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Correlating Laryngoscopic Appearance of Laryngeal Lesions With Histopathology

Mursalin M. Anis, MD, PhD

INTRODUCTION

Early detection of premalignant and malignant lesions of the larynx have long preoccupied head and neck surgeons.1 Progression of laryngeal dysplasia to malignancy is well documented.2,3 The rate of malignant transformation of laryngeal dysplasia with time is directly proportional to the degree of dysplasia.3–6 Ideally, early detection and treatment of dysplastic and malignant lesions will preserve laryngeal function while improving oncologic outcome. White light laryngoscopy (WLL) remains the most widely used method for laryngeal examination, though there have been several advances to complement WLL in the detection of laryngeal dysplasia and carcinoma.7 On WLL, laryngeal keratosis is clearly seen on epithelial surfaces.7 Yet, half of keratotic laryngeal lesions are benign on histopathology.3 Dysplastic and malignant lesions are characterized by neoangiogenesis.8 On laryngoscopy, neoangiogenesis is indicated by alterations in subepithelial vasculature.7 WLL coupled with either contact endoscopy or narrow band imaging (NBI) have increased the sensitivity of detecting laryngeal microvasculature.7 In NBI, the alterations of subepithelial vasculature seen in dysplasia and malignancy are classified as type IV and type V microvascular patterns that appear as brown spots in an area of slight brown tinge.9 These brown spots are due to changes in intraepithelial papillary capillary loops found underneath the basement membrane of epithelium.10 On WLL, the brown spots correspond to vascular stippling.

Identifying vascular stippling in the setting of keratosis may enhance early detection of dysplasia and malignancy. To date, laryngoscopy alone cannot establish the degree of dysplasia or depth of laryngeal lesions without operative biopsy.1 The objective of this study was to determine if WLL could inform clinicians of the likelihood of nonbenign laryngeal pathology that would necessitate operative biopsy. Additionally, suspicious-appearing areas of a lesion under WLL could guide diagnostic sampling to reduce delay in diagnosis.
MATERIALS AND METHODS

Patients

After institutional review board approval was obtained, patients who underwent preoperative flexible WLL, videostroboscopy, and subsequent operative flexible laryngeal biopsies by the author (M.M.A.) were identified in a retrospective chart review between October 1, 2015 and December 31, 2017. Inclusion criteria were patients with laryngeal lesions that had keratosis, vascular stippling, or both on preoperative flexible distal-chip laryngoscopy. Exclusion criteria were patients with prior irradiation to the neck, patients with laryngeal lesions that are not routinely biopsied such as vocal process granulomas, and patients with benign-appearing laryngeal lesions (i.e., polyps, cysts, nodules, or polyoid corditis) that did not have any overlying keratosis or vascular stippling. Patient demographics, tobacco usage history, preoperative laryngoscopic findings, intraoperative findings, laryngeal biopsy methodology and final histopathology were recorded. Patients were identified as cases if they had biopsy-proven laryngeal dysplasia or carcinoma. Patients were identified as controls if they had benign laryngeal pathology.

Approach to Laryngeal Biopsy

Laryngeal biopsies using suspension microlaryngoscopy were done as previously described.11 Briefly, in the operating room, the preoperative videostroboscopy of laryngeal lesions was reviewed. Any area of keratosis and/or vascular stippling on laryngeal lesions was noted. The laryngeal lesion was then scrutinized under high magnification of the operating microscope, especially the suspicious areas seen on preoperative laryngoscopy. In general, biopsies were done to sample the most irregular, abnormal-appearing focus of a laryngeal lesion. The most pronounced keratosis and/or vascular stippling of any lesion were biopsied. This was true for both large exophytic lesions and small lesions confined to one aspect of a vocal fold. To engender optimal phonyatory outcome, sizes of biopsied specimens were ≤6 mm. For Reinke’s edema, polyps, nodules, and cysts (RPNC) with keratosis, the keratosis was excised during the step to trim the epithelium after submucosal excision of the lesion. For large exophytic hyperkeratotic lesions occupying nearly the length of a vocal fold, multiple focal biopsies were done to establish an intraoperative diagnosis for treatment planning. These lesions were friable and did not require a microflap technique for tissue sampling.

For leukoplakia and flat lesions with vascular stippling, a microflap technique was used for operative biopsy.11 The superficial lamina propria (SLP) underlying the laryngeal lesion was expanded with a subepithelial injection of epinephrine in normal saline. A microflap of diseased epithelium was raised medially, taking care to preserve normal-appearing SLP underneath with the aid of high magnification provided by the operating microscope. This microflap of diseased mucosa was then sharply excised at the interface of normal and abnormal-appearing epithelium and sent for permanent histopathology.

