

RESEARCH ARTICLE

Biochemical Response to Vasocclusive Crisis in Sickle Cell Anemia Patients in Enugu South East, Nigeria

Uchechukwu chukwudi Ngwu¹, Silas Anayo Ufelle,^{1,2} Alphonsus Ogbonna Ogbuabor^{3*}

¹Department of Hematology and Blood Transfusion Science, Medical Laboratory Services, Enugu State University of Science and Technology Teaching Hospital Parklane, Enugu State, Nigeria.

²Department of Medical Laboratory Science, Faculty of Health Science and Technology, University of Nigeria, Enugu Campus, Nigeria.

³Department of Medical Laboratory Science, Faculty of Basic Medical Sciences, Enugu State University of Science and Technology, Enugu State, Nigeria.

Corresponding Author: Alphonsus Ogbonna Ogbuabor, Department of Medical Laboratory Science, Faculty of Basic Medical Sciences, Enugu State University of Science and Technology, Enugu State, Nigeria. Tel.: 07032753784, E-mail: Ogbuaborao@yahoo.com

Citation: Uchechukwu chukwudi Ngwu, Silas Anayo Ufelle, Alphonsus Ogbonna Ogbuabor (2023) Biochemical Response to Vasocclusive Crisis in Sickle Cell Anemia Patients in Enugu South East, Nigeria. J Blood Disord Ther 3: 101

Abstract

Biochemical response to vasocclusive crisis were investigated among Sickle Cell Anemia (SCA) patients in Enugu metropolis, Southeast Nigeria. A total of 156 subjects comprising 75 confirmed SCA patients (35 males and 40 females) aged between 16 and 30 years from the Sickle Cell Clinic and 75 apparently healthy age and gender – matched controls participated in the study. Sample size was calculated using simple proportion method. Ethical clearance was obtained from the Health Research and Ethical Committee of the Enugu State University of Science and Technology Teaching Hospital, Enugu State, Nigeria. Informed consent was obtained from subjects. Blood sample (5ml) was collected from each subject, centrifuged, separated and aliquoted into plain bottles for the determination of Alanine Transaminase, Aspartate transaminase, Urea and Creatinine levels using standard methods. Results were analyzed by Statistical Package for Social Sciences using One Way Analysis of Variance at $p < 0.05$ significant level and presented as mean and standard deviations from the mean. Subjects revealed significant increase ($p < 0.05$) in Alanine Transaminase (ALT) ($20.70 \pm 2.24 \mu\text{l/l}$), Aspartate Transaminase (AST) ($25.30 \pm 5.25 \mu\text{l/l}$), Urea ($8.2 \pm 0.6 \mu\text{mol/l}$), Creatinine ($175 \pm 15.3 \mu\text{mol/l}$) during crisis and steady state (ALT) ($12 \pm 6.5 \mu\text{l/l}$) (AST) (15 ± 8.3) Urea (5.0 ± 1.5) Creatinine ($150 \pm 1.5 \mu\text{mol/l}$) compared to controls ALT ($10 \pm 4.5 \mu\text{l/l}$), AST ($12 \pm 3.0 \mu\text{l/l}$), Urea ($3.2 \pm 1.5 \mu\text{mol/l}$) and Creatinine ($95 \pm 10 \mu\text{mol/l}$). This finding provide scientific data for biochemical alteration in sickle cell anemia during vasocclusive crisis.

