The Utility of Histopathology in Identifying Structural Differences Among Layers of the Lamina Propria

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Abstract

Objective

The purpose of this study is to assess the modality of laryngeal histopathology in identifying 1, 2, or 3 layers in the lamina propria.

Study Design

Cross-sectional validation study

Methods

Blinded analysis was performed, with a set of histopathologic slides where the magnification and localized regions shown were all standardized. Two senior pathologists with experience reviewing laryngologic histopathology were asked to assess whether they vocal fold lamina propria evaluated contained 1, 2, or 3 layers. The first pathologist evaluated 17 specimens. Due to poor results, the second received three of these specimens before the evaluation as gold standard references of 1, 2, and 3 layers. Their ability to accurately assess this was calculated.

Results

The first pathologist correctly identified 4 of 17 (24%) specimens. The second identified 8 of 14 (57%) specimens after receiving gold references before the test.

Conclusion

Our results show the difficulty of using histopathology to distinguish layers in the lamina propria even when the reviewers are senior pathologists. These findings imply that more objective modalities for such analyses may be beneficial.

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Introduction

The human vocal fold is a complex, multi-layered structure consisting of epithelial, lamina propria, and deeper muscular layers. In the adult human vocal fold, Hirano et al. described a three-layered lamina propria structure consisting of the superficial, middle, and deep layers (SLP, MLP, and DLP). The human fetal lamina propria consists of a relatively uniform layer of tissue that eventually develops into the bi and then trilaminar structure seen in the adult vocal fold. The majority of work on the structural development of the vocal folds has utilized histopathology in order to observe changes in architecture from infancy to adulthood [1-4]. Yet, there are many issues regarding the ability to draw inferences about developmental timing from histologic samples, which make qualitative assessments of human vocal fold development difficult, and call into question the validity and reliability of the use of this form of data to draw conclusions. The goal of this paper is to highlight the concerns about this dilemma and assess the modality of laryngeal histopathology in identifying 1, 2, or 3 layers in the lamina propria.

Methods

A histological database consisting of adult human, porcine, and human fetal vocal fold tissues was reviewed. Five human fetal, six porcine, and five adult human vocal folds were selected as the best examples of 1, 2, and 3 layers in the lamina propria. All histopathologic images had a standardized 100x magnification and localized region of interest. The images were organized into a test, where they were de-identified and randomized. Each image was placed on a separate page so they could be viewed one at a time to prevent comparisons among specimens.

Table 1: Data for 1st Pathologist

<table>
<thead>
<tr>
<th></th>
<th>Number of Specimens</th>
<th>Specimens Identified as</th>
<th>Specimens Identified as</th>
<th>Specimens Identified as</th>
<th>Specimens Identified as</th>
</tr>
</thead>
<tbody>
<tr>
<td>Human Fetal</td>
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<td>0</td>
<td>1</td>
<td>4</td>
<td>0</td>
</tr>
<tr>
<td>Porcine</td>
<td>6</td>
<td>0</td>
<td>0</td>
<td>6</td>
<td>0</td>
</tr>
<tr>
<td>Human Adult</td>
<td>6</td>
<td>2</td>
<td>0</td>
<td>4</td>
<td>4</td>
</tr>
<tr>
<td>Total</td>
<td>17</td>
<td>2</td>
<td>1</td>
<td>14</td>
<td>4 (24%)</td>
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</tbody>
</table>

Table 2: Data for 2nd Pathologist

<table>
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<th>Specimens Identified as</th>
<th>Specimens Identified as</th>
</tr>
</thead>
<tbody>
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<td>1</td>
<td>3</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
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<tr>
<td>Human Adult</td>
<td>5</td>
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<td>4</td>
</tr>
<tr>
<td>Total</td>
<td>15</td>
<td>1</td>
<td>7</td>
<td>7</td>
<td>8 (53%)</td>
</tr>
</tbody>
</table>

Results

The first pathologist correctly identified 4 of 17 (24%) specimens. As seen in Table 1, all six porcine were mistaken as adult human samples, while 4/5 fetal human specimens were incorrectly marked adult human, with the remaining 1/5 marked as porcine. The second pathologist correctly identified 8 of 15 (53%) specimens. As seen in Table 2, he identified 1/5 fetal human, 3/5 porcine, and 4/5 adult human vocal folds. 3/5 of fetal human specimens were mistaken to be porcine, while 1/5 was marked as human. The 2/5 porcine specimens that were incorrectly identified were both mistaken as adult human samples.

Both pathologists' correct specimen identifications only overlapped with four human histology images. There incorrect identifications overlapped with 3 fetal human, 2 porcine, and 1 human adult specimens.

Conclusion

Our test results show that qualitative analysis of histopathology alone may not be adequate to allow accurate assessment of vocal fold structure and the lamina propria. Quantitative rather than qualitative measures might allow for techniques that involve the utilization of objective data, which could be used to better distinguish between layers of the lamina propria and give greater insight into vocal fold development.

Optical Coherence Tomography (OCT) could represent one possible quantitative solution to an objective method of assessing vocal folds. Hartnick et al. have already shown that this technology can be utilized to distinguish between different vocal fold architecture, and algorithms are currently being developed to accurately distinguish between the merging layered structures of the lamina propria [5].

Despite the pivotal work that has already been done examining the human vocal fold, histopathology alone may need to be coupled with a means of quantitative assessment such as OCT or another technology to allow for more definitive conclusions on the exact timing and pattern of vocal fold maturation in children. Histopathology has been able to clearly show that there is a definitive change in vocal fold structure from infancy to adulthood, yet aspects of this development such as the precise time frame and rate of structural change still remain unknown.

References