AMERICAN JOURNAL OF RESEARCH IN MEDICAL SCIENCES, 2020
VOL 5, NO. 2, PAGE 1 – 11

RESEARCH ARTICLE

Open Access

Effect of Immunozin™ on Sickle Cell Disease in Sub-Saharan Africa. A Pilot Study

Dr. Bamgboye M. Afolabi[1,2*], Dr. Lawal Haliru[3], Ramatu Aliyu Zubair[3], Halima Bello-Manga[3], Dr. Ifeoma Ijei[3], Dr. Dogara Livingstaone Gayus[3], Dr Aliyu Usman[3], Dr. Samira M. Badamasi[3], Ashel Dache Sunday[3], Jebo G. Gaura[3], Aisha Kazaure[3], Shakirah Ajibade[3], Eseyin Johnson[3], Dr. Tolu Fagbile[4]

[1]Health, Environment and Development Foundation, 18 Ogunfunmi Street, Lagos, Lagos State, Nigeria
[2]African, Pan African Health Alliance and Collaborative, Salisbury, North Carolina
[3]Barau Dikko Teaching Hospital/Kaduna State University, Lafiya Road, Kaduna, Kaduna State, Nigeria.
[4]Federal Ministry of Health, National Malaria Elimination Program, Abuja, FCT, Nigeria.

ABSTRACT

Introduction: Sickle cell disease, a genetic blood disorder of public health significance, is associated with high morbidity and mortality among many Nigeria children. There is a gap in the use of local medicinal herbal products in its management. As such, the study intended to document the outcome of the administration of the test drug in Sickle Cell patients in Nigeria.

Objective: To determine the effect of the test drug on hematological, biochemical, and liver enzyme parameters in Sickle Cell patients.

Material and Methods: Thirty-three sickle cell disease (SCD) patients, were recruited into an open-label, one-arm, randomized control pilot study from January to May 2018. After enrollment into the study, subjects were seen on Day 0 (1st visit) and then monthly for 5 months. On each visit, each patient was given age-specific dosage of the test drug and blood was taken for the analysis of serum electrolytes, creatinine, urea, reticulocytes, thrombocytes, white and red blood cells, liver function tests, conjugated and total bilirubin and albumin. STATA 13 was used for statistical analysis.

Results: The means (±sd) of age (years) and weight (Kg) of the study subjects (15 males and 18 females) were 13.6 (6.3) and 33.6 (13.9) respectively. Overall there were significant variations in the means (±sd) of monocyte count (t= -2.86, P-value=0.003), eosinophils (t= -2.71, P-value=0.004), reticulocytes (t= 3.38, P-value=0.0007), bicarbonate (t= -4.51, P-value=0.0001), creatinine (t= -2.86, P-value=0.003) and alkaline phosphatase (t= -4.68, P-value=0.000001) values at recruitment and at end of study after monthly administration of the test drug. Study subjects were 3.5 times more likely to have reduced reticulocyte count after administration of the test drug (χ^2=3.57, P-value=0.06, OR=2.93, 95% CI: 0.95, 9.03). Mean (±sd) creatinine level was significantly higher (44.8 [11.5]) among patients with reticulocyte count <2.5% than among those (34.4 [7.9]) with reticulocyte count ≥2.5% at recruitment but was lower (52.4 [10.7]) among patients with reticulocyte count <2.5% than among those (78.8 [70.4]) with reticulocyte count ≥2.5% at end of study. The significant negative correlation between hemoglobin concentration and reticulocyte (r = -9.96, t= -5.41, P-value=0.00001) and between creatinine and reticulocyte (r = -6.90, t= -3.11, P-value=0.004) at recruitment was not evidenced at end of study.

Conclusion: The study revealed overall pre- and post-hoc leucocytosis, thrombocytosis, hyperkalemia and significant variations in reticulocytes, monocytes, eosinophils, and some liver enzymes which may be due to the administration of the test drug. There is need to conduct a multi-disciple and multi-center study on the test drug in SCD patients to further verify its effect on hematological, electrolytes and liver enzyme parameters in different age groups.

ARTICLE HISTORY

Received 14 November 2020
Accepted 04 December 2020
Published 12 December 2020

KEYWORDS

Sickle Cell Disease, Serum Electrolytes, Reticulocytes, Hemoglobin, Immunozin Therapy, Nigeria.

Contact Dr. Bamgboye M. Afolabi ✉ bmafolabi@gmail.com 🖃 Health, Environment and Development Foundation, 18 Ogunfunmi Street, Surulere, Lagos, Nigeria, Tel: +2348030490729

Keywords

Sickle Cell Disease, Serum Electrolytes, Reticulocytes, Hemoglobin, Immunozin Therapy, Nigeria.

Introduction

Sickle cell disease (SCD) is the commonest monogenetic inherited disorder, initially thought to be limited to the tropical and sub-tropical areas of the world. Both the World Health Organization (WHO) and the African Union (AU) declared it separately as a disease of public health disorder with increasing burden and globalization encouraged by migration [1,2]. Sickle cell disease is described by heterogenicity that may be affected by influences such as socio-economic, ethnicity and race, genetic and epigenetic as well as environmental factors [3].

When a single amino acid substitution occurs in the gene encoding the β-globin subunit, an illness entity known as Sickle cell disease (SCD) occurs and decreased distortion and deformability of the erythrocytes is consequent upon polymerization of deoxygenated sickle hemoglobin [4]. These and other series of events, invariably lead to vaso-occlusive crises (VOCs) due to multicellular aggregation and vascular adherence of erythrocytes, neutrophils and thrombocytes that bring about intermittent obstruction in microcirculation [5].

Ansari et al. [6] earlier described SCD as a destructive syndrome that presents as a multi-organ malady affecting about 100,000 persons in the United States, whose options for therapy were limited and only partially effective, though currently the frontier for management is rapidly changing with many choices. Even with the available therapies - hydroxyurea, blood transfusion, hydration and pain medicines - patients continue to suffer the long-term complications of the disease [7]. It is worth noting that a small proportion of children affected with SCD in sub-Saharan Africa reach adolescence [8]. Nigeria has the highest number of people with SCD with about 150,000 babies born yearly with the disease which claims the lives of 70-90% of those afflicted before they reach 5 years of age in contrast to about 90% of patients with SCD who reach adulthood in high-income countries [9]. As a class of hemoglobinopathies in humans, SCD causes a disruption of the normal activities in different systems [9]. Although it begins with polymerization of red blood cells during the deoxygenating phase, it can erupt into a cascade of potentially debilitating conditions resulting from ischemia, reperfusion injury and inflammation. Sickled erythrocytes and their membranes are susceptible to endogenous free-radical-mediated oxidative damage which correlates with the proportion of irreversibly sickled cells [9]. The phenomenon can result in the formation of oxidative stress as well as limit nitric oxide (NO) bioavailability and decrease antioxidant status [9]. The cumulative effects of these traits cause an increase in other forms of reactive oxygen species (ROS), which in turn intensify the symptoms of SCD and generate a vicious cycle [9].

The suppression of incubation induced oxidative stress by antioxidants, free radical scavengers and an iron chelator suggest that oxidation products of membrane-bound haemoglobin contribute towards the pathology of the disease [10]. It's been shown that anti-oxidants play a role in the inhibition of polymerization and sickling of red blood cell [11] and play a major role in the breaking of the vicious cycle that perpetuates this chronic disease. Fasola et al. [12] concluded that interventions aimed at increasing antioxidant capacity of SCD patients may be beneficial to them. Sickle cell disease management largely focuses on primary prevention, symptomatic treatment and targeting of hemoglobin polymerization and red blood cell sickling. There is a long history of ethno botanical use of herbal extracts in management of sickle cell disease. For example, administration of plant extracts based on their antioxidant and anti-sickling properties has been shown to greatly reduce episodes of vaso-occlusive crisis and other symptoms of sickle cell disease [13]. The underlying problem in sickle cell anemia is that the valine for glutamic acid substitution results in hemoglobin tetramers that polymerize into arrays upon deoxygenation in the tissues. This polymerization leads to deformation of the red blood cell into a sickle-like shape making it relatively inflexible and unable to traverse the capillary beds. This structural alteration in the red blood cell can easily be seen under light microscopy and is the source of the name of this disease. Repeated cycles of oxygenation and de-oxygenation lead to irreversible sickling and vaso-occlusive crisis and painful episodes. More than three decades ago, hydroxyurea received the approval of United States Food and Drug Administration (FDA) for use in the management of SCD and only about 3 years ago, L-glutamine, administered orally as a powder, was also permitted to be used to avert critical complications of SCD. In recent years, studies have been and are still being conducted to find new curative approaches for SCD, a mainly tropical disease that seems to have been neglected [14]. Further insight into the morbidity pattern of SCD was the impetus for the development of innovative mediators that address the underlying pathology of sickle cell disease such as adhesion of red blood cells, inflammatory processes, coagulation, and hemoglobin polymerization, among others [15]. There is paucity of information on clinical trials for the management of SCD with African medicinal herbal products, hence the initiation of this study with the primary objective to assess the efficacy of the intervention test drug, in improving the haematological and biochemical parameters, i.e. complete blood count (CBC), reticulocytes, platelets, serum electrolytes, urea and creatinine as well as liver enzymes and albumin of SCD patients.

