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Sicklable Red Cells as Surrogates for Haemoglobin-S in Patients with Sickle Cell Anaemia Undergoing Exchange Blood Transfusion

¹S.G. Ahmed, ²U.A. Ibrahim and ²A.W. Hassan

¹Department of Haematology,

²Department of Surgery, College of Medical Sciences,
University of Maiduguri, P.M.B. 1069,
Maiduguri, Borno State, Nigeria

³Department of Paediatrics, University of Maiduguri Teaching Hospital,
P.M.B. 1414, Maiduguri, Borno State, Nigeria

Abstract: This study was conducted to determine the potential usefulness of sicklable red cells as surrogate markers for Hb S in monitoring Exchange Blood Transfusion (EBT) in Sickle Cell Anaemia (SCA) patients in resource limited health institutions. A total of 5 patients with SCA who had EBT at the University of Maiduguri Teaching Hospital, Nigeria, were monitored by serial densitometric Hb S quantitation as well as estimation of the proportion of sicklable red cells after each cycle of EBT. The proportion of sicklable red cells were estimated with the use of microscope (x40 objective) from glass slide preparations for sickling test made by standard technique using dithionite. The mean values of Hb S levels and proportions of sicklable red cells that were obtained before the EBT (pre-EBT) and after each cycle of EBT from all the five patients were analyzed using SPSS version 11.0 to determine the coefficient of correlation. The mean levels of Hb S and proportions of sicklable red cells fell in parallel from pre-EBT values of 93 and 100% to 17 and 19% at the end of the 5th cycle, respectively. The proportion of sicklable red cells fell to a target level of less than 20% after cycle-5, by which time the Hb S level was even much lower than 20%. Linear regression analysis between the mean values of Hb S and sicklable red cells revealed a strong positive correlation coefficient, $r = +0.992$ ($p = 0.01$). Hence, sicklable red cells can be used as useful and safe surrogate markers for Hb S in the monitoring of SCA patients on EBT. This may be an attractive model for resource limited health institutions that manage SCA but lacks modern Hb quantitation facilities.

Key words: Sickle cell, haemoglobin-S, exchange blood transfusion

INTRODUCTION

The beneficial effect of Exchange Blood Transfusion (EBT) in Sickle Cell Anaemia (SCA) is due to improvement of microvascular perfusion as a result of decrease in the proportion of haemoglobin-S containing red cells in the circulation (Weatherall, 2001). EBT for SCA can be undertaken electively in preparation of patients for major surgical operation with the aim of preventing the risk of anaesthetic mishaps (Weatherall, 2001). Similarly, SCA patients with bad obstetric history may also be offered EBT during pregnancy in order to reduce sickle cell related morbidity and improve obstetric outcome

Corresponding Author: Dr. S.G. Ahmed, Department of Haematology, College of Medical Sciences,
University of Maiduguri, P.M.B. 1069, Maiduguri, Borno State, Nigeria

(Weatherall, 2001). However, most EBT procedures for SCA patients are undertaken as part of emergency management of severe and life threatening conditions such as acute chest syndrome, cerebrovascular accidents and priapism (Weatherall, 2001).

The ideal types of red cell preparations preferred for EBT are the freshest possible units of whole blood or plasma reduced blood, but not red cell concentrate in OAS the use of which may precipitate or aggravate pre-existing hypoproteinemia because OAS is poor in protein (Davies and Oni, 1997). Moreover, in a setting such as Nigeria where the national prevalence of sickle cell trait (Hb AS) is up to 25% and is reflected in the donor population, every effort must be made to ensure that only Hb AA blood is used for transfusion in the management SCA (Ahmed *et al.*, 2000). Although sickle cell trait (Hb AS) does not disqualify a healthy person from blood donation, the use of Hb AS donor red cells in transfusing SCA patients is not recommended because such red cells contain up to 40% Hb S (Ahmed and Ibrahim, 2006). Hence the use of such red cells in EBT for sickle cell anaemia is counter-productive and less efficacious in bringing down the Hb S levels.

EBT can be performed manually or automatically with a cell separator (Weatherall, 2001). The manual technique though laborious is the only available procedure in most developing countries including Nigeria. The aim of EBT in SCA is to reduce the amount of Hb S to less than 20% of the total while steadily raising the total Hb concentration to 12-14 g dL⁻¹, which will require the transfusion and removal of at least 1.25 times the patient's blood volume (Davies and Oni, 1997). Serial quantitation of the patient's Hb S levels is therefore necessary for effective monitoring of the EBT procedure. Haemoglobin quantitation by manual method is cumbersome and often inaccurate. Computerized densitometry is an easier and more accurate method of haemoglobin quantitation. Unfortunately, most hospitals in Africa, which is the continent that carries the heaviest burden of SCA cannot afford the facilities for densitometry. Nonetheless, most African hospitals can perform simple sickling test and undertake the estimation of sicklable red cells therein. We hypothesize that there should be a strong positive correlation between the Hb S levels and proportions of sicklable red cells in patients undergoing EBT. If this is true, then EBT in SCA patients that are managed in resource limited hospitals can be monitored by serial estimation of the proportion of sicklable red cells to a desired target level that will correspond to Hb S level of less than 20%. In this report we undertook a study to determine the correlation between the levels of Hb S and sicklable red cells in SCA patients on EBT as seen in the University of Maiduguri Teaching Hospital (UMTH), Maiduguri, Nigeria.

