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Original Article

Determination of Red Cell Indices and Sero-prevalence of Transfusion Transmissible Infectious Diseases among Stable Paediatric Sickle Cell Patients

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Abstract

Background: Red blood cell indices are very important tools in screening for anaemia especially in children with sickle cell disease (SCD). Blood transfusion forms an integral part of management of sickle cell disease.

Aim: To determine the red cell indices and sero-prevalence of transfusion-transmissible infectious disease among stable paediatric sickle cell patients.

Methodology: One hundred clinically stable SCD patients that were transfused in the least six months and not in crisis in the last four weeks were recruited into this study. 4ml of venous blood was collected, 2ml was dispensed into K2EDTA for immediate analysis of red cell indices using haematology analyzer, haemoglobin phenotype was also determined using cellulose acetate at alkaline solution. The remain 2ml was dispensed into plain bottles, blood was allowed to clot and centrifugation at 5000 rpm for 5 minutes. Serum was extracted into another plain tubes for serological testing of TTIs using enzyme-linked immunosorbent assay (ELISA).

Results: Mean±SD value of RBC, HGB and HCT among the SCD participants were significantly ($p<0.05$) lower compared with control subjects. However, mean±SD value of MCV, MCH, MCHC and RDW among the SCD participants were significantly ($p<0.05$) higher compared with control subjects. Sero-prevalence of TTIs among blood transfused SCD participants in this study showed zero prevalence in all the TTIs tested using standard technique.

Conclusion: Red blood cell indices values were lower compared with control subjects, nature has it that SCD patients in the steady state have adapted their body system to this anaemic state and live healthy. Blood transfusion does not significantly increase the sero-prevalence of transfusion transmissible infections in our clinically stable SCD participants.

Keyword: Red blood cell indices, Transfusion-transmissible infections, seroprevalence, steady sickle cell disease patients

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Introduction

Sickle cell disease (SCD) is majorly a red blood cell (RBCs) disorder with significant changes observed in certain haematological parameters [1]. This commonest hereditary disorder is characterized by severe haemolytic anaemia results into frequent blood transfusion [2]. Red blood cell indices are very important tools in screening for anaemia in children with and without sickle cell disease, due to chronic haemolysis and increased bone marrow activity in sickle cell disease patients, the red cell indices of children with sickle cell disease is differ from those of children without sickle cell disease [3] .It has been noticed that SCD patients in the steady state have adapted their body system to this anaemic state and live healthy despite low haemoglobin level [4] . Although, SCD patients in crisis (vaso-occlusion) has been reported to have higher haemoglobin level compared with SCD in steady state, this was related to higher blood viscosity in SCD during crisis which further increase VOC [5]. Mean cell volume (MCV), mean cell haemoglobin (MCH) and mean cell haemoglobin concentration (MCHC) are useful parameters in detecting underlying causes of anaemia [9, 6]. There is a significant highred cell distribution width (RDW) reflecting the degree of chronic haemolysis and anaemia in SCD patients [7]. Blood transfusion forms an integral part of management of sickle cell disease. Blood transfusion is also established as a route of transmission of the transfusion-transmissible infectious agents such as hepatitis B virus (HBV), human immunodeficiency virus (HIV), hepatitis C virus (HCV) and syphilisare among the greatest threats to blood safety for blood transfusion recipients and pose a serious public health problem [15]. Blood transfusion in sickle cell disease patients is usually performed when haemoglobin is less than 6g/dl, patients with sickle cell disease usually have haemoglobin level as low as 8g/dl in their steady state. Sickle cell disease (SCD) affects about 2% of Nigerians at birth and it is the commonest genetic disorder in the country. [9] reported, 0.69% prevalence of sickle cell anaemia among the children in Ido-Ekiti.Sickle cell disease is an autosomal recessively inherited disorder resulting in a chronicanaemia

