



Histopathological Changes of Minimal Associated Pathological Lesions of Vocal Folds: Comparative Study between Smokers versus Non-smokers

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Abstract:

Objectives: Investigate the impact of cigarette smoking on the histopathology of resected minimal associated pathological lesions and compare it with the histopathology of the lesions of non-smoker patients for a better understanding of these changes, follow-up and proper management. **Methods:** There are two groups of 60 patients, (group A): 30 smokers and (group B): 30 non-smokers, they were selected from the outpatient clinic, Phoniatics unit of Beni-Suef University Hospital both males and females, and both groups underwent micro-laryngeal surgery then histopathological examination for the resected lesions. **Results:** showed that smokers had a higher incidence of keratinization and dysplasia, as well as basement membrane changes in their lesions compared to non-smokers. Additionally, smokers had more hyaline

degeneration. These findings suggest that cigarette smoke can have harmful effects on the epithelium and lamina propria of minimal associated pathological lesions. **Conclusion:** Cigarette smoking causes histologic alterations in the minimal associated pathological lesions, dysplasia, keratinization, and basement membrane changes, which were significantly higher in the smokers group.

1. Introduction:

The term "minimal associated pathological lesions" (MAPLS) refers to benign superficial lesions of the vocal fold, such as nodules, polyps, cysts, granulomas, Reinke's edema, and polypoid degeneration, that originate from the vocal fold's epithelium and superficial layer of lamina propria. ⁽¹⁾

Vibratory trauma is the main cause of MAPLS, the main risk factors are lifestyle choices and occupational voice demands. The vocal fold mucosa is more susceptible to vibratory trauma from cigarette smoking, acid reflux, allergies, and infections. This can result in damage and the development of morphological abnormalities in the vocal folds, which promotes the formation of lesions. ⁽²⁾

A benign lesion is one that is seen to be largely innocent from both a microscopic and gross perspective, suggesting that it will stay localized and not spread to other areas. ⁽³⁾

Cigarette smoke causes injury to the vocal fold epithelial barrier ⁽⁴⁾, aberrant production and accumulation of keratin in the vocal fold epithelium. It is also regarded as one of the main risk factors for epithelial dysplasia. ⁽⁵⁾

2. Patients and methods:

The study duration was from October 2022 to March 2024. Sixty patients aged 18-60 years, diagnosed clinically by flexible or rigid laryngoscopy to have MAPLS (Polyps, Nodules, cysts, Granuloma, Reinke's edema, and polypoid degeneration).

The study was conducted after approval from the research ethical committee, Faculty of Medicine, Beni Suef University, approval No: **FMBSUREC/04012023/Aboud.** and written informed consent was obtained prior to surgery from all patients.

Study design: Cross-sectional study.

Sample size calculation:

The sample size was determined using G*power (Faul et al., 2007), an open-source calculator. Based on the assumption that Effect size equals 1, the minimal number of participants that should be included was 60 with 30 participants in each arm for power 95% and confidence level 95% t tests - Means: Difference between two independent means (two groups).

Analysis: A priori: Compute required sample size

They were selected from the outpatient clinic, Phoniatrics unit of Beni-Suef University Hospital, their age ranged between 18 and 60 years old, both males and females with variable social class.

Inclusion criteria:

- Patients with benign vocal fold lesions need surgical intervention.
- Age: 18-60 years old.
- Both males and females.

Exclusion criteria:

- Organic vocal fold lesions.
- Congenital laryngeal diseases.

All patients were subjected to the following protocol of assessment:

Full voice assessment being structured and applied in the Phoniatrics Unit, Beni-Suef University Hospital, includes: -

I- Elementary diagnostic procedures:

(1) Patient's interview:

Data were collected about: - The patient's name, age, sex, marital status, address, education, and occupation.

Analysis of the complaint was done as: - Concerning the onset, course, and duration of the symptoms.

Important attention was given to the analysis of the patient's habits of medical importance like smoking cigarettes, cannabis, and alcohol, vocal environmental demands and hazards, and history of COVID-19 infection. Voice abuse and misuse, vocal load, chronic cough, GERD, Covid, Repeated URTI.

