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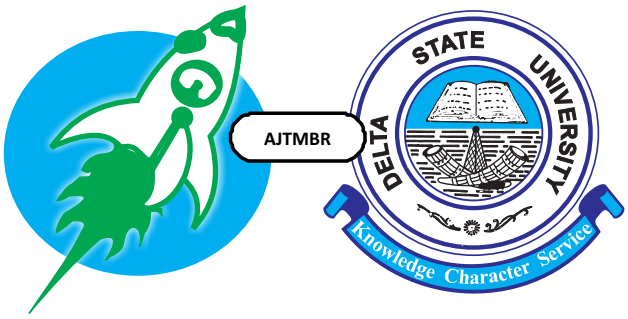

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Table of Contents

Evaluation of Endothelin-1 as a Marker of Endothelial Activation in Patients with Sickle Cell Anaemia in a tertiary Hospital in South-South Nigeria

[1]*Dirisu IM,* [2]*Awodu OA,* [2]*Nwogoh B*

Abstract

Background: Endothelial activation, which often occurs in individuals with sickle cell disease (SCD) patients as a result of oxidative stress, may lead to endothelial dysfunction and acute inflammation. Evaluation of endothelin-1 levels in vaso-occlusive crisis (VOC) may help identify therapeutic targets in the management of SCD.

Materials and Methods: This study aims to assess the role of endothelial activation in the pathophysiology of VOC using endothelin-1 in patients with sickle cell disease managed at the University of Benin Teaching Hospital.
Materials and Methods: This was a longitudinal study conducted at the University of Benin Teaching Hospital, Benin City, Edo State between April 2018 and August 2019. Thirty-five patients with sickle cell anaemia (SCA) were evaluated during VOC and later re-evaluated in steady-state. Thirty-five HbAA subjects, matched for age and sex with the SCA population were recruited as controls. Endothelin -1 (ET-1) concentrations were determined using an enzyme-linked immunosorbent assay. Data were analyzed using SPSS version 21.

Results: The age range of the SCA patients was 18 – 45 years with a mean SD of 27.7 + 6.7 years. The mean endothelin-1 levels in SCA patients in steady state, VOC and control subjects were 9.35mM, 13.79mM and 4.80mM respectively. The mean ET-1 levels in SCA patients in steady-state and VOC was significantly increased compared to the control subjects ($p < 0.001$). There was no significant correlation between ET-1 and number of VOCs per year ($p = 0.108$) and the number of admissions per year ($p < 0.005$).

Conclusion: SCA is associated with increased endothelial activation which may contribute to the pathogenesis of VOC. This finding may be important in determining the role of the use of anti-inflammatory therapies in the management of SCD.

Key words: Endothelin-1, Sickle Cell Anaemia, Vaso- Occlusive Crises, Endothelial activation

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Introduction

Sickle cell disease (SCD) encompasses inherited haemolytic anaemia arising from a single point mutation in the β-globin gene. This results in the formation of the sickle haemoglobin (HbS).[1] In SCD, the phenotypic expressions of patients are complex and is characterized by intermittent vaso-occlusive events, increased susceptibility to

infections, chronic inflammatory disease and microvascular damage in all organs.[2-3]

Since the first description of sickle-shaped erythrocytes, by Herrick in 1910, there has been increasing understanding of the pathophysiology of SCD.[4] New pieces of evidence point to oxidative stress processes as being increasingly related to the SCD pathophysiology.[5]

There has been a better understanding of several mechanisms involved in SCD such as the consequences of HbS polymerization. However, clinical management of the disease is still basic. Although, numerous pieces of evidence give support to the use of periodic red cell exchange blood transfusion and hydroxycarbamide in some circumstances, drugs that target other possible mechanisms of this disease are still understudied.[6]

Sickle cell disease is regarded as a chronic inflammatory condition[7] with studies revealing abnormally high levels of white blood cells (WBC) in SCD patients. Circulating endothelial cells from SCD patients also display an activated state with the expression of adhesion molecules. A genetic profile of blood mononuclear cells from 27 patients in steady-state SCD, showed up-regulation of genes related to oxidative stress and inflammation and global up-regulation of pro-inflammatory markers.[8] Chronic activation and damage of endothelial cells by sickled red blood cells, heme, polymorphonuclear neutrophils (PMNs) and inflammatory mediators contribute to progressive microvascular damage in all organs, including the brain, lungs and kidneys.[9]

