Original Research Article

Haematological Parameters of Sickle Cell Disease Patients on Prophylactic Antimalarial Regimen within Port Harcourt, Nigeria.

ABSTRACT

Introduction: Sickle cell disease and malaria are the worst tropical diseases most prevalent in Nigeria in particular and Africa in general (Diop *et al.*, 2011). The two disease states manifest similar symptoms of severe haemolytic anaemia, fever, weakness and high infant mortality rate (Diop *et al.*, 2011). Malaria is associated with a higher mortality in hospitalized SCD patients compared to hospitalized non-SCD patients (Warley *et al.*, 2018). Prophylaxis against malaria is therefore important in SCD patients, as antimalarial chemoprophylaxis has also been shown to be beneficial in SCD patients, reducing parasitaemia and anaemia, and the requirement for blood transfusion (Gaston *et al*., 2017

AIM: This study evaluated the haematological parameters of sickle cell disease patients on prophylactic antimalarial regimen within Port Harcourt, Rivers State, Nigeria.

METHODS: The study was a cross sectional study which employed was the Haematological auto-analyzer method (Sysmex XN-550 haematology auto-analyzer). Blood samples were collected from a total of 50 Sickle Cell Disease Patients attending different hospitals within Port Harcourt and 50 samples from apparently healthy staffs and students of River State University were used as control.

RESULT: The result show that male (51%) are more exposed to SCD in Port Harcourt than female (49%). There was a statistical significance in the Haemoglobin of Male (8.81±3.61) as compared to that of the female (11.62±3.77) (p=0.0097) while other parameters showed no statistical significance. The result showed a lower mean value for RBC, PCV, HB, MCV, MCH and MCHC of SCD patients on antimalarial regimen when compared to the control subjects. Higher mean value for WBC, Lymphocytes, Monocytes, and Eosinophils where statistically significant when compared with the control subjects. The result gotten emphasised the need for proper management of *P. falciparum* malaria in SCD.

CONCLUSION: This study emphasized that sickle cell disease patients are more of the age range between 18-21 years (50%), this might be attributed to the fact that many of the SCD patients pass away before they get old due to the disorder. This study has also established that Sickle Cell Disease patients on prophylactic anti-malarial regimen have low haemoglobin, haematocrit, RBC, MCV, MCH, and MCHC, but increased WBC, lymphocytes, monocytes and eosinophils. Results from this study can help in the differential diagnosis of malaria infection in HbSS genotype based on haematological parameters in resource limited setting where sickle cell genotyping remains a challenge.

Keywords: Haematological Parameters, Sickle Cell Disease, Prophylactic Antimalarial Regimen

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Introduction

Hemoglobin is a tetramer composed of two α-globin and two non- α -globin chains working in conjunction with heme to transport oxygen in the blood [2]. Normal adult hemoglobin (HbA) is designated αA2βA2 [2]. Variant hemoglobin is derived from gene abnormalities affecting the α-

globin genes (HBA1 or HBA2) or β-globin (HBB) structural genes (exons) [3]. More than a thousand hemoglobin variants have been identified relative to changes in the globin chains. Qualitative changes correspond to amino acid substitutions resulting in hemoglobinopathies [3]. Quantitative changes like amino acid insertions, deletions or mutations in the intervening sequences (introns) correspond to thalassemia and result in decreased globin chain production [3].
Alpha thalassemias are caused by changes (deletions, point mutations, insertions, etc.) in the α-globin genes [3]. Production of α-globin is controlled by the four alleles of HBA1 and HBA2. In the deletional type α-thalassemias, the number of α-globin gene deletions correlates to disease severity. One α-globin gene deletion is unremarkable (also called silent carrier) whereas a two α-globin gene deletion (α-thalassemia trait) and three α-globin gene deletion (HbH disease) have varied clinical and in hematological features. A four α-globin gene deletion (Hb Bart's Hydrops fetalis) is severe and not typically compatible with life [5].

