Available online at http://www.ajol.info

Int. J. Biol. Chem. Sci. 1(3): 287-292, 2007

ISSN 1991-8631

International Journal of Biological and Chemical Sciences

http://indexmedicus.afro.who.int

The moderating effect of haemoglobin A_2 on morbidity in sickle cell anaemia patients

Caroline Edijana OMOTI [1*] and Casimir Erhunmwun OMUEMU [2]

[1] *Department of Haematology, University of Benin Teaching Hospital, P.M.B. 1111, Benin City, Nigeria.*
[2] *Department of Medicine, University of Benin Teaching Hospital, P.M.B. 1111, Benin City, Nigeria.*
[*] *Corresponding author, Phone: 2348056014028, E mail: ediomoti@yahoo.com*

ABSTRACT

The presence of high blood levels of haemoglobin A_2 (HbA_2) has been noted in sickle cell anaemia (SCA) patients. This study was designed to determine if there is a relationship between high HbA_2 levels and a reduction in the occurrence of vaso-occlusive crisis, complications and erythrocyte transfusion requirements in these patients. HbA_2 levels were determined in 330 patients (mean age was 23.69±10.94 years) who presented consecutively to the Haematology clinics of the University of Benin Teaching Hospital, Edo State Central Hospital and Sickle Cell Center in Benin City, Nigeria. Of these, 200 (81.3%) were SCA patients in steady state and 46 (18.7%) having vaso-occlusive crisis, and 84 age and sex matched subjects served as controls. An automated Coulter Counter was used to determine blood count values while the haemoglobin A_2 was estimated by HbS-free microcolumn chromatography. The mean HbA_2 in steady state, vaso-occlusive crisis and control were 4.52% ±1.16, 3.82% ±1.27 and 2.13% ±0.98 respectively. HbA_2 was significantly higher in steady state than during vaso-occlusive crisis ($P<0.01$). Erythrocyte transfusion requirement and occurrence of complications were significantly lower with higher HbA_2 value ($P=0.042$ and $P=0.038$ respectively). High HbA_2 levels are associated with lower morbidity in sickle cell anaemia patients. Treatment strategies, which increase HbA_2, may be beneficial in managing SCA.

Keywords: Haemoglobin A_2; sickle cell anaemia; morbidity.

INTRODUCTION

Sickle cell disease (SCD) is endemic in Nigeria with sickle cell anaemia (SCA) being the commonest genetic disorder of the Black race (Adeodu et al., 2000). It is characterized by a predominance of abnormal haemoglobin S (HbS) that damages and deforms the red blood cells. The disease accounts for over 60% of the world's major haemoglobinopathies and an estimated 2-3 million individuals are affected in Nigeria (Olatunji, 2003).

Normal adult haemoglobin is composed of two *alpha* and two *beta* chains ($\alpha_2\beta_2$). Haemoglobin A_2 (HbA_2) is a minor component of adult haemoglobin characterized by substitution of *delta* (δ) chains for the beta chains and is designated as $\alpha_2\delta_2$ (Ranney et al., 1993). This is similar to the substitution of *gamma* (γ) chains for *beta* chains as occurs in haemoglobin F (HbF) (Schilir'o et al., 1992). The HbA_2 acts as a predominant oxygen deliverer and has two more important features: 1) it is more resistant to thermal denaturation than HbA and 2) it inhibits the polymerization of deoxysickle haemoglobin (Sen et al., 2004).

Trace amounts of HbA_2 are present at birth and rapidly increase to reach near-adult levels by the age of 6 months. It has been reported that HbA_2 concentration in blood is high in various congenital haemolytic disorders but its significance remains unclear (Ranney et al., 1993). Co-inheritance of S-β thalassaemia with a high HbA_2 level results in mild outcome. Similarly, the presence of high levels of HbF may improve morbidity and

mortality and quality of life in SCA (Prower and Weiss, 1984).

