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Clinicopathological parameters associated with histological background and recurrence after surgical intervention of vocal cord leukoplakia

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Abstract

Histological examination of biopsy shows usefulness in the diagnosis of vocal cord leukoplakia; however, in considerable amount of cases, the examination cannot provide definitive diagnosis of malignancy from benign conditions such as hyperplasia and dysplasia. The present work therefore was aimed to identify clinicopathological factors and molecular markers predictive of recurrence and malignant transformation of vocal cord leukoplakia.

Clinical data of 555 cases of vocal cord leukoplakia enrolled from July 1999 to June 2014 were analyzed. The cohort consisted of keratosis (n = 137), hyperplasia (n = 139), dysplasia (n = 177), and primary (n = 10) and invasive (n = 46) carcinoma. Correlations between patients' backgrounds, clinicopathological factors, molecular markers (p53, p16, Ki67, cytokeratin, and proliferating cell nuclear antigen), and histology backgrounds were examined using by Pearson Chi-squared or Fisher exact test. Reflux symptom index (RSI) and reflux finding score (RFS) before and after treatment were compared using Wilcoxon signed-rank test. Risk factors for disease recurrence were identified using Cox proportional hazards models of multivariate analysis. Time to recurrence was analyzed using log-rank test of Kaplan–Meier method.

In the present cohort, alcohol drinking was found associated with GRBAS grade ($P = .0258$) and the site ($P = .0298$) of leukoplakia. For the different disease types, chief complaint ($P = .0179$), GRBAS grade ($P = .0101$), mucosal wave ($P < .0001$), and molecular markers p53 ($P < .0001$) and Ki67 ($P < .0001$) were identified as correlates. RSI and RFS were significantly lowered by surgical intervention. A single side of leukoplakia was predictive of a lower risk of recurrence (odds ratio, 0.378; 95% confidence interval, 0.197–0.723; $P = .0033$). Absence of mucosal wave was associated with a shorter time-to-recurrence ($P = .0357$).

The present work identified clinicopathological factors and molecular markers associated with the different histology of vocal cord leukoplakia, and also the prognostic factor for the low risk of recurrence after surgery.

Abbreviations: CK = cytokeratin, GRBAS = grade of dysphonia, roughness, breathiness, asthenia, and strain, LPR = laryngopharyngeal reflex, PCNA = proliferating cell nuclear antigen, RFS = Reflux finding score, RSI = Reflux symptom index.

Keywords: dysplasia, hyperplasia, keratosis, Ki67, malignant transformation, p53, vocal cord leukoplakia

1. Introduction

Vocal cord leukoplakia is a clinical descriptive diagnosis, indicating a white patch or plaque on the vocal cords.[1] Smoking, alcohol consumption, and infectious conditions are among the common causes of the condition. Vocal cord leukoplakia could range from a benign variant of hyperplasia to an invasive squamous cell carcinoma.[2] In order to ensure that proper treatments are promptly administered to patients, histopathological examination is needed to give a definitive diagnosis, distinguishing benign from premalignant or malignant cases of leukoplakia.

One of the challenges in managing vocal cord leukoplakia is to determine the potential for malignant transformation of the benign and premalignant lesions, and thus to properly assess the need for surgical intervention. Studies have reported that the clinical diagnosis of leukoplakia represents an approximately 6% to 7% chance of progressing into carcinoma.[3] The presence of dysplasia, as revealed by histopathological examination, has been suggested as a prognostic factor of malignant transformation.[4] However, a systematic review of the literatures has revealed that patients with no dysplasia observed at the initial biopsy are also at a considerable risk of developing squamous cell carcinoma,[3] suggesting that histological examination may not accurately predict malignancy. Molecular evaluation of the lesions has been proposed to help further characterize the lesion; however, to date, use of molecular markers in clinical setting is still lacking.

We here conducted a retrospective analysis of a large sample size of 555 cases of vocal cord leukoplakia in our institute, and described clinical characteristics and histopathological examination results of these patients. Also, we determined the prognostic values of several molecular markers, including p53, p16, Ki67, cytokeratin (CK), and proliferating cell nuclear antigen (PCNA).