The duration of dysphonia, voice abuse-misuse, number of years of smoking, and the number of cigarettes smoked per day were also documented (Trupthi et al., 2021).

Phonasthenic symptoms include throat dryness, throat soreness, Frequent throat clearing, sticky secretions, and Globus sensation.

(2) Auditory perceptual assessment:

After careful listening to the patient's voice, changes in the following voice parameters were measured, evaluated and tabulated according to the modified GRBAS scale.

- Overall grade.
- Character.
- Pitch.

- Register.
- Loudness.
- Glottal attacks.
- Associated laryngeal functions.

II- Clinical diagnostic aids

Rigid laryngoscopic equipment set.

Patients who agreed to undergo micro-laryngoscopy with excision of the lesion were included in the study. They were clinically assessed by two experienced phoniatricians and classified into two groups: smokers and nonsmokers. Demographic data like occupation, age, and gender, the onset, course, and duration of dysphonia, Voice abuse and misuse, the duration of using voice, Chronic cough, GERD, COVID, Repeated URTI, and GERD were documented.

All patients underwent micro-laryngoscopy and excisional biopsy were taken under general anesthesia, Hematoxylin and eosin were used to stain the masses after they were embedded in paraffin, fixed in 10% formalin, and sectioned into 4 µm thick slices.

All tissue sections were evaluated using light microscopy by the same pathologist, who was blinded to smoking or non-smoking patients.

Documentation included the histological appearance of the epithelium, the

presence of inflammatory cells, recent or old hemorrhage, and the basement membrane beneath the lamina propria of the lesion.

To characterize the lesions histologically, the following parameters were included in the proposed analytical protocol:

Epithelium, lamina propria, basement membrane, stromal changes, and vascular abnormalities.

When defining these parameters, the histological features of laryngeal nodules, polyps, cysts, Reinke's edema, and granulomas that may be seen histologically based on the stain utilized were taken into consideration.

For the epithelium parameter, the selected alterations were:

Erosion, dysplasia, and hyperplasia, atrophy, and keratinization

Erosion is the shallow ulceration of the epithelium without reaching the lamina propria; **dysplasia** is the epithelial disorganization with cellular atypia;

Hyperplasia is defined as an increase in cell number and epithelium thickness caused by stimulation or trauma; **atrophy** is defined as a reduction in cell number and epithelium thinning; and **keratinization** is the layer

formed by cells that slough off the surface epithelium these conditions can be complete

(orthokeratosis) or incomplete (parakeratosis)

For the lamina propria parameter, the considered alteration are edema, fibrosis and inflammatory infiltrate. Leakage of fluid into the interstitial space was a characteristic of edema; fibrosis was caused by an increase in connective tissue resulting from both normal or excessive healing and inflammatory infiltration by inflammatory cell exudation.

Histological degeneration, hyalinization and myxoid change.

The hyaline type shows masses of eosinophilic hyaline material within the stroma.

Myxoid change is characterized by an abundance of pale-gray fluids beneath the epithelium.

For the basement membrane parameter: it is a laminate structure that sits between the surface layer of the epithelium and the lamina propria. The subsequent elements were chosen for analysis of this parameter: The three types of thickening observed in the

epithelium are diffuse thickening, localized thickening, and finely thinned or without change, which refers to the absence of thickening in any area of the basement membrane.

The vascular changes parameter, finally, we examined the existence of extravasated RBCs, hemosiderin, and ecstatic vessels in the vascular change's parameter.

Statistical analysis:

The average with standard deviation 00B1 is used to express the results. Unpaired t-tests were used to compare the mean values of variables that were different between the two study groups (smokers versus non-smokers). Chi-square tests were used to compare categorical data. Data analysis was performed using IBM SPSS Statistics version 16.0 (Chicago, Illinois, USA). A statistically significant p-value was one that was less than or equal to 0.05, while a very significant p-value was one that was less than or equal to 0.01.

3. Results:

The study population is divided into 2 groups, Table 1 shows that there was no statistically significant difference between them regarding personal age, most of them were professional voice users and had high vocal demand. At the same time, there was a statistically significant difference regarding sex between the studied groups.

