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Point-of-Care Noninvasive Hemoglobin Determination Using Fiber Optic Reflectance Spectroscopy

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Abstract— **Study Objective: Rapidly identifying anemia through qualitative observation of the palpebral conjunctiva for pallor is standard medical practice. We report on the ability of using a fiber optic array probe to collect diffusely reflected light from the palpebral conjunctiva and measure hemoglobin concentration noninvasively. Patients (N=102) admitted to the emergency department and receiving a complete blood count (CBC) were enrolled as a convenience set. The tarsal plate was exposed by averting the lower eyelid and illuminated with a white LED while diffusely reflected light was collected using a fiber optic probe and commercial grating spectrometer. Spectra were analyzed using partial least squares regression to extract hemoglobin concentration information and compare to the CBC result. The probe and algorithm demonstrated correlation coefficient of r = 0.82 and standard error of 1.05 g/dL compared to invasive CBC test. Experimental error is due to variability in distance and position of the probe relative to the conjunctiva. This technique has shown the ability to measure total hemoglobin noninvasively and in real-time at the point-of-care using diffuse reflectance spectroscopy of the tarsal plate, leading to potential inclusion of this class of device as a triage centric tool to identify anemia appearing with internal hemorrhage or other acute conditions.**

I. Introduction

BACKGROUND: Anemia is estimated to affect 3.5 million people in the United States with millions more undiagnosed [1] and, as representative figures, anemia affects 33% of cancer patients, 65-95% of HIV/AIDS patients, and 70% of rheumatoid arthritis patients. Concomitant appearance of anemia with these disorders has been shown to decrease myocardial function, increase peripheral arterial vasodilation, and activate the sympathetic and reninangiotensin-aldosterone system, influencing the progression of heart and kidney failure [2]-[4]. The lack of a medical instrument to reliably identify anemia in vivo leaves the invasive blood draw and in vitro analysis as the requisite method, translating to elevated risks of exposure to blood borne pathogens for phlebotomist, delays in obtaining results, and discomfort for the patient.

Numerous investigations have been conducted on the implementation of total hemoglobin measurement capabilities using spectroscopic transcutaneous devices [5]-[10] with the hopes that this technique can be merged with existing pulse oximeters; however, such an instrument has yet to gain clinical acceptance. Alternative techniques investigated have included spectral imaging of the retina [11] or sublingual mucosa [12], transcutaneous conductance measurements [13]-[14], low coherence interferometry [15], optoacoustic spectroscopy [16]-[17], and reflectance measurements from thick tissue regions [18]-[20]. These evaluations evaluate thick and highly scattering regions, such as the forearm or fingertip, or difficult to sample physiological locations (e.g. the retina) in monitoring total hemoglobin. Our proposed technique builds upon current medical practice, explicitly a physician's observation of the pallor of the tarsal plate of the palpebral conjunctiva, adding a quantitative measurement of the optical properties through reflectance measurements. Sampling from the palpebral conjunctiva is advantageous as (1) it is easily accessible, (2) it has a low concentration of melanosomes, translating to low optical scattering and high uniformity across individuals of varying ethnicity as compared to thick tissue regions, and (3) the mucosal surface is very thin (5-7 cellular layers), transparent, and highly vascular to mediate immunological response.

In an earlier study, we disclosed the ability of a free-space propagation grating spectrometer to measure hemoglobin from the tarsal plate in patients with heads stabilized in a modified slit-lamp (N=30) [21]. Here, we disclose the ability of a fiber optic probe to collect similar information in a larger patient population (N=102), bringing this technique to the bedside and allowing for rapid sampling. We report the precision, accuracy, sensitivity, and specificity of this technique in a emergency department (ED) patient sample set through regression models on collected spectra, along with subset characterization of performance for ethnic groups and anemic, normal, and polycythemic individuals.

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II. Theory and Methods

This is a prospective institutional review board approved nonblinded study comparing the performance of a in-house fabricated device to monitor total hemoglobin concentration to in vitro CBC determined total hemoglobin concentration in a convenience sample of. Two reflectance spectra were collected from each tarsal plate in each eye, processed using regression techniques, and compared to the CBC determined value as a control.

