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Association between Laryngopharyngeal Reflux and Vocal Fold Leukoplakia

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Keywords
Vocal fold leukoplakia · Laryngopharyngeal reflux · Gastroesophageal reflux · pH monitoring

Abstract
Introduction: Vocal fold leukoplakia (VFL) has a risk of malignant transformation, and the underlying mechanisms are currently unrecognized. Some clinical evidence has indicated that laryngopharyngeal reflux (LPR) probably plays a critical role. ***Objective:*** To explore the risk factors associated with the occurrence of VFL and to investigate the importance of LPR in VFL and its different pathological types using 24-h multichannel intraluminal impedance-pH monitoring. ***Materials and Methods:*** Eighty-one patients with VFL and 27 healthy volunteers were recruited. General information and LPR parameters were analyzed. ***Results:*** The monitoring showed that 35.8% (29/81) of patients had acidic LPR and that 43.2% (35/81) had weakly acidic LPR. Heavy drinking (odds ratio = 4.004, $p = 0.037$) and acidic LPR (odds ratio = 4.471, $p = 0.029$) were independent risk factors for the occurrence of VFL. Acidic LPR showed a strong correlation with the Reflux Finding Score ($p < 0.05$) in patients suspected of having LPR based on the scale score. Meanwhile, weakly acidic LPR parameters increased with the severity of pathological degrees which were higher in high-grade dysplasia ($p < 0.05$). ***Conclusion:*** Our study confirms the importance of LPR in VFL. Heavy drinking patients with VFL, particularly those with acidic LPR, should undergo intensive treatment. Meanwhile, weakly acidic LPR may play a critical role in the pathological changes in VFL.

Introduction

Vocal fold leukoplakia (VFL) refers to white plaque-like changes in the vocal fold epithelial surface [1]. According to previous research results, the malignant risks of VFL among patients with mild, moderate, and severe dysplasia reached 11, 33, and 57%, respectively [2]. However, the underlying mechanisms are currently unrecognized. Long-term smoking, alcohol use, viral infection, and inhaled irritant substances can cause long-term and continuous stimulation of the vocal fold epithelium [1, 2]. Recently, laryngopharyngeal reflux (LPR) was recognized to cause numerous upper respiratory symptoms and to provide continuous acid stimulation to the vocal fold epithelium.

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LPR is the retrograde movement of gastric contents that are often returned to the larynx or pharynx [3–6]. Gastric refluxate contains acid and enzymes, particularly pepsin, which breaks down proteins in epithelial cell membranes, resulting in nonspecific inflammation of the mucosa [6]. Indeed, the role of LPR in the occurrence and malignant transformation of VFL is still not clear. The diagnosis of LPR is still challenging because the laryngopharyngeal symptoms are nonspecific, and the symptoms are easily affected by the individual conditions of patients [6]. Interestingly, for the diagnosis of LPR, 24-h multichannel intraluminal impedance-pH (24-h MII-pH) monitoring allows characterization of reflux episodes as acid LPR (AL), weakly acidic LPR (WAL), or weakly alkaline LPR and has been considered as the best tool in recent years [4, 5, 7]. However, data on MII-pH monitoring in VFL are lacking, and much less is known about the prevalence of WAL in VFL. Therefore, exploring the risk factors and characteristics of LPR (AL and WAL) in patients with VFL using 24-h MII-pH monitoring has great clinical significance regarding the choice of treatment strategies and improvement of prognosis.

Materials and Methods

Subjects

Eighty-one patients with VFL who underwent 24-h MII-pH monitoring 2–3 days before surgery under general anaesthesia from March 2018 to September 2020 in the Department of Otorhinolaryngology-Head Neck Surgery, Beijing Tongren Hospital, Capital Medical University were enrolled in the study. Their pathological types were confirmed by pathology, and all pathology samples were classified according to the 2005 WHO head and neck pathology classification criteria as squamous cell hyperplasia, dysplasia (mild, moderate, or severe), and carcinoma in situ (CIS) [1]. This classification was simplified into 2 grades in 2017 [8], dichotomizing low-grade dysplasia (LGD; squamous cell hyperplasia and mild dysplasia) and high-grade dysplasia (HGD; moderate dysplasia, severe dysplasia and CIS). Only patients who fulfilled the inclusion criteria were included in this study. Altogether, 92.6% (75/81) of the patients were male, and the average age was 52.6 ± 7.9 (range 36–70) years. The inclusion criteria were as follows: (1) age >18 years and <80 years; (2) lesions showing unilateral or bilateral white puncta with flaky or exogenous white matter; and (3) normal vocal fold activity. The exclusion criteria were as follows: (1) a history of laryngeal surgery; (2) a history of head and neck radiotherapy or chemotherapy; (3) a history of laryngeal trauma or intubation; and (4) antacid use in the past week.