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2. Patients and methodology

2.1. Study cohort

Clinical data of 562 vocal cord leukoplakia cases receiving surgical intervention in the medical center of Beijing Tongren Hospital from July 1999 to June 2014 were retrieved. The ethical approval was not necessary because the study was retrospective. Out of this cohort, 7 cases were excluded from the study because their resected tissue samples were either too small for sectioning or severely burnt to the extent that did not meet the criteria for immunohistochemistry. The finally enrolled 555 cases had 513 males (92.4%) and 42 females (7.6%) aged from 20 to 84 years, and consisted of 183 keratosis, 139 hyperplasia, 177 dysplasia, and 56 malignancies. The scheme for the management of patients and data collection is depicted as in Fig. 1. The clinicopathological parameters studied included patients' general background, chief complaint, personal history of alimentary disease, cigarette smoking and alcohol drinking, perceptual evaluation of voice quality by GRBAS (grade of dysphonia, roughness, breathiness, asthenia, and strain), mucosal wave, reflux symptom index (RSI), reflux finding score (RFS), laryngopharyngeal reflux (LPR), disease recurrence, and immunohistochemistry (i.e., p53, Ki67, CK, and PCNA).

2.2. Statistical analysis

Correlations between clinicopathological parameters were determined using univariate analysis by Pearson Chi-squared or Fisher exact test. Risk factors for disease recurrence were identified using Cox proportional hazards models of multivariate analysis. RSI and RFS before and after treatment were compared using Wilcoxon signed-rank test. Time to recurrence was analyzed using log-rank test of Kaplan–Meier method. Comparison with P value < 0.05 was regarded statistically significant.

3. Results

3.1. Associations of clinicopathological characteristics between disease conditions

The condition of vocal cord leukoplakia was subdivided into disease types (keratosis, hyperplasia, dysplasia, and malignancy) according to the histopathological results. The patient cohort consisted of 183 keratosis, 139 hyperplasia, 177 dysplasia, and 56 malignancies. Correlations between clinicopathological characteristics and these disease types of vocal cord leukoplakia are summarized in Table 1. There were significant correlations between the disease types and the chief