Materials and Method

This study, conducted between January 11 and May 30 of 2018, was an open-label, single-arm, randomized control pilot study. The study agent in this trial is a commercially available herbal nutritional supplement, which is a combination of *Allium sativum*, *Balanites aegeptiaca, Guiera senegalensis* and *Azadirachta indica.*

Hypotheses to be tested

(i) the test drug does not improve any of the hematological parameters of SCD patients (ii) the test drug does not improve any of the biochemical parameters of SCD patients (iii) the test drug does not improve any of the liver enzyme profiles of SCD patients.

Study area

Based on the 2006 national census figures, Kaduna metropolis, had a population of 760,084 [16] but due to rapid urbanization, its population is now approximately 1,582,102. It is the capital

of Kaduna State in north-west Nigeria, located on River Kaduna and lying between 10.52º North latitude, 7.44º East longitude and 614 meters elevation above sea level. Kaduna State, with over 60 ethnic groups, has two other main urban centers - Zaria and Kafanchan. Kaduna metropolis is a hub of economic activities such as machinery, agriculture, textile and petroleum products industry among others.

Study population

The study population consisted of diagnosed SCD patients attending the pediatric and adult hematology clinics at Barau Dikko Teaching Hospital, Kaduna.

Sample size calculation

A universal formula for selecting the sample size for a clinical trial or research problem based on a level of significance and a chosen margin of error was proposed by Cochran [17] and Levy and Lemeshow [18]. In order to obtain the most efficient, representative sample, for our research, we use the following Cochran's formula for sample size determination.

$$n = \left(\frac{Z_{\alpha/2}\sqrt{\hat{p}(1-\hat{p})}}{\delta} \right)^2 = \frac{Z^2_{\alpha/2}\hat{p}(1-\hat{p})}{\delta^2}$$

Where;

n= minimum sample size required

δ = 0.04 (Predetermined margin of error in the sampling design)

$Z_{\alpha/2}$ = The value of the standard normal ordinate at $\alpha\%$ level of significance

Hence, At the 5% level of significance, $Z_{\alpha/2} = Z_{0.025} = 1.96$. The sample size is finally determined as follows.

In this case; $\hat{p} = 0.25$ (The estimated proportion of sickle cell patients in the population)

$$n = \frac{Z^2_{\alpha/2}\hat{p}(1-\hat{p})}{\delta^2} = \frac{1.96^2 \times 0.25 \times 0.75}{0.06^2} = 200$$

Study design

The sampling design was stratified random sampling with equal allocation into each age group of 0-5, 6-10, 11-15, 16-20, 20 and above. This was a before-and-after study in which participants were evaluated at enrollment and re-evaluated at each monthly administration of the test drug.

Recruitment

Recruitment of participants was carried out at Barau Dikko Teaching Hospital, Kaduna. Each of the 33 recruited SCD patients on the test drug were followed up for 5 visits with an interval of 1 month (approximately 30 days) between each visit. Following completion of all initial checkups, each participant received a monthly dose of the test drug at 500mg (pediatric) and 1000mg (adult) twelve hourly respectively, with a minimum of 120 days of therapy, while standard care was provided by the hospital.

Inclusion criteria

These were (i) diagnosis of Sickle cell disease (ii) provision of signed and dated informed consent form (iii) stated willingness to comply with all study procedures and availability for the duration of the study (iv) aged 5 to 45 years (v) exhibit any clinical signs and symptoms of sickle cell disorder including at list an episode of crisis monthly and (vi) ability to take oral medication and be willing to adhere to the medication regimen.

Exclusion criteria

An individual who met any of the following criteria was excluded from participation in this study: (i) concomitant use of any other medication or medical devices that is not part of the study (ii) known allergic reactions to any of the components of test drug (iii) pregnancy or lactation (iv) is planning to get pregnant within five months after commencement of the study (v) has cardiac, hepatic or kidney disease (vi) febrile illness within 1 month preceding the study (malaria, tuberculosis, measles) (vi) current alcohol or tobacco use 4 months prior to the start of the study.

Screening and baseline data at recruitment into study: On Day 0 (Visit 1), informed consent from and inclusion/exclusion criteria for each participant was verified (urine pregnancy test was conducted for females in reproductive age group), demographic information, medical and medication history as well as tobacco/alcohol use were recorded. Blood was aseptically collected for electrophoresis of hemoglobin genotype, complete blood count, reticulocytes count as well as hepatic and renal parameters. Urinalysis was also performed before each patient was given appropriate dosage of the test drug as specified above (Visit 2).

Endpoint

Important primary endpoint was where a considerable number of the participants demonstrated a significantly lower rate of vaso-occlusive crisis within one hundred and twenty days of the study. Important Secondary Endpoints: Where a significant number of those participants with secondary symptoms or complications exhibited or reported a significant improvement in their symptoms.

Laboratory analyses

Audicom AC 9900 ISE analyser (Ion-selective electrode) was used for the analysis of serum electrolytes; Urease Berthelot method was used for the analysis of urea; Jaffe-Slot Alkaline Picrate Method was used for analysis of Creatinine; Jendrassik and Grof Bilirubin method was used for both Total and Conjugated Bilirubin; Reitman and Frankel Method was used for the liver enzymes; all clinical chemistry parameters were analysed with Selectra Pro S. A complete clinical chemistry Analyser; all Haematology parameters were analysed using Sysmex KX-21N Automated Haematology analyser. The analyser makes use of two key reagents called Cell Pack and Stromatolyser.

Ethical approval

Each study subject (or caregiver/guardian) signed a consent form to participate in the study and was assured that his/her data will be discreet, coded, and unnamed. The study was approved by the Human Research Ethics Committee (HREC) with a reference number 17-0025 and protocol number 17-0027-1.

Data management and statistical analysis

The cleaned and coded data was transferred from Excel

spreadsheet into STATA 13 software which was used for further analysis. For the purpose of this study, age (years) was categorized into <10, 10-19.9 and ≥20. Multivariate regression analysis was performed to determine the association between demographic, hematological and other variables (independent variables) and reticulocyte counts at 1st and 6th visits (dependent variable). Chi-square and Fisher's exact test analysis with odds ratio (OR) at 95% Confidence interval were used to evaluate the differences between two proportions or rates and to determine the probability of low reticulocyte count in different age groups at 1st and 6th visits. Student's t-test was used to evaluate significant differences in means between two continuous variables. One-way analysis of variance (ANOVA) with Bonferroni adjustment or Kruskal-Wallis test was used to compare groups as appropriate. Data were presented as numbers and percentages for categorical variables, as mean with standard deviations for continuous variables and as Tables and Figures for all variables. A P-value <0.05 was regarded as statistically substantial. STATA version 13.0 (STATA Inc., Texas, USA) was used for statistical analysis.

