Research Article ISSN 2639-9512

Stem Cell & Regenerative Medicine

The Role of High-Performance Liquid Chromatography Parameters in Vaso-Occlusive Crisis Frequency Among Sickle Cell Anaemia Patients in OAUTHC Ile-Ife, Nigeria

Amaechi Rose A[1], Awe Adeniyi[2], Arayombo Babatunde Elijah[3]*, Omolajaiye Sunday Abraham, Adeyombo Judith N[2], Oladipo Folashade M[2], Yusuf Oluwole Samson[3], Orisatoberu Omolola R[3], Ademosun Abdulwaheed Adewale, Hassan Mutiyat Adeyoola, Oyebanji Oladayo Oluwabusayo, and Ayanleye Yetunde Funmilola

[1]*Department of Haematology and Blood transfusion, Ambrose Alli University Ekpoma, Edo State, Nigeria.*

[2]*Department of Haematology, Obafemi Awolowo University Teaching Hospital, Complex Ile-Ife, Nigeria.*

[3]*Department of Anatomy and Cell Biology, Obafemi Awolowo University Ile-Ife, Nigeria.*

***Correspondence:**

Arayombo Babatunde Elijah, Department of Anatomy and Cell Biology, Obafemi Awolowo University Ile-Ife, Nigeria.

Received: 04 Jul 2025; **Accepted:** 13 Aug 2025; **Published:** 28 Aug 2025

Citation: Amaechi Rose A, Awe Adeniyi, Arayombo Babatunde Elijah, et al. The Role of High-Performance Liquid Chromatography Parameters in Vaso-Occlusive Crisis Frequency Among Sickle Cell Anaemia Patients in OAUTHC Ile-Ife, Nigeria. Stem Cells Regen Med. 2025; 9(3): 1-7.

ABSTRACT

Introduction: *High-Performance Liquid Chromatography (HPLC) is a technique of separating, identifying and quantifying a mixture and its components via passing through a column packed with a stationary phase, which is achieved by differences in interactions of the components with the mobile and stationary phases under high pressure. Sickle cell anemia is an inherited blood disorder in which red blood cells are shaped abnormally, the higher the fetal haemoglobin (HbF) level the lower the risk of frequency of vaso-occlusive crisis (VOC) and vice-versa.*

Materials and Methods: *The study area is ILE-IFE, with latitudes 7°28'N and 7°46'N, and longitudes 4°36'E and 4°56'E. located in the core of south-western Nigeria. The study recruited 50 participants of Sickle Cell subjects in study group and 50 Haemoglobin AA as control group, of which 23(23.7%) and 27(26.7%) represent male and female in the study group, while 24 (24.2%) and 26 (25.8%) represent male and female in the control group respectively. Sample Size Calculations: Prevalence of 2.0 % Case control study was used, N= Z2 x P (1-P)/d2 ~ 30 and therefore sample size was adjusted to 100. Ethical approval for this study was obtained from the Ethical Committee of Obafemi Awolowo University Teaching Hospitals Complex, Ile-Ife, Osun State. A total of 5mls of blood sample was collected in EDTA bottle from each participant for HPLC parameters. The blood sample from all participants were collected in labelled 5ml EDTA anticoagulant bottle, using - Bio Rad (USA) for the determination of the Hb variants. The data obtained from the experiment were analyzed using one-way ANOVA followed by a student's t-test (post hoc).*

Results: *The mean values of high-performance liquid chromatography of sickle cell patients with frequent and less frequent group (HbSS) parameters were all statistically and significantly different (P<0.05) when compared with the control group (HbAA). The mean values of HbF in sickle cell with frequent vaso occlusive crisis was 4.94±3.8 while control was 2.67±0.40.*

Discussion: *There observed a statistically significant value of HbF (p<0.05) in the sickle cell group when compared with the control group AA. There was however no statistically significant difference but a slight decrease in HbF, when the sickle cells with frequent VOC and those with less frequent VOC crisis were compared. There existed a correlation between HbA2 and HbF in this study. When HbA2 value was low in sickle cell patient with less frequent VOC, there was corresponding increase in the value of HbF and when HbA2 was increased in Sickle cell Patients with Frequent VOC, the HbF was slightly low. The presence of HbA in the Sickle Anaemia patients in this study, though not expected might have been as a result of remnants of HbA from the recent transfusion. The present study found elevated levels of HbF and HbA2 among the SS patients when compared with the control group.*

Conclusion: *It was concluded from this study that, Fetal Heamoglobin (HbF) was slightly low in Sickle cell Anaemia patients with recurrent Vaso Occlusive Crisis compared with Sickle cell Anaemia patient with Less Frequent VOC.*

Keywords
High performance liquid chromatography, Vaso occlusive crisis, OAUTHC, Sickle Cell, Anaemia.

