Biomedical & Translational Science

Original Article

Effect of Immunozin™ therapy on hematological and biochemical parameters of Sickle Cell Disease patients who presented with fatigue, acute bone pain, and acute chest syndrome in Nigeria

Bamgboye M Afolabi[1,2], Ramatu Aliyu Zubair[3], Ezra Gayawan[4], Haruna Usman Nakorji[5], Nana Fatima Sali[6], Abiodun Ogunwale[7], Tolulope Fagbemi[7]

[1]*Health, Environment and Development Foundation, Surulere, Lagos, Nigeria*
[2]*African, Pan-African Health Alliance and Collaborative, Lagos, Nigeria*
[3]*Barau Dikko Teaching Hospital/Kaduna State University, Lafiya Road, Kaduna, Kaduna State, Nigeria*
[4]*Department of Statistics, Federal University of Technology, Akure, Ondo State, Nigeria*
[5]*Ahmadu Bello University, Zaria, Nigeria*
[6]*National Ear Care Center, Golf Course Road, City Centre, Kaduna, Kaduna State, Nigeria*
[7]*Defined Impact Group, Washington DC, USA*
[8]*Federal Ministry of Health, Abuja, National Malaria Elimination Program, Abuja, FCT, Nigeria*

Correspondence
Dr. Bamgboye M. Afolabi
Health, Environment and Development Foundation, 18, Ogunfunmi Street, Surulere,, Lagos, Nigeria
E-mail: bmafolabi@gmail.com

- Received Date: 30 Mar 2021
- Accepted Date: 05 Apr 2021
- Publication Date: 14 Apr 2021

Keywords: Acute Bone Pains, Acute Chest Syndrome, Creatinine, Electrolytes, Fatigue, Nigeria, Sickle Cell Disease, Urea.

Copyright
© 2021 Science Excel. This is an open-access article distributed under the terms of the Creative Commons Attribution 4.0 International license.

Abstract

Introduction: Painful vaso-occlusive crises are frequent complications of sickle cell disease (SCD), which affects about 3% of the Nigerian population with a high mortality in children. Relevance of hematological and biochemical parameters to clinical symptoms of SCD are not often documented. **Aims and Objective:** This study aimed at determining the prevalence of fatigue, acute bone pains (ABP) and acute chest syndrome (ACS) and to assess the associating hematological and biochemical parameters before and after administration of Immunozin™. **Materials and Method:** This study was a double-blind, two-arm, randomized control pilot study with 30 SCD patients presenting with fatigue, acute bone pains (ABP) and ACS, each of whom was given the study agent. At the first visit, after thorough assessment of each patient had been undertaken and hematological parameters were measured, the study drug was administered to each patient accordingly. This process was repeated monthly for five more monthly visits when the study concluded. At each visit, venous blood sample was collected for hematological parameters, electrolytes, urea and creatinine analyses within 2 hours of collection. **Results:** Means (±sd) of age and BMI (Kg/m^2) of the study subjects were 13.0 (5.6) and 16.1 (1.9) respectively with 26 (86.7%) of the patients underweight. At enrollment into the study, 25 (83.3%), 18 (60.0%) and 10 (33.3%) of the patients presented with ABP, fatigue and ACS respectively but at the end of study, post administration of study drug, 11 (36.7%), 8 (26.7%) and 7 (23.3%) presented with ABP, fatigue and ACS. At enrollment, females were less likely to present with moderate fatigue (χ^2=2.03, P-value=0.15, OR=0.23, 95% CI: 0.05, 1.18), moderate ABP (χ^2=0.09, P-value=0.77, OR=0.80, 95% CI: 0.18, 3.46) and moderate ACS (χ^2=0.01, P-value=0.90, OR=0.40, 95% CI: 0.03, 4.96) compared to males. Only one patient each presented with fatigue and ABP at the end of study. The values of many hematological parameters, electrolytes, urea and creatinine significantly varied at the end of the study compared with enrollment values. A significant positive correlation (Pearson's r=0.40, P-value=0.031) was observed between serum urea and ACS at enrollment, and astonishingly between fatigue and ABP (r=0.52, P-value=0.003) but no notable correlation between any of the clinical symptoms and other variables at the end of study. **Conclusion:** The significant reduction in proportion of patients with fatigue, ABP and ACS at the end of study, after administration of test drug may suggest the therapeutic consequence of the test drug among SCD patients. There is urgent need to conduct multi-center and multi-disciplinary studies to corroborate these findings.

Introduction

Sickle Cell Disease (SCD), precipitated primarily by β-globin gene mutation, has fundamental pathophysiological outcomes leading to hemolytic events and the initiation of the inflammatory activities that finally contribute to vaso-occlusion. In addition, these inflammatory procedures also contribute significantly to many acute and severe clinical symptomatologies and complications of the disease such as stroke, acute chest syndrome, acute bone pains, autosplenectomy, pulmonary hypertension, leg ulcers and kidney infarcts [1]. The disease is concomitant with hypercoagulable state in which platelet activation is enhanced and may demonstrate augmented stickiness to inner

Citation: Afolabi BM, Zubair RA, Gayawan E, et al. Some Effect of Immunozin™ therapy on hematological and biochemical parameters of Sickle Cell Disease patients who presented with fatigue, acute bone pain, and acute chest syndrome in Nigeria. Biomed Transl Sci. 2021; 1(2):1-14.

lining of blood vessels (vascular endothelium), a potential causative pathology to the vaso-occlusive process [2]. Sickle cell disease is characterized by the existence of sickle hemoglobin, which has the specific attribute of polymerizing when challenged with deoxygenation, and by dense, dehydrated red cells that are characteristic of the pathophysiology of acute and chronic manifestations of the disease [3]. Recent studies have indicated that SCD is characterized by a hypercoagulable state that contributes to the vaso-occlusive events in microcirculation, leading to acute and chronic sickle cell-related organ damage [4-6]. The disease is often associated with different symptoms of which acute presentation of vaso-occlusive crisis, especially acute bone pains (ABP), is the commonest [7,8]. Chronic pain reported by SCD patients represents not only pain consequent upon vaso-occlusion but pain confirmatory of avascular necrosis of big joints — "*the hips, shoulders and ankles, in decreasing order of frequency*" [9]. Older studies report that approximately 5.2 percent of SCD patients present with up to 10 episodes of painful crises annually [7] though in most of them, the crisis resolves within five to seven days [10]. Factors that may precipitate crisis in SCD patients include dehydration, infection, cold weather, low oxygen tension and infection, among others [11]. Earlier studies reported acute chest syndrome (ACS) as the major cause of death and hospitalization in SCD patients [12-14] and that the pathology is consequent upon fat embolism and fat-laden pulmonary macrophages from bronchoalveolar fluid [15-18]. However, in the past decade, studies have characterized it as "*a new radiodensity on chest radiograph accompanied by fever and/or respiratory symptoms*" with a higher magnitude of disease severity and a higher mortality rate in adults, largely due to a higher incidence of bone marrow and fat emboli in adults [19-21].

Dessap et al [22] also described ACS as a lung injury in SCD patients, consequent upon toxic cycle of "*lung infarction, inflammation, and atelectasis culminating in ventilation–perfusion mismatch, hypoxemia, and acute increases in pulmonary artery and right ventricular pressures*". Further, fatigue, shortness of breath and dizziness are frequent indications of SCD [23] which are likely due to smaller size of the erythrocytes or its distortion or both and as such, cannot convey oxygen efficiently and effectively to all parts of the body as do healthy erythrocytes [23] and may be a predictor of impending crisis, especially fatigue [24]. There is paucity of data on the prevalence of fatigue, ABP and ACS among sub-Sahara Africans with SCD. Even more scarce are data on whether phyto-medicinal interventions, and other African Medicinal Poly-herbal preparations (AMPp) have any therapeutic effect on these symptoms and on hematological and biochemical profiles of patients before and after management. The objectives of this study were to determine the prevalence and severity of fatigue, ABP and ACS among sickle cell patients in Nigeria, and to document the hematological and biochemical parameters of these patients before and at the end of treatment with Immunozin™, so as to determine possible therapeutic effect of the study drug on these symptoms.

Materials and Methods

This double-blind, randomized, control trial, carried out between January and May of 2018, has already been described in a previous publication [25]. Summarily, a commercially procurable herbal nutritional capsule supplement, composed of *Allium sativum, Balanites aegeptiaca, Guiera senegalensis* and *Azadirachta indica* was administered to sickle cell disease patients who also presented with various degrees of severity of fatigue, acute bone pains and acute chest syndrome.

Sample size calculation: Sample size calculation was based on the formula of Cochran [26] and Levy and Lemeshow [27]. Accordingly, 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. Thus sample size calculation in this study has been reported earlier [25].

Study area: The study took place in Kaduna City, Northern Nigeria with a projected population of 1,582,102, according to the 2006 national census outcome [28]

Study population: The study population consists of Sickle Cell Disease patients who reported at the pediatric and adult hematology clinics at Barau Dikko Teaching Hospital, Kaduna City in Nigeria.