Results

A total of 33 SCD patients, 15 males and 18 females, with means (±sd) of age (years) and weight (Kg) as 13.6 (6.3) and 33.6 (13.9) were recruited into the study. The highest proportion of the study participants (19, 57.6%) were aged 10.0-19.9 ranging from 10.0 to 19.0 years and the least (5, 15.1%) were aged 20.0 – 29.9 years ranging from 24.0-27.0 years (Table 1). Only monocytes (baseline 3.4 (0.9)%, end-of-study 4.0 (0.8)%; t = -2.86, P-value=0.003), driven mainly by those aged 10-19.9 years (t-test = -3.28, P-value = 0.001), and eosinophil (baseline 1.7 (0.6)%, end-of-study 2.1 (0.6)%; t = -2.71, P-value=0.004), driven mainly by those aged 10-19.9 years (t-test = -3.08, P-value = 0.002) demonstrated notable increase in mean values while reticulocyte showed significant decrease (baseline 2.5 (0.8)%, end-of-study 1.7 (1.1)%; t = 3.38, P-value=0.0007), driven mainly by those aged <10 years (t-test = 3.12, P-value = 0.003), and those aged ≥20 years (t-test = 3.12, P-value = 0.003) respectively. There was also a significant (t-test = 1.94, P-value = 0.03) reduction in the mean (±) total platelet count from baseline count of 499.6 (220.9) (x109//L) to end-of-study count of 381.1 (148.2) (x109//L) (Table 2). Monthly mean (±) hemoglobin concentration among study groups indicate that those aged <10 years (Figure 1a), attained highest hemoglobin concentration of 8.0 g/L in the 4th month of study (baseline of 7.4g/L), those aged 10-19.9 (Figure 1b) and ≥20 years (Figure 1c) respectively achieved the highest concentrations of 8.4 g/L (baseline of 7.8 g/L) and 8.8g/L (baseline of 8.3 g/L) in the first month of study. There was no consistency in the progression of hemoglobin concentration in any of the age groups. Monthly mean (±) reticulocyte count among study groups indicate that those aged <10 years (Figure 2a), attained highest mean reticulocyte count of 4.0 % in the 2nd month of study (baseline of 2.8 %), those aged 10-19.9 (Figure 2b) and ≥20 years (Figure 2c) reached the highest level of 4.2% in the 3rd month of study respectively before their values declined below the baseline value at end of the study. There was a reduction in the white blood cell count (Figures 3a-3b), significant elevation of the monocytes, reduction in reticulocytes and thrombocyte count (Figure 4) at the end of the study. Hence the hypothesis that the study drug does not improve any hematological parameter is rejected.

There were significant differences (t-test = -1.71, P-value=0.046; t-test = -4.51, P-value=0.0001) in the potassium value of 5.4 (0.9) mmol/l and bicarbonate value of 19.1 (5.5) mmol/l at baseline compared to their values of 5.8 (1.0) mmol/l and 24.1 (3.2) mmol/l respectively at the end of the study (Table 3). However, it is pertinent to note that the mean value of sodium at end-of-study among those aged <10 years at 136.9 (4.4) mmol/l was significantly lower (t-test=2.13, P-value=0.02) than the value at baseline of 140.9±3.5 mmol/l (Table 3), though both values are still within normal range. The Table also shows that mean creatinine value rose significantly (t-test = -2.86,

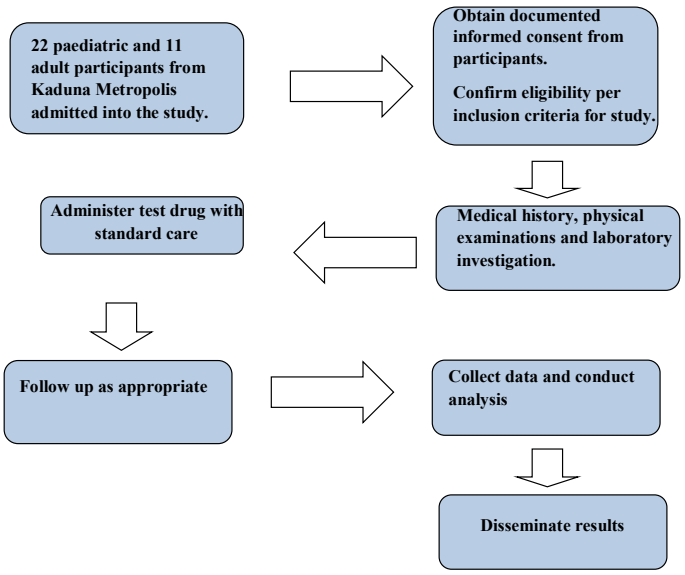
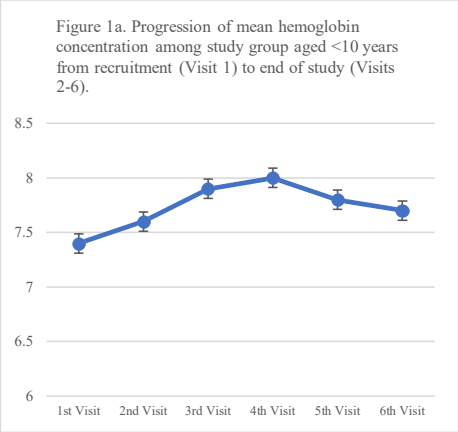
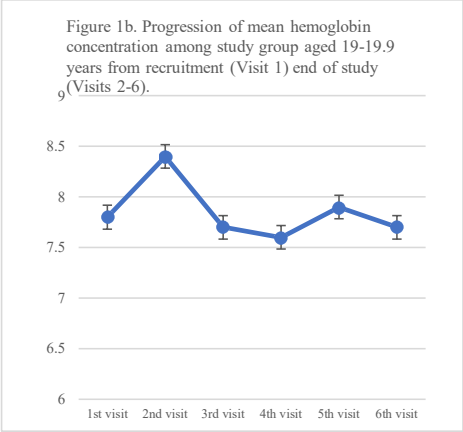


Figure 1. Schematic diagram of study design

Table 1. Demographic characteristics of study participants.

Variable			Study group					
			Freq.	%	Mean	±sd	Minimum	Maximum
Age (years)		All	33	100.0	13.6	6.3	5.2	27
		<10	9	27.3	6.7	1.5	5.2	9.3
		10.0 - 19.9	19	57.6	13.6	2.3	10.0	19.0
		20.0 - 29.9	5	15.1	25.8	1.1	24.0	27.0
Weight (Kg)	If age is	All	33	100.0	33.6	13.9	16.0	75.0
		<10	9	27.3	19.7	3.4	16.0	24.0
		10.0-19.9	19	57.6	33.1	9.3	18.0	54.0
		20.1-30	5	15.1	55.9	8.3	47.0	75.0
Sex		Male	15	45.5	-	-	-	-
		Female	18	54.5	-	-	-	-





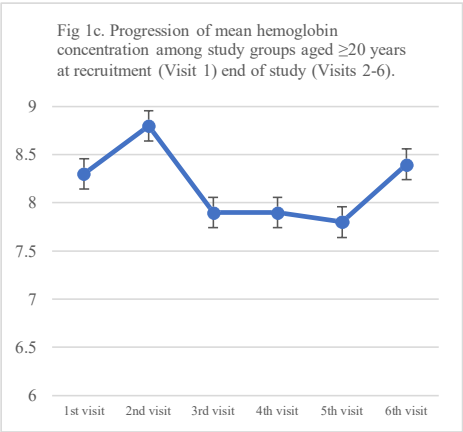
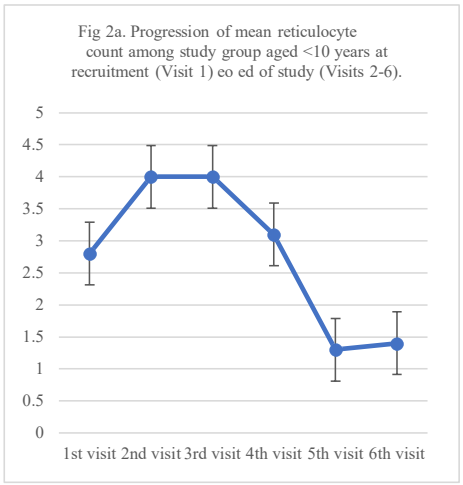


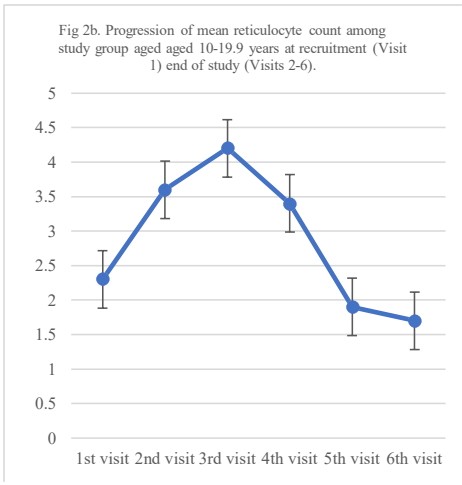
Table 2. Mean (±sd) hematological parameters at recruitment (Visit 1) at end of study (Visit 6) post administration of the test drug relevant to age groups.

