The Journal of Laryngology & Otology (2015), ***129***, *484–488*.

doi:10.1017/S002221511500064X

MAIN ARTICLE

A comparative histopathological study of vocal fold polyps in smokers versus non-smokers

K G EFFAT[1], M MILAD[2]

[1]Department of Otolaryngology, El-Sahel Teaching Hospital, Cairo, and [2]Department of Pathology, Cairo University Hospital, Egypt

Abstract

Background: A large proportion of patients with vocal fold polyps are cigarette smokers. However, prior to this report no comparative study of polyp histopathology in smokers versus non-smokers had been performed.

Methods: A prospective histopathological study of vocal fold polyps excised from 29 patients was undertaken. This comprised a comparative analysis of polyp histopathology in smokers versus non-smokers and a review of the pertinent literature.

Results: Vocal fold polyps were larger in smokers than in non-smokers. Histopathological features significantly associated with the polyps of smokers versus those of non-smokers were increased keratinisation, dysplasia, a basement membrane thinning and hyaline degeneration.

Conclusion: Cigarette smoke has an injurious effect on vocal fold polyp epithelium and leads to increased hyaline degeneration in polyps.

Key words: Vocal fold; Polyps; Smoking; Histopathology

Introduction

A vocal fold polyp is a benign mid-membranous exophytic vocal fold lesion with a smooth surface. It is usually unilateral (but may be bilateral) and can be pedunculated or sessile.[1] Early theories of vocal fold polyp pathogenesis implicated phonotrauma as the inciting event, resulting in damage to the subepithelial capillary walls and protein-rich fluid exudation. Following this, organisation of the exudate may occur, leading to fibrovascular tissue formation. Alternatively, the exudate may undergo hyaline degeneration.[2,3]

The role of mechanical stresses related to phonotrauma as an aetiological factor in vocal fold polyp development was corroborated by computational models. These have identified the most common site of pathogenesis as the centre of the free margin of the membranous vocal fold.[4] Phonotrauma is also reported to upregulate inflammatory biomarkers and repair mechanisms in the stressed vocal fold.[5]

Many patients with vocal fold polyps are cigarette smokers.[6] However, a PubMed and Medline search over the last 35 years identified no comparative study on the vocal fold polyp histopathology in smokers versus non-smokers. This study explored whether cigarette smoking has an impact on the histopathology of resected vocal fold polyps.

Materials and methods

Approval for this study was granted by the local ethics committee. The study period was one year, starting on 1 May 2013. Consecutive patients presenting with persistent hoarseness to the Otolaryngology Clinic at El-Sahel Teaching Hospital, Cairo, underwent video laryngoscopy to investigate laryngeal pathology. During the study period, 29 patients were clinically diagnosed with vocal fold polyps. A comprehensive medical history was taken for each patient, especially focusing on hoarseness duration and smoking status. All patients underwent microlaryngoscopy under general anaesthesia, during which polyp features and vocal fold appearance were documented. Polyps were completely excised at the base and the size was recorded. Polyps were fixed in 10 per cent formalin, embedded in paraffin, cut into 4 µm thick sections and stained with haematoxylin and eosin. Tissue sections were examined by light microscopy by a consultant pathologist with a special interest in laryngology. The pathologist was blinded to the smoking status of each patient.

The histological appearance of the epithelium, basement membrane and underlying lamina propria of the polyps was documented. The appearance of the epithelium was graded according to the Ljubljana classification

Accepted for publication 22 September 2014 First published online 19 March 2015

into normal (atrophic), simple hyperplasia, dysplasia and atypia.[7] The presence of superficial keratosis was noted. The basement membrane was classified as thin or thick. Lamina propria features were categorised as oedema, fibrosis, vascularisation, exudated red blood cells, hyalinisation and the presence of inflammatory cells, with each category being graded according to Hantzakos *et al.* as none, mild changes, moderate changes and marked changes.[8]

Results were expressed as the mean 00B1 standard deviation. The mean values of variables that differed between the two study groups (non-smokers versus smokers) were compared using unpaired *t*-tests. Categorical data were compared using chi-square tests. IBM SPSS Statistics software version 16.0 (Chicago, Illinois, USA) was used for data analysis. A p value less than or equal to 0.05 was considered statistically significant; a p value less than or equal to 0.01 was considered highly significant.