Recruitment, inclusion and exclusion criteria: To be included in the study, a participant must have been diagnosed as an SCD patient, must have provided informed consent (or agreement by parent or care-giver for minors) and explicit willingness to abide by all study procedures as well as availability for the duration of the study. Inclusion criteria also involved (i) manifestation of any clinical signs and symptoms of SCD with atleast one episode of crisis monthly (ii) ability to take oral medication and (iii) compliance with the medication regimen. Exclusion criteria involved (i) concomitant use of any other medication or medical devices not part of the study (ii) known allergic reactions to any of the components of study drug, (iii) pregnant or lactating women or women who were planning to get pregnant within five months after commencement of the study, (iv) patients with cardiac, hepatic or kidney disease, (v) patients who had one or more episodes of febrile illness within 1 month preceding the study (vi) those with concomitant disease such as malaria, tuberculosis, measles and (vii) those who were alcohol or tobacco users 4 months prior to the start of the study.

Study design and protocol: At enrollment into the study, each patient went through clinical examination and baseline blood sample was aseptically collected for hematological analysis and for serum electrolytes and urea. A questionnaire was served to document the severity of fatigue, acute bone pains and chest syndrome in each of the patients. Clean-catch mid-stream urine was collected for urinalysis and for pregnancy test among females in child-bearing age group. Each patient received the first dose of the test drug consisting of 500 mg (pediatric patients 5-18 years) or 1000 mg (adult patients >18 years) given twice daily, (every 12 hours) for a minimum of 120 days, in addition to standard of care practices. Each patient was also re-assessed monthly (approximately 30 days apart) for hematological and biochemical parameters. At the end of the study, a questionnaire with the same set of questions was again served to document the severity of clinical symptoms of interest to this study - fatigue, ABP and ACS.

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.

Definition: Fatigue was defined as a feeling of exhaustion and lack of energy [29]; Acute bone pain was defined as an acute episodic painful crisis that results from microcirculatory obstruction by sickle erythrocytes leading to ischaemic-reperfusion injury of bone and necrosis of bone marrow [30]; Acute chest syndrome was defined as a new radiodensity on chest radiograph accompanied by fever and/or respiratory symptoms [19].

Data management and statistical analysis: Body Mass Index (Kg/m^2) was segregated into <18.5, 18.5-24.9. NCSS software (LLC, Kaysville, Utah, USA) was used for analysis. Student's t-test was used to evaluate significant differences in means between two continuous variables. Pearson's correlation matrix was used to determine association between clinical symptoms and other variables at the beginning and at the end of the study. Chi-square and Odd ratio with 95% Confidence Interval (CI) were used to test the significance of differences between two proportions. Data were presented as absolute numbers, percentages or proportions 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 considered statistically considerable.

Results

General observation (Table 1)

Of the 33 patients with Sickle Cell Disease (SCD) who were enrolled into the study, data for 30 (90.9%) were analyzed in this study as records of acute bone pains in 3 patients were unavailable and thus removed from investigation. The means (±sd) of age (years) and Body Mass Index (BMI) (Kg/m^2) of all study subjects (14 males, 16 females) were 13.0 (5.6) and 16.1 (1.9) respectively. In all, 26 (86.7%) were underweight (BMI<18.5 Kg/m^2) and only 4 (13.3%) were normal weight. At enrollment, SCD patients were 5.7 times more likely to present with ABP (n=25, 83.3%; χ^2=9.64, P-value=0.002, OR=5.71, 95% CI: 1.93, 19.93) than with fatigue (n=18, 60.0%; χ^2=0.02, P-value=0.88, OR=1.07, 95% CI: 0.44, 2.62) or chest syndrome (n=10, 33.3%; χ^2=10.00, P-value=0.0005, OR=0.20, 95% CI: 0.08, 0.51), making ABP the commonest clinical presentation with a prevalence of 83.3%, followed by fatigue with a prevalence of 60.0% and by chest syndrome with a prevalence of 33.3%. At the end of the study, SCD patients were 1.7 times as likely to present with ABP (n=11, 36.7%; χ^2=1.31, P-value=0.25, OR=1.74, 95% CI: 0.68, 4.47) than with fatigue (n=8, 26.7%; χ^2=0.11, P-value=0.74, OR=0.85, 95% CI: 0.32, 2.26) or chest syndrome (n=7, 23.3%; χ^2=0.67, P-value=0.41, OR=0.66, 95% CI: 0.24, 1.80). Thus, the prevalence of ABP decreased to 36.7%, that of fatigue to 26.7% and that of chest syndrome to 23.3% at the end of the study. Of the 18, 25 and 10 SCD patients that presented with fatigue, ABP and Chest syndrome, 17 (94.4%), 23 (92.0%) and 8 (80.0%) were underweight (BMI<18.5 Kg/m^2). Figures 1a and 1b illustrate the histogram and percent distribution of age (years) of the patients in the study, indicating that approximately 32% of the subjects were aged between 12 and 16 years while Figure 2 a and 2b elaborate the histogram and percent distribution of Body Mass Index (Kg/m^2) which indicate that approximately 50% of the patients were between 14-16 Kg/m^2.

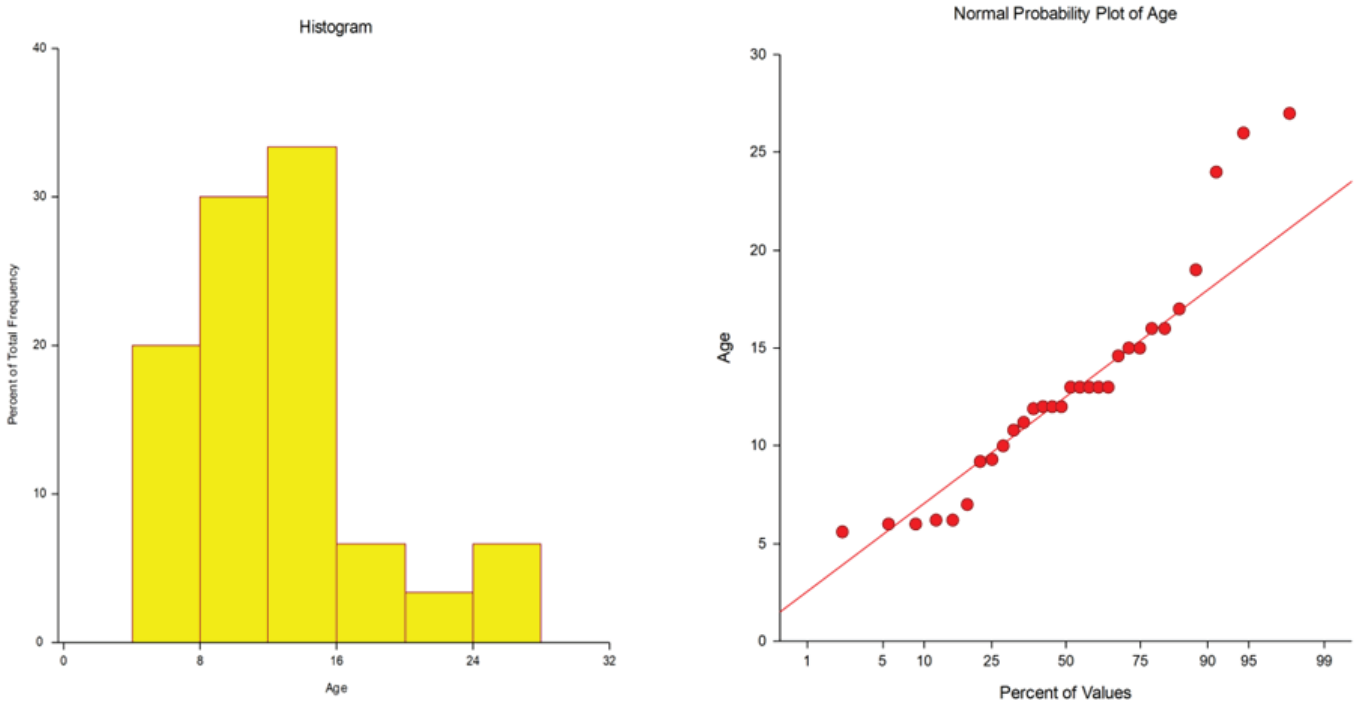

Figure 1. *Histogram (a) and percent distribution (b) of age (years) of study subjects (Shapiro-Wilk W test value=0.90, probability level=0.01, decision (5%) is to reject normality)*

Table 1. Clinical presentations of patients with Sickle Cell Disease relative to age, body mass index and sex