Keywords: Biochemical Parameters; Sickle Cell Anemia; Vasocclusive Crisis; Enugu

Introduction

Sickle Cell Disease (SCD) is the most common genetic blood disease globally.¹ Africa is greatly affected with Sub-Saharan Africa having the highest burden of the disease. It is currently estimated that 75% of all patients of Sub-Saharan African descent and this amount is estimated to increase to 85% by the year 2050. Nigeria particularly has the highest burden of the disease for the region with prevalence that ranges from 2% to 3% [2-4]. A prevalence of 1.6% has been reported for the Enugu population [5]. The high rate of SCD in Enugu is exacerbated by lack of access to comprehensive healthcare, high cost of diagnosis as well as inadequate measures to manage vasoocclusive crisis arising from the condition [6]. Sickle Cell Anemia (SCA) is a group of conditions resulting from the inheritance of abnormal allelomorphs controlling the formation of the beta (β)-chain of hemoglobin (Hb) at least one of which is the sickle gene (HbS) [3]. The common forms of the disease are HbSC, HbSD and HbS β -thalassemia [7]. The homozygous state (HbSS) termed Sickle Cell Anemia (SCA) is the most common and severest form of the disease which is caused by a point mutation (GAG to GTG) in the sixth codon of the amino acid sequence of the beta globin chain (H β) [3,7]. This anomaly results in the replacement of the amino acid glutamic acid for valine with eventual formation of hemoglobin S (HbS) instead of hemoglobin A (HbA) which causes the polymerization of the hemoglobin molecule leading to erythrocytes with less flexible sickle shape different from the normal biconcave disc shape of erythrocytes with normal hemoglobin (HbAA). Patients with sickle cell anemia experience alternating periods of apparent good health (steady state) and acute vasoocclusion (crisis state) which is triggered by conditions such as infection, dehydration and hypoxia [3,9,10]. Vasoocclusion is a direct cause of morbidity and mortality in patients with sickle cell anemia [11]. It is the cause of a myriad of clinical manifestations and complications in the SCA patients which includes leg ulcers, priapism, fatigue, dizziness, osteonecrosis, retarded growth, hepatosplenomegaly, delayed maturity to puberty, dactylitis and end stage organ damage such as the liver and kidney amounting to at least 91% of hospital admissions [12].

Disturbances in metabolism of biochemical variables resulting in organ damage particularly to the kidney and the liver has identified as a common complication in SCA during vasoocclusive crisis¹³. There is currently a paucity of data on the biochemical response to vasoocclusive crisis in SCA patients for the Enugu population. The present study is therefore designed to determine some liver and renal function parameters of SCA patients in vasoocclusive crisis compared to steady state and controls.

Materials and Methods

Study Area

Enugu State is located in the Southeastern part of Nigeria. The state derived its name from its capital and largest city Enugu. It has an area of 7,161 km² with a population of 3,267,837 comprising mainly the Igbo tribe of the Southern Nigeria. It lies between longitudes 6°30'E and 6°55'E and latitudes 5°15'N and 7°15'N. It consists of three senatorial zones namely Enugu East, Enugu West and Enugu North senatorial zones. The Enugu State University of Science and Technology Teaching Hospital is the major tertiary health facility for the state and is located at the center of Enugu metropolis (Parklane) for easy accessibility to Enugu residents [14].

Study Design

The study adopted the survey design. A total of 150 subjects comprising 75 confirmed Sickle Cell Anemia patients (35 males and 40 females) aged between 15 and 30 years from the Sickle Cell Clinic of the Enugu State University of Science and Technology Hospital, Enugu State, Nigeria and 75 apparently healthy age and gender-matched controls participated in the study.

Ethical Consideration

The ethical clearance to conduct this study was obtained from the Ethics Committee of the Enugu State University of Science and Technology Teaching Hospital (ESUTH), Enugu, with reference number: ESUTHP/C-MAC/RA/034/Vol.4/37. Informed consent was obtained from all participants before being recruited for the study.

Sample Size

The sample size calculated using single proportion method.

$$n = \frac{Z^2 (p) (1 - p)}{d^2}$$

Where

n = the desired sample size when the population is more than 10,000

z = standard variation usually set at 1.96 (which corresponds to 95% confidence interval)

p = population proportion of 10% which is 0.1

$$n = \frac{1.86^2 (0.1) (1 - 0.1)}{0.05^2} = \frac{3.861 \times 0.1 \times 0.9}{0.0025} = \frac{0.345744}{0.0025}$$

$$n = 138.3$$

Subjects Inclusion Criteria

Sickle cell anemia patients 16years of age and older in a period of stable clinical condition occurring at least one week before or three weeks after or vasocclusive crisis or three months after a hemolytic crisis requiring a blood transfusion served as the subjects for steady-state, patents in active occlusive pain served as the crisis group while healthy individuals with HbAA genotype who are 16years of age and older served as the control.