Introduction

Sickle cell disease is an inherited hemoglobinopathy stemming from a mutant variant of the β-globin gene (βA) on chromosome 11. This gene is responsible for the assembly of β-globin chains within the hemoglobin A protein. The mutated β-allele (βS) leads to the production of the variant hemoglobin, hemoglobin S. The heterozygous carrier state is referred to as sickle cell trait (SCT). The genetic mutation responsible for sickle cell disease involves a point mutation in the sixth codon of exon 1 in the βA gene, substituting adenine with thymine (guanine adenine-guanine guanine-thymine-guanine). The most prevalent and severe form of SCD, known as HbSS disease, arises from homozygosity for the sickle mutation [1].

Though the measurement of blood viscosity is seldom undertaken in Sickle Cell Anemia (SCA), its link to high-performance liquid chromatography (HPLC) parameters' characteristics in this disease is frequently disregarded [2].

Sickle cell anaemia has a worldwide prevalence, particularly in sub-Saharan Africa, with Nigeria bearing the heaviest burden. All over the World, nearly 50 million people are living with the condition. In Nigeria, an estimated 2% of the population is affected, with approximately 25% carrying the sickle cell trait [3]. This results in a substantial 150,000 life birth being affected yearly, with Nigeria accounting for a large proportion of global SCD births. Comprehensive investigations encompassing the Steady state, Vaso-occlusive state, and HPLC parameters of this disease are yet to be reported. Moreover, a dearth of data and lack of substantial research on HPLC parameters influencing Vaso-Occlusive crises (VOC) among sickle cell patients in the Ile-Ife region and its surroundings necessitate the undertaking of this study to contribute to the existing body knowledge on the subject [3].

The overall aim of this study is to determine HPLC parameters among Sickle cell anaemic subjects with Recurrent Vaso-occlusive crises in OAUTHC, Ile-Ife, Osun State and to know Whether HPLC parameters play a role, in the frequency of the Vaso occlusive crisis among sickle cell subjects.

The severity of sickle cell anemia varies significantly among individuals due to variations in pleiotropic genes. Carriers of the mutation may possess gene variants that either alleviate or exacerbate the associated phenotype [1].

When HbS is deprived of oxygen, the substitution of valine for glutamic acid in the sixth β-globin chain prompts a hydrophobic interaction with the neighboring β-globin chain within the hemoglobin molecule. Consequently, this interaction leads to aggregation and the formation of sizable polymers. This crystallization process generates a polymer nucleus, which grows within the erythrocyte, causing disruption of its architecture, flexibility, and inducing cellular dehydration. These structural changes transform the normal biconcave disc shape of red blood cells into the characteristic sickle or crescent shape [4].

The augmented rigidity of these cells, coupled with changes in their membrane properties, facilitates their adherence to the endothelium and other sickle cells, ultimately forming aggregates that can impede blood flow. This process is the driving force behind vaso-occlusive conditions. The resultant vaso-occlusion often results in tissue ischemia, causing injury and damage to organs and tissues with limited oxygen supply [5].

Sickle cell disease is a complex multisystem disorder originating from a single gene mutation. Virtually every organ within the body can be impacted. The condition is characterized by the presence of abnormal erythrocytes damaged by HbS. This abnormal variant of adult hemoglobin is inherited either from both parents (homozygosity for the HbS gene) or from one parent in conjunction with another hemoglobin variant, such as hemoglobin C (HbC), or with β-thalassemia (compound heterozygosity). In conditions of deoxygenation, HbS undergoes polymerization, leading to erythrocyte damage and loss of cations and water. Such compromised cells exhibit anomalies in the expression of adhesion molecules, contributing to hemolytic anemia and an elevated likelihood of small blood vessel blockages, which subsequently result in vaso-occlusion [6].

Vaso-occlusive Pain Crises (VPC) is defined as the occurrence of prolonged pain lasting two or more hours in regions such as the extremities, back, abdomen, chest, or head. This crisis is a prevalent and distressing complication experienced by adolescents and adults with sickle cell disease. Severe acute episodes of pain, referred to as crises, prompt patients to seek medical attention in hospital emergency departments. Regrettably, pain management often remains inadequate, as physicians sometimes hesitate to administer appropriate doses of narcotic analgesics due to concerns regarding addiction, tolerance, and potential side effects.