Variable			All	Fatigue				Acute bone pains				Cough/Chest pain/Dyspnea			
				At enrollment		At end of study		At enrollment		At end of study		At enrollment		At end of study	
				Present	Absent	Present	Absent	Present	Absent	Present	Absent	Present	Absent	Present	Absent
Age (years)		n (%)	30 (100.0)	18 (60.0)	12 (40.0)	8 (26.7)	22 (73.3)	25 (83.3)	5 (16.7)	11 (36.7)	19 (63.3)	10 (33.3)	20 (66.7)	7 (23.3)	23 (76.7)
		Mean (±sd)	13.0 (5.6)	12.7 (5.3)	13.5 (6.2)	12.4 (5.7)	13.2 (5.6)	12.2 (4.9)	17.0 (7.4)	12.7 (5.2)	13.1 (5.9)	13.4 (5.0)	12.8 (5.9)	12.6 (2.6)	13.1 (6.2)
		Median	12.5	12.5	13.5	11.4	13.0	12.0	17.0	12.0	13.0	12.0	13.0	12.0	13.0
BMI (Kg/m^2)	All	Mean (±sd)	16.1 (1.9)	16.0 (1.7)	16.2 (2.2)	15.9 (2.1)	16.2 (1.8)	15.9 (1.7)	17.4 (2.3)	15.6 (2.0)	16.4 (1.8)	16.2 (2.3)	16.1 (1.7)	15.5 (1.0)	16.3 (2.0)
		Median	15.7	15.7	15.8	15.4	15.8	15.6	17.8	15.1	16.0	15.8	15.6	15.9	15.7
	<18.5	Mean (±sd)	16.0 (1.4) (n=26, 86.7%)	15.8 (1.4) (n=17, 94.4%)	15.2 (1.4) (n=9, 75.0%)	15.3 (1.6) (n=7, 87.5%)	15.7 (1.4) (n=19, 86.4%)	15.5 (1.4) (n=23, 92.0%)	16.0 (1.7) (n=3, 60.0%)	15.2 (1.6) (n=10, 90.9%)	15.8 (1.3) (n=16, 84.2%)	15.3 (1.5) (n=8, 80.0%)	15.7 (1.4) (n=18, 90.0%)	15.5 (1.0) (n=7, 100.0%)	15.6 (1.5) (n=19, 82.6%)
		Median	15.6	15.7	15.1	15.1	15.6	15.6	16.0	14.9	15.8	15.4	15.6	15.9	15.6
	18.5-24.5	Mean (±sd)	19.4 (0.7) (n=4, 13.3%)	19.7 (0.0) (n=1, 5.6%)	19.3 (0.8) (n=3, 25.0%)	19.7 (0.0) (n=1, 12.5%)	19.3 (0.8) (n=3, 13.6%)	19.5 (0.3) (n=2, 8.0%)	19.4 (1.1) (n=2, 40.0%)	19.7 (0.0) (n=1, 9.1%)	19.3 (0.8) (n=3, 15.8%)	19.7 (0.6) (n=2, 20.0%)	19.2 (0.8) (n=2, 10.0%)	0 (0.0) (n=0, 0.0%)	19.4 (0.7) (n=4, 17.4%)
		Median	19.5	19.7	19.2	19.7	19.2	19.5	19.4	19.7	19.2	19.7	19.2	0.0	19.5
Gender	Male	Freq. (%)	14 (46.7)	10 (55.6)	4 (33.3)	5 (62.2)	9 (40.9)	12 (480)	2 (40.0)	5 (45.5)	9 (47.4)	5 (50.0)	9 (45.0)	5 (71.4)	9 (39.1)
	Female		16 (53.3)	8 (44.4)	8 (66.7)	3 (37.5)	13 (59.1)	13 (52.0)	3 (60.0)	6 (54.5)	10 (52.6)	5 (50.0)	11 (55.0)	2 (28.6)	14 (60.9)

Table 2. Severity of clinical symptoms among male and female patients with Sickle Cell Disease

Point in time at study	Severity of symptom	Statistics	Fatigue				Acute bone pain				Acute chest syndrome			
			Male* (n=14)	Female (n=16)	χ^2 (P-value)	OR (95% CI)	Male ! (n=14)	Female (n=16)	χ^2 (P-value)	OR (95% CI)	Male # (n=14)	Female (n=16)	χ^2 (P-value)	OR (95% CI)
At enrollment	None	Freq.	4	8	0.67(0.41)	2.50 (0.55, 11.41)	2	3	0.00 (1.00)	1.62 (0.23, 11.26)	9	11	0.00 (1.00)	1.22 (0.27, 5.59)
		%	28.6	50			14.4	18.8			64.3	68.8		
	Mild	Freq.	3	5	0.04(0.85)	1.67 (0.32, 8.74)	6	7	0.00 (0.96)	1.04 (0.24, 4.41)	2	4	0.08 (0.78)	2.00 (0.31, 13.06)
		%	21.4	31.2			42.8	43.7			14.3	25		
	Moderate	Freq.	7	3	2.03(0.15)	0.23 (0.05, 1.18)	6	6	0.09 (0.77)	0.80 (0.18, 3.46)	2	1	0.01(0.90)	0.40 (0.03, 4.96)
		%	50	18.8			42.8	37.5			14.3	6.2		
	Severe	Freq.	0	0	-	-	0	0	-	-	1	0	0.01(0.96)	0.00 (undefined)
		%	0	0			0	0			7.1	0		
At end of study	None	Freq.	9	13	0.40(0.53)	2.41 (0.46, 12.72)	9	10	0.00 (1.00)	0.93 (0.21, 4.11)	9	14	1.14 (0.29)	3.89 (0.62, 24.52)
		%	64.3	81.2			64.3	62.5			64.3	87.5		
	Mild	Freq.	5	2	1.14(0.29)	0.26 (0.04, 1.62)	5	5	0.00 (1.00)	0.82 (0.18, 3.74)	5	2	1.14 (0.29)	0.26 (0.04, 1.62)
		%	35.7	12.5			35.7	31.2			35.7	12.5		
	Moderate	Freq.	0	1	0.05(0.83)	undefined	0	1	0.00 (1.00)	undefined	0	0	-	-
		%	0	6.3			0	6.3			0	0		
	Severe	Freq.	0	0	-	-	0	0	-	-	0	0	-	-
		%	0	0			0	0			0	0		

*At enrollment, males were 0.40 as likely to present with no fatigue (χ^2=0.68, P-value=0.41, OR=0.40, 95% CI: 0.09,1.83), 0.60 times as likely to present with mild fatigue (χ^2=0.04, P-value=0.85, OR=0.60, 95% CI: 0.11, 3.15) but 4.3 times more likely to present with moderate fatigue (χ^2=2.03, P-value=0.15, OR=4.33, 95% CI: 0.84,22.23) compared to females; !At enrollment, males were 0.72 as likely to present with no acute bone pains (χ^2=0.00, P-value=1.00, OR=0.72, 95% CI: 0.10,5.10), 0.96 times as likely to present with mild mild acute bone pains (χ^2=0.00, P-value=0.96, OR=0.96, 95% CI: 0.23, 4.10) but 1.25 times more likely to present with moderate bone pains (χ^2=0.09, P-value=0.77, OR=1.25, 95% CI: 0.29, 5.41) compared to females: # At enrollment, males were 0.82 as likely to present with no chest syndrome (χ^2=0.00, P-value=1.00, OR=0.82, 95% CI: 0.18, 3.74), 0.50 times as likely to present with mild chest syndrome (χ^2=0.08, P-value=0.78, OR=0.50, 95% CI: 0.08, 3.27) but 2.5 times more likely to present with moderate chest syndrome (χ^2=0.01, P-value=0.90, OR=2.50, 95% CI: 0.20, 31.00) compared to females:

*At end of study, males were 0.42 as likely to present with no fatigue (χ^2=0.40, P-value=0.53, OR=0.42, 95% CI: 0.08, 2.19) but 3.89 times as likely to present with mild fatigue (χ^2=1.14, P-value=0.29, OR=3.89, 95% CI: 0.62, 24.52) compared to females; ! 1.08 times as likely to present with no acute bone pains (χ^2=0.00, P-value=1.00, OR=1.08, 95% CI: 0.24, 4.79) and 1.22 times as likely to present with mild mild acute bone pains (χ^2=0.00, P-value=1.00, OR=1.22, 95% CI: 0.27, 5.59) compared to females.

Fisher's exact test was applied where appropriate

Gender and clinical presentation (Table 2)

At the beginning of the study, females were 2½ times more likely to present with no fatigue (χ^2=0.67, P-value=0.41, OR=2.50, 95% CI: 0.55,11.41), 1.62 times as likely to present with no ABP (χ^2=0.00, P-value=1.00, OR=1.62, 95% CI: 0.23, 11.26) and 1.22 times more likely to present with no ACS (χ^2=0.00, P-value=1.00, OR=1.22, 95% CI: 0.27, 5.59) compared to males. At end of study, females were 2.41 times as likely to present with no fatigue (χ^2=0.40, P-value=0.53, OR=4.41, 95% CI: 0.46, 12.72), approximately 4 times as likely to present with no ACS (χ^2=1.14, P-value=0.29, OR=3.89, 95% CI: 0.62, 24.52) but less likely to present with no ABP (χ^2=0.00, P-value=1.00, OR=0.93, 95% CI: 0.21, 4.11).

