Raman Spectroscopy for Inverted Papilloma: A Proof-of-Concept Study

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Abstract
Inverted papillomas are tumors of the sinonasal tract with a propensity to recur. Raman spectroscopy can potentially identify inverted papillomas from other tissue based on biochemical signatures. A pilot study comparing Raman spectroscopy to histopathology for 3 types of sinonasal tissue was performed. Spectral data of biopsies from patients with normal sinonasal mucosa, chronic rhinosinusitis, and inverted papillomas are compared to histopathology using principal component analysis and linear discriminant analysis after data preprocessing. A total of 18 normal, 15 chronic rhinosinusitis, and 18 inverted papilloma specimens were evaluated. The model distinguished normal sinonasal mucosa, chronic rhinosinusitis, and inverted papilloma tissue with an overall accuracy of 90.2% (95% confidence interval, 0.86-0.94). In conclusion, Raman spectroscopy can distinguish inverted papilloma, normal sinonasal mucosa, and chronically rhinosinusitis tissue with acceptable accuracy.

Keywords
inverted papilloma, Raman spectroscopy, sinonasal tumors, chronic rhinosinusitis, head and neck neoplasm

Materials and Methods

Study Design
Biopsy specimens from patients with an IP, CRS, or normal sinonasal mucosa were compared using RS and histopathology. Ethics approval was obtained from the McGill University Health Centre Research Ethics Board (15-103-MUHC). All patients underwent written informed consent. Patients with other sinonasal masses and pediatric patients were excluded from the study. Normal sinonasal mucosa was obtained from the lateral nasal wall at a distance from the IP. Patients with CRS undergoing sinus surgery had a biopsy taken of polypoid mucosa. Half of each specimen was sent for histopathological analysis and the remainder analyzed by RS. Spectral data from biopsies confirming IP, CRS, or normal sinonasal mucosa on histopathology were retained for analysis.

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Raman Instrumentation and Measurement

The Bruker Raman Senterra (Bruker Optics Ltd, Billerica, Massachusetts) microscope was used to measure spectra of biopsy specimens (Figure 1A). No fixing agents were used and specimens were examined fresh. Using a 785-nm monochromatic laser, the specimens were focused with a 40× magnification lens, and Raman spectra from 400 to 1800 cm⁻¹ were acquired (4 coadditions, 15 seconds of integration time; Figure 1B). A total of 4 random points (1 mm²) on each specimen were measured. It took roughly 4 minutes to collect spectra for each specimen.

Statistical Analysis

Spectra were preprocessed to exclude background noise and normalized at 1000 cm⁻¹ using OPUS software (Bruker Optics Ltd). Principal component analysis (PCA) and linear discriminant analysis (LDA) were used to model the data and calculate accuracy, sensitivity, and specificity. Data analysis was performed in R (R Foundation for Statistical Computing, Vienna, Austria).

Results

A total of 18 normal, 15 CRS, and 18 IP specimens were evaluated by RS and histopathology. No IP specimens contained evidence of carcinoma, although 1 patient with IP showed evidence of mild dysplasia. Both normal sinonasal mucosa, CRS, and IP tissue shared similar spectral peaks at 936, 1000, 1031, 1128, 1444, and 1655 cm⁻¹ (Figure 2). The IP tissue contained additional consistent spectral peaks at 717, 1086, 1265, and 1302 cm⁻¹. Using a PCA-LDA classification of normal sinonasal mucosa, CRS and IP tissues occurred with an overall accuracy of 90.2% (95% confidence interval [CI], 0.86-0.94; Figure 3). The sensitivity and specificity to detect normal and IP tissues were 88.9% (95% CI, 0.83-1.00) and 85.7% (95% CI, 0.74-1.00), respectively. As for CRS and IP tissue, the sensitivity and specificity were 86.7% (95% CI, 0.80-0.92) and 94.4% (95% CI, 0.90-0.98), respectively. The sensitivity and specificity to distinguish normal from CRS tissue were 98% (95% CI, 0.96-1.00) and 93.3% (95% CI, 0.86-0.98), respectively.

Discussion

The optimal management of IPs can be challenging, particularly in recurrent cases. Similar to the light microscope used by pathologists, RS can identify biopsies with high nucleic and amino acid content or otherwise densely populated cells often characteristic of neoplasia. Analogous peaks to IP with differing spectral patterns have been found in colon and lung cancer. It is likely that neoplastic tissue shares certain specific peaks but maintains differences in spectral patterns and intensities. That being said, no difference in spectral peaks was noted in the IP specimens without dysplasia from the 1 patient with mild dysplasia and may be due to sampling error during RS measurements.

RS shows promise for clinical surveillance and operative management of IPs. As others have described, RS can have a significant role in margin assessment intraoperatively. Although frozen section remains the current gold standard for intraoperative assessment of margin status, limitations exist, including time to result as well as sampling and interpretation.

Figure 1. Raman spectroscopy equipment and setup. (A) The Bruker Raman Senterra microscope in the research laboratory. (B) A 1 mm² target is focused at 40× magnification.

Figure 2. The mean Raman spectra among normal sinonasal mucosa, chronic rhinosinusitis, and inverted papilloma tissue.

Figure 3. Principal component analysis–linear discriminant analysis (PCA-LDA) model differentiates normal sinonasal mucosa (normal), chronic rhinosinusitis (CRS), and inverted papilloma (IP). Note the demarcation between plot points, which tend to cluster together, using this model.
errors. Alternative optical diagnostic tools, such as high-resolution microendoscopy (HRME) of the nasal cavity, use systemic or topical contrast agents that stain nuclear membranes of cells and allow for visualization of cellular structure and patterns. Despite the high interobserver reliability, HRME for IP requires training to identify nuclear crowding with sparse cytoplasm patterns. Several hurdles remain to implement RS in clinical practice: the size of the equipment, time to analysis of spectra, and lack of characterization of other sinonasal pathologies. The current setup in a research laboratory and associated cost for the Raman microscope (roughly $50,000 USD) remain active barriers. However, the use of a smaller, handheld probe holds promise for in vivo analysis of tissue within our speciality.

Conclusion

Inverted papilloma can be distinguished from normal sinonasal mucosa and chronic rhinosinusitis using Raman spectroscopy. A larger study with use of a handheld probe is needed to assess its utility in the surgical management of inverted papillomas.

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Author Contributions

Marco A. Mascarella, study design, patient recruitment, data collection, analysis and manuscript preparation; Abdulaziz Alrasheed, study design, patient recruitment, data collection, analysis and manuscript preparation; Naif Fnais, study design, patient recruitment, data collection, analysis and manuscript preparation; Ophelie Gourgas, study design, data collection and analysis, manuscript preparation; Ghulam Jalani, study design, data analysis, manuscript revision; Marta Cerruti, study design, patient recruitment, data analysis, revision of manuscript; Marc A. Tewfik, study design, patient recruitment, data analysis, revision of manuscript.

Disclosures

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References