The vaso-occlusive crisis, also known as the sickle cell crisis, emerges from intricate interactions among sickle cells, endothelial cells, and plasma components. This crisis gives rise to a broad array of clinical complications associated with sickle cell disease, encompassing pain syndromes, stroke, leg ulcers, spontaneous abortion, and renal insufficiency. Central to the vaso-occlusive crisis is the obstruction and reduction of blood flow to vital organs by sickle red blood cells, leading to ischemia, necrosis, and pain. Recurrent episodes contribute to bone infarction and necrosis, alongside gradual degeneration of the bone marrow. Although long bones are most frequently affected, pain episodes can impact any bone marrow-containing structure, including the ribs, sternum, vertebral bodies, and skull.

Vaso-occlusion in sickle cell disease is a multifaceted process characterized by initiation, propagation, and resolution phases. Two primary factors that play a significant role in red blood cell entrapment during crises are diminished deformability of

sickled blood cells and adhesion between endothelial cells and erythrocytes. Episodes of VPC are prevalent and constitute a vital aspect of sickle cell disease. The bones are the typical sites of vaso-occlusion during pain crises. Various triggers for VPC include cold weather, elevated hemoglobin concentration, dehydration, infection, physical activity, damp conditions, inadequate diet, hypoxia, acidosis, emotional stress, and fatigue. The phases of VPC unfold as follows.

(a) Prodromal Phase: About 58% of patients report experiencing symptoms such as numbness, aches, and paresthesias in the extremities one day before the onset of pain. During this phase, there is an observed increase in the number of irreversibly sickled cells (ISCs) and dense cells, alongside reduced erythrocyte deformability compared to the individual's baseline levels.

(b) Initial Phase: Also referred to as the first, evolving, or infarctive phase, this stage is characterized by the emergence of pain accompanied by symptoms like fever, loss of appetite, and anxiety. Notable changes include a relative rise in dense cells, ISCs, and erythrocyte distribution width, coupled with a decrease in platelet count.

(c) Established Phase: This second, inflammatory, or post-infarctive phase typically lasts around four to five days in adults. Severe and persistent pain is a hallmark of this stage. Inflammatory indicators such as fever, leukocytosis, swelling, arthralgias, and joint effusions may be pronounced. Bone infarction usually occurs during this phase. Laboratory assessments often reveal elevated levels of C-reactive protein and lactate dehydrogenase, accompanied by reticulocytosis and decreased hemoglobin concentration compared to steady state values.

(d) Resolving Phase: Pain gradually subsides over one or two days in what is known as the last, healing, recovery, or post-crises phase. During this time, the number of dense cells, ISCs, and erythrocyte deformability returns to steady state levels.

Recurrent Crises: Within one week of the resolving phase, about 20% of individuals experience recurrent crises. This recurrence may result from a subsequent rebound increase in reticulocytes, viscosity, fibrinogen, platelets, and vascular cell adhesion molecule 1(VCAM-1).

Aplastic Crises: In cases of chronic hemolytic anemia, a temporary halt in erythropoiesis can lead to a severe form of anemia referred to as aplastic crises (Enrico and Mark, 2016).

Individuals with sickle cell anemia experience a considerably shorter lifespan for their red blood cells, around twenty (20) days, in contrast to the normal range of 90 to 120 days for healthy red blood cells. This reduced lifespan results from the premature deterioration of red blood cells, a significant consequence of the polymerization of hemoglobin S [7]. The heightened presence of hemoglobin S within red blood cells plays a pivotal role in the pathophysiological advancement of sickle cell anemia, unfolding through three distinct mechanisms. Firstly, in conditions of low oxygen pressure, red blood cells in sickle cell anemia patients lose their natural flexibility, giving rise to ischemia and arterial blockages, culminating in severe pain [8].

Secondly, membrane impairment accelerates the breakdown of red blood cells, leading to both intravascular and extravascular hemolysis. The former diminishes the availability of nitric oxide, promoting elevated vascular tension and pulmonary-artery hypertension. Subsequently, damaged red blood cells assume irregular surface characteristics, fostering heightened adhesion and inflicting harm upon the vascular endothelium [8].

