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Detection of Squamous Cell Carcinoma and corresponding biomarkers using Optical Spectroscopy

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Abstract

Objectives—1) Investigate the use of optical reflectance spectroscopy to differentiate malignant and non-malignant tissues in head and neck lesions; 2) Characterize corresponding oxygen tissue biomarkers that are associated with pathologic diagnosis

Study Design—Prospective non-randomized clinical study

Setting—Tertiary VA Medical Center

Subjects and Methods—All patients undergoing panendoscopy with biopsy for suspected head and neck cancer were eligible. Prior to taking tissue samples, the optical probe was placed at three locations to collect diffuse reflectance data. These locations were labeled “tumor”, “immediately adjacent”, and “distant normal tissue”. Biopsies were taken of each of these respective sites. The diffuse reflectance spectra were analyzed, and biomarker specific absorption data was extracted using an inverse Monte Carlo algorithm for malignant and non-malignant tissues. Histopathological analysis was performed and used as the gold standard to analyze the optical biomarker data.

Results—21 patients with mucosal squamous cell carcinoma of the head and neck were identified and selected to participate in the study. Statistically significant differences in oxygen saturation ($p = 0.004$) and oxygenated hemoglobin ($p = 0.02$) were identified between malignant and non-malignant tissues.

Conclusion—Our study established proof of principle that optical spectroscopy can be used in the head and neck areas to detect malignant tissue. Furthermore, tissue biomarkers were correlated with a diagnosis of malignancy.

Introduction

Head and neck malignancies affect an estimated 50,000 people annually[1]. Early detection of these malignancies results in improved outcomes. However, clinical differentiation between malignant and non-malignant lesions of the upper aerodigestive tract can be challenging.

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Furthermore, prior surgery, trauma or radiation may make a clinical diagnosis even more difficult. In these cases, biopsy of the lesion is performed to establish a diagnosis.

Based on a review at our institution between Jan 2009 and Dec 2009, 256 patients underwent 305 biopsies, of which 192 samples were non-malignant. Thus, nearly 2 out of 3 clinically indicated biopsies were later pathologically determined to be non-malignant. This statistic is likely representative of other tertiary care centers throughout the nation and emphasizes the variability and uncertainty inherent in current clinical examinations.

In addition, surgical biopsy is not without its issues. Some lesions are difficult to access, such as those located in the nasopharynx, oropharynx, hypopharynx and larynx. These biopsies often require general anesthesia and biopsy in the operating room. According to the American Hospital Directory, the national average charge of laryngoscopy with biopsy (CPT 31535) is $1,176 per patient. Significant amounts of labor, facility, and monetary resources are expended on patients who ultimately may have no malignancy. Consequently, patient selection is important to minimize risks and costs and maximize yield. To address this 63% negative biopsy rate at our institution, we've collaborated with the Duke biomedical engineering department to explore an optical method as a potential tool to improve patient selection.

This method is based on reflectance optical spectroscopy technology. Optical spectroscopy is the use of light to evaluate the composition of materials. Spectroscopy is a broad field. In this instance, diffuse reflectance spectroscopy using light in the visible UV-spectrum is utilized. Reflected light is analyzed, providing absorption and scattering data that reflects underlying tissue composition, particular tissue oxygen saturation, total hemoglobin concentration and morphology.

Since carcinogenesis alters the structural and biochemical makeup of cells, the metabolic needs of the tissues are altered, often leading to increased microvascularization and differing oxygen utilization. This in turn affects optical properties of dysplastic and cancerous tissue. Numerous studies [2–4] have demonstrated differences between normal tissue and tumor in their respective diffuse reflectance spectra. These prior studies have relied on qualitative waveform pattern matching across a broad wavelength range.

In contrast, Feld et al. have demonstrated similar quantitative optical spectroscopy techniques *in vitro*[5]. Ramanujam et al. demonstrated the use of quantitative optical spectroscopy in breast cancer[6]. Using a novel inverse Monte Carlo model[7], specific biomarker parameters were extracted from the optical reflectance data. This allowed for a quantitative analysis to measure concentrations of these specific biomarkers and their association with the pathologic diagnoses of disease. This study investigated the use of optical reflectance spectroscopy and the scalable inverse Monte Carlo model to differentiate malignant and non-malignant tissues specifically in head and neck lesions. Furthermore, characterization of corresponding oxygen tissue biomarkers that predict pathologic diagnosis was performed. The hypothesis was that optical reflectance spectroscopy can differentiate malignant and non-malignant tissues in head and neck lesions. Furthermore, these differences can be correlated with tissue oxygen biomarkers.

Subjects and Methods

This IRB approved study was open to all patients who were scheduled for panendoscopy and biopsy for suspected head and neck cancer. Patients were approached and consented to undergo non-invasive evaluation by the optical probe of sites to be biopsied. These sites included "tumor", "immediately adjacent to tumor" and "distant normal".