Results

The study group comprised 29 patients with 31 vocal fold polyps: 14 were non-smokers and 15 were smokers. Of the 14 non-smokers, 10 were female and 4 were male. All 15 smokers were male. Non-smokers ranged in age from 16 to 51 years (mean 35 years), and smokers ranged in age from 32 to 61 years (mean 44 years; $p < 0.05$). The average duration of hoarseness prior to presentation was longer in smokers than in non-smokers. For the non-smoking group, the right vocal fold was affected in nine patients and the left vocal fold in five patients. In the smoking group, 10 polyps were located on the left vocal fold and 3 were on the right; 2 patients had bilateral vocal fold polyps. The mean size of resected polyps was significantly greater in the smoking group than in the non-smoking group ($p < 0.01$). All 14 polyps from non-smokers and 17 polyps from smokers were histologically analysed, and various histopathological features in the epithelium and lamina propria were graded. The pathological features of polyps from non-smokers are shown in Table I and those of smokers in Table II. Significant differences in variables between the two groups are shown in Table III.

Discussion

Vocal fold polyps are almost exclusively found in adult patients.[6] The youngest patient in the current study was 16 years old. There was a significant difference in mean patient age at presentation between groups: 35 years in the non-smoking group and 44 years in the smoking group ($p < 0.05$). In addition, hoarseness duration prior to presentation tended to be longer in the smoking group. These differences may be due to smokers attributing their hoarseness to simple chronic laryngitis, thereby delaying their presentation to the laryngologist.[9] Moreover, persistent squeezing of the polyps by sharp movements of the vocal folds during phonation and coughing may hamper repair

TABLE I
POLYPS PATHOLOGY IN NON-SMOKERS

Pt no	Gender	Age (years)	DoH (months)	Side	Size (mm)	Epithelium					BM	Lamina propria					
						Ker	NAtr	Hyper	Dysp	Aty		Oedema	Fibrosis	Vasc	Hya	RBCs	Inf
1	F	34.0	3.0	Lt	3.0	–	+	–	–	–	thick	–	+	–	–	–	+
2	M	38.0	12.0	Lt	5.0	–	+	–	–	–	thin	+	+	+	–	–	+
3	M	29.0	9.0	Rt	4.0	+	–	+	–	–	thin	–	–	+	+	–	+
4	F	48.0	6.0	Rt	4.0	+	+	–	–	–	thin	+	++	+	+	–	–
5	F	23.0	6.0	Rt	4.0	–	+	–	–	–	thick	++	+	–	–	–	–
6	M	51.0	60.0	Rt	4.0	–	+	–	–	–	thick	++	+	–	–	–	–
7	F	16.0	12.0	Lt	3.0	+	–	+	–	–	thin	+	–	–	–	–	–
8	F	40.0	24.0	Lt	4.0	–	–	+	–	–	thin	–	–	–	–	–	+
9	F	33.0	8.0	Lt	5.0	+	–	+	–	–	thick	–	–	+	+	–	–
10	F	50.0	2.0	Rt	6.0	++	–	+	–	–	thin	+	–	++	–	–	+
11	F	30.0	12.0	Rt	3.0	+	+	–	–	–	thick	+	++	–	–	–	–
12	F	30.0	6.0	Rt	3.0	–	+	–	–	–	thick	–	++	++	–	–	++
13	F	17.0	12.0	Rt	4.0	–	–	+	–	–	thin	+	–	–	–	–	+
14	M	18.0	18.0	Rt	4.0	–	–	+	–	–	thick	–	–	–	–	–	+

–, none; +, mild changes; ++, moderate changes; +++, marked changes. Pt no = patient number; DoH = duration of hoarseness; Ker = keratinisation; NAtr = normal or atrophic; Hyper = hyperplasia; Dysp = dysplasia; Aty = atypia; BM= basement membrane; Vasc = vascularisation; Hya = hyalinisation; RBCs = exudated red blood cells; Inf = inflammatory cells; F = female; Lt = left; M = male; Rt = right

https://doi.org/10.1017/S002221511500064X Published online by Cambridge University Press