It is believed that purposeful alteration of certain red cell parameters may improve the haematological and biological characteristics of SCA patients and consequently improve the clinical course of the disease (Prower and Weiss, 1984). Work has been done on the value of increasing intracorpuscular HbF concentration in SCA patients (Ranney et al., 1993; Omoti, 2005). In contrast, there is a dearth of knowledge regarding the level of intracorpuscular HbA_2 concentration and its relationship to the progression SCA (Ranney et al., 1993). Haemoglobin A_2 as well as foetal haemoglobin can therefore be considered important analytes in the management and follow-up of haemoglobinopathies.

This study was aimed at determining if there is a relationship between high HbA_2 levels and a reduction in the occurrence of vaso-occlusive crisis, complications and erythrocyte transfusion requirements in SCA patients.

MATERIALS AND METHODS

All persons who presented consecutively to the Haematology clinics and Emergency units of the University of Benin Teaching Hospital (UBTH), Edo State Central Hospital and Sickle Cell Center in Benin City, Nigeria between 2002 and 2003 were considered for inclusion in the study. All known SCA patients and those who presented for testing for SCD were included. Subjects with disorders known to affect the haematological values such as renal disease, those who had erythrocyte transfusion within the preceding three months and pregnant patients were excluded from the study.

A total of 330 subjects were recruited into the study. Ethical approval was granted by the Ethical committee of the University of Benin Teaching hospital. Informed consent was obtained from the patients. This study group comprised 200 SCA patients in their steady state (Serjeant et al., 1973), 46 SCA patients in vaso-occlusive crisis (VOC) and 84 age and sex matched persons declared normal after voluntary testing for SCD in the above clinical centers.

In this study, VOC was defined as episodic aches and pains in any part of the body necessitating the use of analgesics, other medical intervention with or without hospitalization after other treatable disorders have been diagnosed and excluded (Charache, 1974). Other tests done to exclude infections were hepatitis BsAg, HCV and HIV 1 and 2 and malarial parasite test. Their requirement for erythrocyte transfusion was based on haemoglobin/haematocrit level below their steady state baseline value and decompensation with features of circulatory collapse.

Information on the demographic profile, the frequency of VOC, number of erythrocyte transfusions and complications of SCA sustained by the patients in the preceding one year was sought using a self-administered questionnaire. Blood samples collected for haematological parameters were analyzed using the automated COULTER® A^C T diff^{TM} Analyzer (1997 model) (Abacus Junior Human, Haematology analyzer, serial no. 588, version ABJ 1.8). Measurement of HbA_2 was done by the chromatographic-spectrophotometric ion-exchange-HbS interference free technique using the Biosystem^R S.A. kit with ISO 9001 certificate registration no. 091006696.

HbA_2 percentage was calculated using the formula (Dacie and Lewis., 1994).

$$\% HbA_2 = \frac{A^{415} HbA_2}{A^{415} HbA_2 + 3 \times A^{415} HbA_O} \times 100$$

A^{415} = Absorbance at 415nm
HbA_0 = Total haemoglobin

The data obtained was analyzed using Instat GraphPad^{Tm} version 2.05a software.

The haemoglobin A_2 (HbA_2) level and erythrocyte transfusion requirement in patients in the steady state, VOC and controls were compared using One-way analysis of variance (ANOVA) and Chi square test. P-value of ≤ 0.05 was considered significant.

RESULTS

A total of 246 SCA patients who met the inclusion criteria, and comprising of 200 (81.3%) subjects in steady state and 46 (18.7%) in VOC state were included in the study. This consisted of 116 males (47.2%) and 130 females (52.8%). The mean age was 23.69 years (S.D±10.94) with ages ranging from 5 to 70 years. The age and sex distribution is shown in figure 1.

