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Research

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Non-Invasive Characterization of Oxygen Transport in Sickle Cell Disease: A Pilot Study

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ABSTRACT

Introduction: Vaso-occlusive (VOC) crisis is, in part, a result of microvascular ischemic insults to tissue causing pain in Sickle Cell Disease patients, which is a common presentation to the Emergency Department (ED). This study simultaneously measured and compared several global and regional indicators of oxygen transport in normal volunteers and subjects with Sickle Cell Disease (SCD).

Materials and Methods: Healthy African American volunteers were compared to SCD patients, assessed at states of clinical non-distress, referred to herein as "baseline". All subjects underwent 10 minutes of non-invasive monitoring to measure cardiac output, oxygen consumption, arterial oxygen saturation (SpO_2), and Cutaneous tissue saturation of oxygenation ($CtSO_2$).

Results: Twenty one patients (9=healthy & 12=SCD baseline) were chosen. The median superficial $CtSO_2$ (healthy *vs.* SCD baseline) was 72% (IQR=10.94) and 56% (IQR=26.86) with a p-value of 0.0011. Traditional measures of hemodynamic performance (heart rate, blood pressure, cardiac index) were not statistically significant between the two groups.

Conclusion: The study shows Sickle Cell Disease to share similarities with sub-clinical compensated state of shock on a microcirculatory level. The values obtained from the study can hopefully shed light into the intricacies of the baseline biophysiology of Sickle Cell Disease; with a foresight to further understand Vaso-occlusive crises pathological processes and sickled cells interactions with its surrounding environment.

KEYWORDS: Oxygen transportation; Microcirculation; Spectroscopy; Hemoglobin.

ABBREVIATIONS: VOC: Vaso-occlusive Crisis; ED: Emergency Department; SCD: Sickle Cell Disease; $CtSO_2$: Cutaneous Saturation of Oxygen; OER: Oxygen Extraction Ratio; IQR: Interquartile ratios; SD: Standard Deviations; CI: Confidence Intervals.

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INTRODUCTION

Sickle Cell Disease (SCD) is a disease manifestation of a set of genetic abnormalities primarily affecting patients of African and Mediterranean descent. It is caused by a substitution of valine for glutamic acid in the sixth position of the beta globin chain on chromosome 11. This alters the surface charge of the molecule and allows sickle hemoglobin (Hb S) tetramers to polymerize inside the red blood cell. The polymer can alter both the red cell shape and membrane properties leading to abnormal and complex interactions with the vascular endothelium. The combination of these effects produces a hemolytic anemia and suspected microvascular dysfunction, which results in severe pain. The mechanisms by which this occurs have not been well delineated, but are likely due in part to abnormalities in oxygen transport. Current concepts suggest several factors may impact oxygen transport including inflammation,[1-4] neurohumoral responses,[5-8] autonomic nervous system adaptations[9,10] and abnormalities in vascular response. These factors may influence vasculature in SCD patients at baseline and during VOC.

In order to gain a deeper understanding of the pathophysiology of SCD and oxygen transportation, better mechanisms to identify the components of oxygen transport are needed. It is now possible to non-invasively monitor several components of oxygen transport. Some method involve measuring cardiac output by a number of means including impedance cardiography, oxygen consumption through indirect calorimetry, arterial hemoglobin oxygen saturation through pulse oximetry, and tissue hemoglobin oxygen saturation through differential absorption spectroscopy. We used these techniques along with conventional hemodynamic parameters such as heart rate and blood pressure to measure and compare whole body and regional tissue oxygen transport and traditional hemodynamic measures in SCD patients at baseline and patients without SCD.

The purpose of this study to begin to understand the relationship between oxygen transport abnormalities in normal, healthy controls and in patients with SCD. The ability to document such abnormalities may provide important diagnostic and therapeutic endpoints allowing for more objective treatments of SCD and VOC.

MATERIALS AND METHODS

Study Population

The study population consisted of two groups. The first group was composed of nine normal, healthy controls of African-American descent with no history of reported Sickle Cell Disease or trait. The second group consisted of twelve patients with a known history of Sickle Cell Disease classified as homozygous Hb SS or doubly heterozygous Hb SC, that at the time of evaluation, did not report pain. There were no statistical analysis of the control group and SCD group demographics due to the control groups being race and age matched to the SCD patients. The Institutional Review Board has approved this study. All patients signed a consent form prior to enrollment in the study as per IRB regulations.