TABLE II
POLYP PATHOLOGY IN SMOKERS

Pt no	Gender	Age (years)	DoH (months)	Side (mm)	Size	Epithelium					BM	Lamina propria					
						Ker	NAtr	Hyper	Dysp	Aty		Oedema	Fibrosis	Vasc	Hya	RBCs	Inf
1	M	40.0	6.0	Lt	9.0	–	–	–	+	–	thick	+++	–	+	++	–	+
2	M	32.0	3.0	Lt	5.0	+	+	–	–	–	thin	–	–	–	–	–	+
3	M	38.0	72.0	Rt	4.0	+	+	–	–	–	thin	–	+++	+	+	–	–
4	M	34.0	48.0	Lt	5.0	+	–	+	–	–	thin	+	+	+	+	–	+
5	M	58.0	180.0	Lt	13.0	–	+	–	–	–	thin	+++	+	+++	+	–	+
				Rt	9.0	–	+	–	–	–	thin	+++	+	+++	+	–	+
6	M	37.0	48.0	Lt	16.0	+	–	+	+	–	thick	+	++	+	+	–	–
7	M	49.0	12.0	Rt	13.0	++	–	–	+	–	thin	++	+	–	+	–	++
8	M	50.0	8.0	Lt	4.0	+	–	–	–	–	thin	–	–	–	+	–	++
9	M	35.0	24.0	Lt	5.0	+	–	+	–	–	thin	+	–	++	+	–	+
10	M	50.0	3.0	Lt	5.0	+	+	–	+	–	thin	–	++	++	+	–	–
11	M	38.0	12.0	Lt	6.0	++	–	–	+	–	thin	–	–	–	–	–	+
12	M	61.0	12.0	Lt	4.0	+	–	–	–	–	thin	+	+	++	+	–	–
				Rt	4.0	+	–	–	–	+	thin	+	+	++		+	–
13	M	35.0	6.0	Lt	5.0	+	–	+	–	–	thin	+++	+	+	+	–	–
14	M	40.0	12.0	Lt	16.0	+	–	+	–	–	thin	+	+	+	+	–	+
15	M	48.0	24.0	Rt	8.0	++	–	+	–	–	thin	++	+	++	–	++	++

–, none; +, mild changes; ++, moderate changes; +++, marked changes. Pt no = patient number; DoH = duration of hoarseness; Ker = keratinisation; Hya = hyalinisation; NAtr = normal or atrophic; RBCs = exudated red blood cells; Hyper = hyperplasia; Inf = inflammatory cells; Dysp = dysplasia; Aty = atypia; BM = basement membrane; Vasc = vascularisation; M= male; Lt = left; Rt = right; F = female

TABLE III
COMPARISON OF POLYP FEATURES IN SMOKERS VERSUS NON-SMOKERS

Feature	Non-smokers ($n = 14$)	Smokers ($n = 17$)	P value
Side of lesion			
– Left	5 (35.7)	12 (70.6)	0.052
– Right	9 (64.3)	5 (29.4)	
Size of lesion (mm)	4.0 ± 0.88	7.71 ± 4.27	0.003**
Keratinisation (yes)	6 (42.9)	14 (82.4)	0.022*
Normal or atrophic (yes)	7 (50.0)	5 (29.4)	0.242
Hyperplasia (yes)	7 (50.0)	6 (35.3)	0.409
Dysplasia (yes)	0 (0.0)	5 (29.4)	0.027*
Atypia (yes)	0 (0.0)	1 (5.9)	0.356
Basement membrane (thin)	7 (50.0)	15 (88.2)	0.020*
Oedema (yes)	8 (57.1)	12 (70.6)	0.436
Fibrosis (yes)	7 (50.0)	12 (70.6)	0.242
Vascularisation (yes)	6 (42.9)	13 (76.5)	0.056
Hyalinisation (yes)	4 (28.6)	14 (82.4)	0.003**
RBCs (yes)	0 (0.0)	1 (5.9)	0.356
Inflammatory cells (yes)	8 (57.1)	11 (64.7)	0.667

Data are expressed as mean ± standard deviation or number (%).*$p < 0.05$; **$p < 0.01$. RBCs = exudated red blood cells

mechanisms acting within polyp tissue, thus leading to recurrent capillary trauma and progression in polyp size.[10] Indeed, in the current study, the mean polyp size was significantly larger in the smoking group than in the non-smoking group ($p < 0.01$).