Table (1): Demographic data of studied groups.

	group A (Smokers) (n = 30)	group B (non-smokers) (n = 30)	P value <0.05 is statistically significant.
Age (year)	44.82±12.41	39.47±13.75	0.119
Sex			
males	24(80%)	16(60%)	0.03
females	6(20%)	14(40%)	
Professional voice user			
Yes	23(76.7%)	24(80%)	0.75
No	7(23.3%)	6(20%)	
Professional vocal demand			
Yes	21(70%)	20(66.7%)	0.78
No	9(30%)	10(33.3%)	

Table (2): Phonasthenic symptoms:

	group A (Smokers) (n = 30)	group B (non-smokers) (n = 30)	P value <0.05 is statistically significant.
Dryness	28(93.3%)	22(73.3%)	0.04
Soreness	27(90%)	20(66.7%)	0.03
Frequent throat clearing	21(70%)	13(43.3%)	0.04
Sticky secretions	17 (56.6%)	9(30%)	0.001
Globus sensation	26(86.7%)	14(46.7%)	0.001

This table shows that the smokers group experienced more Phonasthenic symptoms than non-smokers.

Table (3): Predisposing factors for Voice disorders among studied groups:

	group A (Smokers) (n = 30)	group B (non-smokers) (n = 30)	P value <0.05 is statistically significant
Voice abuse and misuse	25(83.3%)	21(70%)	0.22
NO. of hours using voice	10.57±1.92	9.68±1.83	0.07
Chronic cough	18(60%)	7(23.3%)	0.004*
GERD	16(53.3%)	8(26.7%)	0.04*
COVID	3(10%)	4(13.3%)	0.69
Repeated URTI	5(16.7%)	1(3.3%)	0.09

Regarding the possible etiological factor, both groups reported voice abuse and misuse, number of hours using voice about 9-10 hours a day, there was no statistically significant difference between the studied group regarding COVID-19 and Repeated URTI, at the same time, Smokers group experienced gastroesophageal reflux. and Chronic cough more than the non-smoker group, there was a statistically significant difference between the studied group regarding Chronic cough and GERD.

Table (4): Modified GRBAS scale among studied groups.

		group A (Smokers) (n = 30)	group B (non-smokers) (n = 30)	P value
Overall grade	0	0(0%)	0(0%)	0.797
	1	4(6.7%)	7(23.3%)	
	2	14(46.7%)	12(40%)	
	3	12(33.3%)	11(36.7%)	
irregular	0	4(13.3%)	16(53.3%)	0.002*
	1	11(36.6%)	9(30%)	
	2	15(50%)	5(16.6%)	
				$\leq 0.0001^*$
leaky	0	1(3.33%)	13(43.3%)	
	1	4 (16.6%)	11(36.6%)	
	2	15(50%)	4(16.6%)	
	3	10(33.3%)	2(6.7%)	
Breathy	0	11(36.6%)	13(43.3%)	0.002*
	1	8(23.3%)	10(33.3%)	
	2	9(26.6%)	4(10%)	
	3	2(3.33%)	3(6.7%)	
strained	0	5(16.7%)	11 (36.6%)	0.007*
	1	15(50%)	18 (60%)	
	2	10(33.3%)	1(3.33%)	

This table shows there was no statistically significant difference between the studied group regarding Overall grade, there was a statistically significant difference between the studied groups regarding irregular, leaky, Breathy and strained.

The Minimal associated pathological lesion types differed significantly concerning smoking status Table 5, Reinke's edema affected 23.3% of the smoker's group, while vocal fold nodules affected 33% of the non-smokers group. Concerning vocal fold polyp equally represented in both group 12 (40%) in both groups.