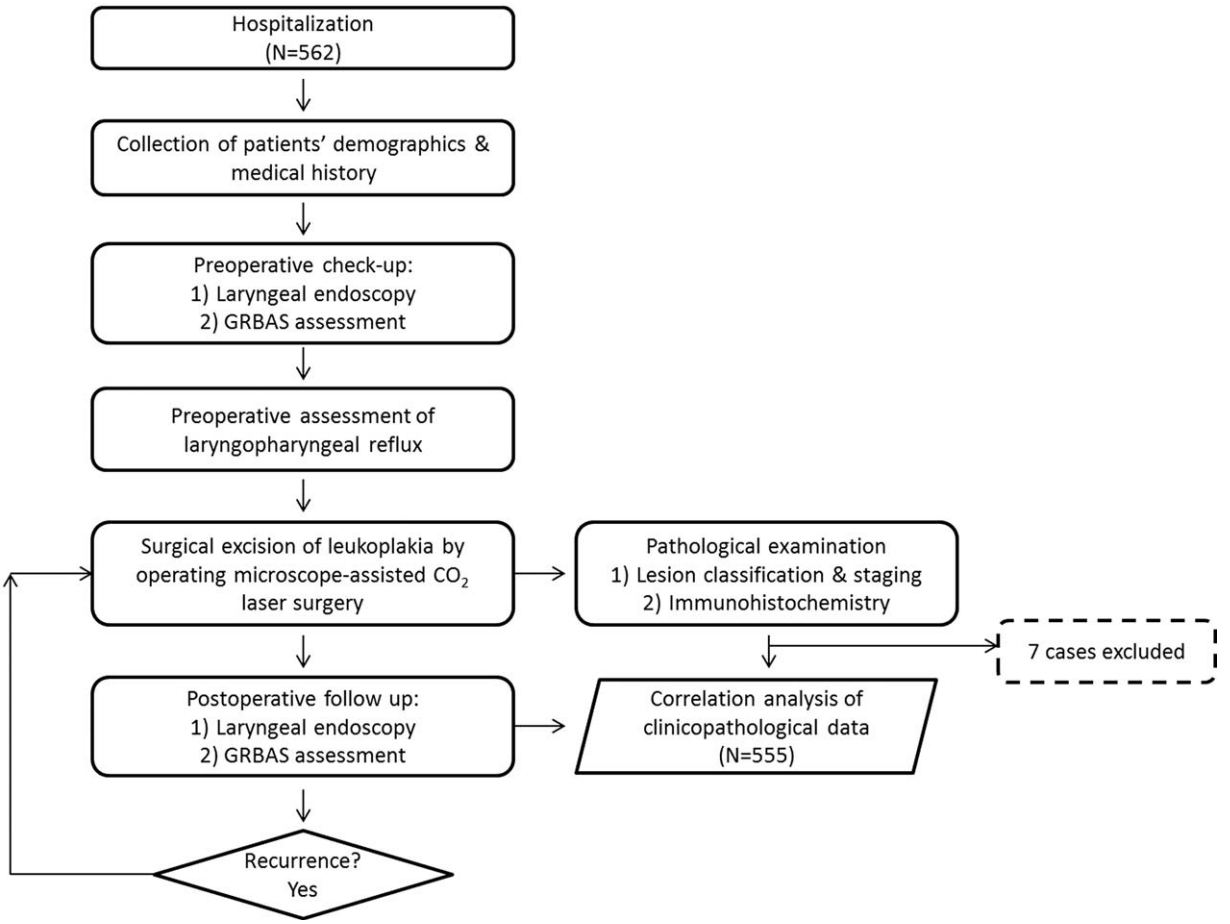

Figure 1. The general scheme for the management of patients and data collection was depicted as in the flowchart. From the hospitalized patients, demographics, medical history, and complaints of symptoms were collected. Before surgery, patients were examined using laryngeal endoscopy, and had their GRBAS and laryngopharyngeal reflux assessed. Surgical excision of leukoplakia was then done by CO_2 laser surgery. Surgically managed patients were followed up, and those with recurrence were treated with surgery again. The excised tissue samples were subjected to pathological examination and immunohistochemistry. Correlations between clinicopathological parameters were statistically analyzed.

Table 1

Correlation of disease types with clinicopathological factors.