In the current study, superficial epithelial keratinisation of polyps was more common in smokers than in non-smokers ($p < 0.05$). Chronic irritation by cigarette smoke is associated with abnormal keratin production and its accumulation in vocal fold epithelium.[11] The presence of keratin on vocal fold epithelium indicates a phenotypic change because normal glottic epithelium is non-keratinising. The presence and degree of epithelial dysplasia is the most important prognostic factor driving conversion to malignancy in cases of superficial keratinisation.[12]

In this study, epithelial dysplasia with or without atypia was not observed in the non-smoking group, but was seen in 35 per cent of vocal fold polyps of smokers ($p < 0.05$). The major risk factor for laryngeal dysplasia development is cigarette smoking, although laryngopharyngeal acid and pepsin reflux may also be implicated.[13] Cigarette smoking was reported to be associated with a higher incidence of reflux.[14]

In a comparative immunohistochemical study of benign vocal fold lesions, the basement membrane was found to be thinner in vocal fold polyps than in nodules.[15] In the present study, vocal fold polyps were significantly associated with thin basement membranes in smokers compared with non-smoker polyps ($p < 0.05$). It is possible that matrix metalloproteinases are upregulated in the inflammatory milieu present in membranous vocal folds exposed to cigarette smoke. These proteases may be responsible for type IV collagen digestion and basement membrane dissolution.[16] Basement membrane thinning is an important step in the progression from carcinoma in situ to microinvasive carcinoma.[17]

Pathophysiological factors related to the development and progression of vocal fold polyps are attributed to capillary breakage in Reinke's space resulting from mechanical stresses caused by voice overuse, misuse or abuse.[18] Subsequent to capillary breakage, extravasation of blood or fibrin-rich oedematous fluid occurs. Ultimately, organisation processes develop, leading to neovascularisation and fibrous tissue deposition, and then to outpouching of Reinke's space.[19] Ongoing shearing forces caused by abnormal phonation and coughing produce high levels of mechanical stress at the polyp base, leading to a self-perpetuating cycle of trauma and inflammation, with subsequent polyp growth.[19–21]

- **A comparative histopathological study of vocal fold polyps in smokers versus non-smokers was performed**
- **There was a higher incidence of keratinisation, dysplasia and basement membrane thinning in smokers' polyps**
- **Smokers' polyps showed increased hyaline degeneration**

Histopathological features of vocal fold polyps have been characterised as the presence of variable amounts of oedema, fibrosis, vascular ectasias, inflammatory cells and hyalinisation.[8,22,23] These are consistent with the pathophysiological mechanism of vocal fold polyp development, as outlined above. The histopathological features observed in this study agree with those of previous studies. However, a highly significant increase in hyalinisation was observed in smokers' polyps compared with those of non-smokers ($p < 0.01$). Hyalinisation is a degenerative change in the fibrous collagen, probably in response to continuous tissue injury caused by cigarette smoke and to abnormal stresses on the polyp caused by phonotrauma and coughing.[24]

Conclusion

In the current study, a comparative analysis revealed that vocal fold polyps were significantly larger in cigarette smokers than in non-smokers. Histopathological evaluation revealed a higher incidence of keratinisation and dysplasia and a thinner basement membrane in polyps of smokers than in those of non-smokers. Moreover, the amount of hyaline degeneration was greater in the polyps of smokers than of non-smokers. These changes probably reflect the injurious effect of cigarette smoke on the epithelium and lamina propria of vocal fold polyps.

https://doi.org/10.1017/S002221511500064X Published online by Cambridge University Press

