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Research Paper

Fetal Hemoglobin is Associated with Peripheral Oxygen Saturation in Sickle Cell Disease in Tanzania

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ABSTRACT

Fetal hemoglobin (HbF) and peripheral hemoglobin oxygen saturation (SpO_2) both predict clinical severity in sickle cell disease (SCD), while reticulocytosis is associated with vasculopathy, but there are few data on mechanisms. HbF, SpO_2 and routine clinical and laboratory measures were available in a Tanzanian cohort of 1175 SCD individuals aged ≥ 5 years and the association with SpO_2 (as response variable transformed to a Poisson distribution) was assessed by negative binomial model with age and sex as covariates. Increase in HbF was associated with increased SpO_2 (rate ratio, RR = 1.19; 95% confidence intervals [CI] 1.04, 1.37 per natural log unit of HbF; p = 0.0004). In univariable analysis, SpO_2 was inversely associated with age, reticulocyte count, and log (total bilirubin) and directly with pulse, SBP, hemoglobin, and log(HbF). In multivariable regression log(HbF) (RR 1.191; 95%CI 1.04, 1.37; p = 0.013), pulse (RR 1.01; 95%CI 1.00, 1.01; p = 0.026), SBP (RR 1.008; 95%CI 1.00, 1.02; p = 0.014), and hemoglobin (1.120; 95%CI 1.05, 1.19; p = 0.001) were positively and independently associated with SpO_2 while reticulocyte count (RR 0.985; 95%CI 0.97, 0.99; p = 0.019) was independently inversely associated with SpO_2. In SCD, improving SpO_2, in part through cardiovascular compensation and associated with reduced reticulocytosis, may be a mechanism by which HbF reduces disease severity.

1. Introduction

Sickle cell disease (SCD) remains the most common hemoglobinopathy worldwide. Clinically, there is great variability amongst individuals with SCD; predictors of severity include hemoglobin F levels, reticulocytosis and alpha globin gene number. A major factor is the wide variation in the innate ability to synthesize fetal hemoglobin (HbF) beyond early childhood. Individuals with high levels of HbF experience milder forms of the disease with lower morbidity and improved survival. HbF level of 10% and above is believed to reduce the risk of major organ failure such as stroke, while much higher levels (20% and above) may be required to prevent recurrent clinical events such as painful crises and pulmonary disorder (Meier et al., 2017). The mechanisms underlying the reduction in the severity of SCD in people with high HbF are not clear. Therefore, studying the associations of HbF and clinical phenotypes of SCD may provide insights into the underlying mechanisms.

Peripheral hemoglobin oxygen saturation (SpO_2), measured non-invasively by pulse oximetry, is related to several disease complications. Lower SpO_2 has been associated with anemia (Quinn and Ahmad, 2005), increase in reticulocytes (Quinn and Ahmad, 2005), hemolysis (Campbell et al., 2009) and increased episodes of acute chest syndrome (Rackoff et al., 1993) and appears to predict central nervous system complications (including stroke (Quinn and Sargent, 2008), higher transcranial doppler velocity (Quinn et al., 2009), number of days per year admitted for pain (Hargrave et al., 2003), tricuspid regurgitant jet velocity (TRV) (Minniti et al., 2009) and diastolic dysfunction (Johnson et al., 2010) in SCD.

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Studies in fetuses, children with cyanotic heart disease and adults ascending to high altitude suggest that HbF synthesis increases in hypoxia (Bard et al., 1994). Furthermore, hemolysis may induce HbF synthesis further (Desimone et al., 1978). There is limited information on the magnitude and direction of any association between HbF and SpO_2 in patients with SCD not on hydroxyurea, but the available data suggest that, as for neonates (Shiao, 2005), SpO_2 is higher in SCD patients with higher HbF (Homi et al., 1997), (Halphen et al., 2014, Cox et al., 2013). In this report, we describe the association between HbF and SpO_2 in individuals with SCD enrolled in the Muhimbili National Hospital cohort in Tanzania.