The contribution of the vascular endothelium to the vaso-occlusive state has been suggested by several studies which demonstrated ~~demonstrating~~ increased adherence between sickled red cells and the endothelium, particularly during a vaso-occlusive crisis.[10] Besides alterations in adherence, instability in local vascular tone via altered metabolism of endothelial-derived vasoactive mediators could contribute to vaso-occlusive events. For example, plasma levels of endothelin-1 (ET-1), has been reported to be elevated in patients with SCD.[11] Endothelin-1 represents the most potent endogenous vasoconstrictor. It is released by activated endothelial and non-endothelial cells in response to hypoxia and reduced nitric oxide bioavailability in several animal models.

Damage resulting from abnormal endothelial activation appears to play significant role in the pathophysiology of SCD. The findings from this study will add to the existing knowledge on the role of endothelial activation in the pathogenesis of SCD.

This study aims to assess the role of endothelial activation in the pathogenesis of VOC using endothelin-1 as a marker in patients with sickle cell disease managed at the University of Benin Teaching Hospital.

MATERIALS AND METHODS

Study design

The study was a hospital-based longitudinal study.

Study area

This study was conducted at the Department of Haematology, University of Benin Teaching Hospital (UBTH). The hospital is a Federal Government-owned tertiary institution with over 800-bed capacity, situated in Egor LGA, Benin City, Edo State. The hospital provides specialized services in the major fields of medicine.

Study Participants:
The study comprised the study population and a control group:

Group I: This consisted of HbSS patients aged 18 - 45 years. They were evaluated for endothelin-1 and haematological parameters in VOC and later followed up and re-evaluated in steady-state (approximately after a period of three months). They were recruited from the Haematology ward and the emergency ward and later followed up in the Haematology outpatient clinic.

Group II: This consisted of apparently healthy HbAA individuals (confirmed by haemoglobin electrophoresis) matched for age and sex, who were consecutively recruited from the blood donor clinic, hospital staff and students.

Inclusion Criteria: Sickle cell anaemia patients who gave consent and apparently healthy age- and sex-matched HbAA adults as controls.

Exclusion criteria: Patients on hydroxyurea due to its anti-inflammatory effects, participants who have been transfused within the last three months, those with coexisting illnesses that could contribute to inflammation.

Sampling techniques and study duration
Patients were recruited consecutively until the sample size was achieved over a period of ten months between April 2018 and August 2019.

Sample collection, storage and analysis
For each patient, the venepuncture site was carefully cleaned with methylated spirit and a total of six milliliters (6ml) of venous blood was collected from the ante-cubital vein with minimal stasis. From this, 3ml of blood was dispensed into an ethylene di-amine tetra-acetic acid (0.47mol/L K3-EDTA) container for basic haematological parameters. Three milliliters of blood, was transferred into a separate plain tube for endothelin-1 assay.

Sample for Endothelin-1 was allowed to clot for two hours at room temperature and centrifuged at 1,000 x g for 20 minutes. Collected serum was stored in aliquots at -80^{0}C for later use.

Test Procedures
Basic Haematological Parameters: Full blood count which included haematocrit, haemoglobin concentration, total white cell count and platelet counts was obtained from the EDTA sample, using an automated blood cell counter (Sysmex Haematology Autoanalyser model KN21). The basic principles underlying these techniques are electronic impedance and light scatter. This was done in the main haematology laboratory, UBTH.
Haemoglobin Electrophoresis: The haemoglobin phenotypes of both subjects and control were confirmed using haemoglobin electrophoresis.

Peripheral Blood Film (PBF): This was done manually for only HbSS subjects in VOC and in steady-state to assess their irreversible sickle cell (ISC) index.

Reticulocyte Count: This was done manually for only HbSS subjects in VOC and steady-state.

Quantitative determination of Endothelin 1: Human ET-1 (Endothelin 1) levels were ~~was~~ assessed using the Elabscience Biotechnology Incorporated ELISA Assay Kit. Catalogue No. E-E-H0064. This study was carried out at the Haematology Laboratory in UBTH, Benin City.