Clinicopathological factors	Frequency	Diagnosed disease types				*P*
		Keratosis	Hyperplasia	Dysplasia	Malignancy	
Gender						
Male	513	165 (90.16)	132 (94.96)	162 (91.53)	54 (96.43)	.2639
Female	42	18 (9.84)	7 (5.04)	15 (8.47)	2 (3.57)	
Etiology						
Excessive use	137	45 (24.59)	36 (25.90)	40 (22.60)	16 (28.57)	.2745
Other	122	46 (25.14)	32 (23.02)	34 (19.21)	10 (17.86)	
Absence	296	92 (50.27)	71 (51.08)	103 (58.19)	30 (53.57)	
Chief complaint						
Hoarseness	521	166 (90.71)	131 (94.24)	169 (95.48)	55 (98.21)	.0179
Other	34	17 (9.29)	8 (5.76)	8 (4.52)	1 (1.79)	
OSAHS						
No	351	129 (92.14)	2 (20.00)	167 (94.35)	53 (94.64)	.2212
Yes	32	11 (7.86)	8 (80.00)	10 (5.65)	3 (5.36)	
History of alimentary disease						
No	535	177 (96.72)	133 (95.68)	173 (97.74)	52 (92.86)	.3620
Yes	20	6 (3.28)	6 (4.32)	4 (2.26)	4 (7.14)	
Smoking						
No	117	44 (24.04)	21 (15.11)	40 (22.60)	12 (21.43)	.3851
Mild	145	48 (26.23)	37 (26.62)	47 (26.55)	13 (23.21)	
Moderate	196	62 (33.88)	62 (44.60)	53 (29.94)	19 (33.93)	
Heavy	97	29 (15.85)	19 (13.67)	37 (20.90)	12 (21.43)	
Alcohol drinking						
No	197	60 (32.79)	47 (33.81)	70 (39.55)	20 (35.71)	.9609
Mild	195	72 (39.34)	50 (35.97)	59 (33.33)	14 (25.00)	
Moderate	118	37 (20.22)	30 (21.58)	34 (19.21)	17 (30.36)	
Heavy	45	14 (7.65)	12 (8.63)	14 (7.91)	5 (8.93)	
Cessation of alcohol drinking						
No	349	119 (96.75)	89 (96.74)	106 (99.07)	35 (97.22)	.4216
Yes	9	4 (3.25)	3 (3.26)	1 (0.93)	1 (2.78)	
GRBAS—Grade						
Normal	15	9 (5.06)	4 (3.01)	2 (1.15)	0 (0.00)	.0101
Mild	324	113 (63.48)	76 (57.14)	105 (60.34)	30 (53.57)	
Moderate	177	50 (28.09)	46 (34.59)	59 (33.91)	22 (39.29)	
Severe	25	6 (3.37)	7 (5.26)	8 (4.60)	4 (7.14)	
GRBAS—Asthenia						
No	476	162 (99.39)	108 (99.08)	159 (99.38)	47 (100.0)	.7453
Yes	3	1 (0.61)	1 (0.92)	1 (0.63)	0 (0.00)	
Site						
Both cords	302	103 (56.28)	84 (60.43)	85 (48.02)	30 (53.57)	.0664
Right side	124	46 (25.14)	29 (20.86)	36 (20.34)	13 (23.21)	
Left side	129	34 (18.58)	26 (18.71)	56 (31.64)	13 (23.21)	
Mucosal wave						
Normal	15	118 (64.48)	64 (46.38)	49 (27.84)	10 (17.86)	<.0001
Mild	241	7 (3.83)	5 (3.62)	2 (1.14)	1 (1.79)	
Moderate	175	42 (22.95)	41 (29.71)	72 (40.91)	20 (35.71)	
Severe	86	10 (5.46)	18 (13.04)	38 (21.59)	20 (35.71)	
Absence	36	6 (3.28)	10 (7.25)	15 (8.52)	5 (8.93)	
p53 immunohistochemistry						
Negative	116	48 (63.16)	27 (50.94)	27 (31.76)	14 (36.84)	.0001
Positive	136	28 (36.84)	26 (49.06)	58 (68.24)	24 (63.16)	
p53 staining grade						
Negative	116	48 (63.16)	27 (50.94)	27 (31.76)	14 (36.84)	<.0001
Weak	129	28 (36.84)	26 (49.06)	55 (64.71)	20 (52.63)	
Moderate	5	0 (0.00)	0 (0.00)	3 (3.53)	2 (5.26)	
Strong	2	0 (0.00)	0 (0.00)	0 (0.00)	2 (5.26)	
p16 immunohistochemistry						
Negative	122	28 (82.35)	27 (81.82)	50 (86.21)	17 (89.47)	.4185
Positive	22	6 (17.65)	6 (18.18)	8 (13.79)	2 (10.53)	
Ki67 staining grade						
Negative	20	14 (18.42)	5 (9.43)	1 (1.18)	0 (0.00)	<.0001
Weak	208	61 (80.26)	47 (88.68)	74 (87.06)	26 (68.42)	
Moderate	19	1 (1.32)	1 (1.89)	8 (9.41)	9 (23.68)	

(*continued*)

Table 1
(continued).

Clinicopathological factors	Frequency	Diagnosed disease types				P
		Keratosis	Hyperplasia	Dysplasia	Malignancy	
Strong	4	0 (0.00)	0 (0.00)	2 (2.35)	2 (5.26)	
Very strong	1	0 (0.00)	0 (0.00)	0 (0.00)	1 (2.63)	
CK immunohistochemistry						
Negative	2	0 (0.00)	0 (0.00)	2 (10.53)	0 (0.00)	.7145
Positive	40	8 (100.0)	5 (100.0)	17 (89.47)	10 (100.0)	
PCNA immunohistochemistry						
Negative	8	3 (17.65)	2 (13.33)	2 (13.33)	1 (11.11)	.6475
Positive	48	14 (82.35)	13 (86.67)	13 (86.67)	8 (88.89)	
Follow-up result						
Malignant transformed	8	1 (1.27)	2 (3.28)	4 (4.65)	1 (3.03)	.7730
Disease recurrence	86	26 (32.91)	17 (27.87)	30 (34.88)	13 (39.39)	
No disease recurrence	165	52 (65.82)	42 (68.85)	52 (60.47)	19 (57.58)	
Reflux symptom index						
Negative	148	35 (77.78)	35 (83.33)	63 (81.82)	15 (68.18)	.6475
Positive	38	10 (22.22)	7 (16.67)	14 (18.18)	7 (31.82)	
Reflux finding score						
Negative	58	11 (24.44)	12 (28.57)	28 (36.36)	7 (31.82)	.2400
Positive	128	34 (75.56)	30 (71.43)	49 (63.64)	15 (68.18)	
Laryngopharyngeal reflux						
Negative	52	10 (22.22)	12 (28.57)	24 (31.17)	6 (27.27)	.4298
Positive	134	35 (77.78)	30 (71.43)	53 (68.83)	16 (72.73)	

CK = cytokeratin, PCNA = proliferating cell nuclear antigen.

complaint ($P = .0179$), GRBAS grade ($P = .0101$), and mucosal wave ($P < .0001$).