MATERIALS AND METHODS

Five cases of SCA were managed by EBT for priapism (4 cases) and cerebrovascular accident (1 case) in the year 2006 at the haematology department of the UMTH, Maiduguri, Nigeria. The patients were adults within the age range of 24-29 years. The patient with cerebrovascular accident was a female. All of the subjects studied in this report were diagnosed as sickle cell anemia (Hb SS) patients at the haematology laboratory of the UMTH, Maiduguri, Nigeria, based on positive sickling tests and haemoglobin electrophoresis at a pH of 8.6 on cellulose acetate paper. Due consent was obtained from each patient before being included in the study. None of the patients studied in this report had history of previous blood transfusion within the past 4 months at the time of this study.

In all cases, the EBT procedure was carried out manually and iso-volemically in five cycles. In each cycle, three units were venesected and three units were transfused using two separate venous accesses to permit simultaneity of the procedures. In each case, only the freshest ABO/RhD compatible whole blood units with Hb AA genotype were used and the EBT procedure was completed within 48 h (Davies and Oni, 1997).

Just before the start of EBT procedure, baseline pre-EBT blood samples were collected in ethylene diamine tetra-acetate containers for estimation of haematocrit, sicklable red cells and scan quantitation of Hb S, F and A₂. Thereafter, the patients were monitored by serial estimation of haematocrit, sicklable red cells and scan quantitation of Hb S at the end of every cycle of EBT. In this study haematocrit was estimated by standard manual micro-haematocrit centrifugation technique (Dacie and Lewis, 1991) and Hb electrophoresis and scan quantitation was conducted by using 24-VISU Densitometer (Helena, France). Sicklable red cells were estimated from glass slide preparations for sickling test made by standard technique using dithionite reagent (Evatt *et al.*, 1992). After 1 h, the slides were examined under dry high power (x40) objective lens (Evatt *et al.*, 1992). The thin areas (where the red cells do not overlap) of the slides were chosen for cell counting. Sicklable red cells (which correspond to the patients' cells) were identified as sickled cells and counted as percentages of the total red cells (both sickled and unsickled) enumerated in each field; the unsickled red cells correspond to the donor cells. For each slide preparation, sicklable red cells were estimated from 10 microscope fields and the average was taken as the actual count.

The mean values of the pre-EBT parameters for the five patients were determined manually and document as baseline values. The mean values of Hb S levels and proportions of sicklable red cells that were obtained after each cycle of EBT from all the five patients were analyzed using SPSS version 11.0 to determine the coefficient of correlation.

RESULTS

The pre-EBT profile (Table 1) of our patients revealed mean values of haematocrit of 0.26 L L⁻¹, sicklable red cells of 100%, Hb S level of 93%, Hb F level of 5% and Hb A₂ of 2%. In the course of EBT (Table 2), the mean levels of Hb S and proportions of sicklable red cells fell in parallel from pre-EBT values of 93 and 100% to 17 and 19% at the end of the 5th cycle, respectively. However, the rate of fall in the levels of both Hb S and sicklable red cells became smaller as the number of cycles rose. The mean values of haematocrit rose steadily from a pre-EBT level of 0.26 to 0.34 L L⁻¹ at the end of the 5th cycle. Scatter and linear regression analysis between the mean values of Hb S and sicklable red cells obtained in the course of EBT as shown on Table 2 revealed a linear relationship (Fig. 1) with a strong and significant positive correlation coefficient, $r = +0.992$ ($p = 0.01$) as determined by SPSS version 11.0.