Subjects Exclusion Criteria

Individuals who are taking any drug as well as those who smoke or drink too much alcohol (14 units per week for females and 21 units per week for males) were excluded from the study (control exclusion criteria). Sickle cell anemia patients with any additional medical conditions such as hypertension or diabetes mellitus, those who smoke or drink excessively (14 units per week for females and 21 units per week for males) or those who have had a blood transfusion within the last three months were excluded from the study.

Sample Collection

Venous blood sample (10ml) was collected from each subject,5ml was dispensed into ethylene diamine tetra acetic acid bottle for determination of subjects' hemoglobin genotype while the remaining 5ml was centrifuged and aliquoted into plain bottles for the estimation of biochemical parameters.

Haemoglobin Electrophoresis (Cellulose Acetate Method)

Principle: Hemoglobin a negatively charged protein migrates to the mode when exposed to an electric field in an alkaline medium which distinguished it from other heme proteins, the rate of migration being directly proportional to the net change in the molecule with different hemoglobin types observed as bands of different hemoglobin variants involving HbA,F,S,C, and E.15

Aspartate Transaminase Estimation

The serum level of aspartate aminotransferase were measured using Randox Commercial Enzyme kits according to Reitman and Frankel's method [16].

Principle: Aspartate aminotransferase (AST/SGOT) catalyses the transfer of the amino group from aspartate to oxoglutarate with the formation of glutamate and oxaloacetate. The latter is reduces to malate by Malate Dehydrogenase (MAD) in the presence of reduced Nicotinamide adenine dinucleotide (NADH). The reaction is monitored kinetically at 340nm by the rate of decrease in absorbance resulting from the oxidation of the reduced Nicotimmide Adenine Dinuclotide (NADH) to oxidized form (NAD⁺), proportional to the activity of AST present in the sample.

Alanine Transaminase Estimation

The serum level of alanine transaminase were measured using Ramdox Commercial Enzyme kits according to Reitman and Frankel's method [16].

Principle: Alanine aminotransferase (ALT/SGPT) reversibly transfers the amino group from alanine to alpha-ketoglutarate, forming pyruvate and glutarmate. The rate of formation of pyruvate is determined by the action of lactate dehydrogenase (LDH) which converts the pyruvate to lactate; the decrease in absorbance at 340nm is measured as reduced nicotinamide adenine dinucleotide (NADH) is oxidized to NAD⁺.

Urea Estimation

The serum urea was estimated spectrophotometrically using Diacetyl Monxime method [17].

Principle: proteins in whole blood, plasma or serum are precipitated with trichloroacetic acid. The urea in the supernatant reacts with diacetylmonoxime in the presence of thiosemicarbazide and cadmiurn ions under acid conditions. The absorbance of the red rose-purple solution is measured at 530nm.

Creatinine Estimation

The serum creatinine was estimated spectrophotometrically by Jaffes Resaction method [17].

Principle: In an alkaline picrate solution, creatinine reacts with picric acid to give a red color (Jaffes reaction) which absorbed at 510nm.

Statistical Analysis

Data was subjected to inferential statistics in the statistical package for social sciences version 2.0 (IBM, Armok, USA) using one-way analysis of variance at 95% confidence interval. Probability value less than 0.05 was considered significant.