At enrollment, females were 1.67 times as likely to present with mild fatigue (χ^2=0.04, P-value=0.85, OR=1.67, 95% CI: 0.32, 8.74), 1.04 more likely to present with mild acute bone pains (χ^2=0.00, P-value=0.96, OR=1.04, 95% CI: 0.24, 4.41), and twice as likely to present with mild ACS (χ^2=0.08, P-value=0.78, OR=2.00, 95% CI: 0.31, 13.06) compared to males. However, at enrollment, females were less likely to present with moderate fatigue (χ^2=2.03, P-value=0.15, OR=0.23, 95% CI: 0.05, 1.18), with moderate acute bone pains (χ^2=0.09, P-value=0.77, OR=0.80, 95% CI: 0.18, 3.46) and with moderate ACS (χ^2=0.01, P-value=0.90, OR=0.40, 95% CI: 0.03, 4.96). The only SCD patient with severe chest syndrome was a male.

At the end of the study, females were 0.26 times as likely to present with mild fatigue (χ^2=1.14, P-value=0.29, OR=0.26, 95% CI: 0.04, 1.62), 0.82 as likely to present with mild acute bone pains (χ^2=0.00, P-value=1.00, OR=0.82, 95% CI: 0.18, 3.74), and 0.26 as likely to present with mild acute chest syndrome (χ^2=1.14, P-value=0.29, OR=0.26, 95% CI: 0.04, 1.62) compared to males. No patient presented with severe fatigue, acute bone pains or acute chest syndrome at the end of the study. (Table 2).

Fatigue (Tables 3 and 4)

While the mean [±sd] reticulocyte count of patients with fatigue at enrollment (2.4 [0.7]) and at the end of the study (1.7 [1.2]) were not significant, the value among patients without fatigue was significantly higher (t-test=3.07, P-value=0.003) at enrollment into the study (2.6 [1.0]) compared to the value at the end of the study (1.5 [1.0]). There were no significant alterations in the means [±sd] of platelets, neutrophils, lymphocytes, eosinophils, total white blood cells or hemoglobin. However the mean [±sd] monocyte count at the end of the study (4.1 [0.8]) was significantly higher (t-test=4.18, P-value=0.0002) than the value at enrollment (2.9 [0.8]). (Table 3). There was no notable alteration in the sodium concentration, though those without fatigue had a significantly higher (t-test= -2.98, P-value=0.003) level of potassium at the end of the study (6.0 [1.0]) compared to the value at enrollment (5.0 [0.9]). Among those with fatigues, the mean [±sd] bicarbonate value was significantly elevated (t-test= -4.22, P-value=0.0002) at the end of the study (25.5 [2.3]) than at enrollment (18.7 [5.9]) but less so among those without fatigue (t-test= -3.00, P-value=0.004). Among those with fatigue, mean [±sd] creatinine value was also raised significantly (t-test= -2.57, P-value=0.01) at the end of the study (53.2 [11.9]) compared to the enrollment value (40.8 [10.1]). The values of bicarbonate and of urea were also significantly raised among those without fatigue.

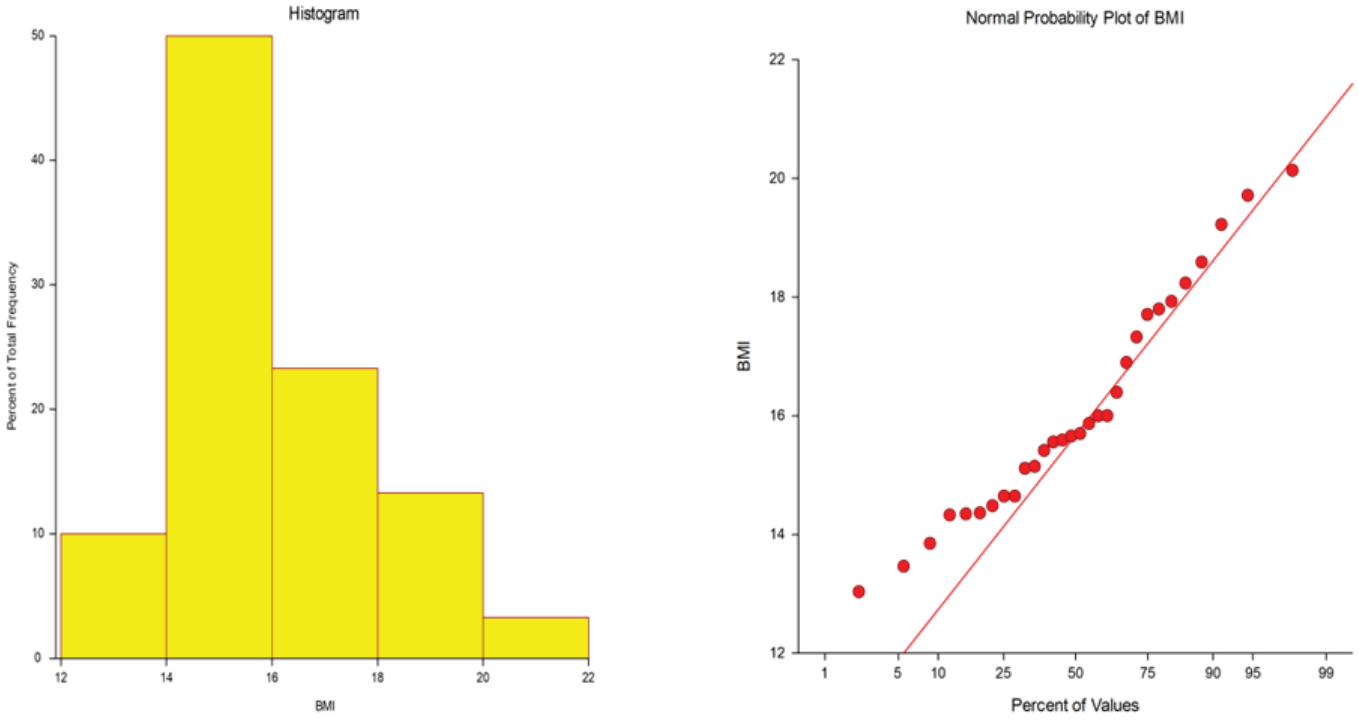

Figure 2. *Histogram (a) and percent distribution (b) of body mass index of study subjects (Shapiro-Wilk W test value=0.95, probability level=0.22, decision (5%) is can't reject normality)*

Variables	Statistics	Fatigue				Acute bone pains				Chest syndrome			
		At enrollment		At end of study		At enrollment		At end of study		At enrollment		At end of study	
		Present * (n=18)	Absent ! (n=12)	Present * (n=8)	Absent ! (n=22)	Present ^ (n=25)	Absent # (n=5)	Present ^ (n=11)	Absent # (n=19)	Present ↓ (n=10)	Absent ↑ (n=20)	Present ↓ (n=7)	Absent ↑ (n=23)
Reticulocytes	Mean (±sd)	2.4 (0.7)	2.6 (1.0)	1.7 (1.2)	1.5 (1.0)	2.4 (0.7)	2.8 (1.4)	1.2 (0.5)	1.8 (1.2)	2.3 (0.7)	2.5 (0.9)	1.3 (0.9)	1.7 (1.1)
Platelets		478.7 (189.9)	471.9 (248.2)	393.1 (157.3)	440.7 (158.0)	478.7 (199.0)	462.2 (294.1)	395.7 (138.8)	447.1 (167.1)	436.2 (219.3)	495.9 (209.6)	478.4 (210.6)	411.4 (137.2)
Monocytes		3.6 (0.9)	2.9 (0.8)	3.9 (0.8)	4.1 (0.8)	3.4 (0.9)	2.8 (0.8)	4.2 (0.8)	4.0 (0.8)	3.3 (0.7)	3.4 (1.0)	3.6 (0.5)	4.2 (0.8)
Neutrophils		53.9 (8.9)	52.8 (9.3)	49.1 (9.0)	51.7 (8.2)	53.4 (9.6)	53.4 (5.1)	51.1 (6.1)	50.9 (9.6)	53.9 (7.2)	53.2 (9.9)	50.3 (7.8)	41.2 (8.7)
Lymphocytes		41.6 (8.3)	42.3 (9.6)	45.1 (9.1)	40.8 (10.7)	41.9 (9.4)	41.8 (4.8)	42.8 (6.0)	41.5 (12.4)	41.2 (6.8)	42.2 (9.7)	44.4 (8.0)	41.2 (11.0)
Eosinophils		1.6 (0.5)	1.5 (0.5)	2.0 (0.5)	2.0 (0.6)	1.6 (0.5)	1.6 (0.5)	2.0 (0.6)	2.1 (0.5)	1.6 (0.5)	1.6 (0.5)	1.7 (0.5)	2.1 (0.6)
Total White Blood Cells		13.3 (4.5)	14.2 (4.8)	9.6 (3.0)	13.0 (3.2)	13.8 (4.3)	13.3 (6.1)	10.7 (3.6)	12.9 (3.2)	11.6 (5.0)	14.7 (4.0)	11.4 (2.5)	12.3 (3.7)
Hemoglobin		7.9 (1.1)	7.8 (1.3)	8.0 (1.4)	7.7 (0.9)	7.8 (1.0)	8.2 (1.8)	8.3 (0.9)	7.5 (1.0)	8.1 (1.2)	7.8 (1.1)	7.7 (0.7)	7.8 (1.1)