Table (5): Laryngoscopic examination among studied groups.

	group A (Smokers) (n = 30)	group B (non-smokers) (n = 30)	P value
Cyst	7(23.3%)	5 (16.66%)	0.514
polyp	12 (40%)	12 (40%)	1
nodules	3(10%)	11 (36.7%)	0.07
Granuloma	1(3.3%)	1 (3.33%)	1
Reinke's edema	7(23.3%)	1 (3.33%)	0.022

Table (6): Histopathology among studied groups.

	group A (Smokers) (n = 30)	group B (non-smokers) (n = 30)	P value
Keratinization			
Ortho	11(36.7%)	7(23.3%)	0.008*
Para	10(33.3%)	3(10%)	
Ortho/Para	6(20%)	6(20%)	
No	3(10%)	14(46.7%)	
Epithelial ulceration			
Present	5(16.6%)	2(6.6%)	0.69
Absent	25(83.3%)	28(93.3%)	
Epithelial hyperplasia			
Present	20(66.7%)	16(53.3%)	0.29
Absent	10(33.3%)	14(46.7%)	
Epithelial atrophy			
Present	10(33.3%)	7(23.3%)	0.389
Absent	20(66.7%)	23(76.7%)	
Dysplasia			
High grade	3(10%)	0%	0.004*

Low grade	10(33.3%)	2(6.7%)	
Absent	17(56.7%)	28(93.3%)	
Basement membrane			
Thin	6(20%)	3(10%)	0.001*
Thick	10(33.3%)	4(13.3%)	
Focally thick	12 (40%)	7(23.3%)	
Normal (Intact)	2(6.7%)	16(53.3%)	
Oedema			
Present	27(90%)	26(86.7%)	0.69
Absent	3(10%)	4(13.3%)	
Fibrosis			
Present	14(46.7%)	9(30%)	0.18
Absent	16(53.3%)	21(70%)	
Ecstatic vessels			
Present	20(66.7%)	17(56.7%)	0.425
Absent	10(33.3%)	13 (43.3%)	
Hemosiderin			
Present	8(26.7%)	7(23.3%)	0.765
Absent	22(73.3%)	23(76.7%)	
Extravasated RBCs			
Present	18(60%)	11(36.7%)	0.07
Absent	12 (40%)	19(63.3%)	
Inflammation			
No	2(6.7%)	5(16.7%)	0.48
Mild	22(73.3%)	18(60%)	
Moderate	4(13.3%)	3(10%)	
Marked	2(6.7%)	4(13.3%)	
Hyalinization			
Present	24(80%)	8(26.7%)	<0.001*

Absent	6(20%)	22(73.3%)	
Myxoid change			
Present	8(26.7%)	5 (16%)	0.347
Absent	22(73.3%)	25(83.3%)	

This table shows no statistically significant difference between the studied group regarding epithelial ulceration, epithelial hyperplasia, epithelial atrophy, edema, fibrosis, ecstatic vessels, hemosiderin, extravasated RBCs inflammation, and myxoid change (Figure 1).

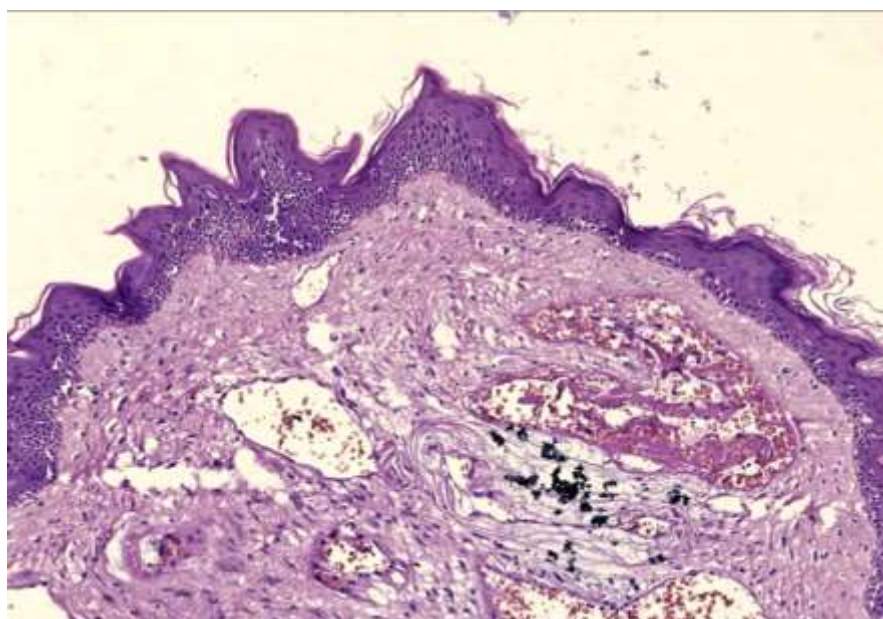


Figure 1: benign vocal fold nodule showing epithelial keratosis, **stromal ectatic blood vessels, and myxoid changes**, *original magnification x 100*.