The mean haemoglobin A_2 (HbA_2) levels of the patients in steady state, VOC and controls were 4.52%±1.16, 3.82%±1.27 and 2.13%±0.98 respectively. The HbA_2 level in steady state was significantly higher than VOC ($P<0.01$) and control ($P<0.01$). The HbA_2 level in SCA patients during VOC was also significantly higher than in the controls ($P<0.01$).

The mean haemoglobin (Hb) levels in steady state, VOC and controls were 7.54g/dl ±2.26, 7.77g/dl ±2.25 and 12.93g/dl ±2.22 respectively (ranges; 4-11g/dl, 5-10.8g/dl and 12-18g/dl respectively). There was no significant difference between Hb of subjects in steady state and during VOC ($P>0.05$). However, the Hb of subjects in steady state and during VOC was significantly less than in control ($P<0.01$).

The relationship between the mean percentage of HbA_2 and the frequency of VOC, erythrocyte transfusions and complications experienced by SCA patients in the preceding year is displayed in table 1. The mean HbA_2 level was significantly higher in those without crisis than in those who had crisis ($P=0.0034$). The mean number of painful crisis experienced was 0.57±0.85. A total of 200 (81.3%) patients did not have any crisis in the preceding one year while the highest number of crisis, 5 in this study was seen in only one patient (0.4%).

Regarding erythrocyte transfusions, the 37 (15%) patients who had need of erythrocyte transfusion had a significantly lower HbA_2 level when compared with patients (85%) who had no erythrocyte

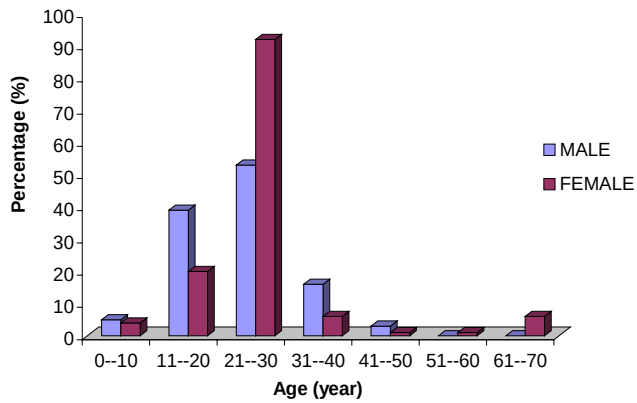

Figure 1: Age and sex distribution of sickle cell anaemia patients

Table 1: Relationship between mean percentage HbA_2 level and painful crisis, blood transfusions and complications.

Clinical outcome	HbA_2 Mean±SD (%)	Number of Patients (%)	P value
Painful crisis			
Present	2.48±0.78	46 (18.7)	
Absent	2.90±0.96	200 (81.3)	0.0034
Erythrocyte transfusion			
Yes	1.43±0.50	37 (15.0)	
No	1.62±0.65	209 (85.0)	0.035
Complications			
Present	2.26±0.64	57 (23.2)	
Absent	2.80±0.81	189 (76.8)	<0.0001

transfusion (P=0.035). However, there was no significant difference between HbA_2 level for those requiring one erythrocyte transfusion and those requiring more erythrocyte transfusions.

Fifty-seven patients (23.2%) had various complications of SCA while 189 (76.8%) patients had none. The mean HbA_2 level for those without complications (2.80±0.81) was significantly higher than the level in those with complications (P<0.0001). The most common complications seen were cholelithiasis (5.3%), leg ulcer (3.7%), osteomyelitis (3.3%), and renal disease (3.3%), while the least common was kyphosis (0.8%).

DISCUSSION

The mean age of the study subjects was 23.69±10.9 years with a peak incidence in the 21-30 year age group. This is similar to the finding in a recent study (Buseri et al.,2006). This is high because the study was carried out in adult clinics. This is because only adult patients attend the haematology clinics while children attend paediatric clinics. Furthermore, haematologists in Nigeria are only trained to handle adult patients. Life expectancy of SCA patients is reported to be short (Leikin et al., 1989; Wierenga et al., 2001). A review of age and sex distribution in this study revealed more females (52.8%) and only females were seen above the age of 50years. This is in agreement with findings that females with SCA may have a longer life expectancy of 48-58years than males whose life expectancy is 42-53 (Wierenga et al., 2001).

