



High-resolution Microendoscope Imaging of Inverted Papilloma and Normal Sinonasal Mucosa: Evaluation of Interobserver Concordance

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Keywords:	Paranasal Sinus Diseases, Endoscopy, Endoscopic Sinus Surgery
Abstract:	<p>Introduction: High-resolution microendoscope (HRME) enables real-time imaging of the surface features of epithelial tissue. The utility of this novel imaging modality for inverted papilloma has not been previously described. This study examines the ability of otolaryngologists to differentiate between images of inverted papilloma and normal sinonasal mucosa obtained with a HRME.</p> <p>Methods: Resected inverted papilloma and normal sinonasal mucosa specimens were stained with a contrast agent, proflavine. HRME images were subsequently captured. Histopathological diagnosis was obtained for each sample. Quality-controlled images were used to assemble a training set. After reviewing the training images, six otolaryngologists without prior HRME experience reviewed and classified test images.</p> <p>Results: Five samples of inverted papilloma and two normal sinonasal mucosa samples were collected. Four representative images from each specimen were used for the twenty-eight image test set. The mean accuracy among all reviewers was 89.9% (95% CI, 84.3%-94.0%). The sensitivity to correctly identify inverted papilloma was 86.7% (95% CI, 79.2%-92.2%), and the specificity was 92.9% (95% CI, 89.0%-100.0%). The Fleiss kappa interrater reliability score was 0.80 (95% CI, 0.70-0.89).</p>

	Conclusion: Inverted papilloma and normal sinonasal mucosa have distinct HRME imaging characteristics. Otolaryngologists can be successfully trained to distinguish between inverted papilloma and normal sinonasal mucosa. As a result, HRME potentially may enable real-time surgical margin determination during surgical excision of inverted papilloma.

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High-resolution Microendoscope Imaging of Inverted Papilloma and Normal Sinonasal Mucosa: Evaluation of Interobserver Concordance

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Abstract

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Methods: Resected inverted papilloma and normal sinonasal mucosa specimens were stained with a contrast agent, proflavine. HRME images were subsequently captured. Histopathological diagnosis was obtained for each sample. Quality-controlled images were used to assemble a training set. After reviewing the training images, six otolaryngologists without prior HRME experience reviewed and classified test images.

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Introduction

Inverted papilloma is a benign neoplasm that arises from the Schneiderian respiratory mucosa of the sinonasal tract, predominately along the lateral nasal wall. These neoplasms represent between 0.5-4.0% of all sinonasal tumors.¹ On clinical examination, inverted papilloma often appears indistinguishable from an inflammatory polyp. However, on histopathological analysis, inverted papilloma displays a characteristic endophytic growth pattern that features inversion into the underlying stroma, distinguishing it from other papillomas.

These locally aggressive tumors have a propensity to recur with rates ranging from 5% to 75%. This high rate of recurrence, regardless of the type of procedure, can be attributed to incomplete excision and multi-centricity of the tumors.² In addition, inverted papilloma can be associated with synchronous or metachronous squamous cell carcinoma differentiation in 5-15% of cases.²

Surgical excision with clear margins is the mainstay of treatment. However, complete excision remains difficult due to the complex anatomy, scarring from previous surgery, potential for multicentric tumors, and associated sinonasal inflammation. Clinical intra-operative examination via the open or endoscopic approach often is inadequate. Currently, surgeons utilize frozen section histopathological analysis to determine margin status intra-operatively in order to obtain clear margins after resection.

Frozen section, however, has its limitations. First, the utility of frozen section is reliant on intra-operative sampling. Given the extensive nature of these tumors, circumferential margin sampling is often difficult. Furthermore, the quality of frozen section analysis is dependent on the pathology expertise available. Finally, frozen section analysis increases intra-operative time, thereby increasing healthcare costs.

Given the importance of complete excision on recurrence rates in inverted papilloma, new modalities for margin differentiation are needed. High resolution microendoscopy (HRME) is an optical imaging technology that permits non-invasive visualization of morphological changes in tissue epithelium.³⁻⁵ HRME has been used to identify cholesteatoma of the middle ear and neoplastic changes in the head and neck and upper gastrointestinal tract.⁶⁻⁹

In our ex-vivo trial, we have shown that inverted papilloma and normal sinonasal mucosa have distinct imaging characteristics with HRME.¹⁰ The purpose of this study is to evaluate the accuracy and inter-rater reliability among otolaryngologists in differentiating inverted papilloma from normal sinonasal mucosa on HRME imaging. We hypothesize that with appropriate training, otolaryngologists without any prior HRME experience will be able to distinguish inverted papilloma from normal sinonasal mucosa with a high degree of accuracy and reliability.