On the other hand, there was a statistically significant difference between the studied groups regarding keratinization (Figure 2), dysplasia (Figure 3 and 4), basement membrane, and hyalinization.

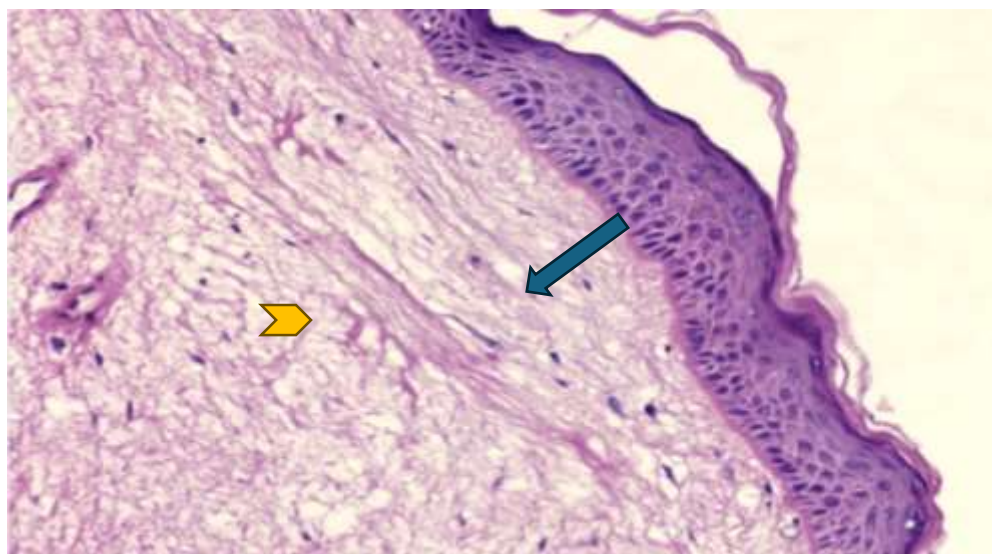


Figure 2: benign vocal fold nodule showing orthokeratosis (arrow), **thick basement membrane (arrowhead)**, and stromal edema. *original magnification x 100.*