Optical Spectroscopy Probe

An optical spectroscopy probe was designed and manufactured to final form in the laboratory of Dr. Nimmi Ramanujam at Duke University. This device is composed of optical fibers that are epoxied together and covered by a stainless steel tube. Probe design details have been described in prior publications by the Ramanujam group [6]. In brief, light from a 450 watt Xenon source is passed through a monochromator, then down a collection of optical fibers within a cable to the probe tip. The monochromator selects for wavelengths in the range 350 – 600 nm. Light reflected off the tissues is then passed along a set of different fibers within the cable to a spectrometer. A laptop computer is used to both control the light source and spectrometer as well as analyze the incoming data. The fiber is 2mm in diameter and the handheld probe is 5mm in diameter and is ensheathed in a stainless steel tube that is amenable to sterilization.

Clinical Study Design

Consented patients had the optical probe placed on the surface of at least three sites ("tumor", "immediately adjacent to tumor" and "distant normal"). By this design, the patients served as their own control. If biopsy of other areas was clinically indicated, then the probe was also used to analyze that site prior to biopsy. Diffuse reflectance scans were performed and data was collected. The data acquisition program allowed for multiple scans to be obtained at the same site, and thus two to four optical scans were routinely obtained at each biopsy site. Immediately after the scan, the probe was removed and the underlying tissue was sampled with biopsy forceps such that the reflectance data could be directly paired with the histopathological diagnosis.

Extraction of tissue biomarkers from optical probe data

A quantitative inverse Monte Carlo model was used to extract scattering and absorption properties of the measured tissues[7]. Diffuse reflectance spectra measured from the tissue were fit over the wavelength range of 450 to 600nm. Using this inverse Monte Carlo model, concentrations of oxygenated hemoglobin (HbO_2), deoxygenated hemoglobin (dHb), and total hemoglobin (THb), as well as oxygen saturation (SO_2%) were calculated in μM. These biomarkers were extracted from the absorption coefficient spectra across all measured subjects and sites. All fits and subsequent data analysis were performed using MATLAB version 7.8, release 2009 (The MathWorks).

Statistical Analysis

For data analysis, tissue samples were grouped into two groups, malignant and non-malignant, based on histopathological diagnosis. Mean concentrations per site for HbO_2, dHb, and THb and SO_2% were then compared using unpaired Wilcox rank-sum test to determine if there were statistically significant differences between malignant and non-malignant tissues.

Results

A total of 21 patients were enrolled into the study, of which all were males During the study, no adverse events were encountered. Patient demographics and biopsy locations are represented in Table 1. A total of 266 scans were performed on 68 tissue biopsy sites, on average four scans per site (range: 1–8 scans/site). Histopathologically, 43 biopsies were normal tissue, 18 biopsies were tumor tissue and 7 biopsies demonstrated dysplasia. These 7 dysplastic specimens represented 23 total scans (Table 2). The dysplasia samples were integrated into the non-malignant category for analysis. Figures A–C demonstrates the calculated molar concentrations of the biomarkers deoxyhemoglobin (dHb), oxyhemoglobin

(HbO_2), and total hemoglobin (THb) for malignant and non-malignant samples. Figure D shows the calculated tissue oxygen saturation (SO_2%). Statistically significant differences (defined as $p < 0.05$) were noted in the HbO_2 concentration ($p=0.02$) and in SO_2% ($p=0.004$).

Discussion

Our study established proof of principle that optical spectroscopy can be used in head and neck lesions to detect tissue biomarkers associated with malignancy. Data demonstrated that tissue reflectance differences between malignant and non-malignant mucosal lesions of the head and neck can be delineated with optical spectroscopy. Specifically, four biomarkers were extracted from the reflectance data using an inverse Monte Carlo model and compared between malignant and non-malignant tissues. Of these, the concentration of oxygenated hemoglobin (HbO_2) and the tissue oxygen saturation (SO_2%) showed a statistically significant difference.

Optical reflectance spectroscopy has previously been demonstrated to show different reflectance, scatter and absorption patterns between malignant and non-malignant tissues [4,6]. This can be attributed to different structural and metabolic properties of the tissues in question[3]. This is in line with many prior studies which have demonstrated significant differences in oxygen tension in normal and metabolically active solid tumor tissues [8,9,10].

In the head and neck, prior studies focused on wave-form pattern matching to demonstrate qualitative differences between normal and malignant tissues[11,12]. For example, Mallia et al. have used laser induced autofluorescence and diffuse reflectance spectroscopy spectral differences with curve-fitting to show differences between dysplastic tissues and squamous cell carcinoma in oral cancers.