In addition, 30 healthy volunteers without laryngeal symptoms were classified into the control group in this investigation. Subjects were excluded if they had a history of dysphonia or laryngeal surgery; the structure of the laryngeal was abnormal; the RSI >13 or RFS >7; or the patient was unable to understand and sign the informed consent form. Each patient was asked to sign the informed consent form prior to examination. During the examination, 3 subjects who were intolerant were excluded. Finally, a total of 27 subjects were included. The study was approved by the Ethics Committee of Beijing Tongren Hospital, Capital Medical University (Protocol No. TRECKY2019-088).

Scale Score

A detailed medical history of all patients was collected, and patients were subjected to strobolaryngoscopy . The Reflux Symptom Index (RSI) was evaluated with self-scoring under the guidance of a physician for the presence and severity of each symptom, and the Reflux Finding Score (RFS) was assessed in a double-blinded fashion by 2 physicians with >2 years of experience in laryngoscopy diagnosis according to strobolaryngoscopy of the subject [9, 10]. The average score was used as the final score. An RSI >13 or an RFS >7 was considered reflux positivity [9, 10].

24-h MII-pH Monitoring Procedure

All subjects voluntarily joined this clinical research project and provided written informed consent. A ZepHr multichannel intracavity impedance-pH portable monitoring system (Sandhill Scientific, Inc., Highlands Ranch, CO, USA) and a single-split electrode (model: ZAI-BL-55, diameter: 2.3 mm) were used. The catheter has 2 pH antimony electrodes and 6 impedance channels, which were introduced nasally with the guidance of a fibrolaryngoscope. The proximal pH sensor was positioned in the posterior cricoid cartilage area and completely covered by the mucosa on the upper edge of the upper oesophageal sphincter (UES). Smit et al. [11] proposed that this method can determine the proximal pH probe above the UES. At this time, the 6 impedance channels were located at 0, 3, 6, 8, 22, and 24 cm below the UES, while the distal pH sensor was located 22 cm below the UES. At the beginning of the procedure, pH monitoring electrodes were calibrated using pH = 4.0 and pH = 7.0 buffer solutions. Patients were monitored for approximately 24 h and encouraged to eat regular meals and participate in routine activities. Changes in position (upright and supine) and symptomatic events were documented by using buttons on the recorder. Data were analyzed using Bioview reflux analysis software (Sandhill Scientific, Inc., Highlands Ranch, CO, USA) and manually corrected.

An AL episode was defined as a decrease in the pH level from an initial value >4.0 to a value <4.0 upon the physical presence of refluxate (as confirmed by the impedance sensors) (Fig. 1a). Incidents in which the proximal pH value ranged from 4.0 to 7.0 were classified as WAL (Fig. 1b), while those with pH ≥7.0 were classified as weakly alkaline (Fig. 1c). A reflux incident had to meet the following conditions: (1) the decrease lasted at least 5 s, as detected by the pH sensor; (2) a proximal pH drop occurred after a distal oesophageal pH drop; (3) the pH drop was accompanied by changes in impedance from the distal side to the proximal side of the oesophageal; (4) the pH drop did not occur when eating and swallowing; and (5) all kinds of artefacts were excluded. LPR positivity was defined as ≥3 pharyngeal reflux events or when the total time of proximal pH decreases was >1% [11, 12].