Results

Results revealed significant increase ($p < 0.05$) in Alanine Transaminase (ALT) (20.70 ± 2.24 iu/l) Aspartate Transaminase (AST) (25.30 ± 5.25 iu/l) Urea ($18.20.6 \mu\text{mol/l}$) and Creatinine ($175 \pm 15.3 \mu\text{mol/l}$) during crisis and steady state (ALT) (16.0 ± 1.5 iu/l), (AST) (15 ± 8.3 iu/l), Urea ($15.0 \pm 1.5 \mu\text{mol/l}$) and Creatinine ($150 \pm 10.5 \mu\text{mol/l}$) compared to controls ALT (10 ± 4.5 iu/l), AST ($12. \pm 3.0$ iu/l), urea ($3.2 \pm 1.5 \mu\text{mol/l}$) and creatinine ($95 \pm 10 \mu\text{mol/l}$).

Parameter	Crisis State	Steady State	Controls
ALT (iu/l)	20.702.24	166.5	104.5*
AST (iu/l)	25.305.25	158.3	123.0*
Urea ($\mu\text{mol/l}$)	18.20.6	15.01.5	8.21.5*
Creatinine ($\mu\text{mol/l}$)	175115.3	15010.5	9510*

Key: ALT-alanine transaminase, AST-aspartate transaminase,*Significant at $p < 0.05$

Discussion

Biochemical alterations has been associated with sickle cell anemia [18]. The results of the present study shows that patients had higher values for urea and creatinine during vasocclusive crisis compared to steady state and controls. This is similar to the findings of some other studies which had reported high levels of urea and creatinine in sickle cell disease patients during vasocclusive crisis [19]. The underlying renal dysfunction leading to increased urea and creatinine in sickle cell disease may include hyposthenuria, hematuria, nephrotic syndrome, acidosis, renal failure and changes in arterial blood pressure [18].

Significant increase in the serum transaminases recorded for the subjects in vasocclusive crisis is similar to the findings of other studies which had reported increased serum transaminase levels in sickle cell patients during vasocclusive crisis [16, 18, 20]. The causes of raised transaminases in SCA may include the acute syndromes associated with the disease such as acute sickle hepatic crisis, acute hepatic sequestration crisis, sickle cell intrahepatic cholestasis resulting from the occlusion of blood vessels by sickled intrahepatic cells.²¹ Raised transaminases may also result from the complications of chronic hemolysis and multiple blood transfusions such as hyperhemolytic syndrome, viral hepatitis, gallstone etc. [21-22]. The small sample size as well as the use of single centre for the present study could be considered a limitation. Further large-scale surveys are needed to support the present findings.

Conclusion

The finding of the present study provides scientific data that supports alterations in biochemical parameters during occlusive crisis in sickle cell anemia and underscores the diagnostic and prognostic importance of routine measurement of biochemical parameters for patient management.