Beta globin variants more commonly seen include HbS, HbC, HbD, HbE and HbG. A mutation in one β-globin subunit results in a combination of variant and normal hemoglobin and denotes carrier or trait status, also known as the heterozygote state [5]. Mutations in both β-globin subunits result in disease based on a homozygous or heterozygous expression. [5]. In the case of sickle cell anemia (HbSS), mutations are homozygous with production of HbS [6]. Other diseases classed under sickle cell disease (SCD), for example HbSE, HbSC and HbSβ-thalassemia are heterozygous expressions.[6]. Regardless of an α-globin or β-globin variant, severity of disease can range from insignificant to serious or life threatening [3]. Therefore, early detection is paramount.[7] .
Sickle cell disease and malaria are the worst tropical diseases most prevalent in Nigeria in particular and Africa in general [7]. The two disease states manifest similar symptoms of severe haemolytic anaemia, fever, weakness and high infant mortality rate [7]. Malaria, on the other hand, is caused by an intracellular protozoan parasite, known as *Plasmodium.* They are usually injected into individuals by female anopheles mosquitoes during their blood meal. The most lethal form of malaria is caused by *Plasmodium falciparum (P.f.)* [11]. It is known that SCD is widely spread in the high malaria endemic areas of Nigeria[11]. Many Nigerians are known not to have understood clearly the generation, presentations and the management of SCD perhaps because of the observed high level of ignorance of the disorder among Nigerians[11]. This ignorance might have contributed to the gross misconception of the presentations of SCD among

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Nigerians, at least Although a linkage exists between the presence of sickle haemoglobin (HbS) and protection from malaria in the heterozygous state [11]. Malaria is a frequent cause of hospitalization and poor outcome among children with SCD in endemic areas, and malaria is associated with a higher mortality in hospitalized SCD patients compared to hospitalized non-SCD patients [12]. Prophylaxis against malaria is therefore important in SCD patients, as antimalarial chemoprophylaxis has also been shown to be beneficial in SCD patients, reducing parasitaemia and anaemia, and the requirement for blood transfusion [9]. The WHO recommends that SCD patients in endemic areas should receive antimalarial prophylaxis [8]; however, the evidence to support the potential beneficial effects of this strategy in SCD patients is limited.

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Materials and Methods

Study Design

This research work was a cross-sectional study. A total of 100 subjects were recruited for this study, of which 50 were registered adult SCD patients diagnosed at haematology clinics of Rivers State University Teaching Hospital (RSUTH), University of Portharcourt Teaching Hospital (UPTH), and other primary health care centers within Rivers State respectively and 50 were apparently healthy staffs and students of Rivers State University (RSU).

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Study Area

This study was carried out in RSUTH, UPTH and other, primary, secondary and private health care centers within Port Harcourt, River state, Nigeria.

Study Population

This study was carried out on 100 subjects of which 50 were Sickle cell Disease patients who visited sickle cell Clinics at RSUTH, UPTH, Meridian Hospital D-line, Maryland Hospital Rumuokwurusi, Model Primary Health Care Centres Rumuokwurusi, Ozuoba, and Rumuigbo all within Port Harcourt, Rivers State. The demographic, prophylactic antimalarial drug regimen, HB electrophoresis and relevant data were obtained from patient case note and interviewer structured questionnaire.

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Eligibility Criteria

Inclusive criteria

Only Sickle Cell Disease Patients on Prophylactic Antimalarial drugs between the age range of 18-90 years were included in this study. Apparently healthy staffs and students of Rivers State University who were not on antimalarial drug were only used as control.

Exclusive criteria

Those who are not SCD patients, Smokers, and alcoholics were excluded from this study.

Recruitment of Participants

A written Informed consent was gotten from the patients before sample collection and socio demographic data and other relevant information were also gotten through an interviewer questionnaire.

Ethical Approval

Ethical clearance was gotten from the Rivers State Hospital Management Board.