Data Collection

General information collected included details of gender, age, BMI, smoking history, and drinking history. Patients were divided into heavy smokers (20 cigarettes or more per day over 20 years) and non-heavy smokers (<20 cigarettes per day over 20 years or non-smoking), and heavy drinkers (250 g of pure alcohol or more

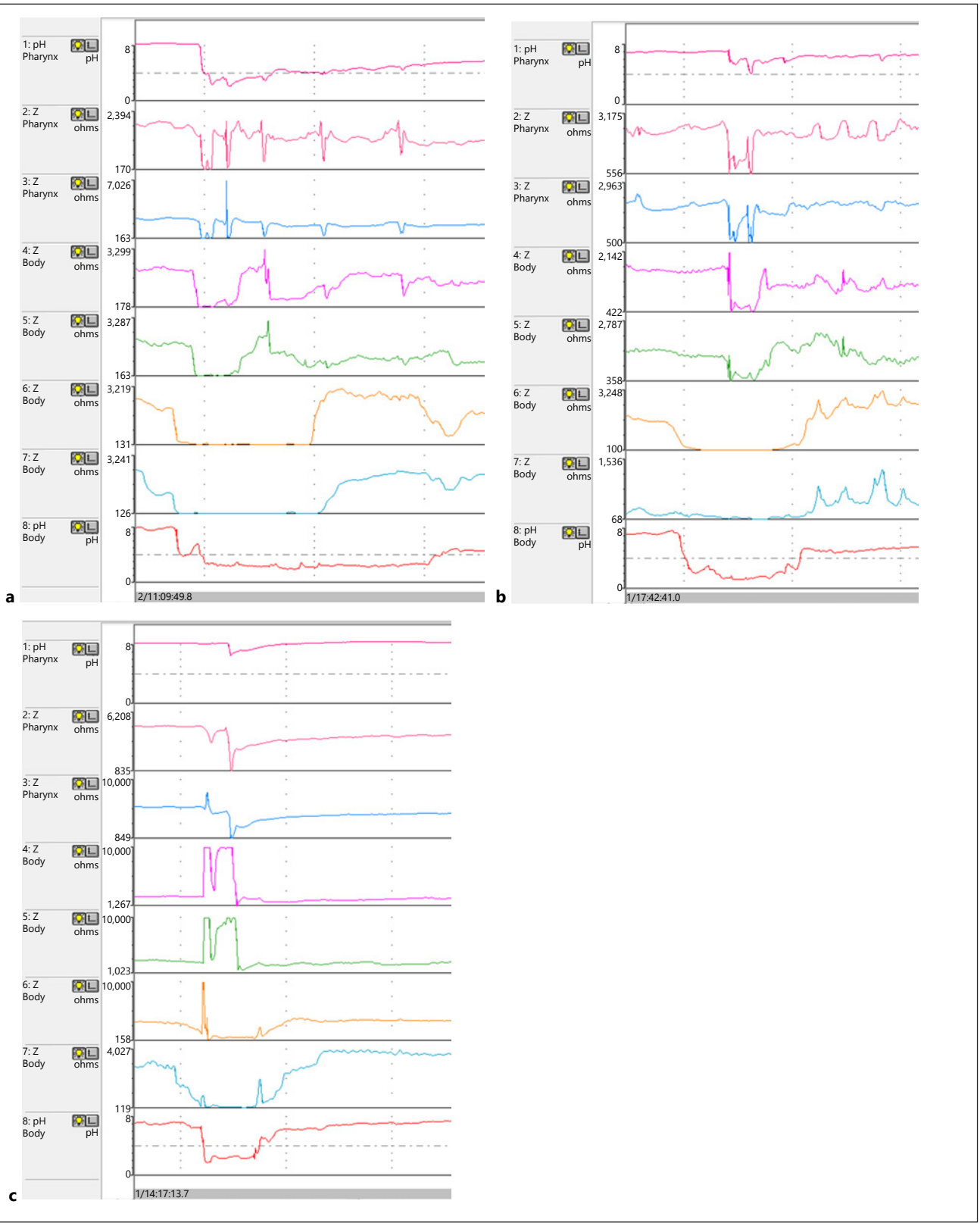

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Fig. 1. Different types of LPR. **a** Acid LPR episode. **b** Weakly acidic LPR episode. **c** Weakly alkaline LPR episode. LPR, laryngopharyngeal reflux.