Non-Invasive Hemodynamic and Oxygen Utilization Measurements

Cutaneous tissue oxygen saturation measurements (CtSO2): Differential absorption spectroscopy was used to measure the aggregate hemoglobin oxygen saturation in a selected volume of tissue. $CtSO_2$ measurements were made with a spectrophotometric[11,12] monitor using a combination of visible and near-infrared light (O_2C: LEA, Inc., Gießen, Germany). A combination of white light and near infrared light was used to detect $CtSO_2$. Oxygen saturation was determined by the differential absorption spectra of oxygenated and deoxygenated hemoglobin to the various light sources as they traverse a certain volume of tissue. The volume of blood in any tissue is approximately 70% venous, 20% capillary, and 10% arterial.[13] The derived $CtSO_2$ is indicative of mainly venous hemoglobin and thus, represents the post-extraction compartment of the tissue. This in turn is indicative of the adequacy of oxygen delivery at the tissue level. Each probe has sensors that can detect superficial as well as deep cutaneous measurements based on optode spacing and the character of light used. Superficial sensors monitor a depth of 2 mm and deep sensors monitor a depth of approximately 7 mm. Two flat probes were secured to the thenar aspect of each individual's palmar surface while recording $CtSO_2$ readings. This was done to minimize pigment interference with the probes while recording data. $CtSO_2$ was measured continuously and values, reported as "percent saturation", were recorded every 5 seconds for averaging over the 10 minute period.

Cardiac index (CI): In order to determine whole body oxygen delivery, cardiac output was measured using an impedance cardiography (Medis Medizinische Meßtechnik, Thueringen, Germany). Eight standard electrodes were placed on each subject; as directed by the manufacturer. Two of these electrodes were placed on each side of the neck and thorax. The electrodes used were standard continuous EKG monitoring electrodes. CI was measured every 5 seconds and these values were used to average CI over the 10 minute period.

Oxygen consumption (VO_2): VO_2 was measured by having patients breathe into a mouthpiece connected to a system that can measure both airflow and the differences between expired and inspired oxygen concentration (BioPac Systems, Gloleta, CA, USA). The patient was instructed to breathe through a disposable mouthpiece and corrugated tubing identical to those used to administer respiratory aerosol treatments. These measurements were made continuously and values taken every 5 seconds were used to average VO_2 over the 10 minute time period.

Arterial hemoglobin oxygen saturation: Arterial hemoglobin

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oxygen saturation (SpO_2) was determined with the use of a pulse oximeter (General Electric Procare Auscultatory 400). SpO_2 was used to substitute for true arterial hemoglobin oxygen saturation. SpO_2 was measured every 5 seconds and averaged over the 10 minute monitoring period.

Oxygen delivery: Oxygen delivery was calculated using the formula $\{DO2=(1.34\times Hgb\times SO_2)+(PO_2\times 0.0031)\}$. Hemoglobin was measured as part of the routine clinic visits or from Emergency Department visits. Control subjects did not have hemoglobin levels drawn. A standard hemoglobin value of 12 mg/dL was used for the control subjects. A Hemoglobin value of 12 mg/dL was chosen for calculating oxygen delivery because this number represents the lower range of normal hemoglobin levels and would therefore underestimate the oxygen extraction ratios when compared to sickle cell patients.

Oxygen extraction ratio (OER): OER can be determined by a number of means. Regional OER was determined by using the $CtSO_2$ and SpO_2 values $(SpO_2\text{-}CtSO_2)/SpO_2$.[14]

Vital signs: Standard vital signs, Heart Rate, Blood Pressure, Temperature and Respiratory Rate, were measured by clinically accepted standards.

Statistical Analysis

Data entry and data analysis was performed using JMP 4.0 (SAS Institute, Cary, NC, USA). A non-parametric median Kruskal-Wallis test was performed to determine any significant differences between the study groups. Comparisons of hemodynamic and oxygen transport measures were made utilizing a non-parametric analysis and group medians. Inter-quartile ratios (IQR) ratios were substituted for Standard Deviations (SD) and Confidence Intervals (CI). The level of significance was set at an alpha of 0.05.