Quality control
The following measures were taken to ensure the accuracy of the result from the assays
1. Samples were appropriately collected and stored accordingly.

2. The temperature of the -80 C freezer was checked daily on the in-built thermometer to ensure optimal function.
3. All reagents were stored according to the manufacturer's instructions.
4. All assays were analyzed following the manufacturer's instructions.

Data analysis

Data were analyzed using Statistical Package for the Social Sciences (SPSS) Version 21 Percentages, means and standard deviations were calculated. Student t-test and Chi-square (or Fischer's exact test) was used for parametric and non-parametric data, respectively. Pearson correlation test was used for correlation between variables. Probability values less than 0.05 ($p < 0.05$) were considered as significant. Results are presented as tables, figures and Box and Whiskers plots. Results of categorized variables such as sex, frequency of VOCs, admissions etc. were summarized using frequencies and percentages. Continuous variables such as age, ET-1, haematological parameters were summarized as mean, standard deviations and ranges.

Et**hical Approval:** The study was approved by the institutional ethical review committee. All participants also gave informed consent.

RESULTS

A total of seventy subjects comprising thirty-five (50%) SCA patients and thirty-five (50%) controls participated in the study. The SCA group consisted of eighteen (51.4%) males and seventeen (48.6%) females while the controls also included eighteen (51.4%) males and seventeen (48.6%) females.

The mean age of SCA patients was 27.7 ± 6.7 years with a range of 18 - 45 years. The controls had a mean age of 24.3 ± 7.3 years with a range of 18 - 46 years. The difference in mean age between the SCA patients and the controls was not statistically significant (P = 0.073). The peak age group of the SCA patients was 20 - 29 years representing 61.4% of the SCA group. This was also similar to those of the control group 20 - 29 but represents 54.3% of the controls. Table 1 shows the demographic parameters of the study population.

Medical History

The median age at diagnosis in SCA patients was 5.0 years with an interquartile range of 1 - 10. They experienced median (IQR) number of 3 (2 - 4) VOCs per year necessitating a median of one hospital admission per year and an interquartile range of 1 - 2.

Sickle cell leg ulcers and visual impairments were the complications documented in 7 (20%) and 1 (2.9%) respectively of the SCA patients.

Endothelin-1, and Haematological Parameters (Crisis vs Steady-state)

Table 2 shows the serum endothelin-1, haematological parameters, reticulocyte count, and sickle cell index. The mean endothelin-1 level in SCA subjects in crisis state was significantly higher than those in steady-state (13.8 ± 7.2mM vs 9.4 ± 5.19mM, $p < 0.001$) (Table 2).

The mean haemoglobin concentration ~~of~~ SCA subjects in crisis was 7.4 ± 1.5g/dl with a range of 3.3 -10.0g/dl and in steady-state was 8.7 - 1.6g/dl and a range of 5.2 - 11.3g/dl. The haemoglobin concentration was significantly lower in the crisis state than in steady-state ($p < 0.001$).

The mean white blood cell count was 15.3 ± 4.2 x 10^9cells/L and 10.4 ± 3.9 x 10^9cells/L for the SCA patients in crisis and steady states respectively. The difference in mean was statistically significant ($p < 0.001$). The mean Absolute Neutrophil

Count (ANC) was 10.3 ± 4.3 x 10^9 cells/L and 6.4 ± 2.8 x 10^9 for SCA patients in crisis and steady-state respectively.

The mean platelet counts of SCA patients in VOC and steady-state were 383.3 ± 152.6 x10^9 252.9 ± 121.4 x 10^9 cells/L respectively.

SCA patients had a mean reticulocyte count of 14.3 ± 3.2% and 9.3 ± 3.4% in crisis and steady-state respectively. The difference in mean was statistically significant ($p < 0.001$). There was also a statistically significant difference in the mean sickle cell index between crisis state and stable state (11.4 ± 5.8% vs 6.1 ± 4.4%, $p < 0.001$).

Table 3 shows the haematological parameters, and serum levels of endothelin-1 in SCA patients in crisis and the control group. The mean ET-1 levels in SCA crisis state was significantly higher than that of the control subjects (13.8 ± 7.2Mm vs 4.80 ± 1.77mM, p 0.001) (Figure 4).

