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 = 48) 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 = .0037). 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,[5] 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).
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](image-url)
| Clinicopathological factors | Frequency | Keratosis (90.16) | Hyperplasia (94.96) | Dysplasia (91.53) | Malignancy (96.43) | \( P \) |
|-----------------------------|-----------|-------------------|---------------------|-------------------|--------------------|------|
| Gender                      | 513       | 165               | 132                 | 162               | 54                 | .2639|
| Female                      | 42        | 18                | 7                   | 15                | 2                  | 3.57 |
| Etiology                    | 137       | 45 (24.59)        | 36 (25.90)          | 40 (22.60)        | 16 (28.57)         | .2745|
| Excessive use               | 122       | 46 (25.14)        | 32 (23.02)          | 34 (19.21)        | 10 (17.86)         | 17.66|
| Absence                     | 296       | 92 (50.27)        | 71 (51.08)          | 103 (58.19)       | 30 (53.57)         |      |
| Chief complaint             | 521       | 166 (90.71)       | 131 (94.24)         | 169 (95.48)       | 55 (98.21)         | .0179|
| Hoarseness                  | 34        | 17 (9.29)         | 8 (5.76)            | 8 (4.52)          | 1 (1.79)           |      |
| OAHS                        | 351       | 129 (92.14)       | 2 (20.00)           | 167 (94.35)       | 53 (94.64)         | .2212|
| Absence of alimentary disease | 32       | 11 (7.86)       | 8 (60.00)           | 10 (6.65)         | 3 (6.36)           |      |
| Smoking                      | 117       | 44 (24.04)        | 21 (15.11)          | 40 (22.60)        | 12 (21.43)         | .3851|
| Alcohol drinking             | 197       | 60 (32.79)        | 47 (33.81)          | 70 (39.55)        | 20 (35.71)         | .9609|
| Cessation of alcohol drinking | 45        | 14 (7.65)        | 6 (3.28)            | 14 (7.91)         | 5 (9.35)           |      |
| GRRAS—Grade                 | 349       | 119 (96.75)       | 89 (66.74)          | 106 (99.07)       | 35 (97.22)         | .4216|
| GRRAS—Asthenia              | 9         | 4 (3.28)          | 3 (3.28)            | 1 (3.28)          | 1 (3.28)           |      |
| Site                        | 302       | 103 (56.28)       | 84 (43.43)          | 85 (48.02)        | 30 (53.57)         | .0664|
| Mucosal wave                | 129       | 34 (25.14)        | 26 (18.71)          | 56 (31.64)        | 13 (23.21)         |      |
| p53 immunohistochemistry    | 116       | 48 (63.16)        | 27 (50.94)          | 27 (51.12)        | 14 (36.84)         | .0001|
| p53 staining grade          | 136       | 28 (36.84)        | 26 (49.06)          | 58 (58.39)        | 24 (63.16)         |      |
| Ki67 staining grade         | 116       | 48 (63.16)        | 27 (50.94)          | 27 (51.12)        | 14 (36.84)         | .0001|
| p16 immunohistochemistry    | 122       | 28 (23.35)        | 27 (81.82)          | 50 (86.21)        | 17 (89.47)         | .4185|
| p16 staining grade          | 22        | 6 (3.28)          | 10 (5.76)           | 15 (8.42)         | 9 (23.68)          |      |

(continued)
vs both cords) (

associated with GRBAS grade (Table 2). On the contrary, alcohol drinking was signi

between cigarette smoking and site of leukoplakia (in this study, despite the fact that there was a weak association

P value cut-off of .05, cigarette smoking had no signi

pathological characteristics of vocal cord leukoplakia. At a

whether these factors would be associated with any clinico-

smoking, and 358 of 555 (64.5%) had alcohol drinking. In this

patient cohort, 438 of 555 (78.9%) of the patients had cigarette

We analyzed disease recurrence in 232 patients of the present

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 1

(continued).