On a global scale, approximately 5-7% of the population carries an abnormal hemoglobin gene defect, with sickle cell disease representing the most widespread form of hemoglobinopathy. The prevalence of sickle cell disease varies between 10 and 45% across diverse regions of sub-Saharan Africa, notably reaching about 2030% in Nigeria. Within Nigeria's population of over 160 million, sickle cell disease affects roughly 2%-3% [9].

Concurrent research conducted by Emechebe, Onyire, and Orji, Achigbu [10] similarly highlighted the presence of sickle cell traits among adult Nigerians at a rate of around 25%, while sickle cell disease manifested in 1-3% of cases. Findings from a study by Idowu, Olufemi, and Ade [11] in Lagos, Nigeria, revealed a population distribution of 2.4% among Yoruba indigenes residing in the city, while the research done by Kumar et al., 2024 stated that Nigeria data revealed a prevalence rate of 2 % for sickle cell anemia.

Materials and Methods

ILE-IFE is the study area, with latitudes 7°28'N and 7°46'N, and longitudes 4°36'E and 4°56'E. located in the core of south-western Nigeria. It is an ancient Yoruba city, an important West African emporium producing sophisticated art forms. The city is located in present-day Osun State, with a population of over 500,000 people. Its strategic location places it at the epicenter of the Yorubaspeaking states in Nigeria. Bordered to the west by Ibadan and to the east by Akure, the town serves as a pivotal gateway to the prominent Yoruba settlements in the eastern direction. Notably, Ile-Ife stands approximately 200 kilometers north-east of Lagos, which served as Nigeria's coastal capital city for more than a century [12].

Sample Size Calculations

Prevalence 2.0 % [13]

Case control study

N= Z2 x P (1-P)/d2 [14]

In the context of statistical analysis, the variables are defined as follows:

N represents the desired sample size, reflecting the number of observations or data points needed to achieve a reliable and representative outcome

Z denotes the statistical value associated with a chosen level of confidence. For instance, at a confidence level of 95%, Z is set at 1.96, signifying the standard deviation from the mean within

which the data points are likely to fall. P signifies the expected prevalence or proportion within the population being studied. In this instance, the anticipated prevalence is estimated to be 2.0%.

d stands for the level of significance, often denoted as α, which indicates the probability of making a Type I error. In this case, a significance level of 0.05 is employed, suggesting a 5% likelihood of incorrectly rejecting the null hypothesis.

$[(1.96)^2 x 2.0/100(1-(2.0/100)]/(0.05)^2$
$=3.8416 X 0.02 (1-0.02) / (0.0025)$
$=3.84 X 0.02(0.98) / 0.0025$
$=3.84 X 0.0196/0.0025$
$=0.075264/0.0025$
$=30$

Hence, size of the sample was adjusted to 100.

One hundred individuals aged 18-48, of both sexes (male and female) were considered, 50 of these were apparently healthy individuals with normal genotype AA, 50 sickle cell anaemia patients, were sub-grouped into: sickle cell anaemia individuals with less frequent VasoOcclusive crisis and sickle cell anaemia patients with recurrent Vaso-occlusive crisis who were attending OAUTHC's Haematology clinic. The crisis was confirmed from their medical records' history, vaso occlusive crisis was categorized based on classification of vaso occlusion frequency [15].

Ethical approval for this study was obtained from the Ethical Committee of Obafemi Awolowo University Teaching Hospitals Complex, Ile-Ife, Osun State. This ensures adherence of the research to ethical standards. A total of 5mls of blood sample was collected from each participant. The blood sample was put in EDTA bottle for HPLC parameters. The 4.0mls of EDTA blood was spun at 500g for 10minutes for plasma viscousity test.

Determination of Haemoglobin Genotype
Westergren Method as Adapted by [16]

Principle

Haemoglobin exhibits a negatively charged characteristic at alkaline pH. Consequently, during the electrophoretic process, haemoglobin migrates towards the anode (+). Distinct structural variants of haemoglobin that exhibit altered surface charge properties at alkaline pH undergo separation from haemoglobin A. It is worth noting that internally located haemoglobin variants may not experience separation, while those with amino acid substitutions that do not impact the overall charge will not be distinguishable through electrophoresis. This phenomenon allows for the differentiation and analysis of various haemoglobin variants based on their charge characteristics under alkaline conditions.