Table 1. Risk factors for VFL

Characteristics	VFL, *n*		Univariate analysis		Multivariate logistic regression analysis		
	with (*n* = 81)	without (*n* = 27)	χ^2 value	*p* value	Wald value	OR (95% CI)	*p* value
Gender			1.653	0.199			
Male	75	22					
Female	6	5					
Age			0.112	0.738			
≤53 years	45	14					
>53 years	36	13					
BMI			0.000	1.000			
≥25	42	14					
<25	39	13					
Smoking			7.937	0.005	1.132	1.955 (0.569–6.723)	0.287
Heavy smokers	40	5					
Non-heavy smokers	41	22					
Alcohol consumption			8.191	0.004	4.344	4.004 (1.086–14.758)	0.037
Heavy drinkers	37	4					
Non-heavy drinkers	44	23					
AL			5.921	0.015	4.755	4.471 (1.164–17.179)	0.029
AL positive	29	3					
AL negative	52	24					
WAL			2.545	0.111			
WAL positive	35	7					
WAL negative	46	20					

VFL, vocal fold leukoplakia; AL, acidic laryngopharyngeal reflux; WAL, weakly acidic laryngopharyngeal reflux; OR, odds ratio; CI, confidence interval.

per day) and non-heavy drinkers (<250 g of pure alcohol per day or non-drinking). The information about LPR collected included the number of episodes, reflux time, and average clearance time of acidic LPR and number of episodes and reflux time of weakly acidic LPR and weakly alkaline LPR.

Statistical Analysis

Continuous variables were compared using the Mann-Whitney U test. Ordered alternatives were compared by the Jonckheere-Terpstra test. Spearman's correlation coefficients were used to examine the correlation between LPR parameters and RSI or RFS. For the univariate analysis, quantitative and qualitative data were compared using χ^2 tests. The parameters with statistical significance in the univariate analysis were subjected to multivariate logistic regression analysis to investigate the independent risk factors for developing VFL, and the odds ratio and 95% confidence intervals were calculated. SPSS 25.0 statistical software was used for all statistical analyses. A *p* value <0.05 was considered to indicate a statistically significant difference.

Results

VFL Risk Factors

During the study period, 81 patients with VFL were eligible for inclusion. BMI values ranged from 16.7 to 33.8 kg/m^2, with an average of 25.5 ± 3.4 kg/m^2. The heavy smoking rate was 49.4%, the heavy drinking rate was 45.7%, and the AL positive rate was 35.8% (29/81). Among the control group, 27 cases were symptom-free, and the average BMI, rate of heavy smoking, and heavy drinking rate were 24.8 ± 3.5 kg/m^2, 18.5%, and 14.8%, respectively (Table 1). The AL positive rate was 11.1% (3/27).

The baseline characteristics of the patients were compared to those of the control group. Univariate analysis indicated that heavy smoking (χ^2 = 7.937, *p* = 0.005), alcohol consumption (χ^2 = 8.191, *p* = 0.004), and AL (χ^2 = 5.921, *p* = 0.015) were significantly correlated with VFL. There was no significant correlation in terms of gender, BMI, a median age of 53 years, and WAL positive rate (*p* > 0.05). Subsequent multivariate analysis indicated that heavy drinking (odds ratio = 4.004, *p* = 0.037) and AL positivity (odds ratio = 4.471, *p* = 0.029) were independent risk factors for the occurrence of VFL.

Correlations between LPR Parameters and the RSI or RFS in VFL

Among patients with VFL, 65 patients had suspected LPR based on scale scores. We found positive correlations

Table 2. Correlation between LPR parameters and RSI or RFS in VFL ($N = 65$)

Parameters	RSI		RFS	
	coefficient	*p* value	coefficient	*p* value
Total reflux episodes, *n*	0.077	0.542	0.294	0.018
Acidic reflux episodes, *n*	0.065	0.608	0.505	0.000
Acidic reflux time	0.106	0.400	0.584	0.000
Average acid clearance time	0.109	0.386	0.548	0.000
Weakly acidic reflux episodes, *n*	0.122	0.333	0.028	0.823
Weakly acidic reflux time	0.142	0.261	0.037	0.768
Weakly alkaline reflux episodes, *n*	0.006	0.960	0.105	0.405
Weakly alkaline reflux time	0.013	0.919	0.110	0.381

RSI, Reflux Symptom Index; RFS, Reflux Finding Score; LPR, laryngopharyngeal reflux; VFL, vocal fold leukoplakia.