3.2. Associations of p53 and Ki67 with disease conditions

We also analyzed immunohistochemistry data of p53, p16, Ki67, CK, and PCNA to see whether there would be any correlation of these markers with disease parameters (Table 1). We found that about half of the patients (136/252) had positive expression of p53, and the p53 staining results (i.e., positive vs negative) correlated with the disease types ($P = .0001$). In addition, the grading of the p53 staining (i.e., negative, weak, moderate, and strong) was also significantly associated with the disease types ($P < .0001$). Another molecular marker, Ki67, was expressed in >90% of the patients. The staining grade of Ki67 was significantly associated with disease types ($P < .0001$). All of the malignant cases in this patient cohort were found positive in Ki67 staining.

3.3. Associations of alcohol drinking with GRBAS and site of leukoplakia

Smoking and alcohol drinking are 2 well known risk factors of vocal cord leukoplakia.[5] As summarized in Table 1, in our patient cohort, 438 of 555 (78.9%) of the patients had cigarette smoking, and 358 of 555 (64.5%) had alcohol drinking. In this context, we further performed statistical analyses to determine whether these factors would be associated with any clinicopathological characteristics of vocal cord leukoplakia. At a P value cut-off of .05, cigarette smoking had no significant association with any of the clinicopathological factors examined in this study, despite the fact that there was a weak association between cigarette smoking and site of leukoplakia ($P = .0702$) (Table 2). On the contrary, alcohol drinking was significantly associated with GRBAS grade ($P = .0258$) and the site (i.e., single vs both cords) ($P = .0298$) of leukoplakia (Table 3).

3.4. Improvements in RSI and RFS after surgical intervention

To evaluate whether patients were benefited from surgical intervention, RSI and RFS before and after treatment were compared using Wilcoxon signed-rank test (Table 4). The mean RSI score before treatment was 10.088 ± 6.02 and decreased after treatment to 8.23 ± 6.08. This decrease was statistically significant ($P = .009$). A total of 3 individual parameters of RSI were found significantly decreased by the treatment, namely hoarseness (difference, -0.47 ± 1.4; $P = .0190$), clearing throat (difference, -0.48 ± 1.37; $P = .0112$), and heartburn (difference, 0.32 ± 1.21; $P = .0332$).

On the contrary, the mean RFS score before treatment was 8.47 ± 2.98 and decreased after treatment to 5.94 ± 2.8 ($P < .0001$) (Table 4). A total of 4 individual parameters of RFS were revealed significantly suppressed by the treatment. These factors included ventricular obliteration (difference, -0.45 ± 0.85; $P = .0013$), erythema/hyperemia (difference, -0.52 ± 1.52; $P = .0268$), vocal cord edema (difference, -0.55 ± 1.21; $P = .0023$), and thick endolaryngeal mucus (difference, -0.52 ± 0.95; $P = .0020$). Notably, the treatment increased the mean score of subglottic edema by 0.35 ± 0.99 ($P = .0245$).

3.5. Risk factor for recurrence and factor associated with time-to-recurrence

We analyzed disease recurrence in 232 patients of the present cohorts. During following up period, a total of 83 recurrence cases were found. Multivariate analysis was performed to identify risk factors for recurrence of vocal cord leukoplakia (Table 5). Among the clinicopathological factors that were analyzed, the site of leukoplakia (i.e., single vs both vocal cords) was significantly associated with the risk of recurrence. Single site of leukoplakia was associated with a lower risk of disease recurrence [odds ratio (OR), 0.378; 95% confidence interval

Table 2

Correlation of cigarette smoking with clinicopathological factors.