2. Materials and Methods

2.1. Study Area and Population

This was a cross sectional study conducted at Muhimbili National Hospital (MNH) in Dar-es-Salaam, Tanzania involving individuals with SCD recruited into the Muhimbili Sickle cohort between March 2004 and December 2013. Recruitment and enrolment of patients and diagnosis of SCD has been previously described (Makani et al., 2011). Informed consent was obtained for each patient upon enrolment. Individuals were identified at pediatric SCD or hematology clinics or during hospitalization and were screened for SCD. A diagnosis of sickle cell anemia (HbSS/HbSβ^0) by alkaline hemoglobin electrophoresis (Helena, Sunderland, Tyne & Wear, UK) was confirmed by high performance liquid chromatography (HPLC) (Variant I analyzer, Bio-Rad, Hercules, CA, USA). Ethical approval was granted by the Muhimbili University Research and Publications Committee (MU/RP/AEC/VOLX1/33).

Individuals were selected into this study if they had HbF values measured at the age of five years or above, since this is the age at which HbF synthesis stabilises. Data were excluded if the patient was on hydroxyurea therapy.

2.2. Clinical Measures

Daytime SpO_2 was determined in clinic when the child was well using a pulse oximeter (Nellcor, Pleasanton, CA, USA). Other clinical information that was collected included pulse rate, systolic blood pressure (SBP), and diastolic blood pressure (DBP).

2.3. Laboratory Measures

Hemoglobin was measured by an automated blood cell analyser (ABX Pentra 60 Analyser, Horiba, Kyoto, Japan) and reticulocytes were counted using the new methylene blue staining method followed by microscopy. HbF measurements were done by HPLC (Variant I, Biorad, Hercules, CA, USA). Routine biochemical analysis included total bilirubin (Roche Cobas Mira, New York, USA or Abbott Architect, New York, USA).

2.4. Statistical Methods

The SpO_2 data were collected as counts, which ranged from 82 to 100%, and could not be transformed into a normal random distribution, and hence a Poisson random distribution was assumed after transformation. To convert the distribution of SpO_2 (Fig. 1A) into a Poisson distribution, 100-SpO_2 transformation was performed, Fig. 1B. The distributions of HbF and bilirubin were positively skewed and hence normalized by natural log transformation. The association of clinical and laboratory variables with SpO_2 (as response variable) was assessed by negative binomial model with age and sex included as covariates, presenting the results as rate ratio (RR) with 95% confidence intervals. A p-value < 0.05 was considered statistically significant. Variables with significant associations with SpO_2 in the univariate analysis were included in the multivariable regression analysis.

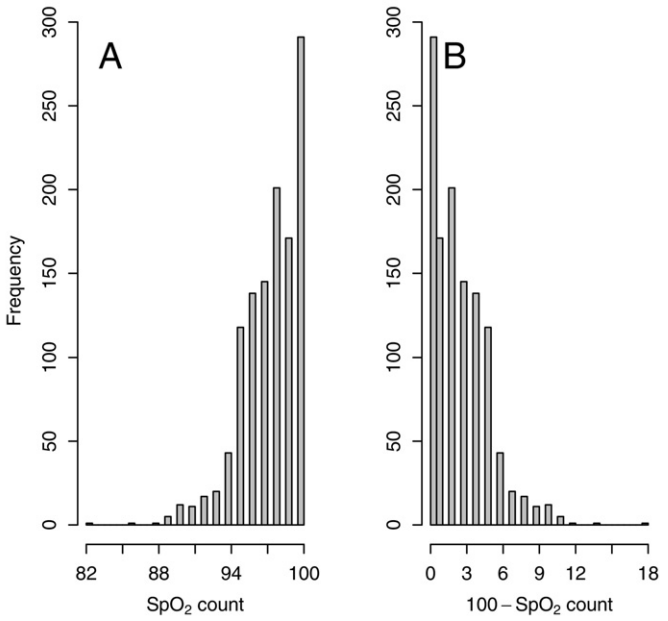

Fig. 1. Distribution of SpO_2 before (A) and after (B) transformation. Conversion of the distribution of SpO_2 (Fig. 1A) into a Poisson distribution, 100-SpO_2 transformation was performed resulting in the distribution shown in Fig. 1B.