The mean haemoglobin concentration of SCA patients in crisis was 7.4 ± 1.5g/dl with a range of 3.3 – 10.0g/dl and in the control group, it was 12.32 ± 1.93g/dl and a range of 5.2 – 11.3g/dl. The haemoglobin concentration was significantly lower in the crisis state when compared to the control subjects. (p 0.001).

The mean white blood cell count was 15.3 ± 4.2 x 10^9 cells/L and 5.6 ± 1.6 x 10^9 cells/L for the SCA patients in crisis and the control subjects respectively. The difference in mean was statistically significant (p 0.001). The mean ANC was 10.3 ± 4.3 x 10^9 cells/L and 2.7 ± 1.1 x 10^9 for SCA patients in crisis and the control subjects respectively. The difference in mean was statistically significant (p 0.001). The mean platelet counts in SCA patients in VOC and control subjects were 383.3 ± 152.6 x 10^9 cells/L and 242.4 ± 115.7 x 10^9 cells/L respectively.

Table 4 shows the serum ET-1 levels, and haematological parameters in SCA patients in stable state and control group. The mean endothelin-1 levels in SCA patients in steady state was 9.35 ± 5.19mM with a range of 4.06 - 22.68mM. The mean ET-1 levels in the control group were 4.08 ± 1.77mM with a range of 2.98 - 22.68mM. (Shown in figure 4). The difference in mean was statistically significant ($p = 0.001$).

The mean haemoglobin concentration in SCA patients in the stable state was 8.69 ± 1.55g/dl with a range of 5.2 - 11.3g/dl and for the control group was 12.32 ± 1.93g/dl and a range of 8.3 - 15.7g/dl. The haemoglobin concentration was significantly lower in the steady-state than in the control group ($p = 0.000$). The mean white blood cell count for SCA patients in steady state was 10.4 ± 3.9 x 10^9 cells/uL with a range of 6.4 – 18.6 x 10^9 cells/uL and 5.6 ± 3.9 x 10^9 cells/μL with a range of 3.4 – 11.3 x 10^9 cells/uL for the control group. The differences in their means were statistically significant ($p= 0.001$). The mean ANC for SCA in steady state was 10.4 ± 3.9 x 10^9. This is significantly higher than the ANC in the control subjects (5.6 ± 1.6 x 10^9). ($p = 0.001$). The mean platelet count for SCA in steady state was 252.9 ± 121.4cells/uL with a range of 109.0 - 655.0 x 10^9 cells/uL while the mean platelet count for the control group was 242.4 ± 91.6 x 10^9 cells/uL with a range of 99.0 – 458.0 x 10^9 cells/uL. The difference in mean was not statistically significant ($p = 0.683$).

Table 5 shows the correlations between ET-1, demographic and clinical parameters in SCA patients. In the steady-state, there was no significant correlation between serum ET-1 and PCV, ($r = 0.004$; $p = 0.980$), ET-1 and number of VOCs per year ($r = -0.094$; $p = 0.593$). In crisis,

there was a weak, but statistically insignificant correlation between ET-1 and PCV (r = -0.177; p = 0.308) as well as between ET-1 and number of VOCs per year (r = 0.188; p = 0.280).

Table 6 shows the correlations between endothelin-1, and haematological parameters in SCA patients. There were positive and statistically significant correlations between endothelin-1 and WBC in SCA patients in steady-state (r = 0.327; p = 0.006), as well as between endothelin-1 and ANC (r = 0.342; p = 0.004). There was a weak, but significant negative correlation between endothelin-1 and haemoglobin concentration (r = -0.353; p = 0.003). There was no significant correlation between ET-1 and haematological parameters in SCA crisis state.

DISCUSSION

Vaso-occlusive crises ~~VOC~~ is the most characteristic feature seen in SCD. The pathogenesis of VOC in SCA is complex and multifactorial. Endothelial activation has been ascribed as a key player in the pathogenesis of VOC in SCA patients but it has not been adequately investigated in our environment. This study was aimed at evaluating endothelin-1 level as a marker of endothelial activation in SCA steady states and during crises.