Clinicopathological factors	Frequency	Smoking status		P
		No	Yes	
GRBAS—Grade				
Normal	15	3 (2.65)	12 (2.80)	.8152
Mild	324	65 (57.52)	259 (60.51)	
Moderate	177	38 (33.63)	139 (32.48)	
Severe	25	7 (6.19)	18 (4.21)	
GRBAS—Asthenia				
0	476	98 (98.99)	378 (99.47)	.5015
1	3	1 (1.01)	2 (0.53)	
Site				
Single cord	253	62 (52.99)	191 (43.61)	.0702
Both cords	302	55 (47.01)	247 (56.39)	
Mucosal wave				
Normal	15	2 (1.72)	13 (2.97)	.5346
Mild	241	49 (42.24)	192 (43.94)	
Moderate	175	33 (28.45)	142 (32.49)	
Severe	86	23 (19.83)	63 (14.42)	
Absence	36	9 (7.76)	27 (6.18)	
Diagnosis				
Malignancy	56	12 (10.26)	44 (10.05)	.2411
Keratosis	183	44 (37.61)	139 (31.74)	
Dysplasia	177	40 (34.19)	137 (31.28)	
Hyperplasia	139	21 (17.95)	118 (26.94)	
Reflux symptom index				
Negative	148	26 (81.25)	122 (79.22)	.7956
Positive	38	6 (18.75)	32 (20.78)	
Reflux finding score				
Negative	58	10 (31.25)	48 (31.17)	.9928
Positive	128	22 (68.75)	106 (68.83)	
Laryngopharyngeal reflux				
Negative	52	9 (28.13)	43 (27.92)	.9814
Positive	134	23 (71.88)	111 (72.08)	

GRBAS = grade of dysphonia, roughness, breathiness, asthenia, and strain.

Table 3

Correlation of alcohol drinking with clinicopathological factors.

Clinicopathological factors	Frequency	Alcohol drinking status		P
		No	Yes	
GRBAS—Grade				
Normal	15	10 (5.18)	5 (1.44)	.0258
Mild	324	120 (62.18)	204 (58.62)	
Moderate	177	53 (27.46)	124 (35.63)	
Severe	25	10 (5.18)	15 (4.31)	
GRBAS—Asthenia				
0	476	170 (99.42)	306 (99.35)	1.000
1	3	1 (0.58)	2 (0.65)	
Site				
Single cord	253	102 (51.78)	151 (42.18)	.0298
Both cords	302	95 (48.22)	207 (57.82)	
Mucosal wave				
Normal	15	4 (2.03)	11 (3.09)	.8241
Mild	241	88 (44.67)	153 (42.98)	
Moderate	175	64 (32.49)	111 (31.18)	
Severe	86	27 (13.71)	59 (16.57)	
Absence	36	14 (7.11)	22 (6.18)	
Diagnosis				
Malignancy	56	20 (10.15)	36 (10.06)	.5664
Keratosis	183	60 (30.46)	123 (34.36)	
Dysplasia	177	70 (35.53)	107 (29.89)	
Hyperplasia	139	47 (23.86)	92 (25.70)	
Reflux symptom index				
Negative	148	47 (78.33)	101 (80.16)	.7729
Positive	38	13 (21.67)	25 (19.84)	
Reflux finding score				
Negative	58	20 (33.33)	38 (30.16)	.6622
Positive	128	40 (66.67)	88 (69.84)	
Laryngopharyngeal reflux				
Negative	52	20 (33.33)	32 (25.40)	.2596
Positive	134	40 (66.67)	94 (74.60)	

GRBAS = grade of dysphonia, roughness, breathiness, asthenia, and strain.

(95% CI), 0.197–0.723; $P = .0033$]. However, recurrence of vocal cord leukoplakia was not associated with gender, alcohol drinking, smoking, GRBAS grade, mucosal wave, etiology, chief complaint, or histopathological features (e.g., keratosis, hyperplasia, dysplasia, and malignancy) ($P > .05$).

We also performed Kaplan–Meier analysis to identify mucosal wave as a factor associated with the time-to-recurrence after intervention; leukoplakia recurred in the fastest rate in patients absent with mucosal wave ($P = .0357$) (Fig. 2). In addition to mucosal wave, time-to-recurrence of leukoplakia was found marginally associated with few clinicopathological parameters, including gender ($P = .0655$), GRBAS-asthenia ($P = .0572$), and GRBAS-strain ($P = .0572$).