Furthermore, many study methods have relied on extrinsic contrast materials such as dyes or stains, or more complex instrumentation in the case of laser induced autofluorescence. Tsui et al. used direct fluorescence visualization to evaluate surgical margins in oral cavity carcinoma[13]. Their study did highlight that optical technologies clearly define surgical margins better than direct visualization. Other studies have used autofluorescence to distinguish normal and abnormal tissues in the oral cavity, but no standardization has been established[2]. These methodologies require additional skill sets or bulky equipment and may rely on subjective interpretation. A quantitative approach, such as the methods described in this study, may aid in defining surgical margins more accurately and objectively.

A limitation of this study is the small sample size. A larger study may better define and identify other biomarkers. Also, it is recognized that determining dysplasia versus malignancy is a more clinically relevant question. To address this, patients are being recruited for a study to expand this dataset with the goal of establishing good specificity and sensitivity data of optical biomarkers to distinguish dysplastic from malignant lesions. Furthermore, we recognize different tissues within the head and neck, such as muscular, mucosal and lymphoid, may likely have different optical signatures. As part of this upcoming larger study, the goal would be to stratify findings by site. Other studies have also identified probe pressure to be a variable that affects spectroscopy readings[14,15]. Probe pressure may affect the contents of vascular channels, artificially reducing measured hemoglobin or oxygen saturation. Standardizing the probe readings in the future will be accomplished by integrating pressure sensors into the probe design.

This study suggests that there are specific biomarkers that can be identified via this method of analyzing optical spectroscopy data. The clinical impact of this approach should improve

patient selection for biopsy. The technology used to demonstrate this has the advantage of being low-cost, portable, non-invasive and, most importantly, quantitative.

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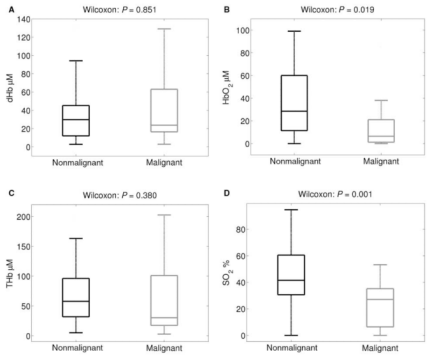

Figure 1.

(A–D) Box plots demonstrating differences between nonmalignant and malignant tissue in terms of molar concentrations for (A) deoxygenated hemoglobin (dHb), (B) oxygenated hemoglobin (HbO_2), (C) total hemoglobin (THb), and (D) oxygen saturation (SO_2%), as determined by optical spectroscopy and an inverse Monte Carlo algorithm. Statistically significant difference is noted in (B) HbO_2 concentration and (D) SO_2% (P = .019 and P = .001, respectively).

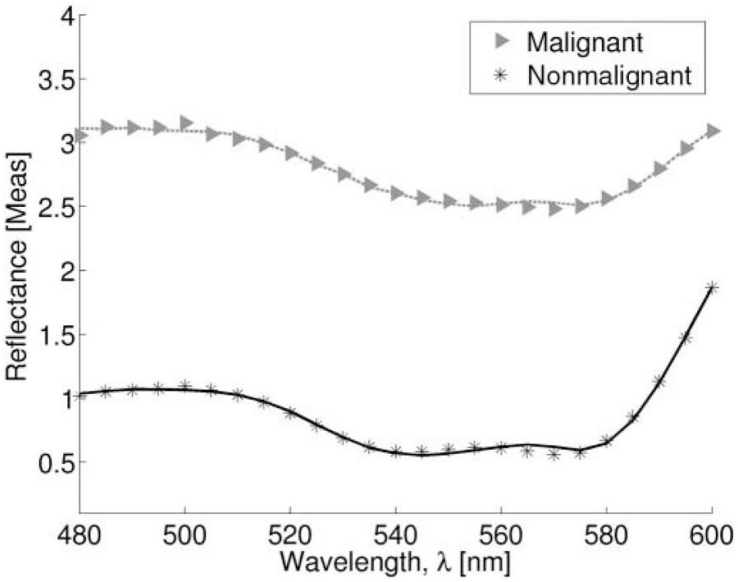

Figure 2.
This is a representative pattern of reflectance from a single scan showing inherent differences in reflectance between malignant and nonmalignant tissues.

Table 1

Patient demographics and biopsy location

Patient Demographics			
Site	**Subsite**	**Non-malignant**	**Malignant**
Larynx		*20*	*10*
	Subglottic	0	0
	Glottic	6	5
	Supraglottic	13	4
	Transglottic	1	1
Pharynx		*23*	*6*
	Oropharynx	7	0
	Hypopharynx	1	0
	Base of Tongue	10	1
	Tonsil	5	5
Oral Cavity		*7*	*2*
	Tongue	5	1
	Floor of Mouth	1	1
	Other	1	0
	Totals	50	18
Age in Years (Range)			
	Mean	60 (46–86)	

Table 2

Summary of biopsy pathology

Tissue	Biopsies	Scans
Non-malignant	w	194
Malignant	18	72
Total	68	266