Procedure

(1) Start by confirming the homogenous lysis of the haemolysate and blood sample through centrifugation.
(2) Prior to connecting the power supply, introduce Tris-buffer into the buffer compartment within the tank. Immerse two chamber wicks in the buffer solution and position them along each divider or bridge support, ensuring proper contact with the buffer.
(3) Dip the cellulose acetate paper into the buffer reservoir, allowing it to soak for approximately 5 minutes prior to use.
(4) Load the sample well plate with 5ul of each diluted sample or control.
(5) Softly position the pre-soaked cellulose acetate paper across the bridge, ensuring optimal contact. Initiate electrophoresis at 220v for a duration of 15 minutes.
(6) After completion of the electrophoresis, promptly transfer the cellulose acetate paper to a solution of Ponceau S, allowing it to fix and stain for a period of 5 minutes.
(7) During staining, eliminate excess stain by immersing the paper in the first acetic acid solution for a 5-minute wash.

This requires a series of steps aimed at achieving efficient electrophoresis and staining of diluted samples or controls on cellulose acetate paper. Properly executed, these steps facilitate the separation and visualization of distinct components, aiding in the identification and analysis of various substances within the sample.

High Performance Liquid Chromatography (HPLC) - Bio Rad (USA) as adapted by Titilope, Oyesola, and Ajoke.

Principle

In this experimental setup, phosphate buffers with varying concentrations are employed as the mobile phase. These buffers are propelled under controlled pressure through an ionic exchange column, serving as the stationary phase. The column is integrated within a temperature-controlled analytical cartridge, housing a specialized resin composed of finely tuned anionic or cationic particles with diameters ranging from 3 to 5 micrometers.

The primary objective of this setup is to facilitate the separation of haemoglobin molecules based on their distinctive interactions with the stationary phase. As the phosphate buffers flow through the column, the haemoglobin molecules undergo specific interactions with the anionic or cationic particles present in the resin.

The separation process relies on the principle of ionic exchange, where haemoglobin molecules with differing charges interact differentially with the charged resin particles. This interaction leads to the distinct migration of haemoglobin species within the column. As a result, haemoglobin components are eluted from the column at varying rates, based on their affinity for the anionic or cationic sites on the resin.

The temperature regulation within the system guarantees optimal separation conditions, minimizing the influence of temperature fluctuations on the separation process. Consequently, the separation of haemoglobin species remains consistent and reproducible, enabling accurate analysis and identification.

Statistical Analysis

The data obtained from the experiment were analyzed using

one-way ANOVA followed by a student's t-test (post hoc). The socio-demographic information gathered from the participants was evaluated utilizing descriptive statistics to provide a detailed summary of the essential characteristics.

Descriptive statistics enabled the organization and presentation of socio-demographic data in a clear and concise manner. Parameters such as mean, median, mode, standard deviation, and range were computed to offer insights into the central tendency, dispersion, and distribution of the socio-demographic variables.

Through this approach, key demographic details such as age, gender, educational background, and occupation were summarized, offering a comprehensive overview of the study participants. Descriptive statistics allowed for a precise portrayal of the socio-demographic landscape, facilitating a better understanding of the sample characteristics, while the haemorheological and HPLC parameters were determined using student t-test. The haemorheological parameters and HPLC in both groups (HbSS and HbAA) were determined using Analysis of Variance (ANOVA)

Results

Table 1 showed the socio-demographic characteristics of the general population of the Sickle Cell Anaemic Patients and the control group. There is no significant difference in age, sex, ethnicity, marital status, religion, and occupation. The mean value of age for sickle cell patients is 30.03±5.73, while apparently healthy HbAA individual is 28.16±4.37 and p>0.05. Hence there is no statistical significant difference.

Table 2 showed the mean values of high-performance liquid chromatography of all sickle cell patients and control group. There is no correlation in HbS between the sickle cell group and the control group. All parameters were significantly different when compared with the control group (P<0.05).

Table 3 showed the mean values of high performance liquid chromatography of sickle cell patients with less frequent and control group (HbAA). All parameters were statistically significantly different when compared with the control group (P<0.05).

Table 4 showed the mean values of high performance liquid chromatography of sickle cell patients with frequent and control group (HbAA). All parameters except HbF were statistically significantly different when compared with the control group (P<0.05) The mean values of HbF in sickle cell with frequent vaso occlusive crisis is 4.94±3.8 while control is 2.67±0.40.

Table 2: Showed HPLC parameters of sickle cell patients (HbSS) control group (HbAA).