Sample Collection and Preparation

5ml of venous blood was antiseptically collected from each individual involved in this study both for the test and control. Each sample was collected and dispensed into ethylenediamine tetra-acetic acid (EDTA) contained bottles and mixed properly. The samples were analyzed for haematological parameters.

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Laboratory Analysis

Analysis of Full Blood Count was carried out Using Sysmex XN-550 Automated CBC Haematology Analyzer.

Statistical Analysis

Statistical analysis was done using Graphpad prism version 5.01. Comparison of haematological parameters between SCD patients on prophylactic antimalarial regimen and apparently healthy staffs and students of Rivers State University was done using students t-test and One way ANOVA. Results were presented as mean $\pm$ SD with statistical significance set at $P<0.05$.

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Results

Demographic Characteristics of Participants.

Table 1 shows that total of 50 Sickle Cell Disease (SCD) patients (males and females) that were on different prophylactic antimalarial regimen were enrolled in this study from different tribes within Rivers State which include Abua, Bonny, Ekpeye, Engenni, Etche, Ikwerre, Kalabari, Ogoni, Ogba and Opobo with a frequency distribution of 0.14, 0.1, 0.08, 0.1, 0.16, 0.2, 0.1, 0.04 and 0.04 respectively. This represents 14%, 10%, 8%, 10%, 4%, 16%, 20%, 10%, 4% and 4% respectively. Of this number 24(48%) were males and the other 26 (52%) subjects were females, within the age range of 18-21, 22-25, 26-29, 30-33, 34-37 and 38-41years with the frequency distribution of 0.5, 0.18, 0.02, 0.18, 0.1 and 0.02 and this represents 50%, 18%, 2%, 18%, 10% and 2% respectively. This emphasize that sickle cell disease patients are more of the age range between 18-21 years (50%), this might be because many of the SCD patients die before they get old due to the disorder.

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The educational status of the study population showed that Primary, Secondary and Tertiary education had the frequency distribution of 0.14(14%), 0.6(60%) and 0.26 (26%) respectively.

Out of the 50 participants 19 were unmarried and 31 were married with the frequency distribution of 0.38(38%) and 0.62(62%) respectively. The participants were recruited from different hospitals within Port Harcourt, Rivers State, Nigeria and used as test samples while 50 samples of apparently healthy students and staffs of Rivers State University (RSU) were used as control of which 27(54%) were male and 23(46%) were females within the period of November, 2020 and March, 2021. The haematological parameters of the subjects and control samples were determined.

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Table 1: Demographic Characteristics of Sickle Cell Disease Subjects

Subjects	No. of Participants	Frequency	Percentage
Tribe			
Abua	7	0.14	14%
Bonny	5	0.1	10%
Ekpeye	4	0.08	8%
Engenni	5	0.1	10%
Etche	2	0.04	4%
Ikwerre	8	0.16	16%
Kalabari	10	0.2	20%
Ogoni	5	0.1	10%
Ogba	2	0.04	4%
Opobo	2	0.04	4%
Age Groups			
18-21	25	0.5	50%
22-25	9	0.18	18%
26-29	1	0.02	2%
30-33	9	0.18	18%
34-37	5	0.1	10%
38-41	1	0.02	2%
Education			
Status			
Primary	7	0.14	14
Secondary	30	0.6	60%
Tertiary	13	0.26	26%
Marital Status			
Unmarried	19	0.38	38%
Married	31	0.62	62%

Comparative Analysis of Haematological Parameters of SCD Patients Against Apparently Healthy Individual

Table 2 Compared the haematological parameters of the test and control sample, it was shown that the White Blood Cell had a statistical significance with mean $\pm$ STD of 5.95 $\pm$ 3.38 and 4.93±1.17 respectively with a P-value of 0.0457, the Red blood cell had a significance value with mean $\pm$ STD of 3.07±1.42 and 4.63±0.62 respectively with a P-value of 0.0001, the Haemoglobin was statistically significant with mean $\pm$ STD of 10.16±3.92 and 13.47±1.11 respectively with P-value of 0.0001, the Haematocrit was statistically significant with mean $\pm$ STD of 28.64±4.73 and 39.72±3.58 respectively and p-value of 0.0001, Mean Corpuscular