and frequently a life-threatening.Sickle red cells assume an abnormal, rigid shape under conditions of reduced oxygen tension and equally adhere to the endothelial walls (in addition with platelets and white cells), leading to vascular occlusion and endothelial dysfunction [10] .Anaemia in sickle cell disease often requires red cell transfusion in its management, complications of blood transfusion in individuals living with SCD are iron overload, red cell alloimmunization and transmission of transfusion-transmissible infections such hepatitis B, hepatitis C, Human Immunodeficiency Virus (HIV) and syphilis. The risk of transfusion-transmissible infections (TTIs)through blood is determined by itsprevalence in the donor population, the problem of seroconversion window (where the donor is infectious but seronegative), the availability and affordability of sensitive diagnostic capabilities. The risk of posttransfusion-transmissible infections in the developed countries is presently extremely low [15]. It is therefore, important that blood given to SCD patients is routinely and properly screened for these transfusion-transmissible infections (TTIs) using standard techniques, especially those on regular blood transfusion to reduce the chances of transmitting transfusion-transmissible infections. This is even made more imperative by the observation that a larger proportion of the blood donor pool in Nigeria is commercial (paid) donors. The reason for this may be attributable to a number of factors, ranging from poor awareness on voluntary blood donation to poverty and illiteracy, particularly among the rural populations of the country [11] .Children with SCD constitute a group that requires frequent blood transfusion, this study was therefore, designed to determine the red cell indices and sero-prevalence of transfusion-transmissible infectious disease among stable paediatric sickle cell patients in the paediatric clinic of Federal Teaching Hospital, Ido-Ekiti, Nigeria.

Materials and Methods

Study design

This study was carried out in Federal Teaching Hospital, Ido-Ekiti among paediatrics sickle cell disease patients insteadystate.The study was approved by the hospital's ethics and research committee. Informed consent was obtained from the parents of the participants. Questionnaire was used to collect the information about demographic data, history of blood transfusion/hospital admission and parents' socioeconomic parameters. Inclusion criteria includes confirmed status of sickle cell patients in steady state within the agegroup between 1 and 15 years. Subjects that were transfused with blood in the last six months and not in crisis in the last four weeks. Exclusion criteria include sickle cell disease patients that does not within the study age group, those that decline from participating in the study and Subjects that were not haemoglobin SS. Control subjects were recruited from apparently healthy children whose haemoglobin phenotype is AA within the age group 1-15 years of both sexes for the purpose of comparing red cell indices parameters with SCD participants. Haemoglobin electrophoresis of subjects werepre-determined using cellulose acetate at alkaline solution (pH 8.6) to confirm the haemoglobin phenotype status of the participants [12] .4ml of venous blood sample was collected from one hundred SCD participants and control subjects, 2ml was dispensed into K_2EDTA for immediate analysis of red cell indices using haematology analyzer, haemoglobin phenotype was also determined using cellulose acetate at alkaline solution. The remain 2ml was dispensed into plain bottles, blood was allowed to clot and centrifugation at 5000 rpm for 5 minutes. Serum was thereafter extracted from the clotted sample into another clean plain tubes for serological testing of TTIs using enzyme-linked immunosorbent assay (ELISA). Data obtained were analyzed using the Statistical Package for Social Sciences, version 22.0 (SPSS 22.0). Statistical significance was set at a p-value less than or equal to 0.05.

Results

Table 1a shows the socio-demographic characteristics of stable sickle cell patients. Age group 9-12 and 1-4 had highest and lowest prevalence respectively among the study SCD participants. In gender distribution, 55 were male and 45 were female. Christians and Yoruba ethnic in religion and tribe variable respectively had highest prevalence in the study group as showed in table 1a. 55.0% of secondary education, 40.0% of family size of three, 38.0% of firstborn children in the family and 34% were transfused with two Units of blood in the last 6months as showed in Table 1b.