Parameters	SSG (n = 60)	Control AA (n=60)	T – Value	P Value
HbA_2 (%)	2.66±0.41	3.38±0.84	5.99	<0.05
HBF (%)	5.8±3.75	1.45±1.45	7.42	<0.05
HBS (%)	90.77±3.64		-1026.18	<0.05
HBA (%)	2.90±0.00	97.02±0.71	7.70	<0.05

KEY; p<0.05-Significant; HbA_2=Haemoglobin A_2, HbF= Fetal haemoglobin, HbS= Sickled haemoglobin, HbA= Adult haemoglobin, SSG=Sickle Cell General, HPLC= High performance liquid chromatography.

Table 1: Showed anthropological parameters of the sickle cell and the control. There were no significant differences between these parameters.

Parameters	SS Mean ± SD	AA Mean ± SD	T-TEST	p Val	Remark	
AGE	30.03±5.73	28.16±4.37	2.007	>0.05	Not sig.	
HEIGHT	1.49±0.12	1.50±0.12	-.684	>0.05	Not sig.	
WEIGHT	63.30±11.73	65.95±9.67	-1.350	>0.05	Not sig.	
BMI	29.02±7.21	29.7±6.65	-.534	>0.05	Not sig.	
	SS	**AA**	**Total**	**X^2**	**P val**	
Gender	Male	28(23.3)	29(24.2)	57(47.5)		
	Female	32(26.7)	31(25.8)	63(52.5)	0.033	
Ethnicity	Yoruba	43(35.8)	51(42.5)	94(78.3)		
	Ibo	10(8.3)	3(2.5)	13(10.3)		
	Hausa	7(5.8)	4(3.3)	11(9.2)		
	Others	-	2(1.7)	2(1.7)	7.269	.64
religion	Christian	4(3.3)	7(5.8)	11(9.2)		
	Islam	50(41.7)	49(40.8)	99(82.5)		
	Traditional	6(5)	2(1.7)	8(6.7)		
	Others	-	2(1.7)	2(1.7)		
marital	Single	24(20)	22(18.3)	46(38.3)	4.828	.19
status	Married	36(30)	38(31.7)	74(61.7)	0.141	.71
OCCUPATION	Civil Servant	3(2.5)	8(5.8)	10(8.3)		
	Trading	21(17.5)	15(12.5)	36(30)		
	Schooling	15(12.5)	14(11.7)	29(24.2)		
	Artisan	7(5.8)	15(12.5)	22(18.3)		
	others	14(11.7)	8(6.7)	22(18.3)	8.180	0.15

KEY; P<0.05=Significant; SS= Haemoglobin SS; AA= Haemoglobin AA.

Table 3: Showed HPLC parameters of sickle cell patients with less frequent VOC and control group (HbAA) in the study area.

Parameters	SS PT WITH LESS FREQ.VOC (N=52)	Control AA (n=60)	T – Value	P Value
HbA_2 (%)	1.14±0.33	3.38±0.84	-18.46	<0.05
HBF (%)	5.90±3.77	2.67±0.40	-6.16	<0.05
HBS (%)	90.63±3.61			<0.05
HBA (%)		97.02±0.71	1026.18	<0.05

KEY; P<0.05-Significant

Hb A_2=Haemoglobin A_2, HbF= Fetal haemoglobin, HbS= Sickled haemoglobin, HbA= Adult haemoglobin, SSG=Sickle Cell General, HPLC= High performance liquid chromatography VOC=Vaso Occlusive Crisis.

Table 4: Showed HPLC parameters of sickle cell anaemic patients with frequent VOC and control group (HbAA) in the study area.

Parameters	SS PT WITH FREQ.VOC (N=8)	Control AA (n=60)	T – Value	P Value
HbA_2 (%)	3.75±0.68	3.38±0.84	-10.33	<0.05
HbF (%)	4.94±3.8	2.67±0.40	-1.69	>0.05
HbS (%)	91.68±3.9			<0.05
HbA (%)		97.02±0.71		<0.05

KEY; P<0.05-Significant

Hb A_2=Haemoglobin A_2, HbF= Fetal haemoglobin, HbS= Sickled haemoglobin, HbA= Adult haemoglobin, SSG=Sickle Cell General, HPLC= High performance liquid chromatography VOC=Vaso Occlusive Crisis.

Table 5 showed the mean values of high performance liquid chromatography of sickle cell patients with less frequent vaso occlusive crisis and sickle cell patients with frequent vaso occlusive crisis. All parameters were not statistically significantly when compared (P>0.05).