After sampling areas with the most pronounced keratosis and/or vascular stippling on any given lesion, remainder of the lesion was photoablated with a potassium titanyl phosphate (KTP) laser. The extent of laser photoablation was informed by intraoperative pathology and the goal of preserving SLP for optimal phonyatory outcome. Significantly, for each biopsy, the specimen was labeled with its surface characterization (keratosis, vascular stippling, or both) and location. For some specimens “vascular stippling” was designated as “papillary” on specimen label. Therefore, each biopsied specimen had a dependent variable of final histopathology and independent variables of surface characterization that were used for logistic regression. The biopsied specimens were not oriented for the pathologist. Histological assessment of the specimens were confirmed by at least three pathologists.

For awake laryngeal biopsies in patients too frail for general anesthesia, patients were placed under monitored anesthesia care. The nasal cavity was decongested and anesthetized with a mixture of oxymetazoline and 4% lidocaine. The oropharynx, hypopharynx, and larynx were anesthetized with aerosolized 4% lidocaine. A flexible bronchoscope was passed through the patient’s nasal cavity until the larynx was visualized. Four percent lidocaine was dripped into the larynx during phonation through the working channel of the bronchoscope. Endoscopic biopsy forceps were then used to biopsy laryngeal lesions with keratosis and/or vascular stippling through the working channel of the bronchoscope. If necessary, hemostasis at the biopsied site was achieved using the KTP laser.

Statistical Analysis

A multivariable logistic regression was done on the recorded data. The dependent variable (end point) was the presence or absence of benign laryngeal pathology, whereas the independent variables were age, gender, tobacco use, preoperative laryngoscopic findings of vascular stippling, keratosis and keratosisc overlying RPNC lesions. For the purpose of the regression, lesions described as dysplastic or malignant on pathology were grouped into one nonbenign group designated as cases. For subgroup analysis exclusively on the dysplastic and malignant lesions, multivariable logistic regression was done using the same independent variables as above, whereas the dependent variable was the presence or absence of mild and mild-to-moderate dysplasia. Moderate dysplasia, severe dysplasia, and carcinoma were all included into one high-grade group. Logistic regression was done in Microsoft Excel 2016 (Microsoft Corp., Redmond, WA) using the Real Statistics add-in.12 Statistical significance of results was chosen a priori to be P ≤ .05. Odds ratios generated from logistic regression were converted to relative risk (RR) due to the high prevalence of dysplasia and malignancy in people with tobacco dependence.13,14

RESULTS

There were 144 laryngeal lesions from 92 patients that met inclusion and exclusion criteria. Fifty percent of the patients were females. The average age of the patients was 55 ± 14 years. Seventy-eight percent of the patients were current or former tobacco smokers. Three patients underwent awake laryngoscopic biopsy under monitored anesthesia care due to frailty and risk of general anesthesia, the rest underwent suspension microlaryngoscopy and biopsy as previously described.11,15 Fifty percent of all the laryngeal lesions biopsied were benign on pathology, whereas the rest were either dysplastic or malignant. All malignancies were squamous cell carcinomas on histopathology. Among the benign lesions on pathology, there were 21 laryngeal lesions described as reactive atypia, focal atypia, or mild atypia. The remainder of the benign lesions were described as hyperplasia and hyperkeratosis.

On WLL, keratosis was present in 128 lesions (89% of all lesions), which varied in morphology from vocal cord leukoplakia to keratotic vocal cord masses (Fig. 1). Thirty-five of these keratotic lesions had keratosis alone without vascular stippling. There were 45 laryngeal
lesions with vascular stippling (31% of all lesions), and some lesions had vascular stippling in association with keratosis. Keratotic RPNC lesions, which included cysts, polyps, nodules, and polypoid corditis, comprised 44% of all lesions (N = 64). None of the keratotic RPNC lesions had vascular stippling on WLL (Fig. 2).

To determine if there is a correlation between laryngoscopic appearance of laryngeal lesions and histopathology, multivariable logistic regression was done (Table I). Laryngeal vascular stippling was associated with nonbenign laryngeal pathology, either dysplasia or malignancy ($P = .0018$). Risk of nonbenign laryngeal pathology was threefold more in the presence of vascular stippling on laryngoscopy than in the absence of vascular stippling (RR = 3.4, 95% CI: 1.8-4.5). On the contrary, there was a significant association between the presence of keratosis on RPNC lesions and the presence of benign diagnosis on pathology ($P = .0023$). Likelihood of benign pathology was nearly fourfold higher in the presence of RPNC lesions on WLL than in the absence of such (RR = 3.9, 95% CI: 1.6-10). Laryngeal keratosis, with all other independent variables held constant, was not associated with histopathology ($P = .059$).