Reticulocyte t-test (P-value): *=1.54 (0.08); != 3.07 (0.003); ^=5.83 (0.000001); #= 1.46 (0.10); ↓=2.46 (0.02); ↑=2.62(0.006): Platelets t-test (P-value): *= 1.20 (0.12); != 0.39 (0.35); ^=- 1.44 (0.08); #=0.11 (0.46); ↓=-0.40 (0.35): ↑=1.53 (0.07): Monocytes t-test (P-value): *=-0.85 (0.20); !=-4.18 (0.0002); ^=-2.66 (0.007);#=-2.98 (0.01); ↓=-1.03 (0.16); ↑=-2.87 (0.003): Neutrophils t-test (P-value):*= 1.26 (0.11); !=0.34 (0.37); ^=0.87 (0.20); #=0.79 (0.22); ↓=1.0 (0.18); ↑=4.19 (0.0001): Lymphocytes t-test (P-value): *= -0.93 (0.19); !=0.42 (0.34); ^=-0.34 (0.37); #=0.08 (0.47); ↓=-0.86 (0.20); ↑=0.32 (0.38) : Eosinophils t-test (P-value): *= -1.88 (0.04); !-2.59 (0.008); ^=-1.94 (0.04); #=-1.99 (0.04);↓=-0.41 (0.35); ↑=-2.98 (0.002): Total WBC t-test (P-value): *=2.47 (0.01);!=0.78 (0.22); ^=2.24 (0.02); #=0.14 (0.45); ↓=0.11 (0.46); ↑=2.03 (0.02) :Hemoglobin t-test (P-value): *= - 0.18 (0.43); != 0.24 (0.47); ^= - 1.48 (0.08); #= 0.84 (0.22);↓=0.86 (0.20);↑=0.00 (1.00).

Table 4. *Blood biochemistry of sickle cell patients who presented with fatigue, acute bone pains and chest syndrome.*

Variables	Statistics	Fatigue				Acute bone pains				Chest syndrome			
		At enrollment		At end of study		At enrollment		At end of study		At enrollment		At end of study	
		Present (n=18)*	Absent (n=12)!	Present (n=8)*	Absent (n=22)!	Present (n=25)^	Absent (n=5)#	Present (n=11)^	Absent (n=19)#	Present (n=10)↓	Absent (n=20)↑	Present (n=7)↓	Absent (n=23)↑
Sodium	Mean (±sd)	140.1 (4.1)	138.3 (5.8)	139.0 (5.4)	137.4 (3.1)	140.0 (3.7)	136.4 (8.4)	138.8 (4.8)	137.2 (3.1)	140.5 (2.9)	138.8 (5.5)	135.5 (5.0)	138.6 (3.1)
Potassium		5.4 (0.9)	5.0 (0.9)	5.7 (0.9)	6.0 (1.0)	5.4 (0.9)	4.9 (0.7)	5.6 (1.0)	6.1 (1.0)	5.3 (1.0)	5.3 (0.8)	6.2 (0.9)	5.8 (1.0)
Chloride		101.9 (3.7)	99.5 (7.1)	101.2 (5.3)	100.7 (2.4)	102.5 (3.5)	93.2 (6.5)	101.3 (4.4)	100.6 (2.7)	101.9 (2.4)	100.5 (6.3)	99.1 (5.7)	101.4 (2.1)
Bicarbon-ate		18.7 (5.9)	18.8 (4.9)	25.5 (2.3)	23.6 (3.5)	18.5 (5.8)	19.8 (3.3)	24.5 (3.2)	23.8 (3.4)	16.0 (4.9)	20.1 (5.2)	23.4 (3.4)	24.3 (3.3)
Urea		2.2 (0.8)	1.8 (0.8)	2.4 (0.9)	2.4 (0.6)	2.0 (0.8)	2.2 (1.0)	2.5 (0.7)	2.3 (0.6)	2.4 (1.0)	1.8 (0.6)	2.6 (0.8)	2.3 (0.6)
Creatinine		40.8 (10.1)	42.9 (11.8)	53.2 (11.9)	51.8 (11.0)	40.6 (9.3)	46.8 (16.3)	53.7 (9.1)	51.3 (12.3)	38.7 (13.6)	43.1 (8.9)	52.3 (14.9)	52.2 (10.0)

Sodium t-test (P-value): *=0.51 (0.31); != 0.50 (0.31); ^=0.74 (0.24); #= -0.21 (0.42); ↓=2.38 (0.02); ↑=0.14 (0.44): Potassium t-test (P-value): *= -0.78 (0.22); != -2.98 (0.003); ^=- -0.57 (0.29); #= -3.09 (0.007); ↓= -1.94 (0.04): ↑= -1.82 (0.04); Chloride t-test (P-value): *=-0.34 (0.37); != -0.57 (0.29); ^=0.80 (0.22); #= -2.49 (0.03); ↓=-1.23 (0.13); ↑= -0.61 (0.27): Bicarbon-ate t-test (P-value):*= -4.22 (0.0002); != -3.00 (0.004); ^= -3.98 (0.0002); #= -2.40 (0.03); ↓= -3.68 (0.001); ↑= -3.11 (0.002): Urea t-test (P-value): *= - 0.54 (0.30); != -2.27 (0.02); ^= -1.89 (0.04); #= -0.21 (0.42); ↓= -0.46 (0.33): ↑= -2.73 (0.005); Creatinine t-test (P-value): *= - 2.57 (0.01); != -2.15 (0.02); ^= -3.95 (0.004); #= -0.58 (0.29); ↓=--1.92 (0.04); ↑= -3.16 (0.002)

Table 5. *Pearson's correlation matrix of some variables at enrollment and their level of significance*

	Age	Sex	Fatigue	ABPs	Chest pain	BMI	Reticulo-cytes	Platelets	Monocytes	Hemoglo-bin	Urea	Creatinine	Potassium	Bicarbon-ate
Age	1	0.08 (ns)	-0.10 (ns)	-0.28 (ns)	0.21 (ns)	0.71 (0.00001)	-0.35 (ns)	0.07 (ns)	0.24 (ns)	0.50 (0.005)	0.04 (ns)	0.43 (0.02)	-0.21 (ns)	0.20 (ns)
Sex	0.08 (ns)	1	-0.31 (ns)	-0.07 (ns)	-0.17 (ns)	0.14 (ns)	-0.05 (ns)	-0.01 (ns)	-0.10 (ns)	0.05 (ns)	0.12 (ns)	0.06 (ns)	-0.08 (ns)	0.05 (ns)
Fatigue	-0.10 (ns)	-0.31 (ns)	1.00	0.52 (0.003)	0.10 (ns)	-0.05 (ns)	-0.04 (ns)	-0.04 (ns)	0.24 (ns)	-0.02 (ns)	0.15 (ns)	-0.12 (ns)	0.25 (ns)	-0.06 (ns)
ABP	-0.28 (ns)	-0.07 (ns)	0.52 (0.003)	1	-0.09 (ns)	-0.16 (ns)	-0.15 (ns)	0.12 (ns)	0.09 (ns)	-0.16 (ns)	-0.29 (ns)	-0.13 (ns)	0.14 (ns)	-0.00 (ns)
Chest pain	0.21 (ns)	-0.17 (ns)	0.10 (ns)	-0.09 (ns)	1.00	0.17 (ns)	-0.20 (ns)	-0.22 (ns)	-0.05 (ns)	0.20 (ns)	0.40 (0.031)	-0.13 (ns)	-0.03 (ns)	-0.30 (ns)
BMI	0.71 (0.00001)	0.14 (ns)	-0.05 (ns)	-0.16 (ns)	0.17 (ns)	1	-0.37 (0.045)	0.04 (ns)	-0.08 (ns)	0.56 (0.001)	0.13 (ns)	0.39 (0.035)	-0.06 (ns)	-0.11 (ns)
Reticulocytes	-0.35 (ns)	-0.05 (ns)	-0.04 (ns)	-0.15 (ns)	-0.20 (ns)	-0.37 (0.045)	1	0.23 (ns)	0.03 (ns)	-0.70 (0.00002)	0.14 (ns)	-0.60 (0.0005)	0.18 (ns)	0.23 (ns)
Platelets	0.07 (ns)	-0.01 (ns)	-0.04 (ns)	0.12 (ns)	-0.22 (ns)	0.04 (ns)	0.23 (ns)	1	0.20 (ns)	-0.45 (0.012)	-0.16 (ns)	-0.17 (ns)	0.29 (ns)	0.27 (ns)
Monocytes	0.24 (ns)	-0.10 (ns)	0.24 (ns)	0.09 (ns)	-0.05 (ns)	-0.08 (ns)	0.03 (ns)	0.20 (ns)	1	-0.17 (ns)	-0.08 (ns)	-0.20 (ns)	0.24 (ns)	0.19 (ns)
Hemoglobin	0.50 (0.005)	0.05 (ns)	-0.02 (ns)	-0.16 (ns)	0.20 (ns)	0.56 (0.001)	-0.70 (0.00002)	-0.45 (0.012)	-0.17 (ns)	1.00	0.11 (ns)	0.66 (0.00002)	-0.34 (ns)	-0.21 (ns)
Urea	0.04 (ns)	0.12 (ns)	0.15 (ns)	-0.29 (ns)	0.40 (0.03)	0.13 (ns)	0.14 (ns)	-0.16 (ns)	-0.08 (ns)	0.11 (ns)	1	-0.19 (ns)	-0.07 (ns)	-0.27 (ns)
Creatinine	0.43 (0.02)	0.06 (ns)	-0.12 (ns)	-0.13 (ns)	-0.13 (ns)	0.39 (0.035)	-0.60 (0.0005)	-0.17 (ns)	-0.20 (ns)	0.66 (0.00002)	-0.19 (ns)	1.00	-0.41 (0.024)	-0.04 (ns)
Potassium	-0.21 (ns)	-0.08 (ns)	0.25 (ns)	0.14 (ns)	-0.03 (ns)	-0.06 (ns)	0.18 (ns)	0.29 (ns)	0.24 (ns)	-0.34 (ns)	-0.07 (ns)	-0.41 (0.024)	1	0.04 (ns)
Bicarbonate	0.20 (ns)	0.05 (ns)	-0.06 (ns)	-0.00 (ns)	-0.30 (ns)	-0.11 (ns)	0.23 (ns)	0.27 (ns)	0.19 (ns)	-0.21 (ns)	-0.27 (ns)	-0.04 (ns)	0.04 (ns)	1