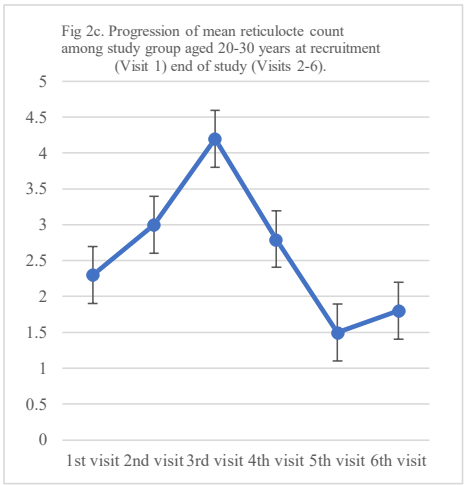
Clinical parameter (%)	Statistics	All				Age <10 years				Age 10-19.9 years				Age ≥20 years			
		Visit 1	Visit 6	t-test	P-value	Visit 1	Visit 6	t-test	P-value	Visit 1	Visit 6	t-test	P-value	Visit 1	Visit 6	t-test	P-value
WBC count (x10^9/L)	n	33	33	1.65	0.05	9	9	0.97	0.17	19	19	1.08	0.15	5	5	0.97	0.17
	Mean	13.7	12.1			15.6	13.7			13.4	12.1			15.6	13.7		
	±sd	4.4	3.4			4.9	3.2			4.1	3.3			4.9	3.2		
	Range	4.9-24.5	6.2-18.4			6.8-24.5	9.3-18.4			7.8-20.3	6.4-17.7			6.8-24.5	9.3-18.4		
Monocytes (%)	n	33	33	-2.86	0.003	9	9	-1.86	0.04	19	19	-3.28	0.001	5	5	-1.86	0.04
	Mean	3.4	4.0			3.1	3.8			3.3	4.1			3.1	3.8		
	±sd	0.9	0.8			0.8	0.8			0.8	0.7			0.8	0.8		
	Range	2-6	3-5			2-4	3-5			2-5	3-5			2-4	3-5		
Neutrophils (%)	n	33	33	0.58	0.28	9	9	0.77	0.23	19	19	0.03	0.49	5	5	0.77	0.23
	Mean	53.6	52.3			54.6	51.3			52.7	52.6			54.6	51.3		
	±sd	8.6	9.6			10.2	7.7			8.9	11.2			10.2	7.7		
	Range	29-71	33-78			42-71	43-67			29-65	33-78			42-71	43-67		
Lymphocytes (%)	n	33	33	0.29	0.39	9	9	-0.25	0.40	19	19	0.65	0.26	5	5	-0.25	0.40
	Mean	41.5	40.8			41.7	42.7			42.2	39.8			41.7	42.7		
	±sd	8.4	11.0			8.9	8.1			9.2	13.1			8.9	8.1		
	Range	29-67	12-61			29-53	26-52			32-67	12-61			29-53	26-52		
Eosinophils (%)	n	33	33	-2.71	0.004	9	9	-2.34	0.02	19	19	-3.08	0.002	5	5	-2.34	0.02
	Mean	1.7	2.1			1.7	2.2			1.5	2.0			1.7	2.2		
	±sd	0.6	0.6			0.5	0.4			0.5	0.5			0.5	0.4		
	Range	1-3	1-3			1-2	2-3			1-2	1-3			1-2	2-3		
Reticulocyte (%)	n	33	33	3.38	0.0007	9	9	3.12	0.003	19	19	2.14	0.02	5	5	3.12	0.003
	Mean	2.5	1.7			2.8	1.4			2.3	1.7			2.8	1.4		
	±sd	0.8	1.1			1.0	0.9			0.7	1.0			1.0	0.9		
	Range	1.2-4.1	0.4-4			1.7-4.1	0.4-3.2			1.5-4.1	0.4-3.9			1.7-4.1	0.4-3.2		
Total Platelet (x10^9/L)	n	33	33	1.44	0.08	9	9	-0.35	0.36	19	19	1.94	0.03	5	5	-0.35	0.36
	Mean	475.7	410.3			427.2	453.3			499.6	381.1			427.2	453.3		
	±sd	206.4	159.0			152.0	160.4			220.9	148.2			152.0	160.4		
	Range	149-928	127-720			149-640	176-702			187-928	127-640			149-640	176-702		
Hemoglobin g/dl	n	33	33	0.00	1.00	9	9	-0.58	0.29	19	19	0.31	0.38	5	5	-0.58	0.29
	Mean	7.8	7.8			7.4	7.7			7.8	7.7			7.4	7.7		
	±sd	1.1	1.1			1.1	1.1			1.0	1.0			1.1	1.1		
	Range	5.9-10.3	6-10.3			5.9-9.6	6-9.4			6.3-10.1	6.1-9.6			5.9-9.6	6-9.4		
Packed Cell Volume	n	33	33	-0.12	0.45	9	9	-0.51	0.31	19	19	0.49	0.31	5	5	-0.51	0.31
	Mean	25.3	25.4			24.2	25.0			25.4	24.9			24.2	25.0		
	±sd	3.6	3.2			3.3	3.4			3.1	3.2			3.3	3.4		
	Range	18-34	19-32			18-30	19-30			20-33	20-30			18-30	19-30		
	SS	df	MS	F	P	SS	df	MS	F	P		SS	Df	MS	F	P	
Between	1.74	5	0.35	0.27	0.93	7.37	5	1.471	1.196	0.316		3.67	5	0.73	0.280	0.921	
Within	62.39	48	1.30			133.08	108	1.232				63.65	24	2.65			
Total	64.13	53				140.45	113					67.35	29				

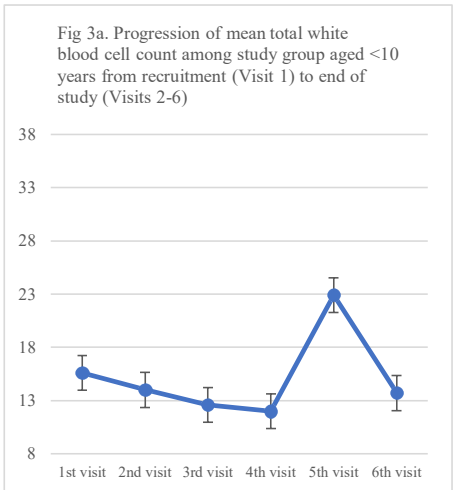
Table 3. Mean (±sd) values of electrolytes, urea and creatinine among study groups at recruitment (Visit 1) and at end of study (Visit 6) after administration of the test drug for the management of Sickle Cell Disease.

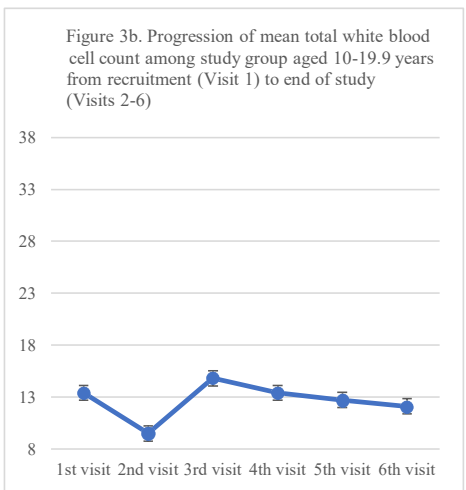
Clinical parameter	Statistics	All				Age <10 years				Age 10-19.9 years				Age ≥20 years			
		Visit 1	Visit 6	t-test	P-value	Visit 1	Visit 6	t-test	P-value	1	6	t-test	P-value	1	6	t-test	P-value
Electrolytes (mmol/l)	n	33	33			9	9			19	19			5	5		
Sodium	Mean	139.1	137.9	1.37	0.13	140.9	136.9	2.13	0.02	138.6	138.0	0.39	0.35	137.6	139.6	-1.08	0.16
	±sd	4.8	3.7			3.5	4.4			5.5	3.7			3.3	2.5		
	Range	122-146	127-144			134-145	130-144			122-146	127-144			132-140	136-143		
	n	33	33			9	9			19	19			5	5		
Potassium	Mean	5.4	5.8	-1.71	0.046	5.5	6.2	-1.65	0.06	5.3	5.9	-1.94	0.03	5.6	5.0	0.93	0.19
	±sd	0.9	1.0			0.9	0.9			0.9	1.0			1.2	0.8		
	Range	3.9-7.4	4-7.6			4.4-7.4	4.8-7.6			3.9-6.9	4.4-7.3			4.3-7.0	4-5.7		
	n	33	33			9	9			19	19			5	5		
Chloride	Mean	100.5	100.7	-0.18	0.43	102.0	100.9	0.54	0.30	100.2	100.6	-0.26	0.40	99.0	100.4	-0.54	0.30
	±sd	5.3	3.3			5.1	3.4			5.6	3.5			5.1	2.7		
	Range	82-108	89-105			93-108	95-105			82-108	89-105			95-108	96-103		
	n	33	33			9	9			19	19			5	5		
Bicarbonate	Mean	19.1	24.1	-4.51	0.0001	18.1	21.7	-1.30	0.11	18.7	24.5	-4.83	0.00001	22.4	26.8	-2.14	0.04
	±sd	5.5	3.2			7.1	4.2			4.7	2.3			4.3	1.6		
	Range	8-3	14-29			8-30	14-27			11-28	20-28			18-29	25-29		
	n	33	33			9	9			19	19			5	5		
Urea (mmol/l)	Mean	2.1	2.7			2.3	2.3			1.8	3.1			2.9	2.1		
	±sd	0.9	2.5	-1.30	0.10	0.9	0.4	0.00	1.00	0.7	3.2	-1.73	0.05	1.1	0.6	1.42	0.10
	Range	1-4.7	1.4-16.2			1.3-3.7	1.6-3			1-4.6	1.5-16.2			1.9-4.7	1.4-2.9		
	n	33	33			9	9			19	19			5	5		
Creatinine µmol/l	Mean	40.7	57.2	-2.86	0.003	35.6	49.7	-2.48	0.01	41.3	61.3	-2.11	0.02	47.6	55.2	-1.07	0.16
	±sd	11.3	31.2			11.3	12.7			10.8	39.8			11.4	11.0		
	Range	14-65	33-221			14-56	33-67			16-63	38-221			33-65	45-72		