Materials and Methods

Imaging System

The HRME system is a high-resolution fiber-optic fluorescence imaging system that has been previously described by Muldoon et al.¹¹ The system is a battery-powered optical imaging system that allows for wide-field imaging of living tissue through a fiber-optic imaging probe. A CCD camera linked to a laptop computer allows for image capture. A light-emitting diode illumination is delivered from the HRME system, through the imaging probe, to the tissue surface. This imaging system is designed for use with proflavine, a fluorescent contrast agent of the acriflavine family. This dye preferentially stains cellular nuclei, binding to DNA in a reversible and non-covalent manner.¹² Specimens were stained with proflavine using a cotton-tip applicator and subsequently imaged. Each image clip is a 3-second real-time movie clip.

Specimen Acquisition

The study protocol was approved by the Icahn School of Medicine at Mount Sinai Institutional Review Board (GCO# 14-1644). Anonymous tissue specimens of inverted papilloma and normal sinus mucosa that were randomly assigned an identification number and stored within the Mount Sinai Biorepository Cooperative were obtained. Each sample was imaged at multiple sites with 2-3 videos at each site. From each three-second video, multiple image stills were obtained using Windows Movie Maker (Microsoft, Redmond, Washington). After image capture, specimens were stored in 10% buffered formalin and histologically processed and prepared for hematoxylin and eosin (H&E) correlation by the Icahn School of Medicine Department of Pathology.

Imaging Database Assembly

Five inverted papilloma specimens and two normal sinus mucosa specimens were imaged. From the collected set of stills, four images were selected from each specimen. Images were selected if nuclei were visible in greater than 50% of the image and greater than 50% of the image was clearly visible (in focus, and not obscured by motion artifact). Four images, two inverted papilloma images and two normal sinus mucosa images, were used for the training set. An example of images from the training set, highlighting important cellular and nuclear features, is shown in Figure 1. Twenty-eight images, four images from each specimen, were used for the testing set.

Interrater Reliability Testing

In order to train each rater, a training module was created for each rater to review. The module consisted of an introduction to HRME and the training set of images with imaging characteristics of each tissue type highlighted. The training set included two images from inverted papilloma samples and two images from normal sinus mucosa samples. After reviewing the training set, the 28-image testing image set was presented and raters were asked to classify each image as either inverted papilloma or normal sinus mucosa. The training module was created on a PowerPoint document. The testing image set was created on Google Forms. Raters were not allowed to review the training module once they began the test. The

results were compared against histopathology of each specimen, which is gold standard for tissue classification.

Statistical analysis was performed using SAS system software (SAS Institute Inc., Cary, NC). Standard procedures were utilized to calculate sensitivity and specificity. 95% confidence intervals were generated using the exact binomial. Multirater kappa (k) statistics, i.e. the chance adjusted measure of agreement, was used to evaluate intraobserver reliability among the 6 reviewers using the SAS macro magree.sas. The range for k is from -1 (complete discordance among reviewers) to 0.0 (random chance) to +1 (perfect concordance among reviewers).¹³ The Kappa values were then interpreted per the initial guidelines set up in the study by Landis and Koch.¹⁴ The parameters were defined as follows: Excellent/Almost Perfect for values of 0.81 to 1.00, Good/Substantial for values 0.61 to 0.80, Moderate for values 0.41 to 0.60, Fair for values 0.21 to 0.40, Slight for values 0.0 to 0.20 and Poor for values under 0.0.

Results

Five samples of inverted papilloma and two samples of normal sinonasal mucosa were identified. Four HRME images from each specimen were included to create a twenty-eight image test. Representative images from HRME and histopathological analysis for normal sinonasal mucosa and inverted papilloma are displayed in Figures 2 and 3, respectively. As seen in these figures, HRME images of inverted papilloma display large prominent nuclei with sparse cytoplasm and minimal inter-

nuclear separation. HRME images of normal sinonasal mucosa conversely are characterized by small, defined nuclei, abundant cytoplasm, and large inter-nuclear separation.

Six otolaryngologists without any prior HRME experience reviewed the training set and rated the images. The mean accuracy among all reviewers in correctly identifying HRME images as either inverted papilloma or normal sinonasal mucosa was 89.9% (95% confidence interval (CI), 84.3%-94.0%). The sensitivity to correctly identify inverted papilloma was 86.7% (95% CI, 79.2%-92.2%), and the specificity was 92.9% (95% CI, 89.0%-100.0%). The Fleiss' kappa score is a statistical measure to evaluate the reliability of agreement among raters. For our study, the Fleiss' kappa interrater reliability score was 0.80 (95% CI, 0.70-0.89).