This study shows that the serum levels of ET-1 in SCA patients were found to be elevated. The serum levels of ET-1 were significantly higher in SCA patients in VOC than in their steady-state. The VOC and steady-state ET-1 levels were significantly higher than those of controls. This is consistent with the findings of Adekunle and colleagues at the University College Hospital, Ibadan.[14] Their study showed that plasma ET-1 alongside other cytokines (TNF-α, IL-8) were markedly higher in the SCA patients in severe bone pain crisis than those in the steady-state and the control groups. Similar findings were also experienced in studies done by Graido – Gonzalez *et al*,[15] Rybicki *et al*,[16] Hammerman [17] and Phelan,[18] and Ergul *et al*.[19] Graido-Gonzalez *et al.* who in each of their studies reported that the plasma levels of ET-1 are increased in SCD patients and remained elevated in the post-crisis period as compared to ET-1 levels in healthy controls. Rybicki and colleagues reported increased plasma ET-1 levels in SCD crisis patients and SCD steady-state patients as compared to aged-matched African American control subjects. Hammerman and Phelan reported increased ET-1 expression and synthesis in cultured endothelial cells exposed to either plasma from acute chest syndrome (ACS) patients or deoxygenated erythrocytes from SCD patients. Ergul and colleagues showed that during an acute crisis, ET-1 levels increased approximately 5-fold as compared to steady-state levels. One week after a crisis, ET-1 levels decreased but remained significantly higher than steady-state levels.

Endothelin-1 constricts large vessels, resistance arterioles and even post-capillary venules, which are the usual site of vaso-occlusion in SCA.[20] Endothelin is produced by endothelial cells at a steady rate that is increased following endothelial injury or activation. The release of ET-1 is modulated by TNF-α and other inflammatory mediators.[21] Therefore, the elevated ET-1 level is expectedly elevated in SCA patients because of the ongoing endothelial activation and elevated pro-inflammatory cytokines. Furthermore, elevated levels of ET-1 results from low oxygen tension in SCA patients because hypoxia acts as a potent stimulus for the production and release of ET-1 by the vascular endothelial cell.[22] These factors become more profound during VOC accounting for the greater elevation of ET-1 during VOCs. Endothelins act via two specific G-protein–coupled membrane receptors, ETR-A and ETR-B, which are on vascular smooth muscle

cells and the smooth muscle contraction results from inositol-triphosphate–mediated increases in intracellular calcium. Though ET-1 is rapidly internalized and cleared from circulation by the lungs within minutes, its vaso-constrictive effect lasts as long as 1 hour.[22] In in vitro assays, endothelin stimulated monocyte production of inflammatory cytokines (TNF-α, IL-1β, IL-6, IL-8 etc.), neutrophil production of platelet-activating factor (PAF). ET-1 also enhances monocyte and neutrophil chemotaxis.[23,24,25]

Endothelin-1 is noted to upregulate endothelial cell expression of ICAM-1, VCAM-1, and E-selectin, which are adhesion molecules that participate in the recruitment of leukocytes to sites of inflammation.[26] Conversely, neutrophil proteases play a crucial role in cleaving bioactive ET-1 from its precursor molecule, thereby leading to the production of active ET and resulting in a vicious cycle with worsening inflammation.[27]

The mean haemoglobin ~~Hb~~ concentration of subjects in VOC was found to be significantly lower compared to steady-state and control subjects. Likewise, the mean haematocrit of subjects in VOC was found to be significantly lower compared to steady-state and control subjects. Similarly, the mean haematocrit in steady state was also significantly lower compared to controls. The mean haemoglobin and HCT values in steady-state, VOC and controls are in agreement with previous studies.[30,31] The chronic haemolysis, nutritional deficiency and proneness to infection that occurs in sickle cell anaemia patients may account for the reduction of the haemoglobin concentration found in this study.