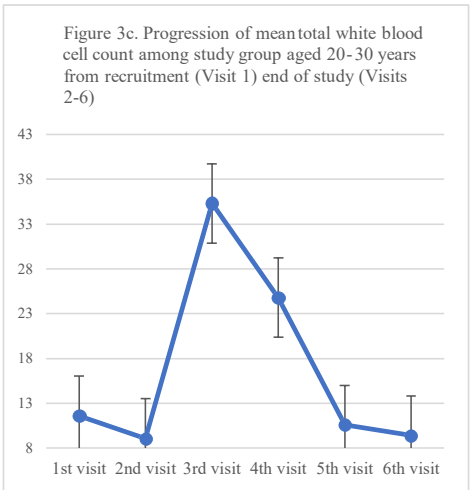












P-value=0.003) from 1st visit value of 40.7 (11.3) µmol/l to 57.2 (31.2) µmol/l at the 6th visit, driven mainly by those aged <10 and 10-19.9 years only. Figures 5a-5c show the monthly values of electrolytes, urea and creatinine among the three age groups indicating a drop in sodium level at 4th month of study among those age <10 years and a deeper drop in the 3rd month among those aged ≥20 years and an almost normal levels of bicarbonate, potassium and urea throughout the study period in all the age groups.

Overall alkaline phosphatase significantly increased (t-test = -4.68, P-value=0.000001) between baseline value of 40.2 (9.6) IU/l and end-of-study value of 54.6 (1.5) IU/l. This increase was most pronounced among those age 10-19.9 years. Mean (±sd) serum albumin was notably raised (t-test = - 2.26, P-value=0.03) from its baseline value of 42.0 (2.5) mg/dl to its end-of-study value of 45.8 (2.8) mg/dl. Mean (±sd) serum conjugated bilirubin was also notably raised (t-test = - 1.91, P-value=0.03) from its baseline value of 6.9 (4.2) µmol/l to its end-of-study value of 9.6 (4.5) µmol/l only among those aged 10-19.9 years (Table 4). At recruitment, only 20 (60.6%) patients recorded a reticulocyte count of <2.5% but this increased to 27 (81.8%) at the end of study. Patients were approximately thrice more likely to have lower reticulocyte count after repeated administration of the test drug (χ^2=3.57, P-value=0.06, OR=2.93, 95% CI: 0.95, 9.03) than at recruitment before administration of study drug, more especially among those <10 years old (χ^2=1.11, P-value=0.30, OR=6.40, 95% CI: 0.55, 74.89), those aged 10-19.9 year (χ^2=1.22, P-value=0.27, OR=3.11, 95% CI: 0.66, 14.60), and males (χ^2=2.54, P-value=0.11, OR=5.69, 95% CI: 0.94, 34.46) (Table 5). Multivariate regression analysis showed that hemoglobin concentration and creatinine were responsible for a significant 48.6% and 23.8% respectively of the variations observed in reticulocyte count at baseline (F=29.28, P-value = 0.000001; F=9.68, P-value=0.004) but were responsible for insignificant 0.1% and 0.7% at the end of the study (Table 6). Significant negative correlation was observed between hemoglobin concentration and reticulocyte count at baseline (R^2 = -9.96, t = -5.41, P-value = 0.00001, 95% CI: -13.71, -6.20) but not at the end of the study (R^2 = -0.03, t = -0.12, P-value = 0.90, 95% CI: -0.53, 0.47). Significant negative correlation was also observed between creatinine and reticulocyte count at baseline (R^2 = -6.90, t = -3.11, P-value = 0.004, 95% CI: -11.43, -2.38) but not at the end of the study (R^2 = -3.28, t = -0.46, P-value = 0.65, 95% CI: -17.89, 11.34) (Table 7).

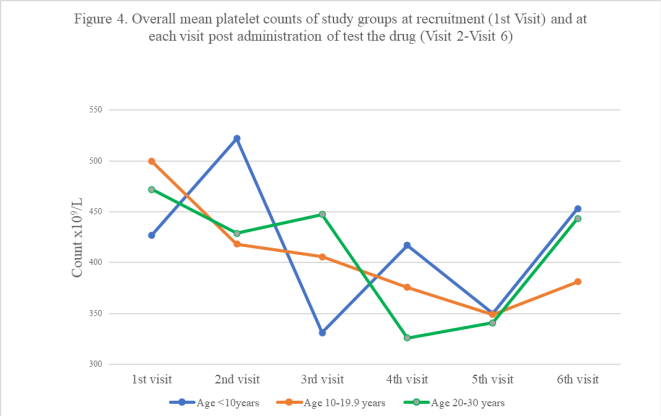


Table 4. Mean (±sd) values of various liver function tests among study groups at recruitment (Visit 1) and at end of study (Visit 6) after the test drug for the management of Sickle Cell Disease.

Clinical parameter	Statistics	All				Age <10 years				Age 10-19.9 years				Age ≥20 years			
		Visit 1	Visit 6	t-test	P-value	Visit 1	Visit 6	t-test	P-value	1	6	t-test	P-value	1	6	t-test	P-value
Alkaline Phosphatase (IU/l)	N	33	32	-4.68	0.000001	9	9	--2.56	0.01	19	19	-3.29	0.001	5	5	-2.95	0.01
	Mean	40.2	54.6			47.1	57.8			39.8	53.8			29.6	51.6		
	±sd	9.6	14.5			5.2	11.4			9.2	16.1			7.7	14.8		
	Range	24-57	23-85			41-54	35-72			26-57	23-85			24-43	37-76		
Alanine aminotransferase (IU/l)	N	33	32	-0.64	0.26	9	9	-1.19	0.13	18	19	-0.25	0.40	5	5	-0.02	0.49
	Mean	24.0	25.9			20.2	24.6			25.4	26.5			25.8	26.0		
	±sd	12.5	11.2			5.7	9.5			14.8	12.1			12.6	12.9		
	Range	7-70	12-64			14-33	12-41			7-70	15-64			8-43	14-40		
Aspartate aminotransferase (IU/l)	N	33	33	0.75	0.23	9	9	0.76	0.23	19	19	0.30	0.38	5	5	0.57	0.30
	Mean	59.0	54.4			61.7	55.2			59.4	56.7			53.0	44.0		
	±sd	28.2	20.0			20.4	15.9			31.5	22.6			32.0	15.3		
	Range	27-156	23-131			37-101	39-81			27-156	29-131			29-108	30-67		
Albumin (mg/dl)	N	33	33	-1.01	0.16	9	9	-1.76	0.05	19	19	0.45	0.33	5	5	-2.26	0.03
	Mean	44.0	45.1			42.9	46.3			45.0	44.3			42.0	45.8		
	±sd	4.5	4.2			5.4	2.1			4.3	5.2			2.5	2.8		
	Range	37-52	31-52			37-52	43-50			38-52	31-52			39-45	42-49		
Conjugated bilirubin (µmol/l)	N	33	33	-1.28	0.10	9	9	-0.60	0.28	19	19	-1.91	0.03	5	5	0.85	0.79
	Mean	7.2	8.6			6.0	7.0			6.9	9.6			10.2	7.4		
	±sd	4.4	4.3			2.7	4.2			4.2	4.5			6.7	3.0		
	Range	1.8-19.5	1.9-22.3			1.8-10.1	1.9-13			1.9-16.1	1.9-22.3			5.1-19.5	3.7-11.2		
Total bilirubin (Âµmol/l)	N	33	33	-0.73	0.24	9	9	0.54	0.30	19	19	-1.02	0.16	5	5	0.93	0.80
	Mean	22.0	28.3			18.1	15.9			21.5	36.1			30.7	20.8		
	±sd	13.1	47.1			7.1	9.9			11.8	61.1			22.6	7.6		
	Range	7.5-62.0	4.9-285			8.5-29.8	4.9-35			7.5-47.1	5.9-285			9.9-62	9.6-30.2		