References

1 Rosen CA, Gartner-Schmidt J, Hathaway B, Blacke Simpson C, Postma GN, Courey M *et al*. A nomenclature paradigm for benign midmembranous vocal fold lesions. *Laryngoscope* 2012;**122**:1335–41
2 Kleinsasser O. Pathogenesis of vocal cord polyps. *Ann Otol Rhinol Laryngol* 1982;**91**:378–81
3 Kambic V, Radsel Z, Zargi M, Acko M. Vocal cord polyps: incidence, histology and pathogenesis. *J Laryngol Otol* 1981;**95**: 609–18
4 Gunter HE.Modeling mechanical stresses as a factor in the etiology of benign vocal fold lesions. *J Biomech* 2004;**37**:1119–24
5 Li NYK, Vodovotz Y, Kim KH, Mi Q, Hebda PA, Abbott KV. Biosimulation of acute phonotrauma: an extended model. *Laryngoscope* 2011;**121**:2418–28
6 Martins RHG, Defaveri J, Domingues MAC, de Albuquerque e Silva R. Vocal polyps: clinical, morphological, and immunohistochemical aspects. *J Voice* 2011;**25**:98–106
7 Gale N, Zidar N, Fischinger J, Kambic V. Clinical applicability of the Ljubljana classification of epithelial hyperplastic laryngeal lesions. *Clin Otolaryngol* 2000;**25**:227–32
8 Hantzakos A, Remacle M, Dikkers FG, Degols J-C, Delos M, Friedrich G *et al*. Exudative lesions of Reinke's space: a terminology proposal. *Eur Arch Otorhinolaryngol* 2009;**266**:869–78
9 Banjara H, Mungutwar V, Singh D, Gupta A. Objective and subjective evaluation of larynx in smokers and non-smokers: a comparative study. *Indian J Otolaryngol Head Neck Surg* 2014; **66**(suppl1):S99–S109
10 Dikkers FG, Nikkels PGJ. Lamina propria of the mucosa of benign lesions of the vocal folds. *Laryngoscope* 1999;**109**: 1684–9
11 Li-jing M, Jun W, Yang X, Jing-ying Y, Wen X, Quing-wen Y. Clinical classification and treatment of leukokeratosis of the vocal cords. *Chin Med J* 2013;**126**:3523–7
12 Isenberg JS, Crozier DL, Dailey SH. Institutional and comprehensive review of laryngeal leukoplakia. *Ann Otol Rhinol Laryngol* 2008;**117**:74–9
13 Sadri M, McMahon J, Parker A. Laryngeal dysplasia: aetiology and molecular biology. *J Laryngol Otol* 2006;**120**:170–7
14 Smit CF, Copper MP, van Leeuwen JAMJ, Schoots IG, Stanojcic LD. Effect of cigarette smoking on gastropharyngeal and gastroesophageal reflux. *Ann Otol Rhinol Laryngol* 2001; **110**:190–3
15 Courey MS, Shohet JA, Scott MA, Ossoff RH. Immunohistochemical characterization of benign laryngeal lesions. *Ann Otol Rhinol Laryngol* 1996;**105**:523–31
16 Hohberger L, Wuertz BRK, Xie H, Griffin T, Ondrey F. TNF-α drives matrix metalloproteinase-9 in squamous oral carcinogenesis. *Laryngoscope* 2008;**118**:1395–9
17 Zhou L, Xie M, Zhou JQ, Tao L. 67-k Da laminin receptor in human laryngeal squamous cell carcinoma. *Laryngoscope* 2006;**116**:28–32
18 Johns MM. Update on the etiology, diagnosis, and treatment of vocal fold nodules, polyps, and cysts. *Curr Opin Otolaryngol Head Neck Surg* 2003;**11**:456–61
19 Altman KW. Vocal fold masses. *Otolaryngol Clin North Am* 2007;**40**:1091–108
20 Jiang JJ, Diaz CE, Hanson DG. Finite element modeling of vocal fold vibration in normal phonation and hyperfunctional dysphonia: implications for the pathogenesis of vocal nodules. *Ann Otol Rhinol Laryngol* 1998;**107**:603–10
21 Zhang Y, Jiang JJ. Chaotic vibrations of a vocal fold model with a unilateral polyp. *J Acoust Soc Am* 2004;**115**:1266–9
22 Wallis L, Jackson-Menaldi C, Holland W, Giraldo A. Vocal fold nodule vs. vocal fold polyp: answer from surgical pathologist and voice pathologist point of view. *J Voice* 2004;**18**:125–9
23 Cipriani NA, Martin DE, Corey JP, Portugal L, Caballero N, Lester R *et al*. The clinicopathologic spectrum of benign mass lesions of the vocal fold due to vocal abuse. *Int J Surg Pathol* 2011;**19**:583–7
24 Walter JB, Israel MS. Connective tissue: its normal structure and the effects of disease. In: Walter JB, Israel MS, eds. *General Pathology*, 5th edn. Edinburgh: Churchill Livingstone, 1979; 59–70

Address for correspondence:
Dr K G Effat
6 Falaky Square,
Cairo, Egypt

E-mail: kamaleffat@hotmail.com

Dr K G Effat takes responsibility for the integrity of the content of the paper
Competing interests: None declared