The mean total white blood count of HbSS in VOC was significantly higher compared to steady-state and control subjects. Leukocytosis, in the absence of infection, is common in SCD patients and predicts other comorbidities including stroke, acute chest syndrome, and overall mortality. Neutrophils and monocytes are abnormally activated in these patients.[2,3] Activated leukocytes further promote vascular inflammation and vessel damage. An increase in several pro-inflammatory mediators including tumor necrosis factor-alpha (TNF-α), interleukin-6 (IL-6), and interleukin-1beta (IL-1β) further characterize sickle cell anaemia. This accounts for the high leucocyte count in HbSS subjects in both VOC and steady-state. The relative increase in leukocyte count in VOC could further be explained by an increased inflammatory response to stress such as infection in this state.[32]

The mean platelets count of subjects in VOC was significantly higher compared to those of the control subjects. There was no statistical significance in the mean steady-state platelet count when compared with controls. This could be explained on the basis that, steady-state platelets are generally normal or increased in number and functionally hyperactive.[33,34,35] The platelet hyperactivity in steady-state reflects the absence of splenic pooling of young active platelets owing to autosplenectomy rather than chronic intravascular activation of platelets in the microcirculation.[36]

In VOC, there is acute intravascular activation of circulating platelets which stimulate endothelial cell activation. This stimulation is performed by direct contact or adhesion of platelets to endothelial cells, and through the nuclear factor kappa beta (NFkB) dependent signaling pathway, thus contributing to the vaso-occlusive process.[37,38,39]

Positive correlations were found between total WBC, ANC, Hb with ET-1 in steady-state. However, these were not statistically significant.

Sickle cell disease is a monogenic disease that has a wide variety of phenotypes with both genetics and environmental factors.[28] Several clinical phenotypes exist.[29.] These are phenotypic variations in clinical presentation and disease outcome characteristic of the disorder. The SCD state is somewhat heterogeneous with some SCA patients manifesting a predominant haemolytic disease while others present with dominant vaso-occlusive crises. A few exhibits a balance of both VOCs and haemolysis.[19] In steady-state all factors and parameters are at equilibrium and optimal. However, during VOC certain factors yet unidentified may contribute to the variation in presentations and outcome observed in this study. Further studies involving SCA patients exhibiting solely VOC may precisely demonstrate the relations between ET-1 and haematological parameters. Furthermore, whole-genome sequencing, epigenetic studies and an assessment of environmental factors might expand our knowledge of SCD heterogeneity.

The following conclusions can be drawn from this study: ET-1 is significantly higher in SCA patients than controls, ET-1 is significantly elevated in SCA in VOC compared to steady-state, and there were positive correlations between ET-1, WBC and ANC in steady state,

LIMITATION OF STUDY

Stringent exclusion criteria led to the elimination of subjects with severe disease. Hence, the study could not evaluate the impact of the measured biomarker in disease severity and organ complications.

TABLES

Table 1: Demographics of the Study Populations.

		SCA n (%)	Control n (%)	Stat test	P-value
Age group					
	< 20	1 (2.9)	8 (22.9)		
	20 - 29	24 (68.6)	19 (54.3)	6.959	0.073
	30 - 39	9 (25.7)	6 (17.1)		
	40 - 49	1 (2.9)	2 (5.7)		
Sex					
	Male	18 (51.4)	18 (51.4)	0.000	1.000
	Female	17 (48.6)	17 (48.6)		
Religion					
	Christian	33 (94.3)	35 (100)	Fisher's exact	0.493
	Islam	2 (5.7)	0 (0.0)		
Educational status					
	Secondary	2 (5.7)	2 (5.7)	Fisher's exact	1.000
	Tertiary	33 (94.3)	33 (94.3)		
Marital status					
	Single	27 (77.1)	29 (82.9)	$\chi^2 = 0.357$	0,550
	Married	8 (22.9)	6 (17.1)		