4. Discussion

The ability to identify vocal cord leukoplakia patients at a high risk of cancer development is crucial for controlling laryngeal cancer.[6] Once identified, high-risk individuals could be offered with an intensive follow-up to monitor the disease progression or be offered with a more aggressive treatment options. The severity of dysplasia has widely been accepted as an important prognostic factor.[7,8] To see whether other clinicopathological factors and molecular markers would also be prognostic, we examined the relationships between clinicopathological factors, molecular markers, and the abnormalities of histology in the present work.

Table 4

RSI and RFS of 62 patients before and after treatment.

RSI and RFS factors	After	Before	Difference	P
Hoarseness	1.95 ± 1.44	2.42 ± 1.35	−0.47 ± 1.4	.0190
Clearing throat	1.32 ± 1.26	1.81 ± 1.47	−0.48 ± 1.37	.0112
Excessive throat mucus	0.73 ± 1.28	0.82 ± 1.21	−0.1 ± 1.25	.5739
Difficulty in swallowing	0.65 ± 0.94	0.5 ± 0.95	0.15 ± 0.95	.3777
Coughing	0.47 ± 0.84	0.66 ± 1.1	−0.19 ± 0.98	.1726
Difficulty in breathing	0.37 ± 0.75	0.48 ± 0.94	−0.11 ± 0.85	.6308
Troublesome or annoying cough	0.66 ± 1.31	0.68 ± 1.14	−0.02 ± 1.23	.9367
Sensation of something sticking in throat	1.44 ± 1.33	1.73 ± 1.56	−0.29 ± 1.45	.2642
Heartburn	0.66 ± 1.1	0.98 ± 1.31	−0.32 ± 1.21	.0332
Subglottic edema	1.16 ± 0.99	0.81 ± 0.99	0.35 ± 0.99	.0245
Ventricular obliteration	0.29 ± 0.71	0.74 ± 0.97	−0.45 ± 0.85	.0013
Erythema/hyperemia	1.13 ± 1.43	1.65 ± 1.6	−0.52 ± 1.52	.0268
Vocal cord edema	0.95 ± 1.06	1.5 ± 1.34	−0.55 ± 1.21	.0023
Diffuse laryngeal edema	0.03 ± 0.18	0.03 ± 0.18	0 ± 0.18	1.0000
Posterior commissure hypertrophy	1.23 ± 0.78	1.18 ± 0.78	0.05 ± 0.78	.6287
Granuloma	0.16 ± 0.55	0.1 ± 0.43	0.06 ± 0.49	.6875
Thick endolaryngeal mucus	0.97 ± 1.01	1.48 ± 0.88	−0.52 ± 0.95	.0020
Reflux symptom index	8.23 ± 6.08	10.08 ± 6.02	−1.85 ± 6.05	.0099
Reflux finding score	5.94 ± 2.8	8.47 ± 2.98	−2.53 ± 2.89	<.0001

RFS = Reflux finding score, RSI = Reflux symptom index.

Table 5

Risk factors for recurrence analyzed by multivariate analysis.

Clinicopathological factors	Estimated odd ratio	95% CI lower	95% CI upper	*P*
Age	0.993	0.964	1.023	.6520
Alcohol drinking (no vs heavy)	1.734	0.454	6.619	.3057
Alcohol drinking (little vs heavy)	1.444	0.376	5.542	.6772
Alcohol drinking (medium vs heavy)	1.070	0.257	4.461	.6150
Gender (male vs female)	2.280	0.541	9.605	.2614
GRBAS—Grade (normal vs severe)	1.114	0.146	8.512	.9913
GRBAS—Grade (mild vs severe)	1.062	0.257	4.386	.8572
GRBAS—Grade (moderate vs severe)	1.339	0.307	5.828	.6075
Mucosal wave (normal vs absence)	20.318	1.234	334.554	.0587
Mucosal wave (mild vs absence)	6.822	0.717	64.887	.3995
Mucosal wave (moderate vs absence)	6.106	0.653	57.100	.5857
Mucosal wave (heavy vs absence)	3.737	0.346	40.328	.5519
Etiology (excessive use vs no reason)	0.728	0.335	1.578	.9954
Etiology (other vs no reason)	0.532	0.247	1.145	.2168
Site (single vs both)	0.378	0.197	0.723	.0033
Cigarette smoking (no vs heavy)	1.276	0.423	3.849	.5402
Cigarette smoking (little vs heavy)	0.859	0.308	2.395	.4829
Cigarette smoking (medium vs heavy)	1.102	0.434	2.803	.8423
Diagnosis (malignancy vs hyperplasia)	3.229	0.495	21.068	.3264
Diagnosis (keratosis vs hyperplasia)	1.180	0.535	2.602	.3097
Diagnosis (dysplasia vs hyperplasia)	1.938	0.856	4.388	.6041
Chief complaint (other vs hyperplasia)	1.678	0.492	5.720	.4082

95% CI = 95% confidence interval, GRBAS = grade of dysphonia, roughness, breathiness, asthenia, and strain.