Discussion

This study is a continuation of our previous investigations evaluating the utility of HRME in identification of inverted papilloma. Our prior ex-vivo trial, the first to evaluate the efficacy of HRME for sinonasal neoplasms, revealed that inverted papilloma and normal sinonasal mucosa have distinct imaging characteristics on HRME, allowing them to be easily distinguished from each other.¹⁰ HRME imaging characteristics of inverted papilloma include nuclear crowding with small inter-nuclear separation and sparse cytoplasm, while normal sinonasal mucosa demonstrates abundant cytoplasm with small, bright nuclei.

Given their distinct characteristics, this study evaluates the feasibility of training otolaryngologists without any prior HRME experience to distinguish between inverted papilloma and normal sinonasal mucosa. Raters were able to accurately identify images as either inverted papilloma or normal sinonasal mucosa in 89.9% (95% CI, 84.3%-94.0%), indicating significant accuracy after reviewing only 4 training images. Furthermore, the sensitivity to correctly identify inverted papilloma was 86.7% (95% CI, 79.2%-92.2%), and the specificity was 92.9% (95% CI, 89.0%-100.0%). Finally, the Fleiss kappa interrater reliability score was 0.80 (95% CI, 0.70-0.89), signifying substantial agreement among raters.¹⁴

Surgical resection is the mainstay of treatment for inverted papilloma. Surgical approaches may include open, endoscopic, or combined techniques. In recent years, the endoscopic approach has emerged as the primary method for surgical resection.¹⁵ Despite its advantages in limiting morbidity, shortening inpatient stays, and enhancing visualization, recurrence rates with endoscopic approaches have not significantly changed.^{16,17} Recurrences arise due to incomplete resection typically at the site of origin.^{18,19}

New technologies are needed to improve intra-operative margin differentiation, diminish reliance on frozen section histopathology analysis, and reduce recurrence rates. As a result, HRME holds significant promise in its ability to decipher inverted papilloma from normal sinonasal mucosa. In addition, the cost of the device is relatively affordable compared to other optical imaging platforms. Furthermore,

this study suggests that otolaryngologists can be easily trained to accurately identify inverted papilloma on HRME imaging, creating the potential for broad applicability and easy adoption.

Given the potential for recurrence and malignant transformation, inverted papilloma requires long-term follow up. Woodworth et al. found an average time to recurrence of 23 months.²⁰ In their review of 2407 cases, Mirza et al. found an average interval of 52 months (6-180 months) to development of metachronous carcinoma development.²¹ At present, surveillance consists of endoscopic examination and interval imaging studies. In addition to its intra-operative utility, HRME has potential as a surveillance tool to enable earlier detection of recurrence and reduced follow up imaging studies.

This study is limited by the limited number of samples of inverted papilloma and normal sinonasal mucosa available. In addition, all samples used in the training set were imaged ex-vivo, and not in-vivo, which is how the HRME is ultimately intended to be utilized. Our group is currently pursuing an in-vivo trial to determine the feasibility, safety, and efficacy of intra-operative margin differentiation for inverted papilloma with HRME. Further studies to evaluate the utility of optical imaging technology for other sinonasal neoplasms are also needed.

Conclusions

HRME can be used to distinguish inverted papilloma from normal sinonasal mucosa. Otolaryngologists without any prior experience with HRME can be successfully trained to differentiate between inverted papilloma and normal sinonasal mucosa. As a result, HRME represents a cost-effective clinical tool that may enable real-time surgical margin differentiation during surgical excision of inverted papilloma with the potential for broad applicability and easy adoption.

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Figure 1: (A) HRME image of normal sinus mucosa; black arrow shows cytoplasm, dashed arrow shows small, bright nuclei, solid line represents the internuclear separation **(B)** HRME image of inverted papilloma; black arrow shows cytoplasm, dashed arrow shows large, prominent nuclei. Notice small internuclear separation compared to Figure 1A.