Volume was significant with mean ± STD of 69.52±10.8 and 83.81±8.08 respectively with p-value of 0.0001, Mean Corpuscular Haemoglobin was significant with mean ± STD of 23.98±4.90 and 33.63±1.58 respectively with p-value of 0.0001, Mean Corpuscular Haemoglobin Concentration was also significant with mean ± STD of 28.37±2.99 and 33.63±1.58 respectively with p-value of 0.0001, Lymphocyte was significant with mean ± STD of 49.09±29.68 and 33.68±5.31 respectively with p-value of 0.0005, monocyte was also significant with mean ± STD of 10.11±4.04 and 5.20±2.33 respectively with p-value of 0.0001 and Eosinophils was also significant with mean ± STD of 5.16±0.69 and 3.02±1.36 respectively with p-value of 0.0001. Other parameters like the platelet, Neutrophil and Basophils were statistically non-significant all set at a significant level of $p<0.05$ as shown in table 2 below.

Table 2: Comparative Analysis of Haematological Parameters of SCD Patients Against Apparently Healthy Individual

Parameters	SCD Patients (Test)	Apparently Healthy Individuals (Control)	p-value	T-value	Remark
WBC($x10^3$/ul)	5.95 ± 3.38	4.93±1.17	0.0457	2.024	S
RBC($x10^6$/ul)	3.07±1.42	4.63±0.62	0.0001	7.117	S
HB(g/dl)	10.16±3.92	13.47±1.11	0.0001	5.75	S
HCT(%)	28.64±4.73	39.72±3.58	0.0001	13.21	S
MCV(fl)	69.52±10.8	83.81±8.08	0.0001	7.49	S
MCH(pg)	23.98±4.90	33.63±1.58	0.0001	11.01	S
MCHC(g/dl)	28.37±2.99	33.63±1.58	0.0001	11.01	S

PLT($x10^3$/ul)	210.7±80.94	196.1±39.37	0.2541	1.147	NS
NEUT(%)	59.96±12.07	56.72±6.10	0.0935	1.694	NS
LYM(%)	49.09±29.68	33.68±5.31	0.0005	3.614	S
MONO(%)	10.11±4.04	5.20±2.33	0.0001	7.457	S
EOSINO(%)	5.16±0.69	3.02±1.36	0.0001	9.905	S
BASO(%)	0.29±0.26	0.40±0.49	0.1847	1.336	NS

KEYS: S=Significant, NS=Not Significant. WBC=White Blood Cell, RBC= Red Blood Cell, HCT= Haematocrit, MCV= Mean Corpuscular Volume, MCH= Mean Corpuscular Haemoglobin, MCHC= Mean Corpuscular Haemoglobin Concentration, PLT= Platelet, NEUT= Neutrophil, LYM= Lymphocytes, MONO= Monocytes, EOSINO= Eosinophils, BASO= Basophils

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Discussion

This study was carried out to investigate the haematological parameters of Sickle Cell Disease patients on antimalarial regimen within Rivers State, Nigeria. A total of 100 samples were collected for this study of which 50 (50%) were from SCD patients on prophylactic antimalarial regimen and were used as test samples while 50 (50%) samples were from apparently healthy staffs and students of Rivers State University and used as control samples. Out of this 100 samples 51(51%) were from male and 49 (49) were from female. Of the 50 test samples, 24 (48%) were from male and 26 (52%) were from female. And for the 50 control samples, 27 (54%) were from male and 23 (46%) were from female.