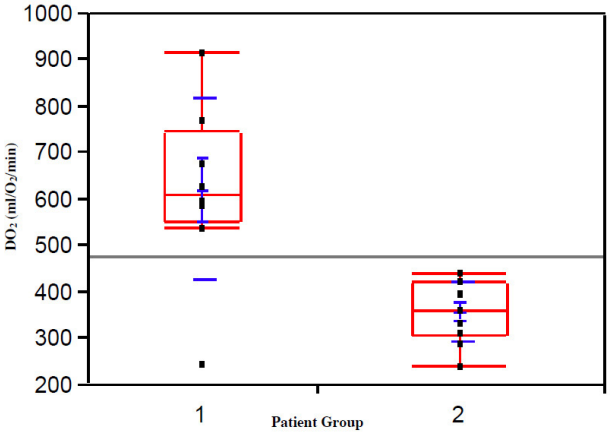
RESULTS

There were 9 self-reported, healthy African-American control subjects, and 21 SCD patients. The median age for the healthy controls was 29±6 years and the median age for the SCD patients was 34±11 years (Table 1). The majority of SCD patients were Hgb SS followed by Hgb SC (Table 1). The majority of the control subjects were male and there was a nearly even gender distribution in the SCD patients (Table 1). DO_2 and VO_2 measurements were measured for healthy control subjects and SCD patients at baseline. The DO_2 for the control subjects was 525.5 ml/O_2/min and 326.8 ml/O_2/min for the SCD group at baseline (Table 2). There were significant differences of DO_2 between the healthy control subjects *vs*. SCD patients at baseline (Figure 1 and Table 2). The VO_2 for the control group was 214.5 ml/min and 202.5 ml/min for the SCD group at baseline which showed no statistically significant differences in VO_2 (Table 2). Superficial and Deep $CtSO_2$ differences between the groups are shown in Figure 2 and Table 2. The median superficial $CtSO_2$ for the Control group and the SCD group was 72% (IQR=10.94) and 56% (IQR=26.86) respectively (p=0.0011). The median deep $CtSO_2$ (control *vs*. SCD baseline) was 73% (IQR=3.74) and 63% (IQR=12.1) respectively and was also significantly different (p=0.0033). Global measures of oxygen delivery (CO, CI, SV, SI and Hgb) were similar with no statistical differences existing between the groups except cardiac output (Table 3). Cardiac index and other global measures of cardiac function were not statistically significant therefore cardiac output was not interpreted to be clinically significant. We found no statistical difference in standard vital sign parameters (Blood Pressure, Heart Rate, Temperature, Respiratory Rate, and SpO_2) between healthy controls and sickle cell patients at baseline. There were significant differences in the OER between the Control group and the SCD group at baseline. The results of Regional Superficial OER were 26% and 42% respectively, with a p-value of 0.0013. The results of the Regional Deep OER were 25% and 34% respectively with a p-value of 0.0037 (Table 2).

Variable	Control Group	SCD Group
Age (years)	29±6	34±11
Hgb SS	Normal Hgb.	16
Hgb SC		4
Hgb Sβ-Thal		2
Gender (M/F)	7/2	11/11

SCD: Sickle Cell Disease; Hgb: Hemoglobin; Thal: Thalassemia.

Table 1: Demographics of control group *vs*. SCD group.



SCD: Sickle Cell Disease; DO_2: Delivery of Oxygen; PT Group: Patient Group.
*Significant at p-value<0.05; DO_2 p=<0.0001.

Figure 1: Oxygen Delivery of control group(1) (n=9) *vs*. SCD(2) (n=12) individuals.

DISCUSSION

The current study demonstrates that non-invasive oxygen transport monitoring is possible in SCD patients. Based on both $CtSO_2$ and VO_2 measurements in the SCD baseline state, it does not appear that SCD patients, despite the chronic nature of the disease, have down regulated their metabolism to compensate for this chronic decrease in DO_2. Due to the anemia that is commonly seen in SCD patients, despite full hemoglobin satura-