A. Concept and Theory

Total hemoglobin concentration can be determined using visible broadband (white) light at the palpebral conjunctiva, as the blood vessels are visible through a translucent mucosal surface. The tarsal plate is the most vascular location on the palpebral conjunctiva containing blood vessels from palpebral branches of nasal and lacrimal arteries throughout the squamous epithelium and capillary beds running in underlying dense fibrous tissue interspersed with sebaceous meibomian glands. Light (photons) impinging on the conjunctiva will diffuse throughout the conjunctiva, tarsal plate, and underlying orbicularis muscle and dermis, interacting through absorption with hemoglobin molecules in intact red blood cells (RBC's) and free hemoglobin and subsequently modifying the relative amounts of each wavelength of light. Assuming the diffusion and scattering properties are comparable in different patients, as is acceptable with the low concentration of melanosome granules at the palpebral conjunctiva, light scattering back from the conjunctiva following interaction with blood vessels will have a spectrum dependent primarily on the amount of hemoglobin encountered in the diffusion process. A calibration model can then be developed based on reflection signals and used to determine unknown hemoglobin concentrations using the same configuration. It is also assumed that a large spot (>1 mm) is necessary to ensure the interaction of diffusing photons with numerous blood vessels, creating an averaging effect such that spectra will not be dependent on local variations in blood vessel density, but rather total hemoglobin concentration. Lastly, as oxy- and deoxyhemoglobin have similar absorption spectrum in the visible regime (as opposed to NIR utilized in pulse oximetry), it allows a measurement of total hemoglobin to take place without a priori knowledge of saturation in active sites of metabolism at capillary beds. The possibility of elevated local hematocrit during systole is minimized by the superficial penetration of light through soft tissue and would interact with capillary beds primarily.

B. Study

The setting of this study is the ED of RIH, a large tertiary-care facility. Patients admitted to the adult ED were identified as study candidates following initial consultation with the ED physician and subsequent determination of the need for a CBC in the course of their treatment within 6 hours of data collection. Patients with a history of substance or alcohol abuse were excluded, as were patients with active bleeding in the ocular region. Patients with hemoglobin concentrations or hematocrit levels outside of the World Health Organization defined limits of normal values (male 13-17 g/dL, female 12-16 g/dL) and patients of darker skin pigments were given preference in enrollment to establish a large variance in hemoglobin/hematocrit and in the skin pigmentation levels. For this reason, the demographic represented is not purely indicative of the patient population in a large urban ED. The study was approved by the IRB of Rhode Island Hospital.

C. Data Collection

Study volunteers were situated in a prone position. As a one research assistant everted the lower eyelid by pulling downward while directing the patient to look upward, fully exposing the mucocutaneous junction, tarsal plate, and fornix. A metal ferrule with six 200 μm fibers oriented in a hexagonal pattern around a single 200 μm collection fiber and collimating lens affixed to the end of the probe (R-200-7, Ocean Optics, Dunedin, Fl) (see Figure 1) was oriented such that the tarsal plate was irradiated. Collimating optics establish a consistent spot size of 2 mm independent of fluctuation in the separation from probe to conjunctiva.

Fig. 1. Photograph of data collection technique from tarsal plate of the palpebral conjunctiva.

Illumination was achieved using a white LED (Ocean Optics, Dunedin, FL) and collection of reflection spectra was performed using a commercial miniature static grating spectrometer (USB2000, Ocean Optics, Dunedin, FL) to minimize instrument size and durability. Overhead fluorescent lights were turned off to remove contributions of sharp fluorescent emissions in spectra. The miniature grating spectrometer was interfaced to a PDA (Hewlett Packard) where the integration time was set to 50 ms per spectrum. Two reflection spectra were collected from the tarsal plate from each eye of the study volunteer, resulting in four spectra collected for each patient. The probe was removed and patients were instructed to blink between each collection to maximize comfort. Subsequent to data collection, age, sex, ethnicity, chief complaint, CBC results, pulse, Sp02, and blood pressure were recorded by a research assistant or author (JM). Patients were classified as normal (Hgb 13-17 g/dL male, 12-16 g/dL female), anemic (Hgb < 13 g/dL male, < 12 g/dL female), severely anemic (Hgb < 10 g/dL male, < 9 g/dL female), or polycythemic (Hgb > 17 g/dL

male, > 16 g/dL female) based upon CBC determined values.

D. Data Processing

Prior to analysis, spectra were normalized to 679 nm to eliminate variations in overall light intensity levels and data clipped from 440-680 nm to remove noisy signal where white LED intensity is low. One spectra from each eye with variance between the two minimized were selected and averaged to produce one signal to analyze for each patient while minimizing sampling artifacts. Spectral processing was performed in the Mat Lab software package (The Mathworks, Natick, NJ). To extract hemoglobin concentration from reflectance signals, a linear regression calibration model was applied to the reflectance spectra. The partial least squares (PLS) regression algorithm [22]-[23], was chosen as a technique to calibrate the reflectance spectra to hemoglobin concentration. PLS is a good choice in this method as it is a soft modeling technique capable of identifying regions of the spectra highly correlated with the analyte of interest in the presence of other unknowns and random fluctuations in the spectra (such as variations in optical scattering, bilirubin concentration, and, to a lesser extent, saturation and melanin concentration). The spectra