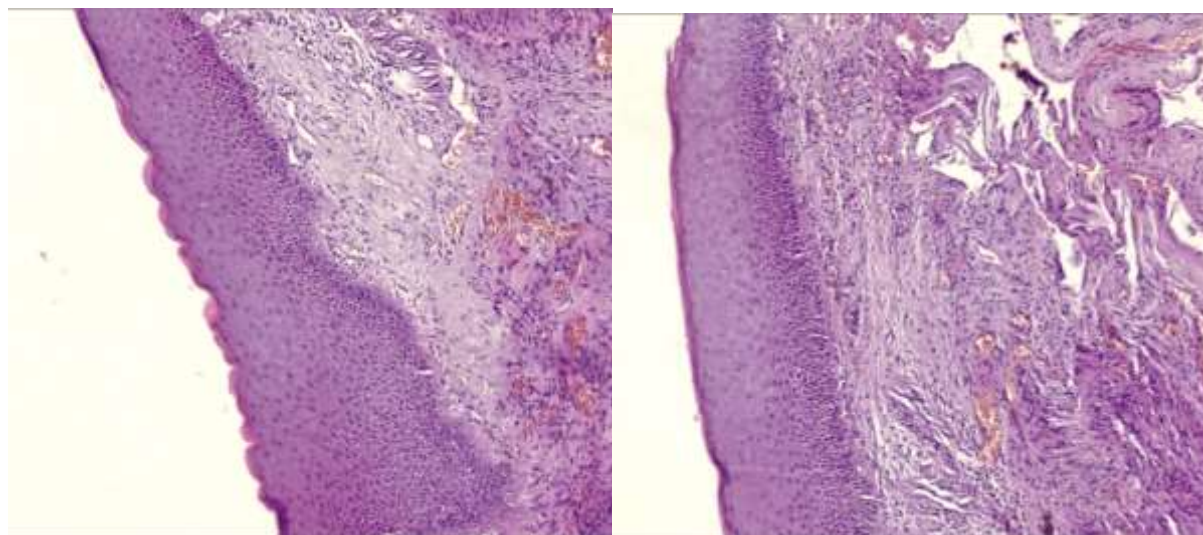


Figure 3: benign vocal fold polyp showing **low-grade dysplasia** *original magnification x 100.*
H&E.

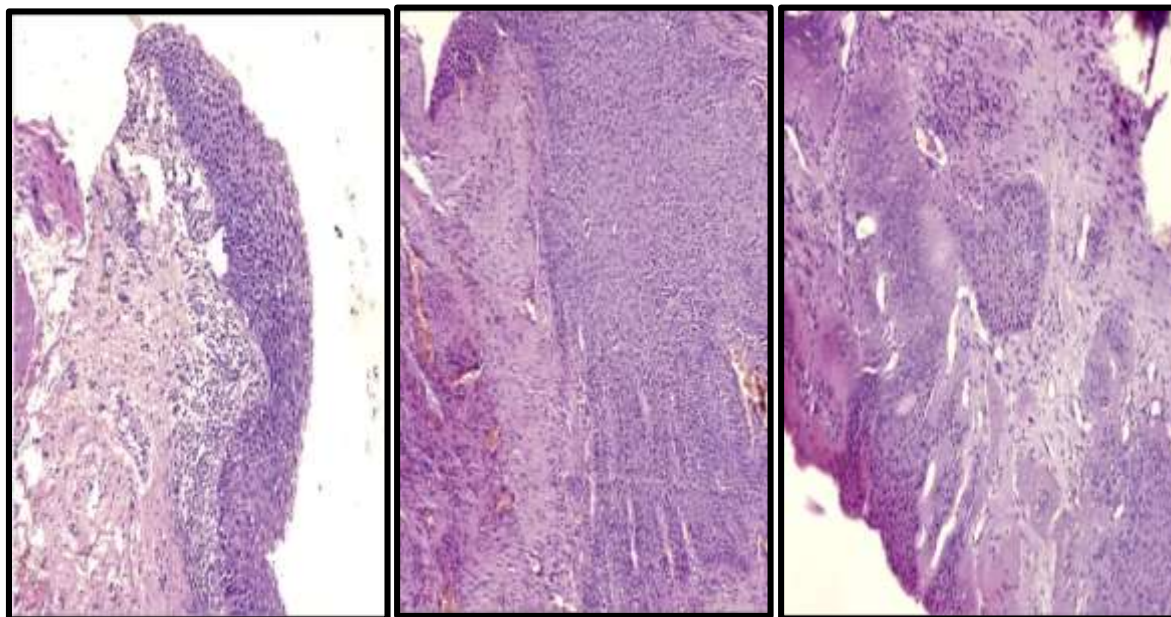


Figure 4: benign vocal fold polyp showing **high-grade dysplasia** *original magnification x 100*.

4. Discussion:

In the current study Table 1, males represented 80% of the smokers group. There was a significant difference (P value 0.03) between the two groups regarding sex.

According to the Egyptian Demographic and Health Survey, 43% of men and 1% of women use tobacco, with men being the primary smokers in the country. The low frequency among females may be explained by Egyptian cultural customs and the fear of shame. ⁽⁶⁾

People who depend on their voices to fully execute daily functions are called voice professionals. ⁽⁷⁾ Teachers, Quran reciters and Tele-operators, Singers, actors, salespersons,

and lawyers are considered Professional voice users.

Studies indicate that voice disorders are higher and prevalent in professional voice users than in the overall population. ⁽⁸⁾ The study population included in this study primarily voice professionals.