References

1. Siransy LK, Dasse RS, Adou H, Koualou P, Kouamenan S et.al (2022) Are IL-1 family cytokines important in management of sickle cell disease in sub-sharan Africa patients. *Frontiers in Immunology* 14: 1-10
2. Fome AD, Sangeda RZ, Balandya E, Mgaya J, Soka D et.al (2022) hematological and biochemical reference ranges for the population with sickle cell disease at steady state in Tanzania. *Hemato* 3: 82-92.
3. Abubakar Y, Ahamd HF, Faruk JA (2019) hematological parameter of children with sickle cell anemia in steady and crisis state in Zaria. *Annale of Tropical Pathology* 10: 122-125.
4. Chukwu BF, Ezenwosu OU, Eke CB, Chinawa JM, Ikefuna AN et.al (2014) What factors influence the age at diagnosis of sickle cell anemia in Enugu Nigeria. *Journal of Blood Disorders and Transfusion* 5: 1-4
5. Achigbu KI, Odetunde OI, Chinawa JM, Achigbu EO, Ikefuna AN et.al (2015) Pulmonary function indices in children with sickle cell anemia in Enugu, South East, Nigeria. *Saudi Medical Journal* 36: 928-934
6. Adigwe PO, Onoja SO, Onavbavba G (2023) A critical review of sickle cell disease burden and challenges in Sub-Saharan Africa. *Journal of Blood Medicine* 14: 367-76
7. Luliano PMM, Albuquergue XMCC (2023) Interleukin-6 gene polymorphism influencing in hematological indices from sickle cell anemia patients. *Brazilian Journal of Development* 9: 6581-94
8. Alaka AA, alaka OO, Lyanda (2023) Nitric oxide and zinc levels in sickle cell hemoglobinopathics: a relationship with the markers of disease severity. *Pomeranian Journal of Life Science* 69: 6-12
9. Cardoso, EC, Silva-Neto PV, Hounkpe BW, Chenou F, Akbuquergue XMCC et.al (2023) Changes in heme levels during acute vaso-occlusive crisis in sickle cell anemia. *Hematology/onocolgy and Stem Cell therapy* 16: 1-11
10. Sesti-Costa R, Costa FF, Conran N (2022) Role of macrophages in sickle cell disease erythrophagocytosis and erythropoiesis. *International Journal of Molecular sciences* 24: 1-15
11. Lagos MD, Okoh JB, Pilot UY, Vioamdem NY, Ofojekwu MJN et.al (2018) Some hematologic parameters of blood donors at the National Blood Transfusion Service (NBTS), Jos, Nigeria. *Journal of Blood Disorders and Transfusion* 10: 1-5
12. Nwabuko CO, Onwuchekwa U, Iheji O (2022) An overview of sickle cell disease from the socio-demographic triangle – a Nigerian single institution retrospective study. *Pan African Medical Journal* 42(161):1-14
13. Nanitelamio LPE, Mokono SO, Mbani CJ, Atipo-Tsiba OG, Niama FR (2021) Hematological and biochemical profile of sickle cell patients in critical and inter-critical periods in Brazzaville, republic of Congo. *Open Journal of Blood Diseases* 11: 57-65
14. Ogbuabor AO, Arji NG, Ngwu CU (2022) Knowledge and determinants of hepatitis β virus testing and vaccination status among sickle cell disease patients. *International Journal of Pathgen research* 11: 1-6
15. Siransy LK, Yapo-Crazoit CCA, Diane MK, Goore S, Kabore S (2018) Th1 and Th1 cytokines pathern among sickle cell disease patients in coted'ivoire. *Clinical Immunology and Research* 2: 1-4
16. Uche CL, Isaiah AO, Ezirim EO, Airaodion AI (2022) Sickle cell anemia contributes to liver abnormality. *International Journal*

of Research and Reports in Hematology 5: 122-34

17. Ikaraoha IC, Ogwurike CO (2022) Variations in serum levels of uric acid urea, creatinine, potassium and sodium in exposed male paint workers in Owerri, Nigeria. *Asian Journal of Research in nephrology* 5: 1-8
18. Olaniyan MF (2016) Investigation of some biochemical parameters in subject with HbSC and HbSS in Saki West Local Government – a Rural Community in Nigeria. *American Journal of Biochemical Sciences* 8: 237-46
19. Airhomwanbor KO, When IC, Okparaku BO, Dic-Ijewere EO, Ehimare RI (2018) Renal function of sickle cell subjects in Edo State - Nigeria. *Archives of Nephrology and Urology* 1: 001-8
20. Akaba K, Enang O, Bassey BO, Babatope O, Riman O (2019) Biochemical assessment of the liver in SCD in a tertiary hospital in South-South, Nigeria. *Journal of Advances in Medicine and Medical research* 29: 1-6
21. Odeghe EA, Lesi OA, Otegbayo JE (2016) Prevalence of raised transaminase and associated risk factors in stable sicklers in Lagos, Nigeria. *Nigeria Journal of Gastroenterology and Hepatology* 8: 15-24
22. Ogbuabor AO, Achulwu PU, Ufelle SA, Ogbuabor DC (2020) Hyperhemolytic syndrome due to hemoglobinopathy in Sub-Saharan Africa. *Journal of Applied hematology and Blood Transfusion* 2: 104