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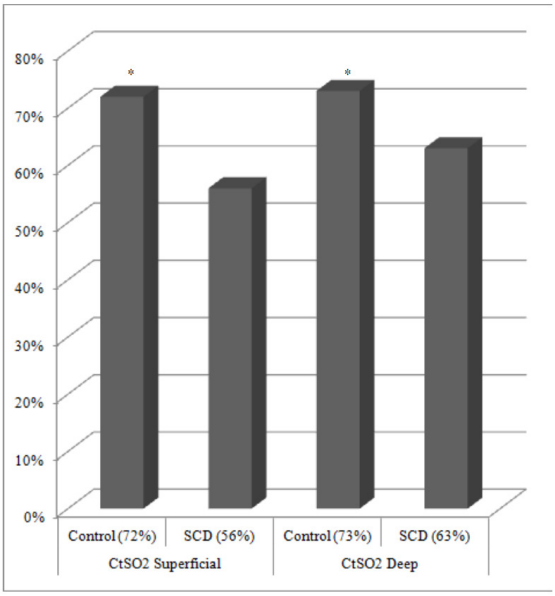
Variable	Control Group-Median (IQR)	SCD Group-Median (IQR)	P-Values
$CtSO_2$ Superficial (%)*	72%(10.94)	56%(26.86)	0.0011
$CtSO_2$ Deep (%)*	73%(3.74)	63%(12.1)	0.0033
VO_2 (ml/min)	214.5(143.3)	202.5(88.75)	0.6742
DO_2 (ml/O2/min)*	525.5(166.6)	326.8(239.3)	<0.0001
Regional Superficial OER (%)*	26%	42%	0.0013
Regional Deep OER (%)*	25%	34%	0.0037

*Significant at p-value<0.05.
SCD: Sickle Cell Disease; $CtSO_2$: Cutaneous Saturation of Oxygen; VO_2: Oxygen Consumption; DO_2: Delivery of Oxygen

Table 2: Comparison of Oxygen Delivery, Oxygen Consumption, Oxygen Extraction Ratio and Cutaneous Saturation of Oxygen.



*Significant at p-value<0.05; Superficial $CtSO_2$ p=0.0011; Deep $CtSO_2$ p=0.0033.
$CtSO_2$: Cutaneous Saturation of Oxygen; SCD: Sickle Cell Disease.

Figure 2: Superficial and deep $CtSO_2$: Control group (n=9) *vs.* SCD group (n=12).

Variable	Control Group-Median (IQR)	SCD Group-Median (IQR)	P-Values
CO (L/min)	7.20(3.21)	5.39(2.22)	0.0430
CI (L/min/mm)	3.34(1.06)	2.96(0.97)	0.0611
SV (ml/min)	97.49(28.23)	77.32(30.45)	0.7250
SI (ml/min/mm)	49.62(17.03)	41.87(19.30)	0.6560
Hgb	12(N/A)	9.4(2.8)	-

SCD: Sickle Cell Disease; CO: Cardiac Output; CI: Cardiac Index, SV: Stroke Volume; SI: Stroke Index; Hgb: Hemoglobin

Table 3: Comparison of global measures of oxygen delivery between control group and SCD group. *Significant at p-value<0.05. CI, SV and SI were not statistically significant, therefore CO was not interpreted to be clinically significant.

tion with oxygen, it is not surprising that their DO_2 is significantly lower than patients without SCD. What may not be as obvious is that there does not appear to be any significant increase in cardiac output to compensate for this reduction in hemoglobin content.

As discussed in the *Methodology* section of this paper, the values of $CtSO_2$ by differential absorption spectroscopy are representative of the venous hemoglobin oxygen saturation values, since venous blood dominates the majority of blood volume of the analyzed tissue. Thus, a measure of tissue hemoglobin oxygen saturation reflects the post-extraction compartment of tissue in terms of oxygen delivery and utilization. In our study, SCD patients compared to non-SCD controls exhibit evidence of increased regional oxygen extraction, even when not reporting a VOC. Patients in this cohort revealed that they have high extrac-

tion ratios at baseline. This decrease in microvascular delivery may in turn be caused by a combination of further rheologic and/or microvascular problems. Whether or not this can be termed "dysfunction" at the microvascular level is unclear, as this may represent appropriate compensation at this level. Given our data, sickle cell disease might be viewed as a sub-clinical compensated state of shock as defined by decreases in tissue oxygen delivery on a microcirculatory level.[15-18] Similar to other states of shock, regional oxygen transport changes are possible without changes in global oxygen transport or vital signs.