ns= not significant

Table 6. *Pearson's correlation matrix of some variables at end of study and their level of significance*

	Age	Sex	Fatigue	ABPs	Chest pain	BMI	Reticulo-cytes	Platelets	Monocytes	Hemoglo-bin	Urea	Creatinine	Potassium	Bicarbon-ate
Age	1	0.08 (ns)	-0.04 (ns)	0.04 (ns)	-0.04 (ns)	0.71 (0.00001)	0.07 (ns)	-0.10 (ns)	0.01 (ns)	0.51 (0.005)	0.02 (ns)	0.25 (ns)	-0.36 (0.056)	0.14 (ns)
Sex	0.08 (ns)	1	-0.10 (ns)	0.07 (ns)	-0.27 (ns)	0.14 (ns)	-0.05 (ns)	0.21 (ns)	-0.00 (ns)	0.01 ((ns)	-0.18 (ns)	0.11 (ns)	0.05 (ns)	-0.10 (ns)
Fatigue	-0.04 (ns)	-0.10 (ns)	1.00	0.28 (ns)	-0.02 (ns)	-0.05 (ns)	0.22 (ns)	-0.06 (ns)	-0.22 (ns)	0.00 (ns)	-0.05 (ns)	0.04 (ns)	-0.03 (ns)	0.18 (ns)
ABP	0.04 (ns)	0.07 (ns)	0.28 (ns)	1	0.03 (ns)	-0.12 (ns)	-0.30 (ns)	-0.12 (ns)	0.09 (ns)	0.33 (ns)	0.15 (ns)	0.05 (ns)	-0.33 (ns)	0.13 (ns)
Chest pain	-0.04 (ns)	-0.27 (ns)	-0.02 (ns)	0.03 (ns)	1	-0.19 (ns)	-0.17 (ns)	0.19 (ns)	-0.36 (0.057)	-0.07 (0.057)	0.20 (ns)	0.00 (ns)	0.20 (ns)	-0.12 (ns)
BMI	0.71 (0.00001)	0.14 (ns)	-0.05 (ns)	-0.12 (ns)	-0.19 (ns)	1.00	0.04 (ns)	-0.12 (ns)	-0.00 (ns)	0.37 0.048)	-0.09 (ns)	0.33 (ns)	-0.32 (ns)	0.01 (ns)
Reticulo-cytes	0.07 (ns)	-0.05 (ns)	0.22 (ns)	-0.30 (ns)	-0.17 (ns)	0.04 (ns)	1	0.28 (ns)	-0.61 (0.0005)	-0.22 (ns)	-0.14 (ns)	-0.19 (ns)	-0.02 (ns)	0.12 (ns)
Platelets	-0.10 (ns)	0.21 (ns)	-0.06 (ns)	-0.12 (ns)	0.19 (ns)	-0.12 (ns)	0.28 (ns)	1	-0.17 (ns)	-0.38 (0.045)	0.11 (ns)	-0.31 (ns)	0.22 (ns)	-0.33 (ns)
Monocytes	0.01 (ns)	-0.00 (ns)	-0.22 (ns)	0.09 (ns)	-0.36 (0.057)	-0.00 (ns)	-0.61 (0.0005)	-0.17 (ns)	1.00	0.08 (ns)	0.12 (ns)	-0.01 (ns)	0.09 (ns)	-0.06 (ns)
Hemoglobin	0.51 (0.005)	0.01 (ns)	0.00 (ns)	0.33 (ns)	-0.07 (0.057)	0.37 (0.048)	-0.22 (ns)	-0.38 (0.045)	0.08 (ns)	1	0.21 (ns)	0.65 (0.0001)	-0.54 (0.002)	0.21 (ns)
Urea	0.02 (ns)	-0.18 (ns)	-0.05 (ns)	0.15 (ns)	0.20 (ns)	-0.09 (ns)	-0.14 (ns)	0.11 (ns)	0.12 (ns)	0.21 (ns)	1	0.18 (ns)	0.01 (ns)	-0.30 (ns)
Creatinine	0.25 ((ns)	0.11 (ns)	0.04 (ns)	0.05 (ns)	0.00 (ns)	0.33 (ns)	-0.19 (ns)	-0.31 (ns)	-0.01 (ns)	0.65 (0.0001)	0.18 (ns)	1	-0.38 (0.045)	-0.01 (ns)
Potassium	-0.36 (0.056)	0.05 (ns)	-0.03 (ns)	-0.33 (ns)	0.20 (ns)	-0.32 (ns)	-0.02 (ns)	0.22 (ns)	0.09 (ns)	-0.54 (0.002)	0.01 (ns)	-0.38 (0.045)	1	-0.23 (ns)
Bicarbonate	0.14 (ns)	-0.10 (ns)	0.18 (ns)	0.13 (ns)	-0.12 (ns)	0.01 (ns)	0.12 (ns)	-0.33 (ns)	-0.06 (ns)	0.21 (ns)	-0.30 (ns)	-0.01 (ns)	-0.23 (ns)	1.00

ns= not significant

Acute Bone Pains (Tables 3 and 4)

Sickle cell disease patients with ABP recorded a mean [±sd] reticulocyte count of (2.4 [0.7]) at the beginning which was reduced significantly (t-test = 5.83, P-value = 0.000001) to (1.2 [0.5]) at the end of the study. There was no noticeable difference in the mean reticulocyte count among SCD patients without ABP at the enrollment and at the end of the study. The mean values of platelets, neutrophils, lymphocytes and hemoglobin were not altered at enrollment compared to the values at the end of study among SCD patients with ABP. However, the mean [±sd] monocyte count of those with ABP at enrollment (3.4 [0.9]) was significantly elevated (t-test = -2.66, P-value = 0.007) at the end of the study (4.2 [0.8]) and the enrollment value of those without ABP (2.8 [0.8]) also significantly increased (t-test = -2.98, P-value = 0.01) at the end of the study (4.0 [0.8]). There were also noteworthy changes in eosinophil and total white blood cell values among those with and without ABP at enrollment and at the end of the study. Means of potassium, chloride and bicarbonate concentrations of those without ABP were significantly higher (t-test = -3.09, P-value = 0.007; t-test = -2.49, P-value = 0.03; t-test = -2.40, P-value = 0.03) at the end of the study compared to the value at enrollment. However, means of urea and creatinine were notably higher among those with ABP when values at the end of study were compared to those at enrollment.