In general, 47% of all keratotic laryngeal lesions had either dysplasia or malignancy. In contrast, 82% of lesions with vascular stippling were either dysplastic or malignant on histopathology. Sensitivity and specificity of

![Laryngoscopic findings in laryngeal lesions. RPNC = Reinke’s edema, polyps, nodules, and cysts; VS = vascular stippling.](Image)

Fig. 2. Laryngoscopic findings in laryngeal lesions. RPNC = Reinke’s edema, polyps, nodules, and cysts; VS = vascular stippling. [Color figure can be viewed in the online issue, which is available at www.laryngoscope.com.]

<table>
<thead>
<tr>
<th>LARYNGOSCOPIC FINDINGS IN 144 LARYNGEAL LESIONS</th>
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<tbody>
<tr>
<td>Keratosis</td>
</tr>
<tr>
<td>24%</td>
</tr>
<tr>
<td>Keratosis + VS</td>
</tr>
<tr>
<td>20%</td>
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<tr>
<td>Keratosis + RPNC</td>
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<td>45%</td>
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![Figure 2](Image)

Fig. 1. Laryngoscopic findings. (A) Leukoplakia alone. (B) Leukoplakia on RPNC (polypoid corditis). (C) Vascular stippling alone. (D) Leukoplakia and vascular stippling. Vascular stippling highlighted by ovals. Leukoplakia indicated by arrowheads. RPNC = Reinke’s polyps, nodules, and cysts. [Color figure can be viewed in the online issue, which is available at www.laryngoscope.com.]

**TABLE I.** Association of Laryngoscopic Findings With Histopathology Using Multivariable Logistic Regression in 144 Laryngeal Lesions

<table>
<thead>
<tr>
<th>Laryngoscopic Finding</th>
<th>$P$</th>
<th>RR</th>
<th>95% CI</th>
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<tbody>
<tr>
<td>Vascular stippling</td>
<td>.0018</td>
<td>3.4</td>
<td>1.8-4.5</td>
</tr>
<tr>
<td>Keratosis</td>
<td>.059</td>
<td>2.9</td>
<td>1.0-4.4</td>
</tr>
<tr>
<td>RPNC + keratosis</td>
<td>.0023</td>
<td>3.9</td>
<td>1.6-10</td>
</tr>
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Hyperplasia, hyperkeratosis, reactive atypia, focal atypia, mild atypia are in one dependent group for regression. All degrees of dysplasia and carcinoma are in another dependent group for regression.

CI = confidence interval; RPNC = Reinke’s polyps, nodules, and cysts; RR = relative risk.
vacular stippling for presence of dysplasia or malignancy were 51% and 89%, respectively (Table II). Twenty-five percent of RPNC lesions with keratosis had some degree of dysplasia. All but one dysplastic RPNC lesion with keratosi was in the setting of former or current tobacco use. No malignancies were noted among the keratotic RPNC lesions. Sensitivity and specificity of keratosis on RPNC lesions for presence of benign pathology were 66% and 79%, respectively (Table II).

Finally, a subgroup analysis was done on the lesions classified on pathology as either dysplastic or malignant. A logistic regression was done examining the ability of vascular stippling to discriminate mild and mild-to-moderate dysplasia from moderate dysplasia, severe dysplasia, and carcinoma (Table III). There was no association between laryngoscopic findings on WLL and the degree of dysplasia. Laryngoscopic evidence of vascular stippling did not discriminate between different degrees of dysplasia (P = .25). Additionally, keratosis on WLL could not differentiate degrees of dysplasia on histopathology (P = .33). However, five keratotic lesions without any vascular stippling on WLL were found to have significant dysplasia and malignancy on histopathology. These lesions occupied at most one-fourth of the length of the vocal folds and were not suspected of harboring high-grade dysplasia on surface appearance.

**DISCUSSION**

In this retrospective pilot study, laryngeal vascular stippling on white light laryngoscopy was associated with nonbenign laryngeal pathology, either dysplasia or malignancy. Thus, finding lesions with vascular stippling on laryngoscopy warrant operative biopsy for tissue diagnosis to avoid treatment delay. In contrast, keratosis on RPNC lesions was associated with benign histopathology. This confirmed previous findings of primarily benign pathology in tobacco-induced polyoid corditis. Overall laryngeal keratosis did not predict histopathology, as previous studies had demonstrated that nearly half of laryngeal keratosis is due to benign etiology. Finally, there was no association between laryngoscopic findings on WLL and the degree of dysplasia in nonbenign laryngeal lesions.