Regarding the phonasthenia Table 2, the smoker's group experienced a statistically significant difference in dryness, Soreness, and frequent throat clearing ($p < 0.05$) This could be explained by the hydrophilic effects of the inhaled smoke cause the laryngeal mucosa to dry and delay the liquidity of cyclic vocal fold vibrations during the process of voice production. ⁽⁹⁾ a highly statistically significant difference regarding the Feeling of sticky mucus and Globus

sensation most probably attributed to chronic smoking which causes in a rise in goblet cells, an increase in upper respiratory tract secretions, and metaplasia of the respiratory mucosa. ⁽¹⁰⁾

The group of smokers experienced more GERD and a persistent cough than the non-smokers. Tobacco smoke can irritate the laryngeal mucosa to participate in chronic contact inflammation, erythema, dryness, and itching because of long-term tobacco use or exposure to smoke. It's typical to experience secondary cough reflexes along with a globus sensation. ⁽⁹⁾

The association between GERD and Smoking, in addition to decreasing the amount of saliva produced that is high in bicarbonate, which is necessary for clearing acid and buffering the esophagus PH, smoking tobacco also lowers the lower oesophageal sphincter (LOS) pressure by 41% in just one to four minutes. ⁽¹¹⁾

The Minimal associated pathological lesion types differed significantly concerning smoking status Table 5, Reinke's edema affected 23.3% of the smoker's group, while vocal fold nodules affected 33.3% of the non-smokers group.

Reinke's edema occurrence is closely related to tobacco smoking. The clinical manifestation of this disease seems to be

correlated with the number of cigarettes smoked each day, even though the type and severity of the epithelial lesion appear to be related to the length of time spent exposed to tobacco smoke (the longer exposure time results in a higher degree of histologic damage. ⁽¹²⁾

Vocal fold nodules represented 33.3% of the non-smokers most probably attributed to the female sex, representing 40% of the non-smokers group.

In the superficial layer of the lamina propria, female vocal folds usually have lower quantities of hyaluronic acid this is according to **Butler et al., 2001**. As a result, there is a reduction in the tissue's capability to heal and a reduction in its ability to absorb stress from vibratory trauma during vocalization. ⁽¹³⁾

Hirano et al., 2024 hypothesized that the mechanical pressure from high-pitched vibration is focused on the vocal fold's edge, leading to the development of nodules through the stimulation of subepithelial fibroblasts and the increase in collagen fiber deposition. ⁽¹⁴⁾

According to our study histopathological changes of the benign lesions of the smoker's group which are highly significant are keratinization, dysplasia, and basement membrane changes,

this is concordant with **Montgomery and White, 2011** findings, who investigated 937 patients between 1998 and 2011 and found that 131 (14%) had laryngeal dysplasia, 57% were smokers, 19% were ex-smokers, smoking status was unknown for 12 patients and 12% had never smoked.⁽¹⁵⁾

The study conducted by **Duarte et al. (2006)** examined the histological alterations in the vocal fold epithelium of rats that were subjected to 30 cigarettes per day for 25, 50, and 75 days. The rats showed signs of hyperplasia, and fewer cigarettes per day also caused notable disruptions in the epithelial structure.⁽¹⁶⁾

According to **Isik et al. (2004)**, rats exposed to cigarette smoke for two hours each day for sixty days showed signs of epithelial hyperplasia, decreased desmosome counts, and enlargement of the paracellular pathways in addition to disturbed epithelial stratification, desquamation, and disorganized micro ridges.⁽¹⁷⁾

In **2020, Park and colleagues** employed Raman spectroscopy to investigate the initial biochemical alterations in mouse vocal folds following time-limited exposure to cigarette smoke. A closer look at the coronal slices showed that the foundation membrane, underlying lamina propria, and epithelium of the vocal fold cover had

undergone morphological alterations. After two and four weeks of cigarette smoking, they saw thickening of the vocal fold epithelium, which is consistent with hypercellularity or hyperplasia.⁽¹⁸⁾

According to our study there was a highly significant increase in hyalinization in smokers' MAPLs compared to those of the non-smoker's group ($p < 0.01$). a degenerative alteration in the fibrous collagen is called hyalinization, this is due to continuous tissue insult by cigarette smoke, phonotrauma, and chronic cough, these results agreed with **Effat and Milad, 2015** results who observed hyaline changes caused by smoking in vocal fold polyps.⁽¹⁹⁾

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