Acute Chest syndrome (Tables 3 and 4)

Significant differences were observed in the mean [±sd] reticulocyte count of those with (t-test = 2.46, P-value = 0.02) and without (t-test = 2.62, P-value = 0.006) chest syndrome when values at enrollment were compared with values at the end of the study. Among those without chest syndrome, the mean values of monocytes, neutrophils, eosinohils and total white blood cells at enrollment were notably varied (t-test = -2.87, P-value = 0.003; t-test = 4.19, P-value = 0.0001; t-test = -2.98, P-value = 0.002; t-test = 2.03, P-value = 0.02) compared to the values at the end of the study. Apart from the serum level of sodium which significantly decreased (t-test = 2.38, P-value = 0.02) at the end of the study (135.5 [5.0]) compared to the value at enrollment (140.5 [2.9]) among those with chest syndrome, the serum levels of potassium (5.3 [1.0]; 6.2 [0.9]), bicarbonate (16.0 [4.9]; 23.4 [3.4]) and creatinine (38.7 [13.6]; 52.3 [14.9]) significantly increased (t-test = -1.94, P-value = 0.04; t-test = -3.68, P-value = 0.001; t-test = -1.92, P-value = 0.04) among this group of patients. Incidentally, among those without chest syndrome, the means of potassium, bicarbonate, urea and creatinine also increased significantly at the end of the study in comparison with the values at enrollment.

Correlation coefficients (Tables 5 and 6)

Surprisingly, a significant positive correlation (Pearson's r = 0.40, P-value = 0.031) was observed between serum urea and chest pain at enrollment, and astonishingly between fatigue and ABP (r = 0.52, P-value = 0.003). Other correlation coefficients and values of their level of significance are as shown in Table 5. There was no noteworthy correlation between any of the clinical symptoms and other variables at the end of the study though other correlation coefficients and values of their level of significance could be found in Table 6.

Discussion

This study was designed to investigate the prevalence of fatigue, acute bone pains and chest syndrome at enrollment and at the end of the study. In addition, the study also examined the hematological and biochemical parameters of patients presenting with these three symptoms of sickle cell disease and compared enrollment values with the values obtained at the end of the study, after the trial drug had been given to patients over a period of six months. The study aimed to determine whether the proportion of SCD patients with fatigue, acute bone pains and chest syndrome would decrease, remain the same or increase at the end of the study. Our intention was to avoid presupposing that the study drug has therapeutic efficacy, allowing the data to present its own internal representation of the observation. Sickle cell disease is an illness that is usually associated primarily with abnormality of the red blood cells (sickle hemoglobin) which deform when deprived of oxygen, causing obstruction to the flow of blood it passes through, especially in very small veins or arteries. The disease is also affiliated with abnormalities in the coagulation system, disruption of platelet activation and accumulation, poor functioning of the inner wall of blood vessels, and with cell inflammatory responses, among others [3].

This study includes relatively new information about the prevalence of fatigue, ABP and ACS as well as the hematological and biochemical parameters associated with these clinical symptoms before and after the administration of a novel study drug among Black Africans. One of the key findings in this study is that acute bone pains had the highest prevalence than fatigue and ACS among the patients, probably due to bone marrow ischaemia or infarction. The 94% prevalence of ABP observed in this study is higher than the 70% reported by Shah et al [31]. The higher reticulocyte count among SCD patients with and without ABP may indicate attempt by the bone marrow to counterbalance consequential anemia associated with SCD. Contrary to what Canalli et al [32] reported, eosinophil level was not elevated at enrollment in this study. Evidence is scarce on possible functional changes of eosinophils and their derived mediators in SCD, especially with the administration of the study drug, though these cells are known to participate in body's inflammatory processes. It is not certain what role eosinophil plays in vaso-occlusion of SCD in Africa population. It is speculated that the insignificant increase of eosinophil count at the end of the study may indicate a probable activation leading to an attempt to ameliorate adhesion of sickle erythrocytes to the vascular endothelium but this should be a topic for another study, considering therapeutic management with the study drug.

The 60.0% prevalence of fatigue observed among the patients in this study is similar to the 65% reported by Ahmadi et al [33] from a study in Iran. In the present study of adolescents with SCD, mild to moderate fatigue may be devastating when it interferes with patient's ability to study, have a retentive memory, engage in peer-group activities, or in performing their daily chores. Further, fatigue could reveal a poorer quality of life as it does in other chronic diseases such as cancer and diabetes [34-37]. Adolescent SCD patients have disturbance of sleep and cognitive function [38] and significantly lower levels of energy [39] compared to healthy peer and population, respectively. This corroborates finding

in this study that, at enrollment, approximately 94% of those who presented with fatigue were also underweight with a BMI of <18.5, a figure that is much higher than the 48% reported by Odetunde et al [40]. It is observed that SCD patients in developed countries are living longer, thanks to new therapies, and it is not unusual to find this group of patients in their sixth, seventh and eight decade of life [41] with obesity as a confounding factor to SCD [42]. Woods et al [43] documented an increase in the prevalence of obesity among SCD patients. Thus, if SCD patients in Africa have access to affordable therapy, they may also have a longer life expectancy. In this study, fatigue was not associated with age, in line with what Ameriga et al reported [23]. However, at enrollment, fatigue was significantly associated with ABP, which is a novel finding. The only possible explanation that can be adduced to this association is that probably, both symptoms are directly related to each other in that acute bone pains cause fatigue and fatigue exacerbates acute bone pains. The pathophysiology of this relationship requires further studies. At the end of the study, there was no significant association between fatigue and ABP or with any other variable, an observation that calls for an important study of the mechanism of action of the study drug. Data on acute chest syndrome (ACS) among sub-Sahara Black Africans with SCD is very rare and as such, this may be one of the first studies, if not the first, to report the prevalence of this syndrome, an aggregation of symptoms that take place when sickled cells are clustered in the lungs. Data are also scarce on the hematological and biochemical characteristics associated with ACS among Africans with SCD.

The prevalence (33%) of ACS reported in this study is much lower than the 45% suggested in other studies [21, 44, 45]. The prevalence, prognosis, recurrence and mortality of ACS in different African countries or in different parts of any African country or region cannot be verified at this time until a multi-center and muti-disciplinary study is conducted. Acute chest syndrome may be a more common complication of SCD in sub-Sahara Black African children or adults with SCD. One surprising observation is that the proportion of SCD patients with chest syndrome dropped from 33% at enrollment to 23% at the end of the study. This drop may seem insignificant but it might be a pointer to the mechanism of action of the study drug which, it is speculated, may unclog the micro-circulation in yet unexplained mechanism of action or may prevent adhesion of various cells or may prevent release of inflammatory substances. It is also pertinent to say that those with ACS at enrollment were comparatively older than those with fatigue or with ABP. Also the proportions of those with mild and with moderate ACS were lower that those with mild or moderate ABP or with mild or moderate fatigue. This may indicate that ACS is not as common as the other symptoms but could be more lethal.

Study limitations

This study used questionnaire to determine fatigue. There could have been a bias as the responses were subjective. A Multidimensional Fatigue Symptom Inventory-Short Form (MFSI-SF), a 30-item survey that assesses fatigue over the past month prior to the study would have presented a more robust measure of fatigue [46, 47]. However, the 4-point Likert-type format with response options ranging from 0 (Not at all) to 3 (Severe) was used and deemed adequate for this study. Also, questionnaire was used by patients to subjectively rate their pain on a numeric rating, subjectively describing their pain during the past 24 hours prior to the study. Brief Pain Inventory (BPI) would have been used to assess pain [48,49] had it been a multi-center, multi-disciplinary study. For a small sample size of 30 and a preliminary study, questionnaire documentation was decided and considered to be adequate. Records on length of stay in hospital and ICU were available for few and not all the patients and for this reason, they were not documented in this paper. Further, prognosis and mortality were unavailable. Lung function test were not performed. This would have been a valuable test to determine the involvement of the lungs, especially among patients with ACS. A future study will take these into consideration.

Conclusion

New therapeutic agents for improved outcome of SCD are needed and scientists are daily studying novel approaches to this end. Gutsaeva et al [50] recently reported an anti-P-selectin aptamer to inhibit adhesive interaction between circulating sickle red blood cells (RBC), leukocytes and endothelial cells. In this study, it was observed that there was an increase in eosinophil level at the end of the study compared to at enrollment and it is speculated that eosinophil may be activated, in the presence of the study drug, to also prevent adhesion of RBC and leukocytes. Acute chest syndrome among SCD patients in sub-Sahara Africa is mostly undocumented, though it might be the cause of significant morbidity and mortality. There is no evidence of whether its prevalence, recurrence and mortality are high or low in comparison to other parts of the world. It is a frequent hindrance in SCD which often calls for frequent hospital admissions. Clinicians caring for SCD should be acutely made aware for early clinical signs of ACS so that the best prophylactic measures are given and appropriate procedures, such as chest X-ray and other laboratory protocols are promptly undertaken. It goes without saying that, because a large number of Africans within the continent and in diaspora suffer from SCD and its variants, studies should be conducted throughout Africa to concentrate on various clinical symptoms, immuno-diagnostic tools, pathophysiology, hospital and home management as well as on consequence of not only ACS but other symptoms of SCD in Africans.

Declaration: The authors declare no conflicting interest.

Acknowledgement: This study was sponsored by Rahma Integrated Concept Ltd, Kaduna, Nigeria.