Out of the 50 Sickle Cell Disease (SCD) patients (males and females) that were on different prophylactic antimalarial regimen enrolled in this study were from different tribes within Rivers

State which include Abua, Bonny, Ekpeye, Engenni, Etche, Ikwerre, Kalabari, Ogoni, Ogba and Opobo with a frequency distribution of 0.14, 0.1, 0.08, 0.1, 0.16, 0.2, 0.1, 0.04 and 0.04 respectively. This represents 14%, 10%, 8%, 10%, 4%, 16%, 20%, 10%, 4% and 4% respectively. Of this number 24(48%) were males and the other 26 (52%) subjects were females, within the age range of 18-21, 22-25, 26-29, 30-33, 34-37 and 38-41years with the frequency distribution of 0.5, 0.18, 0.02, 0.18, 0.1 and 0.02 and this represents 50%, 18%, 2%, 18%, 10% and 2% respectively. This emphasize that sickle cell disease patients are more of the age range between 18-21 years (50%), this might be because many of the SCD patients die before they get old due to the disorder.

The educational status of the study population showed that Primary, Secondary and Tertiary education had the frequency distribution of 0.14(14%), 0.6(60%) and 0.26 (26%) respectively.

Out of the 50 participants 19 were unmarried and 31 were married with the frequency distribution of 0.38(38%) and 0.62(62%) respectively.

This study observed that male (51%) are more exposed to SCD than female (49%) in Port Harcourt, Rivers State. The age range with the highest SCD rate in Rivers State is From 18-21 years (50%). This may be due to the fact that this genetic abnormality is mostly diagnosed within the above age range and the patients become more aware of their condition within that period. There is a statistical significance in the Haemoglobin of Male (8.81±3.61) as compared to that of the female (11.62±3.77) (p=0.0097) while other parameters showed no statistical significance in this study.

White Blood Cell had a statistical significance increase in SCD patients compared to the control 5.95 $\pm$ 3.38 and 4.93±1.17 respectively with a P-value of 0.0457, the Red blood of SCD patients cell had a significance decrease of 3.07±1.42 as compared to the control 4.63±0.62 with a P-value of 0.0001, the Haemoglobin was statistically significant with values of 10.16±3.92 and 13.47±1.11 respectively with P-value of 0.0001, the Haematocrit was statistically significant with values of 28.64±4.73 and 39.72±3.58 respectively and p-value of 0.0001. The Mean Corpuscular Volume, Mean Corpuscular Haemoglobin, and Mean Corpuscular Haemoglobin Concentration, and other white blood cell differential values of SCD patients, all showed a

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significant decrease when compared to the control, except Platelets, Neutrophils and Basophils, that showed no significant different. All parameters were set at a significant level of $p<0.05$ as shown in table 2.

The result on Table 2 can be attributed to increased consistent hemolysis by the ingestion of anti-malarial drugs as well as the pathogenesis of SCD which turns the RBC into a crescent shape. This result is in agreement with the study carried out by [1], Antwi-Boasiako *et al.* [4] and Kosiyo *et al.* [10] which states that carriage of HbSS was associated with reduced haemoglobin, reduced haematocrit, reduced RBC count, reduced MCHC, increased leucocytosis and increased monocytosis. The increased WBC, Lymphocytes, Monocytes and Eosinophils may be due to the fact that the body keeps fighting against infections as the SCD patients are always exposed to infections. This contradicts the study carried out by Kosiyo *et al*, [10] which states that carriage of HbSS leads to reduced monocytosis. There was no statistical significance in the platelet count, neutrophil and basophil counts. This agrees with several studies carried out including that of Kosiyo *et al.*[10].

Conclusion

This study has established that Sickle Cell Disease patients on prophylactic anti-malarial regimen have low haemoglobin, haematocrit, RBC, MCV, MCH, and MCHC, but increased WBC, lymphocytes, monocytes and eosinophils. Results from this study can help in the differential diagnosis of malaria infection in HbSS genotype based on haematological parameters in resource limited setting where sickle cell genotyping remains a challenge.

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