Findings of this study uniquely demonstrate that the widely available diagnostic modality of WLL approaches the specificity of advanced laryngoscopic methods such as NBI in distinguishing lesions suspicious for dysplasia and malignancy. When laryngeal lesions are meticulously scrutinized for alterations in subepithelial microvascular patterns, specificity for nonbenign lesions on WLL is 89%, whereas specificity for similar lesions on NBI is 87% to 92%. Though the sensitivity of WLL of 51% is less than the reported sensitivity of NBI of 61% to 91% in the larynx, WLL is often the first laryngoscopic modality used in laryngeal examination.

The enhanced sensitivity of NBI derives from the filtered blue and green incident light, maximally absorbed by hemoglobin, along with advanced image processing. However, the limitations of NBI are the same as that for WLL in being able to discern the subepithelial vasculature. With significant overlying laryngeal keratosis, there is an umbrella effect that prevents visualization of the subepithelial microvascular pattern by the white plaque.

In this retrospective pilot study there were at least five cases of hyperkeratotic plaque that prevented visualization of underlying subepithelial vasculature, whereas all five lesions were reported as having severe high-grade dysplasia. Another limitation of WLL is the inability to inspect the medial aspect of the vocal folds orthogonally so that mostly tangential views of medial vocal folds are obtained. Because the type IV and type V intraepithelial papillary capillary loops, which are indicative of dysplastic and neoplastic lesions, are seen best as brown spots when viewed orthogonally, the corresponding vascular stippling on the medial aspect of the vocal folds will be missed on tangential views. But dysplastic or malignant disease is rarely found exclusively on the medial phonyatory surface with no component on the superior ventricular surface of the vocal folds. Thus, inspection for vascular stippling on the superior ventricular surface of vocal folds should be sufficient to decide whether a microscopic exam of the glottis under anesthesia is warranted.

Vascular stippling was prominent in nine human papillomavirus (HPV)-induced papillomatous laryngeal lesions. Half of these neoplastic papillomatous lesions had some degree of dysplasia on pathology, whereas none had malignancy. Though as previously reported, vascular stippling in HPV-induced lesions appear different from the vascular stippling seen in dysplasia or malignancy; inclusion of HPV-induced lesions may have caused spurious association between vascular stippling and nonbenign laryngeal pathology. Therefore, a second regression analysis was done excluding HPV-associated squamous papillomas. Vascular stippling remained significantly
associated with dysplastic and malignant laryngeal pathology \( (P = 0.0053) \) (data not shown). However, the status of HPV infection in the nonpapillomatous laryngeal lesions was not known. Routine testing for HPV infection in laryngeal dysplastic and malignant lesions was not done.

Aside from limitations inherent in retrospective studies, this study was not blinded. Further studies would employ blinded reviews of preoperative laryngoscopy to address this bias. The goal of this retrospective pilot study was to determine if there is an association between certain laryngoscopic features and histopathology to shorten any delay in proceeding with operative biopsy. Finding an exact one-to-one correlation between surface appearance and histopathology necessitates a prospective study and was beyond the scope of this article. Not finding such an exact one-to-one correlation is highlighted by the fact that there were five laryngeal lesions with keratosis alone on WLL, which were surprisingly found to have significant dysplasia on histopathology. A larger sample size would enable better characterization of these hyperkeratotic lesions to distinguish them from nearly half of keratotic lesions that have benign pathology. This would increase clinical awareness for these lesions and shorten any delay in biopsy. Additionally, a larger sample size would lend confidence to the subgroup analysis done to evaluate the ability of WLL to discriminate degrees of dysplasia (Table III). There was no association found between laryngoscopic findings of vascular stippling, keratosis, or keratosis on RPNC lesions and degrees of laryngeal dysplasia \( (P = 0.25, 0.33, \text{ and } 1.0, \text{ respectively}) \). Lack of association could be due to insufficient sample size or it could signify that these laryngoscopic findings do not predict degree of dysplasia.

**CONCLUSION**

Vascular stippling seen on WLL differentiates benign laryngeal disease from nonbenign epithelial diseases that necessitate operative biopsy. During laryngeal biopsy, sampling of tissue with vascular stippling may increase the likelihood of diagnostic biopsy to gauge the degree of dysplasia along with the depth of involvement.\(^1\) Degree of dysplasia may not be predictable from laryngoscopic findings alone. Keratosis on RPNC lesions is likely to indicate benign pathology. Further studies of a greater number of lesions using well-characterized laryngoscopic findings by blinded reviewers are necessary to gain confidence in discriminating lesions that warrant operative biopsy during initial laryngoscopic examination.

**BIBLIOGRAPHY**