We found that mucosal wave, p53 staining grade, and Ki67 staining grade are highly associated ($P < .0001$) with the histological features of leukoplakia, suggesting that molecular markers in addition to clinical and histology examinations may offer further characterization of the lesions. p53 is a well-known tumor suppressor, and it is often mutated in cancers, resulting in loss of action of the *p53* gene.[9,10] The protein product of the mutated *p53* gene is stable and easily detectable by immunohistochemistry. Barbatis et al[11] studied 41 cases of invasive squamous cell carcinoma of the larynx and 28 cases of dysplasia, and the group found that p53 was highly expressed in cancer and in dysplasia tissues compared with controls, and the p53 expression was found correlated with grade of dysplasia. Indeed, in our patient cohort, high expression of p53 (moderate/strong staining grade) was only found in dysplasia and malignancy tissues. Ki67 is one of the widely used biomarker for cell proliferation.[12,13] Ashraf et al[14] found that Ki67 expression increased with severity of dysplastic changes in the laryngeal epithelium, which is consistent with our findings that high expression (strong/very strong staining grade) of Ki67 was found only in the dysplasia and malignancy tissues, and only 1 of 85 dysplasia tissues showed negative Ki67 signal. The findings suggested that p53 and Ki67 may be used as biomarkers in addition to histological examination.

We analyzed different clinicopathological factors that are associated with risk of leukoplakia recurrence. There were no statistically significant associations with smoking status or alcohol drinking, although both are considered risk factors for vocal cord leukoplakia. The analysis showed that the site of leukoplakia (single vs both vocal cords) is the only clinicopathological factor associated with risk of recurrence, indicating the extent of the lesions is predictive of the recurrence. Indeed, Lee et al[4] also found that the extent of vocal cord leukoplakia (<50% or ≥50%) is predictive for the risk of recurrence. Therefore, more intensive monitoring programs may be offered for patients with leukoplakia appearing on both vocal cords.

In the present study, surgical treatment was shown to be able to ameliorate reflux-related symptoms. This may due to the fact that some patients with laryngopharyngeal reflux were administered proton-pump inhibitor, for example, omeprazole after the surgery. Some of the patients presented improvements in reflux symptoms because they ceased smoking and changed their eating habits with less intakes of irritating foods. In addition, in surgery, the lesion was surgically resected, so reducing the postoperative RFS.

To summarize, the present work has identified clinicopathological factors and molecular markers (i.e., p53 and Ki67) associated with the different histology of vocal cord leukoplakia, and also the prognostic factor for the low risk of recurrence after surgery. Histology examination of biopsy is sometimes not accurate enough to differentiate malignant from benign conditions. Our findings provide insights into the development of diagnostic tools using molecular markers such as p53 and Ki67,

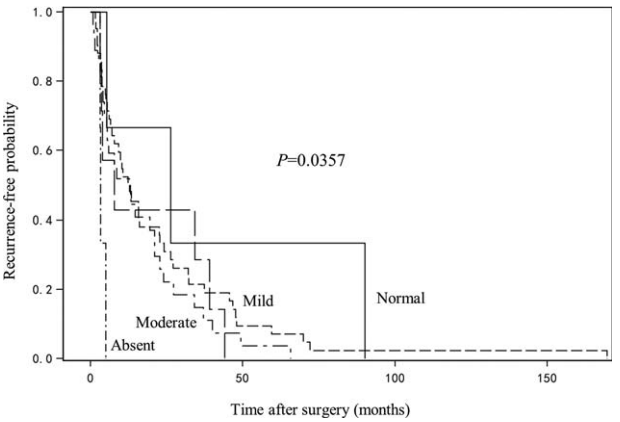

Figure 2. Kaplan–Meier analysis showing the association of recurrence time with mucosal wave in patients with vocal cord leukoplakia.

and also the establishment of surveillance guidelines for patients under high risk of leukoplakia recurrence.

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