Variable	Participants (%) N = 100	Control (%) N = 100
Age group (in years)		
1 – 4	10 (10.0)	5 (5.0)
5 – 8	32 (32.0)	15 (15.0)
9 – 12	46 (46.0)	30 (30.0)
13 – 15	12 (12.0)	50 (50.0)
Mean age ± SD	10.6 ± 5.1	8.9 ± 3.9
Age range	2 – 15	4– 15
Gender		
Male	55 (55.0)	60(60.0)
Female	45 (45.0)	40(40.0)
Religion		
Christianity	88 (88.0)	75(75.0)
Islam	12 (12.0)	25(25.0)
Tribe		
Yoruba	96 (96.0)	80(80.0)
Ibo	2 (2.0)	15(15.0)
Hausa	2 (2.0)	5(5.0)

Table 1a: Socio-demographic characteristics of stable sickle cell participantsand control.

Variable	Participants N (%) N = 100
Current educational level	
None yet	4 (4.0)
Primary	38 (38.0)
Secondary	55 (55.0)
Tertiary	3 (3.0)
No of children in the family	
1	12 (12.0)
2	26 (26.0)
3	40 (40.0)
4	15 (15.0)
5	5 (5.0)
6	2 (2.0)
Position	
1st	38 (38.0)
2nd	32 (32.0)

3rd	20 (20.0)
4th	6 (6.0)
5th	3 (3.0)
6th	1 (1.0)
Units of blood transfused in the last 6 months	
1	26 (26.0)
2	34 (34.0)
3	20 (20.0)
4	12 (12.0)
5	8 (8.0)

Table 1b: Socio-demographic characteristics of the stable sickle cell participants

The mean ± SD value of RBC, HGB and HCT among the SCD participants were significantly ($p<0.05$) lower compared with control subjects. However, mean ± SD value of MCV, MCH, MCHC and RDW among the SCD participants were significantly ($p<0.05$) higher compared with control subjects as showed in table 2a.

Variable	Participants N = 100	Control N = 45	Statistical test
	Mean ± SD	Mean ± SD	p-value
RBC **HGB**	3.31 ± 0.72 8.58 ± 1.35	5.27 ± 0.83 13.24 ± 5.86	**0.02** **0.01**
HCT	24.87 ± 5.08	39.51 ± 4.03	**0.01**
MCV	84.18 ± 9.69	81.31 ± 7.18	**0.04**
MCH	29.42 ± 4.35	25.60 ± 3.10	**0.01**
MCHC	36.23 ± 1.73	30.23 ± 4.33	**0.02**
RDW	22.87 ± 2.41	15.34 ± 2.52	**0.01**

Table 2a: Mean± SDof red cell indices among stable sickle cell participants and control.

KEY:

RBC – Red Blood Cell ($10^6/\mu L$)

HGB – Haemoglobin (g/dL)

HCT – Haematocrit (%)

MCV – Mean Cell Volume (fL)

MCH – Mean Cell Haemoglobin (pg)

MCHC – Mean Cell Haemoglobin Concentration (g/dL)

RDW– Red Cell Distribution Width (fL)

Mean± SD of red cell indices in SCD participants and control subjects among the gender was higher in male compared with female although the difference was not significant ($p>005$) except in RDW among the control subjects where the difference is significant as showed in table 2b.

Sero-prevalence of TTIs among blood transfused SCD Participants in this study showed zero prevalence in all the TTIs tested using standard technique as showed in table 3.

Discussion

One of the characteristic features in the pathophysiology of SCD is chronic hemolytic anemia causing increased tendency for RBC lysis and adhesion which shortened red cell survival, there is also low erythropoietin response to anaemia [5,11] .These facts are associated with low red cell indices in SCD patients during steady state as

Variable	Participants			Control		
	Male N = 55	Female N = 45	p-value	Male N = 60	Female N = 40	p-value
RBC HGB	3.13±0.74 8 .47±1.38	3.03±0.49 8.01±1.25	0.48 0.79	5.10±0.35 13.43±8.43	4.97±1.44 12.56±4.75	0.40 0.84
HCT	25.59±5.37	24.92±4.53	0.46	39.12±5.78	38.81±4.05	0.91
MCV	85.49±6.53	84.98±7.39	0.38	81.31±8.05	80.57±6.32	0.17
MCH	30.22±3.49	29.96±5.40	0.45	25.19±3.26	24.37±3.24	0.08
MCHC	36.32±1.28	35.94±1.39	0.82	30.54±3.76	30.14±2.33	0.22
RDW	22.71±3.30	22.15±3.50	0.63	15.26±5.78	16.34±1.31	0.04

Table 2b: Comparism of Mean±SD of red cell indices among the genders SCD participants in steady state and control.