Table 5: Showed HPLC parameters between sickle cell with less frequent VOC and sickle cell patients with frequent VOC.

Parameters	SS PT WITH LESS FREQ. VOC (N=52)	SS PATIENT WITH FREQ. VOC(N=8)	T – Test	P Val
HbA_2 (%)	1.14±0.33	3.75±0.68	-1.23	>0.05
HBF (%)	5.90±3.77	4.94±3.80	0.67	>0.05
HBS (%)	90.63±3.61	91.68±3.90	-0.71	>0.05
HBA (%)	2.90±0.00			>0.05

KEY; p<0.05-Significant

Hb A_2=Haemoglobin A_2, HbF= Fetal haemoglobin, HbS= Sickled haemoglobin, HbA= Adult haemoglobin, SSG=Sickle Cell General, HPLC= High performance liquid chromatography VOC=Vaso Occlusive Crisis.

Discussion

The aim of this study was to evaluate the High Performance Liquid Chromatography (HPLC) parameters among Sickle Cell Subjects with recurrent Vaso Occlusive crisis in OAUTHC, Ile Ife Osun State, Nigeria. Progress has been made in the last decades in the understanding of the pathophysiological mechanisms involved in Sickle cell anaemia patients but not much has been done on HPLC parameters in this environment especially in Ile-Ife.

The study employed 50 participants of Sickle Cell subjects for the test group and 50 Heamoglobin AA as control group, out of which 23(23.7%) and 27(26.7%) represented male and female in the study group respectively, while 24 (24.2%) and 26 (25.8%) represented male and female in the control group respectively. The age range were 31.02±4.63 and 27.25±3.72 for the test group and control groups respectively as in Table 1.

The study showed further in Table 1 that there were no statistically significant differences between the sickle cell anemia subjects and the control groups observed for clinical parameters such as Age, Height, Weight and BMI. Also, there were no statistically significant differences in all the socio-demographic data between the groups, this was in keeping with the research done by Chinawa et al., 2016 who submitted that there was no gender difference when anthropometrical variables which were were compared with haematological profiles of children with sickle cell anemia. Contrary reports were however documented by Zemel et al., who noted female preponderance in his study. However, Singhal et al., reported increase among the male counterpart. These variations of results could be due to differences in geographical and racial construct.

Table 2 showed the mean values of high-performance liquid chromatography of all sickle cell patients and control group. There was no correlation in HbS between the sickle cell group and the control group. All parameters were significantly different when compared with the control group (p<0.05). There observed a statistically significant value of HbF (p<0.05) in the sickle cell group when compared with the control group AA. There was however no statistically significant difference but a slight decrease in HbF, when the sickle cells with frequent VOC and those with less frequent VOC crisis were compared. The reason for the insignificance between these two groups might be that Frequent VOC group which is expected to have a low HbF may have been on treatment with Hydroxyurea (HU) which normally increases the HbF in Sickle cell. Also, the slight decline of HbF among the patients with frequent vaso occlusive crisis may be a cause and an indication for their recurrent episodes.

Table 3 showed the mean values of high performance liquid chromatography of sickle cell patients with less frequent vaso-occlusive crisis and control group (HbAA). All parameters were statistically significantly different when compared with the normal control group (p<0.05).

There existed a correlation between HbA2 and HbF in this study. When HbA2 value was low in sickle cell patient with less frequent VOC, there was corresponding increase in the value of HbF and when HbA2 was increased in Sickle cell Patients with Frequent VOC, the HbF was slightly low. The low level of HbF with consequential increased value of HbA2 might be an indicator to the recurrent VOC. The presence of HbA in the Sickle Anaemia patients in this study, though not expected might have been as a result of remnants of HbA from the recent transfusion. Table 4 showed the mean values of high-performance liquid chromatography of sickle

cell patients with frequent and normal control group (HbAA). All parameters except HbF were statistically significantly different when compared with the control group ($p<0.05$). The mean values of HbF in sickle cell with frequent vaso-occlusive crisis is 4.94±3.8 while control is 2.67±0.40.

Table 5 showed the mean values of high performance liquid chromatography of sickle cell patients with less frequent vaso occlusive crisis and sickle cell patients with frequent vaso occlusive crisis and here all parameters were not statistically significantly when compared ($p>0.05$).