| Clinicopathological factors   | Frequency | Keratosis | Hyperplasia | Dysplasia | Malignancy | P     |
|------------------------------|-----------|-----------|-------------|-----------|------------|-------|
| Strong                       | 4         | 0 (0.00)  | 0 (0.00)    | 2 (5.26)  | 2 (5.26)   |       |
| Very strong                  | 1         | 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 (69.47)| 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 (25.00)   | 4 (46.67)| 1 (6.90)   | .7730 |
| Disease recurrence           | 86        | 26 (32.91)| 17 (27.97)  | 30 (44.44)| 13 (39.39) |       |
| No disease recurrence        | 165       | 52 (65.82)| 42 (62.86)  | 52 (60.47)| 19 (57.58) |       |
| Reflux symptom index         |           |           |             |           |            |       |
| Negative                     | 148       | 35 (23.78)| 35 (23.78)  | 63 (41.82)| 15 (68.18) | .6475 |
| Positive                     | 38        | 10 (22.22)| 7 (18.42)   | 14 (18.42)| 7 (18.42)  |       |
| Reflux finding score         |           |           |             |           |            |       |
| Negative                     | 58        | 11 (24.41)| 12 (24.41)  | 28 (56.92)| 7 (12.73)  | .2400 |
| Positive                     | 128       | 34 (75.60)| 30 (75.60)  | 49 (43.08)| 15 (87.27) |       |
| Laryngopharyngeal reflux     |           |           |             |           |            |       |
| Negative                     | 52        | 10 (22.22)| 12 (23.53)  | 24 (46.23)| 6 (27.27)  | .4298 |
| Positive                     | 134       | 35 (77.78)| 30 (77.78)  | 53 (53.77)| 16 (72.73) |       |

OK = cytokeratin, PCNA = proliferating cell nuclear antigen.
(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.\(^{[10]}\) 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.\(^{[17,21]}\) To see whether other clinicopathological factors and molecular markers would also be prognostic, we examined the relationships between clinicopathological factors, molecular markers, and the abnormality of histology in the present work.

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**Table 2**

Correlation of cigarette smoking with clinicopathological factors.

| Clinicopathological factors | Frequency | Smoking status | \( P \) |
|----------------------------|-----------|----------------|------|
| Normal                     | 15        | No             | .8152|
| Mild                       | 324       | Yes            |      |
| Moderate                   | 177       | No             |  .5015|
| Severe                     | 25        | Yes            |      |
| Mucosal wave               | 476       | No             |  .0702|
| Normal                     | 3         | Yes            |      |
| Mild                       | 253       | No             |  .5346|
| Moderate                   | 302       | Yes            |      |
| GRBAS—Grade                | 476       | No             |  .5015|
| Normal                     | 3         | Yes            |      |
| Mild                       | 253       | No             |  .5346|
| Moderate                   | 302       | Yes            |      |
| GRBAS—Asthenia             | 147       | No             |  .7956|
| Normal                     | 38        | Yes            |      |
| Mild                       | 122       | No             |  .7956|
| Moderate                   | 32        | Yes            |      |
| Dysplasia                  | 148       | No             |  .7956|
| Normal                     | 184       | Yes            |      |
| Mild                       | 120       | No             |  .7956|
| Moderate                   | 92        | Yes            |      |
| Hyperplasia                | 119       | No             |  .7956|
| Normal                     | 177       | Yes            |      |
| Mild                       | 139       | No             |  .7956|
| Moderate                   | 107       | Yes            |      |
| Reflux symptom index       | 148       | No             |  .7956|
| Normal                     | 126       | Yes            |      |
| Mild                       | 101       | No             |  .7956|
| Moderate                   | 72        | Yes            |      |
| Laryngopharyngeal reflux   | 52        | No             |  .7956|
| Normal                     | 134       | Yes            |      |
| Mild                       | 111       | No             |  .7956|
| Moderate                   | 70        | Yes            |      |

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

**Table 3**

Correlation of alcohol drinking with clinicopathological factors.