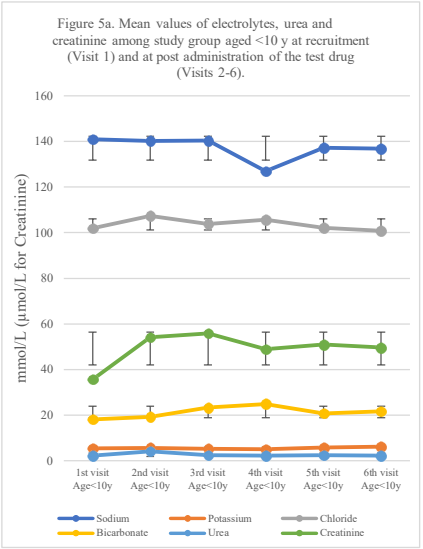
Table 5. Determination of low reticulocyte count at baseline (Visit 1) and at end of the study (Visit 6) relative to age groups and gender

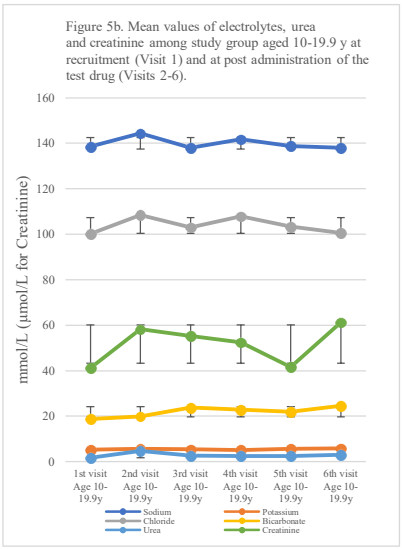
Variable		Corrected reticulocyte count at recruitment (Visit 1)						Corrected reticulocyte count at end of study (Visit 6)									
		<2.5%		≥2.5%		t-test	P-value	<2.5%		≥2.5%		t-test	P-value	χ^2	P-value	OR	95% CI
		Freq (%)	Mean (±sd)	Freq. (%)	Mean (±sd)			Freq. (%)	Mean (±sd)	Freq. (%)	Mean (±sd)						
Age in years (Mean±sd)	All	20 (60.6)	13.5 (6.4)	13 (39.4)	13.6 (6.5)	-0.04	0.48	27 (81.8)	12.9 (5.9)	6 (18.2)	16.7 (7.7)	-1.14	0.15	3.57	0.06	2.93	0.95, 9.03
	<10	5 (25.0)	6.0 (0.7)	4 (30.8)	7.7 (1.8)	-1.96	0.045	8 (29.6)	6.8 (1.6)	1 (16.7)	6.2 (0.0)	-	-	1.11	0.30	6.40	0.55, 74.89
	10-19.9	12 (60.0)	13.6 (2.1)	7 (53.8)	13.4 (2.8)	0.16	0.56	16 (59.3)	13.4 (2.4)	3 (50.0)	14.3 (2.3)	-0.62	0.29	1.22	0.27	3.11	0.66, 14.60
	≥20	3 (15.0)	25.7 (1.5)	2 (15.4)	26.0 (0.0)	-	-	3 (11.1)	26.0 (0.0)	2 (33.3)	25.5 (2.1)	-	-	0.00	1.00	1.00	0.08, 12.56
Gender	Male	8 (40.0)	-	7 (53.9)	-	-	-	13 (48.2)	-	-	2 (33.3)	-	-	2.54*	0.11	5.69	0.94, 34.46
	Female	12 (60.0)	-	6 (46.1)	-	-	-	14 (51.8)	-	-	4 (66.7)	-	-	0.14*	0.71	1.75	0.40, 7.70

SCD patients were approximately 3 times more likely to have reticulocyte count <2.5% after administration of the test drug (Visits 2-6) compared to when they were recruited into the study (Visit 1) (χ^2=3.57, P-value=0.06, OR=2.93, 95% CI: 0.95, 9.03).

Table 6. Multiple regression analysis on associations between reticulocyte counts at 1st and 6th visits as dependent variables and demographic and other serum quantity as independent variables

Equation	Visit 1				Visit 6			
	Obs.	R^2	F	P-value	Obs.	R^2	F	P-value
Age	33	0.020	0.633	0.432	32	0.021	0.649	0.427
Height	33	0.030	0.944	0.339	32	0.026	0.799	0.378
Weight	33	0.022	0.683	0.415	32	0.021	0.651	0.426
BMI	33	0.022	0.683	0.415	32	0.021	0.651	0.426
Total platelets	33	0.047	1.527	0.226	32	0.001	0.036	0.851
Hemoglobin	33	0.486	29.282	0.000001	32	0.001	0.015	0.903
White Blood Cell Count	33	0.073	2.454	0.127	32	0.060	1.912	0.177
Urea	33	0.045	1.459	0.236	32	0.010	0.309	0.583
Creatinine	33	0.238	9.683	0.004	32	0.007	0.209	0.651
ALT	33	0.005	0.149	0.703	32	0.016	0.478	0.495
ALK	33	0.000	0.003	0.958	32	0.022	0.670	0.420
Aspartate transaminase (AST)	33	0.075	2.506	0.124	32	0.000	0.002	0.961
Albumin	33	0.001	0.033	0.856	32	0.034	1.062	0.311
Conjugated bilirubin	33	0.053	1.738	0.197	32	0.024	0.737	0.398
Total bilirubin	33	0.060	1.964	0.171	32	0.030	0.933	0.342
Sodium	33	0.064	2.125	0.155	32	0.008	0.253	0.618
Potassium	33	0.047	1.530	0.225	32	0.000	0.005	0.942
Chloride	33	0.003	0.095	0.760	32	0.041	1.285	0.266
Bicarbonate	33	0.055	1.789	0.191	32	0.009	0.279	0.601





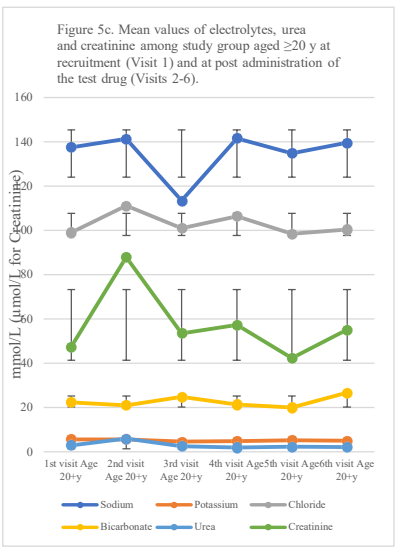


Table 7. Correlation coefficient outcomes between reticulocytes at 1st and 6th visits as dependent variable and other serum quantity as independent variables