3. Results

We investigated 1175 individuals (52.1% female, median age 11.2 [IQR: 7.9–16.7] years) with both HbF and SpO_2 measurements. Median HbF was 4.4% [IQR: 2.4–7.2] and median SpO_2 was 98% [IQR: 96–99]. Table 1 shows the results for the univariable and multivariable analyses. The distribution of HbF in relation to SpO_2 is presented in Fig. 2; which shows the increase in oxygen saturation by one unit is associated with increase in mean of log(HbF) by 0.031 (95%CI: 0.013, 0.049), $p = 0.020$) while other variables are held constant (Table 1). In univariable analysis, SpO_2 was directly associated with pulse rate, systolic blood pressure (SBP), hemoglobin, and log(HbF) and inversely with age, reticulocyte count, and log (total bilirubin) (Table 1). In multivariable regression, log(HbF), pulse rate, SBP, and hemoglobin were positively and independently associated with SpO_2, while reticulocyte count was inversely and independently associated with SpO_2 (Table 1).

4. Discussion

This study reports the association of HbF with SpO_2 in individuals with SCD. The study involves one of the largest single-centre SCD population to date and the first study to be conducted in an African population where the environment is different. This population is composed of a Central African Republic (CAR) sickle haplotype of predominatly β^S/β^S genotype, a genetic background associated with a more severe disease. In addition, the population under study is hydroxyurea naïve and there are limited resources for interventions. These factors make it pertinent for the study to be conducted in this population in order to identify disease-modifying factors that may be amenable to treatment. We have confirmed the association between HbF and SpO_2 (Homi et al., 1997). Both HbF and total hemoglobin (Hb) were associated with SpO_2 independently suggesting that there may be separate mechanisms by which increasing Hb and HbF improve SpO_2. The association between HbF and SpO_2 should augment efforts to develop and evaluate interventions that increase both HbF and SpO_2. Although the mechanism of this association is not known, the oxygen dissociation curve properties for HbS and HbF are different. Therefore, the decreased oxygen affinity of hemoglobin S (Ueda et al., 1979) may partially contribute to the low SpO_2 in SCD. On the other hand, higher levels of

Table 1
Association between SpO2(%) and clinical and laboratory parameters in sickle cell disease.

Parameters	SpO2 (%) univariable				SpO2 (%), multivariable, N = 632		
	N	RR	95%CI	P	RR	95%CI	P
Age (years)	1175	0.99	0.89, 0.99	**0.024**	0.99	0.98, 1.01	0.422
Sex	1175	0.93	0.83, 1.04	0.191	0.91	0.78, 1.07	0.265
Pulse rate (beats/min)	1170	1.00	1.00, 1.01	**0.020**	**1.01**	**1.00, 1.01**	**0.026**
Systolic blood pressure (mm Hg)	1167	1.01	1.00, 1.01	**0.009**	**1.008**	**1.00, 1.02**	**0.014**
Log(HbF [%])	1175	1.19	1.08, 1.31	**0.0004**	**1.191**	**1.04, 1.37**	**0.013**
Hemoglobin (g/dL)	1136	1.07	1.07, 1.12	**0.001**	**1.120**	**1.05, 1.19**	**<0.001**
Reticulocyte count ($\times 10^9$/L)	661	0.99	0.98, 1.00	**0.043**	**0.985**	**0.97, 0.99**	**0.019**
Log(Bilirubin total [mg/dl])	228	0.65	0.54, 0.77	**<0.001**			

Bolded values represent significant associations with p values of less than 0.05.

HbF, which has a higher affinity for oxygen, results in a higher SpO_2, in neonates (Shiao, 2005). In addition, compounds, including an aromatic aldehyde agent, 5-hydoxymethyl-2-furfural (5-HMF, also known as Aes-103), has been found to increase oxygen affinity of sickle hemoglobin and as a result reducing hypoxia-induced sickling *in vitro* and protects sickle cell mice from the effects of hypoxia (Safo and Kato, 2014).