Table 3. LPR parameters in patients with different pathological types ($N = 81$)

Group	SCH ($n = 30$)	Mild dyspl ($n = 18$)	Mod dyspl ($n = 8$)	Sev dyspl ($n = 16$)	CIS ($n = 9$)	*p* value
Acidic LPR						
Positivity rate, *n* (%)	8 (26.7)	8 (44.4)	4 (50.0)	4 (25.0)	5 (55.6)	J-T = 1.063, $p = 0.288$
Episodes, *n*	1.0 [0, 3.0]	0 [0, 4.3]	3 [0.3, 4.8]	0.5 [0, 5.0]	3.0 [0, 4.0]	J-T = 0.619, $p = 0.536$
Reflux time, s	35 [0.0, 92.0]	0 [0, 122.3]	70.8 [1.9, 212.2]	9.0 [0, 96.3]	66 [0, 114.6]	J-T = 0.287, $p = 0.774$
Average clearance time, s	14.3 [0, 34.9]	0 [0, 26.4]	16.9 [1.9, 46.9]	4.5 [0, 24.3]	16.0 [0, 28.5]	J-T = 0.439, $p = 0.661$
Weakly acidic reflux						
Positivity rate, *n* (%)	6 (20.0)	8 (44.4)	4 (50.0)	10 (62.5)	7 (77.8)	J-T = 3.119, $p = 0.002$
Episodes, *n*	0 [0, 2.0]	2.0 [0.8, 7.5]	1.5 [0, 5.5]	3.5 [2.0, 5.8]	5.0 [1.5 9.0]	J-T = 3.607, $p < 0.000$
Reflux time, s	0 [0, 17.3]	29.4 [11.1, 67.2]	9.3 [0.0, 74.1]	31.1 [13.8, 59.2]	77 [12.5, 174.8]	J-T = 4.119, $p < 0.000$

Data are reported as mean (SD) for normal distribution and M [quartiles] for abnormal distribution. LPR, laryngopharyngeal reflux; SCH, squamous cell hyperplasia; dyspl, dysplasia; mod, moderate; sev, severe; CIS, carcinoma in situ.

between RFS and total refluxes or AL parameters (Spearman coefficients 0.294, 0.505, 0.584, and 0.548; $p < 0.05$) but little correlations with other parameters. No correlation was found between reflux parameters and RSI score ($p > 0.05$) (Table 2).

LPR Parameters among Different Pathological Types or Grade Dysplasia of VFL

According to pathological diagnostic classification of VFL, we observed squamous cell hyperplasia in 30 patients (37.0%), mild dysplasia in 18 patients (22.2%), moderate dysplasia in 8 patients (9.9%), severe dysplasia in 16 patients (19.8%), and CIS in 9 patients (11.1%). Interestingly, when the pathology of VFL was more severe, the parameters of weakly acidic reflux (the positivity rate, number of episodes, and reflux time) increased ($p < 0.01$). However, we found few differences in parameters of acid reflux among the different pathological types of VFL (Table 3). In addition, we divided the cases into 2 groups: LGD and HGD. The parameters of weakly acidic reflux in the HGD group were higher than those in the LGD group ($p < 0.05$) (Table 4). Meanwhile, the parameters of acid reflux showed little difference between the 2 groups ($p > 0.05$).

Discussion

Acidic LPR, as detected by pH monitoring, has been demonstrated to cause a wide variety of extra-oesophageal diseases [3, 13]. With the advent of 24-h MII-pH monitoring, characterizing reflux episodes as acid or

Table 4. Characteristics of patients according to different grade dysplasia

Characteristics	Overall (n = 81)	LGD (n = 48)	HGD (n = 33)	p value
Total LPR episodes, n	4 [1, 8.5]	2 [1, 7.8]	6 [2.5, 9.5]	$Z = 1.986$, $p = 0.047$
Acidic LPR				
Positivity rate, n (%)	29 (35.8)	16 (33.3)	13 (39.4)	$\chi^2 = 0.313$, $p = 0.576$
Episodes, n	1 [0, 4.0]	0.5 [0, 3.0]	1.0 [0, 4.0]	$Z = 0.788$, $p = 0.431$
Reflux time, s	18 [0, 103.2]	15 [0, 96.0]	18 [0, 127.2]	$Z = 0.502$, $p = 0.615$
Average clearance time, s	8 [0, 28.8]	3.5 [0, 32.8]	9.0 [0, 26.4]	$Z = 0.000$, $p = 1.000$
Weakly acidic LPR				
Positivity rate, n (%)	35 (43.2)	14 (29.2)	21 (63.6)	$\chi^2 = 9.469$, $p = 0.002$
Episodes, n	2 [0, 5.0]	1.0 [0, 4.8]	4.0 [1.0, 6.0]	$Z = 2.504$, $p = 0.012$
Reflux time, s	18.5 [0, 46.6]	12.2 [0, 38.0]	28.4 [11.1, 83.3]	$Z = 2.761$, $p = 0.006$
Weakly alkaline LPR				
Episodes, n	0 [0, 0]	0 [0, 0]	0 [0, 0]	$Z = 0.282$, $p = 0.778$
Reflux time, s	0 [0, 0]	0 [0, 0]	0 [0, 0]	$Z = 0.379$, $p = 0.705$