| Clinicopathological factors | Frequency | Alcohol drinking status | \( P \) |
|----------------------------|-----------|-------------------------|------|
| Normal                     | 15        | No                      | .0258|
| Mild                       | 324       | Yes                     |      |
| Moderate                   | 177       | No                      |  .0258|
| Severe                     | 25        | Yes                     |      |
| Mucosal wave               | 476       | No                      |  .0258|
| Normal                     | 3         | Yes                     |      |
| Mild                       | 253       | No                      |  .0258|
| Moderate                   | 302       | Yes                     |      |
| Dysplasia                  | 148       | No                      |  .0258|
| Normal                     | 184       | Yes                     |      |
| Mild                       | 120       | No                      |  .0258|
| Moderate                   | 92        | Yes                     |      |
| Hyperplasia                | 119       | No                      |  .0258|
| Normal                     | 177       | Yes                     |      |
| Mild                       | 139       | No                      |  .0258|
| Moderate                   | 107       | Yes                     |      |
| Reflux symptom index       | 148       | No                      |  .0258|
| Normal                     | 126       | Yes                     |      |
| Mild                       | 101       | No                      |  .0258|
| Moderate                   | 72        | Yes                     |      |
| Laryngopharyngeal reflux   | 52        | No                      |  .0258|
| Normal                     | 134       | Yes                     |      |
| Mild                       | 111       | No                      |  .0258|
| Moderate                   | 70        | Yes                     |      |

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

**Table 4**

RSI and RFs of 62 patients before and after treatment.

| RSI and RFs factors     | After | Before | Difference | \( P \) |
|-------------------------|------|-------|------------|------|
| Hearsension              | 2.42±1.35 | 2.42±1.35 | -.047±1.4 | .0190|
| Clearing throat          | 1.81±1.47 | 1.81±1.47 | -.048±1.37 | .0112|
| Excessive throat mucus   | 0.82±1.21 | 0.82±1.21 | -.01±1.25 | .5739|
| Difficulty in swallowing| 0.65±0.94 | 0.65±0.94 | 0.5±0.95 | .3777|
| Coughing                | 0.47±0.84 | 0.47±0.84 | -.01±0.98 | .1726|
| Difficulty in breathing  | 0.48±0.94 | 0.48±0.94 | -.01±0.95 | .6083|
| Troublesome or annoying cough | 0.68±1.31 | 0.68±1.31 | -.02±1.23 | .9367|
| Sensation of something sticking in throat | 1.73±1.56 | 1.73±1.56 | -.02±1.45 | .6242|
| Heartburn               | 0.98±1.31 | 0.98±1.31 | -.032±1.21 | .0332|
| Subglottic edema         | 0.81±0.99 | 0.81±0.99 | 0.35±0.99 | .2455|
| Ventricular obliteration | 0.74±0.87 | 0.74±0.87 | -.05±0.85 | .0013|
| Erythema/hyperplasia     | 1.65±1.52 | 1.65±1.52 | -.05±1.52 | .0268|
| Vocal cord edema         | 1.5±1.35 | 1.5±1.35 | -.05±1.21 | .0023|
| Diffuse laryngeal edema  | 0.03±0.18 | 0.03±0.18 | 0±0.18 | 1.0000|
| Posterior commissure     | 1.16±0.78 | 1.16±0.78 | 0.05±0.76 | .6287|
| Hyperplasia              | 0.15±0.55 | 0.15±0.55 | 0±0.49 | .6875|
| Granuloma                | 0.13±0.88 | 0.13±0.88 | 0±0.95 | .0020|
| Thick endolaryngeal mucus | 1.48±0.88 | 1.48±0.88 | 0±0.95 | .0020|
| Reflux symptom index     | 8.47±2.88 | 8.47±2.88 | -.25±2.89 | <.0001|

RSI = Reflux finding score, RSI = Relex finding 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)       | 3.078               | 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        | 3697 |
| Diagnosis (hyperplasia)     | 1.938               | 0.856        | 4.388        | 6041 |
| Gender (male vs female)     | 6.106               | 0.653        | 57.100       | 5857 |
| Alcohol drinking (medium vs heavy) | 1.070               | 0.257        | 4.461        | 6150 |
| Alcohol drinking (little vs heavy) | 1.444               | 0.376        | 5.542        | 6772 |
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| Etiology (other vs no reason) | 0.532               | 0.247        | 1.145        | 2168 |
| Site (single vs both)       | 3.078               | 0.197        | 0.723        | 0033 |
| Cigarette smoking (no vs heavy) | 1.276               | 0.423        | 3.849        | 5402 |
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| Diagnosis (malignancy vs hyperplasia) | 3.229               | 0.495        | 21.068       | 3264 |
| Diagnosis (keratosis vs hyperplasia) | 1.180               | 0.535        | 2.602        | 3697 |
| Diagnosis (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.[15] 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.
and also the establishment of surveillance guidelines for patients under high risk of leukoplakia recurrence.

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