Variable	Corrected reticulocyte count at recruitment					Corrected reticulocyte count at end of study				
	Visit 1					Visit 6				
	Coeff.	Std. Err.	t	P	95% CI	Coeff.	Std. Err.	t	P	95% CI
Age	-1.12	1.41	-0.80	0.43	-3.98, 1.75	-1.16	1.44	-0.81	0.43	-4.11, 1.79
Height	-4.26	4.39	-0.97	0.34	-13.27, 4.69	-4.02	4.50	-0.89	0.38	-13.22, 5.17
Weight	-2.55	3.08	-0.83	0.42	-8.83, 3.74	-2.6	3.17	-0.81	0.43	-9.03, 3.91
BMI	-2.54	3.08	-0.83	0.42	-8.83, 3.74	-2.56	3.17	-0.81	0.43	-9.03, 3.91
Total Platelets	55.77	45.13	1.24	0.23	-36.28, 147.81	-6.88	36.29	-0.19	0.85	-81.00, 57.24
Hemoglobin SI unit	-9.96	1.83	-5.41	0.000001	-13.71, -6.20	-0.03	0.24	-0.12	0.90	-0.53, 0.47
White Blood Cell Count	1.47	0.94	1.57	0.13	-0.44, 3.39	-1.03	0.74	-1.38	0.18	-2.54, 0.49
Urea	0.24	0.20	1.21	0.24	-0.17, 0.65	-0.32	0.57	-0.56	0.58	-1.48, 0.85
Creatinine	-6.90	2.22	-3.11	0.004	-11.43, -2.38	-3.28	7.16	-0.46	0.65	-17.89, 11.34
ALT	1.08	2.80	0.39	0.70	-4.63, 6.79	1.77	2.56	0.69	0.50	-3.46, 7.00
ALK	-0.12	2.15	-0.05	0.96	-4.51, 4.28	-2.66	3.25	-0.82	0.42	-9.29, 3.97
AST	9.62	6.08	1.58	0.12	-2.78, 22.03	-0.23	4.59	-0.05	0.96	-9.61, 9.15
Albumin	0.18	1.00	0.18	0.86	-1.86, 2.23	0.99	0.96	1.03	0.31	-0.97, 2.95
Conjugated bilirubin	1.25	0.95	1.32	0.20	-0.68, 3.19	-0.84	0.98	-0.86	0.40	-2.84, 1.16
Total bilirubin	3.98	2.84	1.40	0.17	-1.81, 9.76	-10.32	10.69	-0.97	0.34	-32.15, 11.50
Sodium	1.51	1.03	1.46	0.16	-0.60, 3.61	0.43	0.86	0.50	0.62	-1.32, 2.18
Potassium	0.25	0.20	1.24	0.23	-0.16, 0.66	0.02	0.22	0.07	0.94	-0.43, 0.46
Chloride	0.37	1.19	0.31	0.76	-2.06, 2.79	0.83	0.73	1.13	0.27	-0.67, 2.33
Bicarbonate	1.59	1.19	1.34	0.19	-0.83, 4.01	-0.39	0.74	-0.53	0.61	-1.91, 1.12

Discussion

There are limited prior studies that have examined the impact of novel African medicinal herbs on sickle cell disease. Two early Nigerian study documented the effect of the roots of *"Fagara zanthoxyloides"* (Orin ata in Yoruba language, Chewing stick in English) which, according to the study, seemed to possess anti-sickling activity, on red blood cells [19] and specifically on sickle cell anemia [20]. Adebiyi et al. [21] also reported that crude extract on "*F. zanthoxyloides*" has relatively high antioxidant activity. Use of the test drug as a therapy in the management of SCD is unique in that it is predominantly from African medicinal herbarium, which is not included in the current and novel therapies for the prevention of vaso-occlusive crisis in sickle cell disease documented by Osunkwo et al. [5].

The 5.8 ± 1.0 post hoc serum potassium reported was higher than what Jaitly et al. [22] reported as a case report on an African American with a SCD and that reported by Agoyero and Nwanze [23]. Hyperkalemia may be an indication of cell dehydration, a distinctive feature of sickle cell disease and an essential contributor to disease pathophysiology [24]. Earlier studies suggested that excessive potassium loss results in cellular dehydration and deoxygenation [25] and that dehydration and hypoxia are two of the characteristic features of crisis state. This may account for the potassium losses from the cell into the extracellular fluid which resulted in an elevated plasma potassium concentration [26].

The overall low hemoglobin level of 7.8 ± 1.1 g/dl throughout this study is coherent with the findings in another study conducted in south of Nigeria that reported a mean hemoglobin level of 7.93 ± 1.47 g/dl but lower than the 8.53 ± 1.6 g/dl (male) or the 8.31 ± 1.57 g/dl (female) reported from Ghana [27].

Data from this study also indicates the presence of thrombocytosis in all patients. This finding is consisted with the reports of Ahmed et al. [28] in a Saudi Arabia study and that of Onwukeme et al. [29] in a Nigeria study, who attributed thrombocytosis to the background hemolytic anemia and auto-splenectomy. However, data from this study revealed that only SCD patients aged 10-19.9 years had normal value of thrombocytes at the end of the study.

The finding of elevated white blood cell counts (WBC) in the study patients was coherent with an earlier studies, which shows that leucocytosis is a consistent event in SCD even when there are no infections, an observation thought to reflect redeployment of granulocytes from peripheral to circulating pool [30]. The total white blood cell count described in this study is also consistent with what was reported in an earlier study in Zaria, also in Kaduna State, North of Nigeria [31] and in Lagos, South of Nigeria [32].

Monocyte series of the white blood cells are hardly reported in the hematological profiles of SCD in sub-Saharan Africa [27,31,33]. Monocytes play a vital role in scavenging dead cells and sickled red cells. The monocytosis among SCD patients in this study is probably part of the "patrolling monocytes" which protect against vascular damage by scavenging cellular debris and ameliorate VOC in SCD [34]. In this study, the increased population of monocytes, post-administration of the test drug, probably supports in repairing tissues that are already damaged as a result of reduced perfusion by stopping the inflammatory process and evacuating necrotic cells from the site of tissue damage and possibly cytokine production. Belcher et al [32] are of the opinion that monocytes, not lymphocytes or platelets, are the mononuclear cells responsible for activating endothelial cells triggering endothelial nuclear factor-kappa B (NF-kappaB) nuclear translocation. Cell-to-cell contact of monocytes and endothelium enhanced, but was not required for, activation. They speculated that activated monocytes and endothelium, caused by vascular inflammation, play considerable part in the pathophysiology of VOC.

Another interesting finding is the increase in eosinophil population post administration of the test drug in all age

groups in the study, especially in those aged 10-19.9 years. Eosinophils produce an array of cytokines and growth factors linked with numerous immuno-modulatory tasks. The increase in eosinophil population in the study could have been for tissue repair, general homeostasis, glucose metabolism and a host of other preservative functions [36]. In a study carried out in Northern Nigeria, Ahmed and Uraka [37] hypothesized that eosinopenia may be a more reliable marker of infection in SCA patients which could indicate that participants in this study had some infection at baseline, as reflected in the relatively low eosinophil count and relative leucocytosis in all age groups at recruitment into the study.

Another key finding is that mean reticulocyte count was reduced after administration of the test drug in all the study subjects and in each age group an observation consistent with what Borba et al. [38] reported in Brazil. Thachil [39] noted that excess reticulocytes may contribute to the promotion of thrombus formation in patients with sickle cell. The reticulocyte count in SCD patients differentiates a sequestration crisis from an aplastic crisis.

Conclusion

At enrollment, SCD was associated with leucocytosis, reticulocytosis, thrombocytosis, lower level of eosinophil, hyperkalemia, raised level of alkaline phosphatase, raised level of Aspartate Amino transferase (AST), and slightly raised level of conjugated bilirubin. At end of the study there was a marginally significant reduction in leucocytosis, significant reduction in reticulocytosis to within normal range, insignificant reduction in thrombocyte count, no change o hemoglobin and PCV values, significant increase in the value of serum potassium, significant increase in alkaline phosphatase, insignificant reduction in ASTGOT and insignificant increase in conjugated bilirubin. Taken together, these findings suggest the occurrence of decreased hemolytic process in patients undergoing the test drug treatment. Nevertheless, complete hematological profile of SCD patients on the test drug should still be monitored for hyperkalemia and liver enzymes because of high probability for thrombosis and vaso-occlusive crisis and for unfavorable response of the heart to high potassium level. A multi-center, multi-disciplinary study is certain to provide a more robust data which can throw more light and better understanding of the mechanism of the test drug in SCD patients.

Limitations and strengths

This study has some limitations that should be noted. First, the sample size represents restricted group of SCD patients in one location of the country most likely of low socioeconomic status who attend an urban health facility for the management of their condition. Replication of the study with diverse SCD populations in different geo-political zones of the country is essential. Also, the sample size is small and thus the effect size may not be adequate to make a valid assumption of the true effect of the test drug. Although the intervention seems to be effective, the effect may still be considered to be modest in size, though the results seem to be compelling. The study also has some strong points. It was carried out by experts in the fields of Pediatrics, Hematology, Internal Medicine, Statistics and Laboratory Science. The facility where the study was carried out is one of the best in the country and has very modern equipment and dedicated staff that have been seeing pediatric and adult SCD patients as well as other compromised health conditions for over a two decades or more.

Funding

This study was sponsored by Rahma Integrated Concepts Limited, National Board for Technology Incubation (TIC Kaduna).

References

[1] Weatherall DJ, Clegg JB. *Distribution and population genetics of the thalassaemias*. 4th ed. Oxford: Blackwell Science; 2001. Chapter 6.