Data are reported as mean (SD) for normal distribution and M [quartiles] for abnormal distribution. LGD, low-grade dysplasia; HGD, high-grade dysplasia; LPR, laryngopharyngeal reflux.

non-acid has become feasible, and several studies have reported that not only acidic reflux but also weakly acidic reflux may cause laryngopharyngeal symptoms and findings [4, 5, 7]. Furthermore, based on a study of pepsin, both acid and weak acid stimulation are believed to be able to destroy the local environmental steady state, leading to the malignant transformation of cells [14].

Evaluation by 24-h MII-pH monitoring in 81 patients with VFL showed that 35.8% of the VFL patients had AL and 43.2% had WAL. To explore the risk factors associated with the occurrence of VFL, we found that heavy smoking, alcohol consumption, and AL were significantly correlated with VFL compared to the control group by univariate analysis. Subsequent multivariate analysis indicated that heavy drinking and AL were independent risk factors for the occurrence of VFL. In addition, significant positive correlations were found between RFS and acidic LPR parameters in patients with suspected LPR based on scale scores, including total refluxes, the number of acidic reflux episodes, acidic reflux time, and average acid clearance time. However, WAL had little correlation with the occurrence of VFL in this investigation. Interestingly, when the pathology of vocal leukoplakia was more severe, the parameters of WAL (the positivity rate, number of episodes, and reflux time) increased. Moreover, patients in the HGD group had higher WAL parameters.

In recent years, gastroesophageal reflux has been thought to cause reflux oesophagitis, and gastric acid, pepsin, or duodenal juice in the refluxate induces the development of Barrett's oesophageal metaplasia [15]. Therefore, whether LPR can be considered a risk factor for precancerous lesions and laryngeal cancer remains controversial. Lewin et al. [3] performed 24-h dual-probe pH monitoring on 40 patients with moderate-severe dysplasia and early laryngeal cancer and found that 85% of patients had LPR; therefore, they suggested that gastroesophageal reflux may be associated with laryngeal dysplasia and laryngeal cancer, but the degree of pathological malignancy and the severity of reflux were not necessarily related. Interestingly, Sezen et al. [16] treated 24 patients with VFL with proton pump inhibitors for 6 months and found that 7 patients (29.2%) showed complete lesion regression, 12 patients (50%) showed partial lesion regression, and 5 patients (20.8%) showed no response to treatment. Regrettably, their study also did not determine the pathological type of VFL. These findings suggest a certain correlation between acid stimulation and VFL. However, some scholars have also proposed the opposite hypothesis that acidic LPR is not a risk factor for laryngeal cancer. For example, Ozlugedik et al. [13] collected information from LPR patients with laryngeal cancer (group I), LPR patients with normal laryngeal findings (group II), and LPR patients with related laryngeal pathology (group III). They found that the incidence of LPR did not support the hypothesis that LPR is an independent risk factor for the development of laryngeal cancers. In addition, many scholars have new understandings of the main types of LPR. Duricek et al. [7] reported that an unbiased comprehensive approach did

