Published in final edited form as:

Hematology. 2009 February ; 14(1): 46–48. doi:10.1179/102453309X385133.

Near-infrared spectra absorbance of blood from sickle cell patients and normal individuals

M. Nahavandi¹, J. P. Nichols¹, M. Hassan², A. Gandjbakhche², and G. J. Kato¹

¹Pulmonary and Vascular Medicine Branch, NHLBI, Critical Care Medicine Department, NIH, Bethesda, MD, USA

²Medical Biophysics, National Institute of Child Health and Human Development, NIH, Bethesda, MD, USA

Abstract

Limited data are available regarding the physicochemical dynamics of tissue hypoxia in sickle cell disease. Studies using near-infrared spectroscopy (NIRS) have reported that patients with sickle cell disease (SCD) have lower cerebral oxygen saturation values (rSO₂) than normal individuals. The reason SCD patients have subnormal rSO₂ values is not known. It may be related to the degree of anaemia, sickle haemoglobin, disease complications and the possibility of SCD different NIRS absorbance spectra than normal. This study compared NIRS absorbance spectra of blood with adult haemoglobin AA, sickle haemoglobin SS, and AS. Venous blood was collected from SCD (SS and AS) and non-SCD patients (AA). Whole blood, cell free haemoglobin samples were scanned through the wavelength range of 600–1000 nm. The results showed no different NIRS spectra absorbance between the haemoglobin's AA, SS. It thus appears that lower brain oxygen saturation in sickle cell anaemia patients is related to impaired oxygen carrying capacity or delivery by sickle haemoglobin.

Keywords

Near-infrared spectroscopy; sickle cell disease; hemoglobin; cerebral oxygen saturation

Introduction

Vaso-occlusion involving both the microvasculature and large blood vessels is central to the pathophysiology of sickle cell disease (SCD). Vaso-occlusive crises are characterized by obstruction of the microcirculation, impaired tissue oxygen delivery, tissue hypoxia, ischemia, and infarction, which contribute to the morbidity and mortality of SCD.¹ All tissues may be damaged by oxygen deprivation, but when the brain is affected, serious neurological consequences may occur. It is well known that the brain has a high demand for oxygen and a low tolerance for oxygen deprivation in normal individuals, but there is limited information concerning the brain's oxygen supply and demand in SCD. Cerebral oximetry or near-infrared spectroscopy (NIRS) is a useful tool for the non-invasive monitoring of brain oxygenation. NIRS estimates the balance between the brain's oxygen delivery, demand and utilization employing dual-wavelength spectrophotometer at 733 and 809 nm that measures oxygen saturation of the mixed arterial and venous blood in the cerebral circulation. Oxygenated and deoxygenated haemoglobin and cytochrome have distinct absorption characteristics in the near

infrared spectrum.^{2–4} NIRS has been used for monitoring cerebral oxygenation in various clinical scenarios and in other organ such as muscle, liver and kidney.^{5–7} We previously reported the first use of NIRS to measure cerebral oxygen saturation (% rSO₂) in adult SCD patients and found that they had a significantly lower rSO₂ than normal individuals.⁸ Similar results were also found in children with SCD.⁹ The precise cause of the lower % rSO₂ is not known. This study compared the absorbance spectra of sickle haemoglobin with AA haemoglobin using NIRS to look for an explanation for SS patients' lower % rSO₂.

Materials and methods

After Institutional Review Board approval and informed consent were obtained, nine African-America subjects with homozygous SCD³ (SS), heterozygous sickle cell trait³ (AS) and healthy control subjects³ (AA) were enrolled in the study. All sickle cell subjects had their diagnosis of haemoglobin documented by haemoglobin electrophoresis or high performance liquid chromatography, and complete blood count data were measured in the clinical laboratory. The haemoglobin concentration (Hb g/dl and HbAA%, HbSS% and HbAS%) were obtained from each individual. Additional venous blood samples were collected in 5 ml EDTA tubes from each individual. A portion of whole blood samples were diluted in PBS (Hb=0.029–0.0447 g/dl). Plasma was separated from red blood cells by centrifugation (14,000 rev/min at 4°C for 2 min). Cell free haemoglobin was obtained by freezing red blood cells by liquid nitrogen and thawing at room temperature. Cell free haemoglobin was diluted in PBS (Hb= 0.3333 g/dl) (Table 1). Samples were scanned through the bottom of glass vials over the wavelength range of 600–1000 nm on spectrophotometer

Results

The patient number, the mean ($n=3$) of haemoglobin concentration g/dl in whole blood, diluted whole blood and red cell free hemoglobin (Hb) with HbAA%, HbSS% and HbAS% samples collected from patients are shown in Table 1. The mean ($n=3$) of NIRS spectra absorbance in diluted whole blood was lower in HbSS blood samples than HbAA and HbAS because of lower Hb concentration (0.029 versus 0.041 g/dl) (Fig. 1). The mean ($n=3$) of NIRS spectra absorbance in red cell free hemoglobin had no different in blood samples with HbAA, HbAS and HbSS because of similar haemoglobin concentration (0.333 g/dl) (Fig. 2).