References

1. Conran N, Belcher JD. Inflammation in sickle cell disease. Clin Hemorheol Microcirc., 2018;68(2-3):263-299.
2. Proença-Ferreira R, Brugnerotto AF, Garrido VT, et al. Endothelial activation by platelets from sickle cell anemia patients. PLoS One 2014;9(2):e89012.
3. De Franceschi L, Cappellini MD, Olivieri O. Thrombosis and sickle cell disease. Semin Thromb Hemost. 2011;37(3):226-36.
4. Kaul DK, Finnegan E, Barabino GA. Sickle red cell-endothelium interactions. Microcirculation. 2009;16(1):97–111.
5. Hebbel RP. Adhesion of sickle red cells to endothelium: myths

and future directions. Transfus Clin Biol. 2008;15(1-2):14–18.

6. Turhan A, Weiss LA, Mohandas N, Coller BS, Frenette PS. Primary role for adherent leukocytes in sickle cell vascular occlusion: a new paradigm. Proc Natl Acad Sci U S A. 2002;99(5):3047–3051.
7. Platt OS, Thorington BD, Brambilla DJ, et al. Pain in Sickle Cell Disease. N Engl J Med. 1991; 325 (1):11-6.
8. Adewoyin AS. Management of Sickle Cell Disease: A Review for Physician Education in Nigeria (Sub-Saharan Africa). Anemia. 2015;2015:21.
9. Okpala I, Tawil A. Management of pain in sickle-cell disease. J R Soc Med 2002;95:456–458
10. Shapiro BS. The management of pain in sickle cell disease. Pediatr Clin North Am. 1989;36:1029–45.
11. Yale SH, Nagib N, Guthrie T. Approach to the vaso-occlusive crisis in adults with sickle cell disease. Am Fam Physician. 2000;61(5):1349-56.
12. Vichinsky E. Comprehensive care in sickle cell disease: its impact on morbidity and mortality. Semin Hematol. 1991;28:220-6.
13. Castro O, Brambilla DJ, Thorington B, et al. The acute chest syndrome in sickle cell disease: incidence and risk factors: the Cooperative Study of Sickle Cell Disease. Blood. 1994;84:643-9.
14. Platt OS, Brambilla DJ, Rosse WF, et al. Mortality in sickle cell disease: life expectancy and risk factors for early death. N Engl J Med. 1994;330:1639-44.
15. Castro O. Systemic fat embolism and pulmonary hypertension in sickle cell disease. Hematol Oncol Clin North Am. 1996;10:1289-303.
16. Vichinsky E, Williams R, Das M, et al. Pulmonary fat embolism: a distinct cause of severe acute chest syndrome in sickle cell anemia. Blood. 1994;83:3107-12.
17. Godeau B, Schaeffer A, Bachier D, et al. Bronchoalveolar lavage in adult sickle cell patients with acute chest syndrome: value for diagnostic assessment of fat embolism. Am J Respir Crit Care Med. 1996;153:1691-6.
18. Hutchinson RM, Merrick MV, White JM. Fat embolism in sickle cell disease. J Clin Pathol. 1973;26:620-2
19. Ballas SK, Lieff S, Benjamin LJ, et al. Definitions of the phenotypic manifestations of sickle cell disease. Am J Hematol. 2010;85:6.
20. Dang NC, Johnson C, Eslami-Farsani M, Haywood LJ. Bone marrow embolism in sickle cell disease: a review. Am J Hematol. 2005;79:61.
21. Vichinsky EP, Neumayr LD, Earles AN, et al. Causes and outcomes of the acute chest syndrome in sickle cell disease. National Acute Chest Syndrome Study Group. N Engl J Med. 2000; 342:1855.
22. Dessap AM, Leon R, Habibi A, et al. Pulmonary hypertension and cor pulmonale during severe acute chest syndrome in sickle cell disease. Am J Respir Crit Care Med 2008; 177:646–653
23. Ameringer S, Elswick RK, Smith W. Fatigue in Adolescents and Young Adults with Sickle Cell Disease: Biological and Behavioral Correlates and Health-Related Quality of Life. J Pediatr Oncol Nurs. 2014; 31(1):6–17.
24. Jacob E, Beyer JE, Miaskowski C, Savedra M, Treadwell M, Styles L. Are there phases to the vaso-occlusive painful episode in sickle cell disease? J Pain Symptom Manage. 2005; 29(4):392-400.
25. Afolabi BM, Haliru L, Zubair RA, et al. Effect of Immunozin™ on Sickle Cell Disease in Sub-Saharan Africa: A Pilot Study. Am J Res Med Sci. 2020;5(1):1-11
26. Cochran WG. Sampling techniques (3rd ed.), 1977. New York: John Wiley.
27. Levy SP, Lemeshow S. Sampling of population (4th ed.), 2008. New York: John Wiley.
28. Nigerian Population Commission. 2006 Population Census, Abuja, Nigeria
29. Is fatigue caused by sickle cell disease? Sickle-cell.com (Accessed on 25th February 2021).
30. Alagbe AE, Olaniyi JA, Aworanti OW. Adult sickle cell anaemia patients in bone pain crisis have elevated pro-inflammatory cytokines. Mediterr J Hematol Infect Dis 2018;10(1):e2018017.
31. Shah N, Bhor M, Xie L, Paulose J, Yuce H. Sickle cell disease complications: Prevalence and resource utilization. PLoS ONE, 2019;14(7):e0214355.
32. Canalli AA, Conran N, Fattori A, Saad STO, Costa F. Increased adhesive properties of eosinophils in sickle cell disease. Granulopoiesis, 2004;32(8):728-734
33. Ahmadi M, Poormansouri S, Beiranvand S, Sedighie L. Predictors and Correlates of Fatigue in Sickle Cell Disease Patients. Int J Hematol Oncol Stem Cell Res. 2018;12(1):69-76.
34. Bakshi R. Fatigue associated with multiple sclerosis: diagnosis, impact and management. Multiple Sclerosis. 2003;9:219–227.
35. Falk K, Swedberg K, Gastonjohansson F, Ekman I. Fatigue is a prevalent and severe symptom associated with uncertainty and sense of coherence in patients with chronic heart failure. European Journal of Cardiovascular Nursing. 2007;6:99–104.
36. Kralik D, Telford K, Price K, Koch T. Women's experiences of fatigue in chronic illness. Journal of Advanced Nursing. 2005;52:372–380.
37. Ream E, Richardson A. Fatigue in patients with cancer and chronic obstructive airways disease: a phenomenological enquiry. International Journal of Nursing Studies. 1997;34:44–53.
38. Dampier C, Lieff S, LeBeau P, et al. Health-related quality of life in children with sickle cell disease: A report from the Comprehensive Sickle Cell Centers Clinical Trial Consortium. Pediatric Blood & Cancer. 2010; 55:485–494.
39. Dampier C, LeBeau P, Rhee S, et al. The Comprehensive Sickle Cell Centers Clinical Trial Consortium Site, I. Health-related quality of life in adults with sickle cell disease (SCD): A report from the comprehensive sickle cell centers clinical trial consortium. American Journal of Hematology. 2011; 86:203–205.
40. Odetunde OI, Chinawa JM, Achigbu KI, Achigbu EO, Pak J. Body mass index and other anthropometric variables in children with sickle cell anaemia.Med Sci. 2016;32(2):341-6.
41. Ballas SK, Pulte ED, Lobo C, Riddick-Burden G. Case series of octogenarians with sickle cell disease. Blood. 2016;128:2367-2369.
42. Ballas SK. The Sixth Vital Sign: Body Mass Index in Patients With Sickle Cell Disease. J Clin Med Res. 2017;9(10):889-890.
43. Woods KF, Ramsey LT, Callahan LA, et al. Body composition in women with sickle cell disease. Ethn Dis. 2001;11(1):30- 35.
44. Poncz M, Greenberg J, Gill FM, Cohen A. Hematological changes during acute chest syndrome in sickle cell disease. Am

J Pediatr Hematol Oncol 1985;7:96-9.

45. Johnson CS, Verdigem TD. Pulmonary complications of sickle cell disease. Semin Respir Med 1988;9:287-96.
46. Stein KD, Jacobsen PB, Blanchard CM, Thors C. Further validation of the multidimensional fatigue symptom inventory-short form. Journal of Pain and Symptom Management. 2004;27(1):14-23
47. Stein KD, Martin SC, Hann DM, Jacobsen PB. A multidimensional measure of fatigue for use with cancer patients. Cancer Pract. 1998;6(3):143-152
48. Watt-Watson J, Chung F, Chan VWS, McGillion M. Pain management following discharge after ambulatory same-day surgery. Journal of Nursing Management, 2004;12:153–161
49. Daut RL, Cleeland CS, Flanery RC. Development of the Wisconsin Brief Pain Questionnaire to Assess Pain in Cancer and Other Diseases. Pain, 1983;17:197-210.
50. Gutsaeva DR, Parkerson JB, Yerigenahally SD, et al. Inhibition of cell adhesion by anti-P-selectin aptamer: a new potential therapeutic agent for sickle cell disease. Blood. 2011;117(2):727–735.