Although it is unlikely that the subcutaneous tissues were dysoxic or ischemic, changes in $CtSO_2$ measured at this level are consistent with homeostatic changes seen in organ systems that are at risk of damage by states of shock, such as the splanchnic bed.[19,20] Previous studies by Cheung, et al.[21,22] have demonstrated the ability to use surrogate sites, such as conjunctival vessels, as a marker of active VOC. The study however, did not look at global measurements of oxygen transport and compare them to regional measurements of oxygenation. The study instead focused on the use of intravital microscopy to objectively quantify conjunctival vessels in SCD patients at baseline, during crisis, and post crisis.

Diverting blood flow from cutaneous tissue beds to more essential organ systems, to maintain oxygen delivery, is a known event in hemorrhagic, cardiogenic and at several stages of septic shock.[23-25] With further investigative studies, the paradigm of SCD physiology may be shown to more closely resemble shock syndromes. The introduction of regional measurement techniques has highlighted the inadequacy of the information being garnered by global measurements of hemodynamic oxygenation such as DO_2, VO_2, and arterial hemoglobin oxygen saturation as well as traditional physical examination findings such as blood pressure and heart rate. Therefore, consideration should be given to emphasizing the underlying microcirculation,[26,27] as reflected in tissue oxygenation as both a diagnostic and therapeutic endpoint.

The pathophysiology of sickle cell is complex and involves many organ systems as a result of episodic microcirculatory insults which are believed to result in end-organ ischemic damage and pain.[28-30] Currently approved clinical tools are limited in their ability to detect localized changes in oxygen transport. The use of non-invasive tools will allow for increased understanding of microcirculatory oxygen delivery and utilization of SCD along with the many factors that are likely to impact it at this level in a clinical environment.[31,32] For example, one could envision using this type of monitoring to explore the impact of such interventions as vasodilators or blood substitutes on their ability to improve regional oxygen delivery and to correlate this with the outward manifestation of pain. The effects of treatment on these parameters were not measured; however, future studies should incorporate these in a temporal fashion.

CONCLUSION

SCD patients have decreased levels of $CtSO_2$ at baseline when compared to healthy controls suggesting an increased rate of oxygen extraction, which may be secondary to decreases in tissue oxygen delivery, as represented by the DO_2 values of both study populations. SCD may share microvasular similarities to compensated shock and can be measured. Novel non invasive techniques should be evaluated and may allow for further understanding of SCD microvasculature.

CONFLICTS OF INTEREST

The authors declare that they have no conflicts of interest.

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AUTHOR'S CONTRIBUTIONS

Dr. Imoigele P. Aisiku had full access to all the data in the study and had final responsibility for the decision to submit for publication.

Study Concept and Design: Imoigele P. Aisiku, Wally R. Smith, Lynn T. Penberthy and Kevin R. Ward.

Acquisition of Data: Imoigele P. Aisiku, Wally R. Smith and Kevin R. Ward.

Analysis and Interpretation of Data: Imoigele P. Aisiku, Wally R. Smith, Lynn T. Penberthy and Kevin R. Ward.

Drafting of Manuscript: Imoigele P. Aisiku, Osama R. Kandalaft, Wally R. Smith, Lynn T. Penberthy, Raghu R. Seethala, Peter C. Hou, and Kevin R. Ward.

Critical Review of Manuscript for Important Intellectual Content: Imoigele P. Aisiku1, Osama R. Kandalaft, Wally R. Smith, Lynn T. Penberthy, Raghu R. Seethala, Peter C. Hou, and Kevin R. Ward.

Statistical Analysis: Imoigele P. Aisiku, Wally R. Smith, Lynn T. Penberthy, and Kevin R. Ward.

Obtained Funding: Imoigele P. Aisiku, Wally R. Smith and Kevin R. Ward.

Administrative, Technical, or Material Support: Imoigele P. Aisiku, Osama R. Kandalaft, Wally R. Smith, Lynn T. Penberthy, Raghu R. Seethala, Peter C. Hou, and Kevin R. Ward.

Study Supervision: Imoigele P. Aisiku, Wally R. Smith and Kevin R. Ward.

CONSENT

No consent for publication is required as all patients signed a consent form to be part of the study and no identifying data is presented in the manuscript.

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