[2] Weatherall DJ, Clegg JB. Inherited haemoglobin disorders: an increasing global health problem. Bull World Health Organ. 2001; 79: 704-12.

[3] Ojewunmi OO, Adeyemo TA, Ayinde OC, Iwalokun B, Adekile A. Current perspectives of sickle cell disease in Nigeria: changing the narratives. Expert Rev Hematol. 2019; 12(8):609-620.

[4] Manwani D, Frenette PS. Vaso-occlusion in sickle cell disease: pathophysiology and novel targeted therapies Blood. 2013; 122(24): 3892–3898.

[5] Osunkwo I, Manwani D, J. Current and novel therapies for the prevention of vaso-occlusive crisis in sickle cell disease. Ther Adv Hematol. 2020; 11: 2040620720955000.

[6] Ansari J, Moufarrej YE, Pawlinski R, Gavins FNE. Sickle cell disease: a malady beyond a hemoglobin defect in cerebrovascular disease. Expert Rev Hematol. 2018; 11(1):45-55. DOI: 10.1080/17474086.2018.1407240. Epub 2017 Dec 5. PMID: 29207881; PMCID: PMC6117162.

[7] Okam MM, Ebert BL. Novel approaches to the treatment of sickle cell disease: the potential of histone deacetylase inhibitors. Expert Rev Hematol. 2012; 5(3):303-11. DOI: 10.1586/ehm.12.20. PMID: 22780210.

[8] Fraiwan A, Hasan MA, An R, Rezac AJ, Kocmich NJ, Oginni T, Olanipekun GM, Hassan-Hanga F, Jibir BW, Gambo S, Thota P, Obaro SK, Gurkan UA. Advancing Healthcare Outcomes for Sickle Cell Disease in Nigeria Using Mobile Health Tools. Blood. 2019; 134 (Suppl 1):2173.

[9] Erica N. Chirico and Vincent Pialoux, "Role of Oxidative Stress in the Pathogenesis of Sickle Cell Disease", IUBMB Life, 2012; 64(1): 72–80.

[10] Rice-Evans C, Omorphos SC, Baysal E. "Sickle cell membranes and oxidative damage" Biochem. J. 1986; 237: 265-269.

[11] Ren H, Ghebremeskel K, Okpala I, Lee A, Ibegbulam O, Crawford M, "Patients with sickle cell disease have reduced blood antioxidant protection.", Int J Vitam Nutr Res. 2008; 78(3):139-47.

[12] Fasola FA, Adedapo K, Anetor J, Kuti MA. Total antioxidants status and some hematological values in sickle cell disease patients in steady state. Journal of the National Medical Association. 2007; 99(8): 891-4.

[13] Junichiro Takasu, Rolando Uykimpang, Maria Alenor Sunga, Harunobu Amagase and Yutaka Niihara, "Aged garlic extract therapy for sickle cell anemia patients", BMC Blood Disorders 2002, http://www.biomedcentral.com/1471-2326/2/3

[14] Neumayr LD, Hoppe CC, Brown C. Sickle cell disease: current treatment and emerging therapies. Am J Manag Care. 2019; 25(18 Suppl):S335-S343.

[15] Kapoor S, Little JA, Pecker LH. Advances in the Treatment of Sickle Cell Disease. Mayo Clin Proc. 2018; 93(12):1810-1824.

[16] Nigerian Population Commission. 2006 Population Census, Abuja, Nigeria

[17] Cochran WG. Sampling techniques (3rd ed.), 1977. New York: John Wiley.

[18] Levy SP, Lemeshow S. Sampling of population (4th ed.), 2008. New York: John Wiley.

[19] Isaac-Sodeye WA. Preliminary clinical evaluation of crude extracts of Fagara zanthoxyloides, preceedings of the Symposium, Fagara and the red blood cells. University of Ife Press. 1973: 88-90.

[20] Isaac-Sodeye WA, Sofowora EA, Williams AO, Marquis VO, Adekunle AA, Anderson CO. Extract of Fagara zanthoxyloides root in Sickle Cell Anemia. Acta Haematol. 1975; 53:158-164.

[21] Adebiyi AO, Koekemoer T, Adebiyi AP, Smith N, Baxter E, Naude RJ, van de Venter M. Antimicrobial and antioxidant activities of crude extracts of two Nigerian chewing sticks. Pharmaceutical Biology. 2009; 47(4):320-327.

[22] Jaitly M, Mohan S, Park CM, Anderson HL, Cheng J, Pogue VA. Hypokalemia During Sickle Cell Crises Apparently Due to Intermittent Mineralocorticoid Excess. American Journal of Kidney Diseases. 2008; 51(2):319-325.

[23] Agoyero FO, Nwanze N. Plasma sodium and potassium changes in sickle cell patients. International Journal of Genetics and Molecular Biology. 2010; 2(2):014-019.

[24] Brugnara C. Sickle cell dehydration: Pathophysiology and therapeutic applications. Clin Hemorheol Microcirc. 2018; 68(2-3):187-204.

[25] Clark MR, Guatell JC, White AT, Shohet SB. Study of the dehydrated effect of the red cell Na+ /K+ pump in treated cells with varying Na+ and water content: Biochem. Biophys. Acta. 1981; 646: 422 – 432.

[26] Harvey S. Sickle cell disease: Editor in chief well connected reports: Associate professor of medicine, Harvard medical school. Massachusetts General Hospital. Available from: https://www.researchgate.net/publication/229003923_Plasma_sodium_and_potassium_changes_in_sickle_cell_patients [accessed Nov 26 2020].

[27] Antwi-Boasiako C, Ekem I, Abdul-Rahman M, et al. Hematological parameters in Ghanaian sickle cell disease patients. J Blood Med. 2018; 9:203-209.

[28] Ahmed SG, Ibrahim AU, Hassan AW. Haematological parameters in sickle cell anaemia patients with and without priapism. Ann Saudi Med. 2006; 26:439–443

[29] Onwukeme KE. Haematological indices of Nigerians with sickle cell anaemia. Nig Med Pract. 1993; 25:25–28.

[30] Boggs DR, Hyde F, Srodes C. An unusual pattern of neutrophil kinetics in sickle cell anemia. Blood. 1973; 41:59–62.

[31] Ahmed SG, Ibrahim AU, Hassan AW. Haematological parameters in sickle cell anaemia patients with and without priapism. Ann Saudi Med. 2006; 26:439–443.

[32] Abubakar Y, Ahmad HR, Faruk JA. Hematological parameters of children with sickle cell anemia in steady and crisis states in Zaria, Nigeria. Ann Trop Pathol. 2019; 10:122-5.

[33] Akinbami A, Dosunmu A, Adediran A, Oshinaike O, Adebola P, Arogundade O, et al. Haematological values in homozygous sickle cell disease in steady state and haemoglobin phenotypes AA controls in Lagos, Nigeria. BMC Res Notes 2012; 5:396.

[34] Musonda T, Zulu M, Samutela M, et al. Leucocytosis and Asymptomatic Urinary Tract Infections in Sickle Cell Patients at a Tertiary Hospital in Zambia. Anemia. 2020; 2020:3792728.

[35] Liu Y, Zhong H, Bao W, Mendelson A, An X, Shi P, Chou ST, Manwani D, Yazdanbakhsh K. Patrolling monocytes scavenge endothelial adherent sickle RBCs: a novel mechanism of inhibition of vaso-occlusion in SCD. Blood. 2019; 134(7):579-590.

[36] Belcher JD, Marker PH, Weber JP, Hebbel RP, Vercellotti GM. Activated monocytes in sickle cell disease: potential role in the activation of vascular endothelium and vaso-occlusion. Blood. 2000; 96(7):2451-9.

[37] Wen T, Rothenberg ME. The Regulatory Function of Eosinophils. Microbiol Spectr. 2016; 4(5):

[38] Ahmed SG, Uraka A. Eosinopenia as a marker of infection in patients with sickle cell anaemia: A preliminary report. Inter J Biomed Hlth Sci. 2010; 6(1):57-61.

[39] Borba R, Lima CSP, Grotton HZW. Reticulocyte Parameters and Hemoglobin F Production in Sickle Cell Disease Patients Undergoing Hydroxyurea Therapy. Journal of Clinical Laboratory Analysis. 2003; 17:66–72.

[40] Thachil J. The possible role of reticulocytes in sickle cell disease associated thromboembolism. Hematology. 2008;13(1):68-70.

[41] International Journal of Biomedical and Health Sciences 0794-4748/2010 $12.00 + 0.00 Vol. 6, No. 1, June 30, 2010