not reveal any relationship between AL and the symptoms or laryngeal injury attributed to LPR. Oelschlager et al. [4] reported that gastroesophageal reflux was acidic, while LPR episodes were predominately non-acid reflux (pH >4). Pavic et al. [5] thought that both acid and non-acid reflux seem to play significant roles in the pathogenesis of LPR in children with suspected LPR. Lee et al. [17] found that subglottic oedema was specific for non-acid reflux episodes, while granuloma was specific for acid reflux episodes among the laryngoscopic findings used in the RFS. Our study confirmed the importance of acidic LPR in VFL using 24-h MII-pH monitoring. Compared with healthy people, acidic LPR and heavy drinking were independent risk factors for the occurrence of VFL. Laryngeal reflux findings may be caused by acid stimulation. More importantly, AL was not correlated with pathological changes, whereas WAL may be a critical factor. To our knowledge, this is the first time that this finding has been described in the literature. The pathogenetic mechanism is unknown. Studies have shown that ethanol and acetaldehyde in alcohol can participate in the occurrence and development of cancer by disrupting DNA synthesis and repair and inducing DNA hypomethylation [18]. We speculate that there is a certain relationship between heavy drinking and reflux. For heavy drinkers, acidic LPR worsens, further damaging the vocal fold mucosa membranes and causing VFL. When VFL occurs, relatively long-term and frequent weakly acidic reflux will aggravate the progression of mucosal lesions. Acidic LPR is common in patients with VFL. Heavy drinking patients with VFL should undergo intensive monitoring.

The histopathology of VFL differs widely. The occurrence and development of VFL are related to the long-term effects of many pathogenic factors and are associated with a certain tendency for malignant transformation [2]. A meta-analysis including 940 laryngeal pre-carcinoma patients noted that the overall malignant rate was 14%, the rate of mild and moderate dysplasia was 10.6%, and the rate of severe dysplasia was 30.4% [19]. In our previous study, we obtained similar results. After analyzing the histopathological features of 138 patients with VFL, we found that squamous cell hyperplasia was present in 61.6% and mild, moderate, and severe hyperplasia or CIS occurred in 13.0, 7.2, and 10.9%, respectively [1]. The degree of leukoplakia lesions can dynamically change with time; the course of the disease is reversible, and the disease can progress or revert to different levels of dysplasia [20]. Since the different pathological types of VFL require different treatments and have varying prognoses, we suggest that the causes and predisposing factors of malignant transformation should be considered in the process of treatment. For example, heavy drinking patients with VFL, particularly those with acidic LPR, should undergo intensive treatment. 24-h MII-pH monitoring is helpful. If the monitoring finds frequent weakly acidic reflux among patients with VFL, early surgical treatment or active acid suppression treatment is necessary.

Some potential limitations in our study should be mentioned. First, patients with mild dysplasia were observed in the outpatient clinic, which reduced the number of patients in the inflammation and simple hyperplasia groups, which may have impacted the results. Second, the cases that we collected predominantly involved males and were subjected to selection bias, but this circumstance is similar to those in other epidemiological investigations. Third, the evidence level that WAL will aggravate the progression of mucosal lesions appears to be low in this investigation. This needs further research to confirm.

Conclusion

In summary, our study provides evidence that through the detection of acidic, weakly acidic, and weakly alkaline LPR episodes, 24-h MII-pH monitoring should be recommended for patients with VFL, especially for patients with heavy drinking. Heavy drinking and AL were independent risk factors for the occurrence of VFL. Our findings confirm that the importance of LPR in VFL and laryngeal reflux finding may be caused by acid stimulation. More importantly, WAL may be a critical factor in pathological changes. However, further investigations are needed to identify the mechanisms underlying the pathogenesis of laryngopharyngeal damage in VFL patients with LPR and to establish the most appropriate diagnostic test for LPR. This knowledge would help in developing new therapeutic options for VFL.

Statement of Ethics

The study was approved by the Ethics Committee of Beijing Tongren Hospital, Capital Medical University (Protocol No. TRECKY2019-088).

Conflict of Interest Statement

The authors have no conflicts of interest to disclose.

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Author Contributions

Guowei Lu contributed to the conception of the study and the data analyses and wrote the manuscript. The first author and Xiu Ding performed the experiment together. The corresponding author contributed significantly to the analysis and manuscript revision.