The mean haemoglobin (Hb) values in steady state, VOC and controls were similar to previous studies (Leslie et al., 1975; Serjeant, 1974; Osaghae, 1987). The haemoglobin values in steady state and VOC were significantly lower than control (P<0.01). Although the Hb/Hct values during VOC were higher than during the steady state period, the difference was not significant (P>0.05). This may be due to the fact that the anaemia created a form of erythropoietic stress, which caused a physiological increase in the erythropoietin level (Osaghae, 1987). The paradox is found in the results for the controls where the mean Hb value of 12.9g/dl recorded was lower as against Ezeilo's report of 15.6g/dl (Ezeilo.,1971). This could be as a result of poor nutrition and other related factors. Understanding causes of anaemia and potential mechanisms are crucial to our ability to intervene to reduce this burden (Tolentino and Friedman, 2007).

The presence of HbA_2 in erythrocytes is known to have a protective effect against damage in SCA by reducing the minimum gelling concentration of haemoglobin S (Nagel et al., 1979). The mean HbA_2 level in steady state, VOC and controls were similar to those found in the study by Hall and Malia (1984) who reported a mean HbA_2 of 5% with a range of 3.6-7.0%. Similar findings of 3.48%±0.50 for males and 3.38%±0.62 for females were reported by Serjeant et al. (1981). The mean HbA_2 levels in steady state and during VOC were significantly higher than in controls (P=0.01). This is in agreement with previous studies (Craver et al., 1997). The HbA_2 value in the steady state was significantly higher than during VOC (P<0.01) giving an insight into the probable protective effect of HbA_2. It is thought that HbA_2 may have a protective effect against complications in SCA patients through its physiological functions.

The most common complication observed was cholelithiasis which is due to the chronic haemolysis resulting in excess pigment in bile (Macsween, 1985). Leg ulceration occurring in 3.7% was slightly lower than previous reports of 5.4-9.6% (Konotey-Ahulu, 1974; Durosinmi et al., 1991). It was found that patients with complications had significantly lower HbA_2 levels than patients without complications (P<0.0001).

The pain rate (number of episodes per year) had been used previously for the purpose of analysis. Unfortunately, it is subject to error as the patients may not remember the required information and give false estimates (Platt et al., 1991). To limit this error the number of painful crisis of sufficient severity that required strong analgesics at home or which the patient considered to be typical of pain crisis with or without hospital admission was used. It was discovered that significantly higher HbA2 values (p=0.0034)

were found in patients who had no painful crisis during the study period.

Interestingly, all the patients (100%) who had no blood transfusions had significantly higher HbA_2 levels compared to patients who had blood transfusions (2-6) (P=0.035). Those who had need of 3-6 blood transfusions occurred when complications arose, e.g. leg ulcer, priapism etc. All the patients who had more than one blood transfusion in the preceding year actually had HbA_2 values less than 2%. These results support previous reports that high HbA_2 levels may improve prognosis in SCA (Craver et al., 1997). Also, the percentage of HbA_2 may be a strong predictor of a patient's need for hospitalization or blood transfusion, with patients with lower HbA_2 being more likely to be hospitalized (Craver et al., 1997).

In conclusion, SCA patients had significantly lower HbA_2 levels during VOC than in steady state. Erythrocyte transfusion requirements and occurrence of complications were higher in SCA patients with lower HbA_2 levels. Hence, strategies to increase HbA_2 levels would be beneficial in reducing the morbidity outcome in SCA subjects. A HbA_2 level that is protective and beneficial in SCA subjects as well as its possible drawbacks will need further research.

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