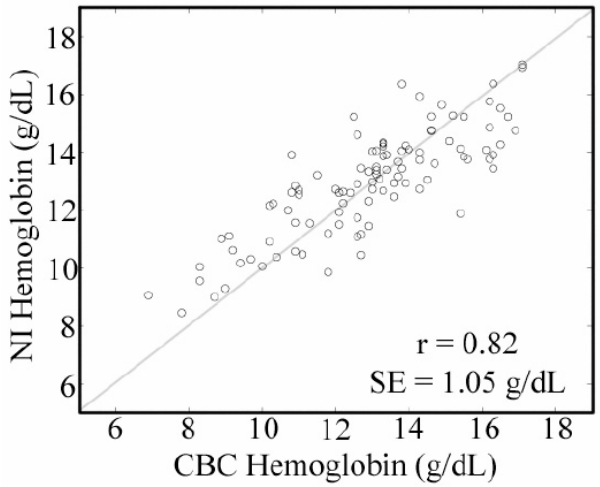

Fig. 2. Regression analysis of conjunctiva reflectance signals versus CBC determined total hemoglobin concentration values. This technology shows significant premise to render hemoglobin involvement a non-invasive and point-of-care process.

area analyzed is a leave-one-out cross validation; one spectra is removed, a calibration model is generated from all other spectra, the model is used to determine hemoglobin concentration of removed spectra, and the algorithm repeated for all data. The results are compared to the CBC determined values via Pearson correlation coefficients, sensitivity and specificity determinations

The outcome measures are the spectroscopically determined total hemoglobin concentration, and sensitivity/specificity of noninvasive detection of WHO defined anemia and polycythemia.

III. Results

One hundred and two patients were enrolled in this study of which 60% (N = 61) were female. The average age was 54 years (range 18-92, SD ± 21.4), average hemoglobin concentration was 12.9 g/dL (range 6.9-17.1, SD ± 2.4), with 66% (N=67) in the normal hemoglobin range, 31% (N=32) with in the anemic range with subset of 7% (N=7) with severe anemia, and 3% (N=3) in the polycythemic range. The breakdown in ethnicity among the 102 patients is 14% (N=14) African-American, 14% (N=14) Hispanic, 2% (N=2) Asian, and 60% (N=62) Caucasian.

A plot of the fiber optic determined hemoglobin concentration versus the invasive CBC determined hemoglobin is shown in Figure 2. The Pearson correlation coefficient is r = 0.82 with a standard error of 1.05 g/dL (range 0.00 - 3.52 g/dL SD ± 0.82 g/dL).

Sensitivity and specificity are calculated based on predicted versus real observation of each subclass of hemoglobin concentration value. Normal value patients (N=67) are predicted with sensitivity of 88 % (95% CI: 84 % to 93 %) and specificity of 71 % (95% CI: 66 % to 77 %), anemic patients (N=32) predicted with sensitivity of 78% (95% CI: 75% to 81%) and specificity of 90% (95% CI: 87% to 94%), subset of severely anemic patients (N=7) predicted severely anemic with sensitivity of 57 % (95% CI: 42% to 57%) and specificity 98% (95% CI: 95% to 100%), and polycythemic patients (N=3) predicted polycythemic with sensitivity of 33% (95% CI: 0% to 66%) and specificity of 99 % (95% CI: 98% to 100%). Ethnicity does not appear to affect the experimental accuracy of the technique.

IV. Discussion

The variation in accuracy of this study from an initial study [25] is dependent on experimental configuration. In the initial study, the conjunctiva was fixated in a stabilization apparatus and spectral sampling performed using an objective lens grating spectrometer, allowing for high interpatient reproducibility of measurement conditions. The introduction of a fiber-optic probe in this contribution allows for this technique to be performed at the bedside, but introduces significant variation in data collection, likely the primary source of error in this study. Follow-up clinical studies are being designed for two alternative data capture techniques. First, reflectance signals can be collected using a spectral imaging device with a two dimensional photodetector such that reflectance data is resolved in the vertical position dimension along one axis of the photodetector and in the wavelength regime along the other dimension. Second and alternatively, a single point fiber optic spectrometer can capture reflectance signals in kinetics mode while the probe is physically scanned vertically. The signal from the optimal positioning on the tarsal plate can be identified by the strongest hemoglobin absorption signal and the data processing algorithm applied to this signal only.

V. Conclusions

In conclusion, this study shows the capability of a noninvasive technique to measure total hemoglobin concentration at the bedside in an ED setting across a diverse patient population. While the accuracy of this methodology has yet to compare to that of invasive blood testing, refinement in data collection techniques to ensure reproducibility can enable this test to become a screening tool for anemia.

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