References

1 Zhu H, Xu W, Li Y, Cheng L. Observation of clinicopathological characteristics of vocal fold leukoplakia and laryngopharyngeal reflux. Zhonghua Er Bi Yan Hou Tou Jing Wai Ke Za Zhi. 2014;49(5):368–73.
2 Manterola L, Aguirre P, Larrea E, Arestín M, Gaafar A, Elorriaga K, et al. Mutational profiling can identify laryngeal dysplasia at risk of progression to invasive carcinoma. Sci Rep. 2018;8(1):6613.
3 Lewin JS, Gillenwater AM, Garrett JD, Bishop-Leone JK, Nguyen DD, Callender DL, et al. Characterization of laryngopharyngeal reflux in patients with premalignant or early carcinomas of the larynx. Cancer. 2003;97(4):1010–4.
4 Oelschlager BK, Quiroga E, Isch JA, Cuenca-Abente F. Gastroesophageal and pharyngeal reflux detection using impedance and 24-hour pH monitoring in asymptomatic subjects: defining the normal environment. J Gastrointest Surg. 2006;10(1):54–62.
5 Pavić I, Babić I, Čepin Bogović J, Hojsak I. The importance of combined 24-hour multichannel intraluminal impedance-pH monitoring in the evaluation of children with suspected laryngopharyngeal reflux. Clin Otolaryngol. 2017;42(3):544–9.
6 Tan JJ, Wang L, Mo TT, Wang J, Wang MG, Li XP. Pepsin promotes IL-8 signaling-induced epithelial-mesenchymal transition in laryngeal carcinoma. Cancer Cell Int. 2019;19:64.
7 Duricek M, Banovcin P, Halickova T, Hyrdel R, Kollarik M. Acidic pharyngeal reflux does not correlate with symptoms and laryngeal injury attributed to laryngopharyngeal reflux. Dig Dis Sci. 2019;64(5):1270–80.
8 Gale N, Poljak M, Zidar N. Update from the 4th edition of the World Health Organization Classification of head and neck tumours: what is new in the 2017 WHO blue book for tumours of the hypopharynx, larynx, trachea and parapharyngeal space. Head Neck Pathol. 2017;11(1):23–32.
9 Belafsky PC, Postma GN, Koufman JA. The validity and reliability of the reflux finding score (RFS). Laryngoscope. 2001;111(8):1313–7.
10 Belafsky PC, Postma GN, Koufman JA. Validity and reliability of the reflux symptom index (RSI). J Voice. 2002;16(2):274–7.
11 Smit CF, Tan J, Devriese PP, Mathus-Vliegen LM, Brandsen M, Schouwenburg PF. Ambulatory pH measurements at the upper esophageal sphincter. Laryngoscope. 1998;108(2):299–302.
12 Plocek A, Gębora-Kowalska B, Białek J, Fendler W, Toporowska-Kowalska E. Esophageal impedance-pH monitoring and pharyngeal pH monitoring in the diagnosis of extra-esophageal reflux in children. Gastroenterol Res Pract. 2019;2019:6271910.
13 Ozlugedik S, Yorulmaz I, Gokcan K. Is laryngopharyngeal reflux an important risk factor in the development of laryngeal carcinoma? Eur Arch Otorhinolaryngol. 2006;263(4):339–43.
14 Rohani N, Hao L, Alexis MS, Joughin BA, Krismer K, Moufarrej MN, et al. Acidification of tumor at stromal boundaries drives transcriptome alterations associated with aggressive phenotypes. Cancer Res. 2019;79(8):1952–66.
15 Souza R. Reflux esophagitis and its role in the pathogenesis of Barrett's metaplasia. J Gastroenterol. 2017;52(7):767–76.
16 Sezen Goktas S RD, Yenigun A, Calim O, Ozturan O, Tugrul S. A new approach to vocal cord leukoplakia and evaluation of proton pump ınhibitor treatment. Eur Arch Otorhinolaryngol. 2019;276(2):467–71.
17 Lee YC, Kwon OE, Park JM, Eun YG. Do laryngoscopic findings reflect the characteristics of reflux in patients with laryngopharyngeal reflux? Clin Otolaryngol. 2018;43(1):137–43.
18 Kawakita D, Matsuo K. Alcohol and head and neck cancer. Cancer Metastasis Rev. 2017;36(3):425–34.
19 Weller MD, Nankivell PC, McConkey C, Paleri V, Mehanna HM. The risk and interval to malignancy of patients with laryngeal dysplasia: a systematic review of case series and meta-analysis. Clin Otolaryngol. 2010;35(5):364–72.
20 Ahn A, Wang L, Slaughter JC, Nguyen AM, Ossoff RH, Francis DO. Serial full-thickness excision of dysplastic vocal fold leukoplakia: diagnostic or therapeutic? Laryngoscope. 2016;126(4):923–7.