Pulse rate, SBP and hemoglobin were also positively associated with SpO_2 in multivariable analysis. The direct association of hemoglobin with SpO_2 has been described previously and hence confirmed in the population that we have studied. The direct association of pulse rate and SBP suggests that one of the mechanisms for maintaining an adequate SpO_2 is cardiovascular compensation. The HbF levels for this population are low compared to other populations with different sickle haplotypes; despite this, an association with SpO2 was established and can be further examined when interventions to increase HbF are in place. Treatment trials could examine whether improving SpO_2, with agents that increase HbF or other methods of improving oxygenation, reduce SBP, a risk factor for stroke in adults and children with SCA.

An association between increased hemolysis and low SpO_2 has been reported (Nouraie et al., 2013). In this study, we report an inverse association between SpO_2 and total bilirubin and reticulocyte count, which are markers of hemolysis, on univariate analysis, suggesting higher SpO_2 in the presence of lower hemolysis or conversely, increased hemolysis if SpO_2 is lower. The effect of therapies designed to improve SpO_2 or reduce hemolysis may determine whether the initiating mechanism for the association involves either low SpO_2 or hemolysis. However, total bilirubin may also reflect liver compromise and reticulocytosis may be related to the response of the bone marrow to non-hemolytic anemia. On multivariable analysis, the reticulocyte count was independently associated with SpO_2, which suggests an effect on erythropoiesis which may or may not involve hemolysis. We could not investigate the relative importance of hemolysis further as we only had total bilirubin for one fifth of the patients; future studies should include measurement of indirect bilirubin, as a more specific marker of hemolysis, in all patients.

This study reports the association of HbF with SpO_2two variables with strong clinical significance in individuals with SCD. The underlying mechanism of this association and the optimal range for HbF, measures cardiac function such as blood pressure and pulse, and SpO_2 for good health in SCD needs to be established. This information will aid in the development and improvement of HbF-augmenting agents. The findings from this study may be applied to other SCD populations that may be similar.

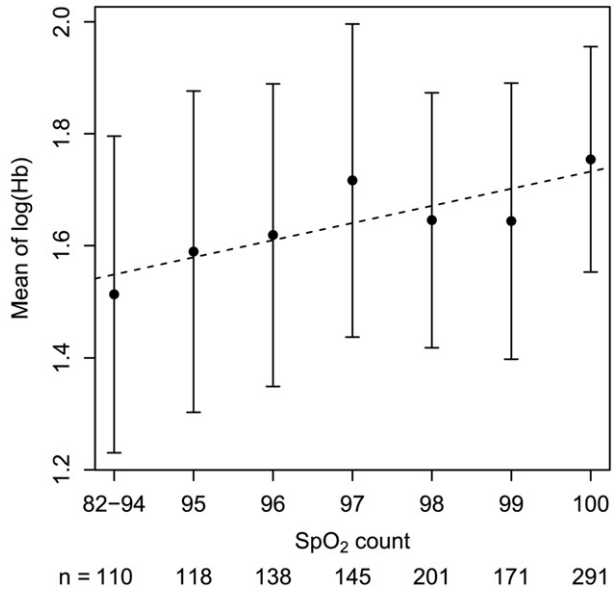

Fig. 2. Distribution of mean log(HbF) levels by oxygen saturation. The dotted line show the linear fit of the mean levels, which shows positive association with the increase with the oxy sat. The increase in oxy sat by one unit is associated with increase in mean of log(HbF) by 0.031 (95%CI: 0.013, 0.049), p = 0.020.

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Conflict of Interest

The authors declare no competing financial or other interests.

Author Contributions

J. Makani, F.J.K, B.P. M and S.N.M. designed the study. J.Mgaya, collected the data. B.P.M. performed the analysis. S.N.M, J.Makani, B.P.M, S.C., C.R.N. and F.J.K wrote the manuscript and all authors commented on the drafts of the manuscript.

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