Figure 2: (A) Hematoxylin and eosin stain of normal sinus mucosa specimen (200x magnification) **(B)** HRME image normal sinus mucosa specimen

Figure 3: (A) Hematoxylin and eosin stain of inverted papilloma specimen (200x magnification) **(B)** HRME image of inverted papilloma specimen

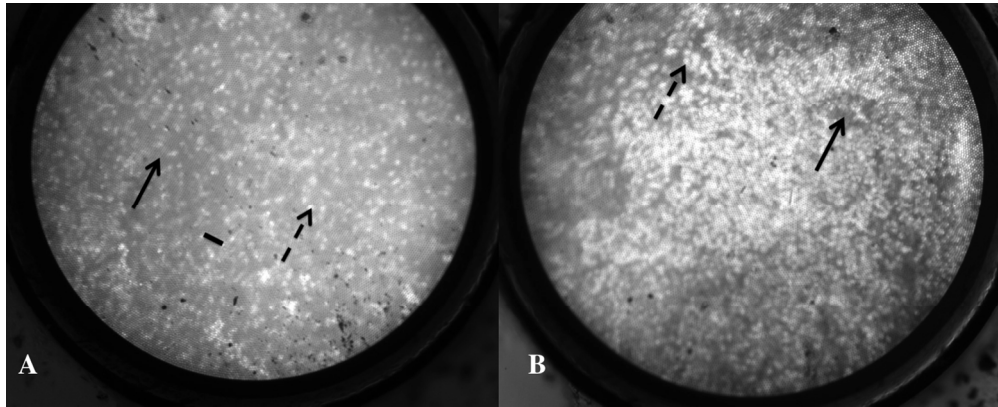


Figure 1: (A) HRME image of normal sinus mucosa; black arrow shows cytoplasm, dashed arrow shows small, bright nuclei, solid line represents the internuclear separation (B) HRME image of inverted papilloma; black arrow shows cytoplasm, dashed arrow shows large, prominent nuclei. Notice small internuclear separation compared to Figure 1A.
254x102mm (150 x 150 DPI)

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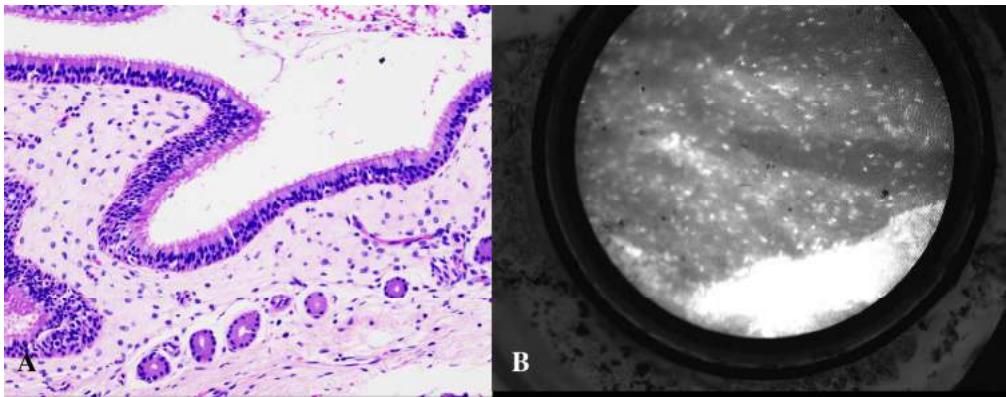


Figure 2: (A) Hematoxylin and eosin stain of normal sinus mucosa specimen (200x magnification) (B) HRME image normal sinus mucosa specimen 254x99mm (150 x 150 DPI)

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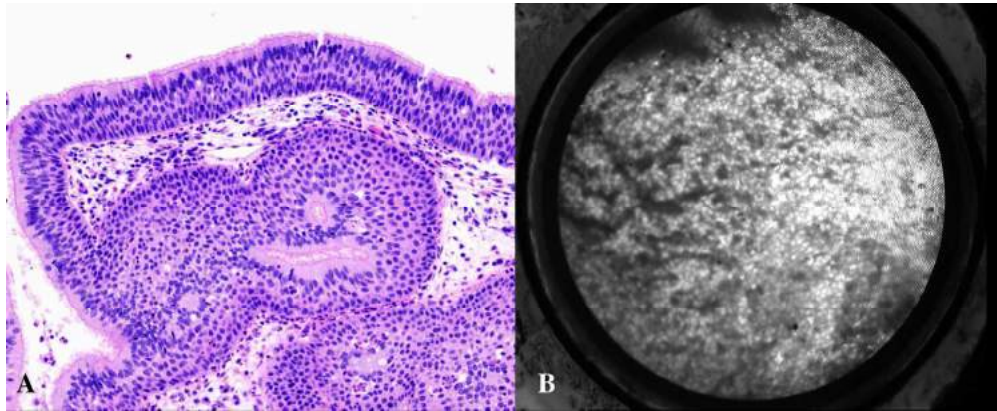


Figure 3: (A) Hematoxylin and eosin stain of inverted papilloma specimen (200x magnification) (B) HRME image of inverted papilloma specimen 259x105mm (150 x 150 DPI)

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