Discussion

This study found no different in NIRS spectra absorbance in blood samples from patients with SCD and normal individuals. The result of this study is potentially important, because it helps to support the validity of the use of NIRS for detection of brain oxygenation in SCD patients, an area of considerable clinical importance. It also supports the previous reports using NIRS to measure brain oxygenation in patients with SCD patients^{8–10} and red blood cell transfusion and hydroxyurea therapy response in SCD patients.^{8,11,12} In our previous simple transfusion studies, we found a strong positive correlation of brain oxygen saturation with HbAA%, haematocrit and negatively with HbSS ($r=0.8$, $p<0.001$).⁸ This means that lower rSO₂ values in SCD patients mainly are related to impairment of oxygen delivery and/or carrying capacity of sickle blood which contributes to tissue hypoxia and ischemia. Additional complicating factors in SCD such as anaemia, decreased nitric oxide bioavailability, functional arteriovenous shunting, systemic hypoxemia, alterations in cerebral blood flow, haemoglobin oxygen affinity, red cell 2,3-diphosphoglycerate and brain metabolic rate might also influence on rSO₂ values in SCD patients. Further investigations are needed to clarify the relationship of rSO₂ with SCD complications. This finding further supports the potential use of cerebral oximetry by NIRS as a noninvasive and rapid method for assessing brain oxygen saturation and the effect of circulating normal red cells in sickle cell patients after therapies.

Acknowledgement

This research was supported by intramural funding from the National Heart, Lung and Blood Institute, National Institute of Child Health and Human Development, and the Clinical Center at the National Institute of Health, Bethesda, MD, USA.

References

1. Embury, SH.; Hebbel, RP.; Mohandas, N., et al. Sick cell disease: basic principles and clinical practice. New York: Raven Press; 1994.
2. Madsen PL, Wardle SP, William P, Weindling AM. Determination of cerebral functional oxygen extraction using near-infrared spectroscopy in preterm neonates. *J Cerebral Blood Flow Metab* 2000;20:272–279.
3. Nemoto EM, Yonas H, Kassam A. Cerebral oximetry: cerebral oxygen supply and demand. *J Neur Anesth* 1998;266:401–408.
4. Madsen PL, Secher NH. Near-infrared oximetry of the brain. *Prog Neurobiol* 1999;58:541–560. [PubMed: 10408656]
5. Pollard V, Prough DS, DeMelo AE, Deyo DJ, Uchida T, Widman R. Validation in therapeutic aphaeresis infrared spectroscope for monitoring brain oxygenation *in vivo*. *Anesth Analg* 1996;82:269–277. [PubMed: 8561326]
6. Madsen P, Pott F, Olsen B, Nielsen HB, Burcev I, Secher NH. Near-infrared specrophotometry determined brain oxygenation during fainting. *Acta Physiol Scand* 1998;162:501–507. [PubMed: 9597118]
7. Skak C, Rasmussen A, Kirkegarrrd P, Secher NH. Cerebral oxygen saturation and blood flow during liver transplantation. *Anesth Analg* 1997;84:730–733. [PubMed: 9085947]
8. Nahavandi M, Tavakkoli F, Hasan SP, Wyche MQ, Castro O. Cerebral oximetry in patients with sickle cell disease. *Eur J Clinic Invest* 2004;34:143–148.
9. Raj A, Bertolone SJ, Mangold S, Edmonds HL Jr. Assessment of cerebral tissue oxygenation in patients with sickle cell disease: effect of transfusion therapy. *J Pediatr Hematol Oncol* 2004;26:312–314. [PubMed: 15111785]
10. Raj A, O'Brien LM, Bestroiones SJ, Gozal D, Edmonds HL Jr. Cerebral oximetry improves detection of sickle cell patients at risk for nocturnal cerebral hypoxia. *Pediatr Pulmonol* 2006;41:1088–1094. [PubMed: 16998929]
11. Nahavandi M, Tavakkoli F, Wyche MQ, Castro O. Effects of Hydroxyurea treatment on cerebral oxygenation in adult patients with sickle cell disease: an open-label pilot study. *Clin Ther* 2005;27:1083–1088. [PubMed: 16154487]
12. Nahavandi M, Tavakkoli F, Wyche MQ, Trouth AJ, Tavakkoli N, Perlin E. Effect of transfusion on cerebral oxygenation, flow velocity in a patient with sickle cell anemia and moyamoya disease: a case report. *Hematology* 2006;11(56):379–381.

Figure 1.

The mean ($n=3$) near-infrared spectra absorbance diluted of whole blood samples with oxyhaemoglobin SS and AS and AA. HbSS blood samples had lower NIRS spectra absorbance than HbAA and HbAS because HbSS had lower Hb concentration (0.029 versus 0.41 g/dl)

Figure 2.

The means ($n=3$) of NIRS spectra absorbance ($n=3$) in diluted red cell free oxyhemoglobin (Hb=0.333 g/dl) SS, AS and AA. There was no different spectra absorbance among HbSS and HbAS and HbAA

Mean (*n*=9) of oxygenated haemoglobin concentration (Hb g/dl) in whole blood, in diluted whole blood and diluted red cell free haemoglobin, HbAA%, HbSS% and HbAS% in blood samples from the African-American patients with sickle cell anaemia (SCD) and without SCD. Hb concentration in SS patients was lower than AA and AS patients

Table 1

Patient no.	Hb (g/dl)	(WB) Hb (g/dl)	Red cell free Hb (g/dl)	HbAA%	HbSS%
1	8.7	0.029	0.333	5.700	81.600
2	10.800	0.036	0.333	18.000	76.000
3	10.200	0.034	0.333	17.000	79.000
Mean	9.900	0.033	0.333	14.567	78.300
4	12.500	0.042	0.333	63.000	32.000
5	13.200	0.044	0.333	58.000	37.000
6	11.600	0.039	0.333	61.000	34.000
Mean	12.433	0.041	0.333	60.667	34.333
7	12.500	0.042	0.333	93.000	0.000
8	11.700	0.039	0.333	94.700	0.000
9	13.700	0.046	0.333	95.000	0.000
Mean	12.633	0.042	0.333	94.23	0.000

Hb: haemoglobin, WB: whole blood.