KEY:

RBC – Red Blood Cell ($10^6/\mu L$)

HGB – Haemoglobin (g/dL)

HCT – Haematocrit (%)

MCV – Mean Cell Volume (fL)

MCH – Mean Cell Haemoglobin (pg)

MCHC – Mean Cell Haemoglobin Concentration (g/dL)

RDW– Red Cell Distribution Width (fL)

Parameters	SCD Participants	
	Positive (%)	Negative (%)
HbsAg (n=100)	0.00	100
HCV (n=100)	0.00	100
HIV (n=100)	0.00	100
VDRL (n=100)	0.00	100

Table 3: Sero-prevalence of TTIs among blood transfused SCD Participants.

reported in this present study. The values of red cell indices obtained in this study was similar to the results of previous studies conducted in this country, findings in this study confirmed the features of chronic haemolytic disorder in SCD patients [14] .Chronic haemolysis in sickle cell disease patients stimulate haemopoiesis, haemopoietic activity thus providing a more rapid supply of young red blood cells causing mean cell volume to be high despite the anaemia, this shows that haemoglobin value is true measurement of anaemia and not packed cell volume.High MCHC and MCH reported in this study can be supported with the fact that increase in haemoglobin concentration per red cell is associated with cellular dehydration in SCD patients causing red cells of SCD patients to be less deformity in hypoxic state, making it difficult for sickle cells to traverse the microvasculature thus promoting vaso-occlusive crisis.High Red cell Distribution Width (RDW) recorded in this study confirmed the fact that red cell distribution width (RDW) is an index of the variation in the size of the red blood cells which can be used to detect subtle degrees of anisocytosis [15], since SCD is associated with marked anisocytosis morphologically due to rapid erythropoiesis, sickling of red cell and different stages of maturation. The prevalence rates of HBsAg, HCV, HIV and Syphilis in the study were 0 (0.0%), 0 (0.0%), 0 (0.0%), and 0 (0.0%) respectively. It was reported in the previous study that HIV

prevalence in SCD patients varies between 0% and 11.5% which is similar to the findings in this study [1]. Supporting the findings in this study, might due to proper screening of the donor using standard techniques.However, majority of our SCD participants sourced the blood for transfusion at our hospital facility blood bank alone where a higher standard of ensuring safe blood transfusions is ensured,another possible reason for zero prevalence of TTIs in this study may be due to family replacement donor or voluntary donors that normally come to donate especially for the SCD patients because of possible multiple transfusion [15] . However, frequent blood transfusion among the participants in this study was low, this may contribute to zero prevalence of TTIs reported in this study.All SCD participants were vaccinated with hepatitis B vaccine since it is component of the national programme on immunization for children, this might be due to possible cause of zero prevalence in HBV.Supporting the findings in this study, Bolarinwareported thatoverall risk of HBV infection in SCD patients was not clearly increased by blood transfusion, he further stated that the prevalence of viral markers was not significantly different in SCD participants that had received blood transfusion and those who did not [8].

Conclusion

Based on the findings in this study, red blood cell indices values were lower compared with control subjects, nature has it that SCD patients in the steady state have adapted their body system to this anaemic state and live healthy. Blood transfusion does not significantly increase the seroprevalence of transfusion transmissible infections in our clinically stable SCD participants; this may be related to the low blood transfusion rate observed in the patients, coupled with the standard donor screening procedures applied in our facility.

Recommendations

It is recommended that screening of donated blood for TTIs using standard techniques should be continued to prevent risk of transmitting these infections via blood transfusions. Further strengthening of timely birth-dose vaccination will be important for reducing chronic HBV infection. A booster dose should be given to those who did not mount a response to vaccination.

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