Table 2. Endothelin-1, and Haematological Parameters

	SCA Crisis		SCA Steady		P-value
Parameters	Mean ± SD	Range	Mean ± SD	Range	
Endothelin (mM)	13.79 ±7.20	4.58 - 28.89	9.35 ± 5.19	4.06 - 22.68	< 0.001
WBC (x10^9/L)	15.3 ± 4.2	4.5 - 23.5	10.3 ± 3.9	4.8 ± 18.6	< 0.001
ANC (x 10^9/L)	10.3 ± 4.2	2.4 -16.9	6.4 ± 2.8	2.2 - 12.2	< 0.001
Haemoglobin (g/dl)	7.44 ± 1.54	3.3 - 10	8.69 ± 1.55	5.2 - 11.3	< 0.001
PCV (%)	20.7 ± 4.44	11.8 - 28.2	25.3 ± 4.00	13.4 - 31.4	< 0.001
Platelet (x 10^9 /L)	383.3 ±152.6	109.0 -709.0	252.9 ±121.4	109.0 -655.0	< 0.001
Reticulocyte (%)	14.32 ± 3.16	7.8 - 23.5	9.25 ± 3.44	4.4 ± 16.8	< 0.001
Sickle cell index (%)	11.43 ±5.78	2.0 - 26.6	6.09 ± 4.42	0.8 - 25.8	< 0.001

Table 3. Endothelin-1, Glutathione and Haematological Parameters (crisis vs control)

	SCA Crisis		Control		t-test	*P-value*
Parameters	Mean ± SD	Range	Mean ±SD	Range		
Endothelin (mM)	13.79 ± 7.20	4.58 -8.89	4.80 ± 1.77	2.98-9.41	7.16	*0.000*
WBC (x10^9/L)	15.3 ± 4.23	4.5 - 23.5	5.6 ± 1.6	18 – 46	12.71	*0.000*
ANC (x10^9/L)	10.3 ± 4.2	2.4 - 23.5	2.7 ± 1.1	1.2 -6.3	10.35	*0.000*
Haemoglobin (g/dl)	7.4 ± 1.54	3.3 - 10	12.3 ± 1.93	8.3 - 15.7	-11.71	*0.000*
PCV (%)	20.7 ± 4.44	11.8 -28.2	38.7 ± 5.15	27.7- 49.1	-15.64	*0.000*
Platelet (x10^9/L)	382.9 ±152.6	109.0 -709.0	242.4±915.7	99.0 -458.0	4.67	*0.000*

Table 4. Comparison of Endothelin 1, and Haematological Parameters in Steady -State and Control

	SCA Steady state		Control		t-test	*P-value*
Parameters	Mean ± SD	Range	Mean ± SD	Range		
Endothelin (mM)	9.35 ±5.19	4.06-22.68	4.80 ± 1.77	2.98-22.68	4.903	*0.001*
WBC (x10^9/L)	10.3 ± 3.9	6.4 -18.6	5.6 ± 1.6	3.4 -11.3	6.711	*0.001*
ANC (x10^9/L)	6.4 ± 2.8	2.2 -12.2	2.7 ± 1.1	1.2 - 6.3	7.137	*0.001*
Haemoglobin (g/dl)	8.69 ± 1.54	5.2 - 11.3	12.32 1.93	8.3 - 15.7	-8.695	*0.005*
PCV (%)	25.3 ± 4.00	13.4 - 31.4	38.7 ± 5.15	27.7 - 49.1	-12.15	*0.001*
Platelet (x10^9/L)	252.9± 121.4	109.0 -655.0	242.4 ± 91.6	99.0 -458.0	0.410	*0.683*

Table 5: Correlations between Endothelin-1, Demographic and Clinical Parameters of SCA patients in Steady-state and Crisis

		Steady-state Endothelin(mM)	Crisis state Endothelin(mM)
Steady PCV (%)	R	0.004	-0.177
	P-value	0.980	0.308
VOC/year	R	-0.094	0.188
	P-value	0.593	0.280
Admissions/year	R	0.089	0.249
	P-value	0.612	0.149

Table 6: Correlations between Endothelin-1 and Haematological Parameters in SCA Patients and Controls

		Steady-state Endothelin (mM)	Crisis state Endothelin (mM)	Control
WBC	r	0.327	0.071	-0.081
	P-value	0.006	0.684	0.644
ANC	r	0.342	0.082	-0.068
	P-value	0.004	0.640	0.699
Haemoglobin	r	-0.353	-0.110	0.221
	P-value	0.003	0.528	0.202
Platelets	r	-0.004	-0.114	-0.319
	P-value	0.976	0.513	0.062
SCI	r	0.016	-0.036	
	P-value	0.929	0.839	
Reticulocyte	r	0.036	0.196	
	P-value	0.838	0.260	

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