The present study found elevated levels of HbF and HbA2 among the SS patients when compared with the control group. Low levels of HbF, HbA2 might be a pointer to recurrent vaso occlusive crises among the sickle cell anaemia patients, this is in keeping with the work of Chinawa et al., 2016 who stated that children with sickle cell anaemia have significantly lower haemoglobin concentration, which may be due to premature haemolysis and reduced red blood cell lifespan in these subjects. There is no doubt that these recent developments in association with collaborative efforts between scientists from different fields should allow further improvement of the clinical condition of Sickle cell patients.

Conclusion

It was concluded from this study that, Fetal Heamoglobin (HbF) was slightly low in Sickle cell Anaemia patients with recurrent Vaso Occlusive Crisis compared with Sickle cell Anaemia patient with Less Frequent VOC. Also the presence of HbA in sickle cell Anaemia with less frequent VOC group may be a result of previous blood transfusion. The Low Fetal Haemoglobin with High Heamoglobin A2 may be an indication of recurrent Vaso Occlusive crisis in Sickle Cell Anaemia Patients.

Recommendation

i. Periodic measurement of HbF is recommended to be able to predict the tendency of crisis,
ii. Sickle cell patients' needs to be educated on the importance of proper hydration which will reduce the viscosity and rheology of their blood. Hydration will reduce the process of Sickling by increasing Plasma volume and thereby decreasing blood Viscousity
iii. Prophylaxis against platelet aggregation or clot formation due to high platelet count and high blood viscousity is recommended.
iv. Further studies could be done by researchers on the possibility of genetic modification of pluripotent cell to continue to produce HbF in Sickle Cell Patients which will stall the transition of Heamoglobin (S) HbS to at least a less complicated percentage.

References

1. Wjdan AA, Hani AA, Mohammed Z, et al. Techniques for the Detection of Sickle Cell Disease: A Review. Micromachines (Basel). 2021; 12: 519.
2. Yann L, Xavier W, Samir BK, et al. Haemolysis and abnormal heamorheology in sickle cell anaemia. Br J Haematol. 2014; 165: 564-572.
3. Izulwa G, Akpoluzor JO, Okpokam DC, et al. Haemorheologic and Fibrinolytic activities of HbSS, HbAS, and HbAA subjects in Abuja. N J Med Sci. 2016; 16: 32-37.
4. Julia ZX, Swee LT. Revisiting anaemia in Sickle cell disease and finding the balance with therapeutic approaches. Blood. 2022; 139: 3030-3039.
5. Renaudier P. Sickle cell pathophysiology. Transfus Clin Biol. 2014; 21: 178-181.
6. Frederic PB, Martin SH, David CR, et al. New England Journal of Medicine. 2017; 376: 1561-1573.
7. Tamara R, Daryl N, Jamal B, et al. Advances in the management of sickle cell disease Journal of Pharmacy. 2020; 45: 8-12.
8. Prithu S, Mark TG, Erinco MN, et al. Pathophysiology of sickle cell disease. Annu Rev Pathol. 2019; 14: 263-292.
9. Ademola SA. Management of Sickle Cell Disease. A review for physician Education in Nigeria (sub-Sahara Africa). Anaemia. 2015; 791498.
10. Emechebe GO, Onyire NB, Orji ML, et al. Sickle Cell Disease in Nigeria, A Review. IOSR J Dental Med Scie. 2017; 16: 87-94.
11. Idowu AT, Olufemi AO, Ade OD, et al. Frequency of Sickle cell genotype among the Yorubas in Lagos: Implications for the level of awareness and genetic counselling for sickle cell disease in Nigeria. J community genet. 2011; 2: 13-18.
12. Joseph O, Raphael OO, Hubert Y, et al. Geospatial analysis of Urban sprawl in Ile-Ife, Nigeria. South African Journal of Geomatics. 2014; 3: 128-144.
13. Kumar Alok, Bhattacharya Sudip. Sickle cell disease: a comparative perspective on global and national initiatives. Frontiers in Hematology. 2024; 3: 1457158.
14. Jaykaran C, Tamoghana B. How to calculate sample size for different study designs in medical research. Indian J psychol med. 2013; 35: 121-126.
15. Deepika SD, Onyinye O, Mehdi N, et al. Markers of severe Vaso Occlusive Painful frequency in Children and Adolescents with Sickle cell anaemia. J Paediatr. 2012; 160: 286-290.
16. Ramesh K, Wilbert AD. Cellulose acetate Electrophoresis of Heamoglobin: Methods Mol Biol. 2019; 1855: 81-85.