Table 1: Pre-EBT haematocrit, sicklable red cells and Hb profile of SCA patients

Parameters	Mean±2SD
Haematocrit (L L ⁻¹)	0.26±0.03
Sicklable red cells (%)	100.00±0.00
Hb S level (%)	93.00±5.00
Hb F level (%)	5.00±1.50
Hb A ₂ level (%)	2.00±0.50

Table 2: Values of Hb S, sicklable red cells and haematocrit in SCA patients on EBT

Timing	Cumulative No. of units exchanged	Mean level of Hb S (%)	Mean proportion of sicklable red cells (%)	Mean haematocrit (L L ⁻¹)
Pre-EBT	0	93	100	0.26
After 1st cycle	3	77	79	0.28
After 2nd cycle	6	50	63	0.30
After 3rd cycle	9	34	38	0.32
After 4th cycle	12	19	25	0.33
After 5th cycle	15	17	19	0.34

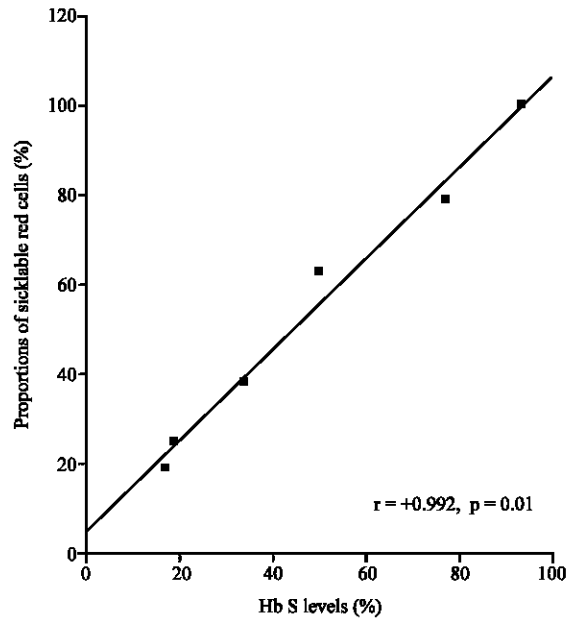


Fig. 1: Hb S levels and proportions of sicklable red cells in SCA patients on EBT

DISCUSSION

The pre-EBT profile of our patients revealed a moderately severe anaemia, normal Hb A₂ level with mildly elevated level of Hb F, which was consistent with previous findings among Nigerian patients with SCA (Akinyanju, 1989; Ahmed *et al.*, 2006). The finding of 100% pre-EBT sicklable red cells in present patients was consistent with the fact that none of them had any blood transfusion in the last four months before being admitted with priapism or cerebrovascular accident for EBT. This finding would indicate total absence of residual donor cells from previous transfusions and that all the red cells were endogenous to the patients before the EBT was initiated. However, despite the finding of 100% sicklable red cells, the pre-EBT profile revealed an Hb S level of only 93%. Hence, from the outset the level of Hb S was lower than the corresponding value of sicklable red cells by 7%, which was accounted for by the minor hemoglobinous F and A₂.

The initiation of EBT showed a progressive fall in both Hb S and sicklable red cell levels. However, the rate of fall for both parameters was higher in the earlier than in the later cycles of the EBT procedure. This was because of the progressive dilutional effect of donor blood on the levels of Hb S and sicklable red cells in the patients after each cycle (Ahmed *et al.*, 2002). Consequently, the earlier cycles caused relatively greater fall in both parameters as compared with the later cycles. Nonetheless, there was a steady rise in the level of haematocrit after every cycle. This was due to the fact that the SCA patients were anaemic from the onset. Therefore, the blood that was removed had lower haematocrit than the blood that was transfused in the EBT procedure, a situation that led to a net gain in patients' haematocrit after each cycle (Davies and Oni, 1997; Ahmed *et al.*, 2002). However, because the differential between patient and donor haematocrit became narrower after each cycle, the net gain was higher in the earlier than in the later cycles (Davies and Oni, 1997; Ahmed *et al.*, 2002).

The result of this study revealed a positive correlation between the level of Hb S and the proportion of sicklable red cells throughout the EBT procedure. However, the data showed that the level of Hb S fell to the target level of less than 20% one cycle earlier than the sicklable red cells. This was interpreted to be a reflection of the pre-EBT quantitative difference between the two parameters,

which was also reflected at every stage of the EBT procedure. Taking our data into consideration, the Hb S fell to a target level of 19% after cycle-4, at a moment when the proportion of sicklable red cells was still 25%. The differential between the two parameters at that moment was 6% (25-19%), which roughly equals the initial difference between the two parameters observed in the pre-EBT profile. Nonetheless, when the proportion of sicklable red cells fell to less than 20% after cycle-5, the Hb S level was well below 20%. This trend implied that by the time the proportion of sicklable red cells reached the target level of less than 20%, the corresponding level Hb S would be even much lower and well into the 10-20% range. Therefore, the proportion of sicklable red cells, with a target of less than 20%, can be safely used as surrogate for Hb S level in monitoring SCA patients on EBT.

CONCLUSION

The proportions of sicklable red cells correlated strongly with Hb S levels in SCA patients on EBT. Hence, sicklable red cells can be used as useful and safe surrogate markers for Hb S in the monitoring of SCA patients on EBT. This may be an attractive model for resource limited health institutions that manage SCA but lacks modern Hb quantitation facilities.

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