستنتاج: تبرز هذه النتائج الدور الحاسم لعدوى الملوية البوابية بالتزامن مع التدخين في تطور الاورام من خلال إعادة تشكيل المادة الخلالية خارج الخلية بواسطة مصفوفة الميتالوبروتينايز-٩ و الالتهاب و تعديل الجهاز المناعي.

الكلمات المفتاحية: جرثومة المعدة، MMP-9، سرطان الخلايا الحرشفية في الحنجرة، أورام حميدة في الحنجرة.

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