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Cytokine Response to Vasoclusive Crisis in Sickle Cell Anemia Patients in Enugu, South East, Nigeria

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Abstract

Cytokine response to vasoclusive crisis were investigated among Sickle Cell Anemia (SCA) patients in Enugu metropolis, South East Nigeria. A total of 150 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 (5.0ml) was collected from each subject, centrifuged, separated and aliquoted into plain bottles for determination of pro-inflammatory, anti-inflammatory and hemopoietic cytokines by Enzyme linked Immunosorbent Assay. 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. Anti-inflammatory cytokines revealed significant decrease ($p < 0.05$) in IL-1 during crisis (5.6+0.82 pg/ml) and during steady state (6.2+0.44pg/ml) and in IL-10 during crisis (3.92+0.78pg/ml) and during steady-state (4.75+0.39pg/ml) compared to controls IL-1 (3.5+0.53pg/ml) and IL-10 (8.5+0.45pg/ml). Pro-inflammatory cytokines revealed significant increase ($p < 0.05$) in TNF-α during crisis (15.17+0.91pg/ml) and during steady-state (11.63+0.45pg/ml), in IL-3 during crisis (20.92+8.92pg/ml) and during steady-state (15.70+3.61pg/ml) while haemopoietic cytokines revealed non-significant increase in ($p > 0.05$) in EPO during crisis (15.32+6.85iμ/l) and during stead-state (13.05+3.60iμ/l) and in lL-6 during crisis (13.2+0.5pg/ml) and during steady-state (11.87+ 0.91pg/ml) compared to control (10.63+0.45pg/ml). This finding provides scientific data for cytokine derangement in sickle cell anemia, Enugu.

Keywords: Cytokines; Sickle cell anemia; Crisis state; Steady state; Enugu

Abbreviations: SCD: Sickle Cell Disease; SCA: Sickle Cell Anemia.

Introduction

Sickle Cell Disease (SCD) is the most common genetic blood disease globally [1]. Sub-Saharan African has the highest burden of the disease where currently 75% of all patients resides and is estimated to increase to 85% by the year 2050 with Nigeria particularly having the highest burden for the region [2,3]. It is a group of conditions resulting from the inheritance of abnormal allelomorphic genes 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 HbSβ- thalassemia [4]. 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 (Hβ) [3,5]. 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 which are different from the normal biconcave disc shape of erythrocytes with normal hemoglobin (HbAA). Patients with sickle cell anemia experiences alternating periods of apparent good health (steady state) and acute vasoclusion (crisis state) which are triggered by conditions such as infection, dehydration and hypoxia [3,6,7] Vasoclusion is a direct cause of morbidity and mortality in patients with sickle cell anemia [8].

Cytokines are a general term for a large family of secreted proteins involved in cell-to-cell signaling during immune response [9]. Alternations in cytokines has been linked to various mechanisms leading to vasoclusion in sickle cell disease [8,10]. There is currently a paucity of data on the cytokine response to vasoclusive crisis in sickle cell anemia patients for the Enugu population. The present study is therefore designed to determine the cytokine profile of sickle cell anemia patients in vasoclusive crisis compared to steady state and healthy controls.

Materials and Methods

Study Setting

Enugu State is located in the south eastern part of Nigeria. The State derived its name from its capital and largest city Enugu. It has an area of 7,161km^2 with a population of 3,267,837 comprising mainly the Igbo tribe of the southern Nigeria. It lies between longitudes 6o30'E and 6o55'E and latitude 5o15'N and 7o15'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 (ESUT) 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 residents [11].

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 clearance was obtained from the Hospital Ethics Committee. Informed Consent was obtained from subjects prior to the study.

Sample Size

The sample size was calculated using the 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 internal).

p = population proportion of 10% which is 0.1

$$n = \frac{1.86^2 (0.1)\ (1-0.1)}{0.05^2} = \frac{3.861\ x\ 0.1\ x\ 0.9}{0.0025} = \frac{0.345744}{0.0025}$$
$$= 138.3$$

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 vasoclusive 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 the determination of subjects hemoglobin genotype while the remaining 5ml was centrifuged and aliquoted into plain bottles for cytokine estimation.

Haemoglobin Electrophoresis (Cellulose Acetate Method)

Principle: Hemoglobin, a negatively charged protein migrates to the anode when exposed to an electric field in an alkaline medium which distinguishes it from other hemo proteins. The rate of migration being directly proportional to the net charge in the molecule with different hemoglobin types observed as bands of different hemoglobin variants such as HbA, F, S, C, D and E [12].

Procedure: A hemolysate was prepared from whole blood by mixing with water. The hemolysate is spotted to a cellulose acetate paper as bands using an applicator. The cellulose acetate paper was transferred into an electrophoretic chamber containing citrate buffer at PH 8.4.The electrophonetic tank was set at 220 volts and allowed for 15 minutes for band separation.

Cytokine Estimation (Elisa Method)

The various cytokines were estimated using Enzyme Linked Immunosorbent Assay kits purchased from Sulong Diagnostic Company Ltd, China.

Principle: The enzyme linked immunosorbent assay is an antibody-based assay designed to detect analytes by assessing the conjugated enzyme activity through incubation with a substrate to produce a measurable product [13].

Procedure: Standard solution and sample (100ml each) were added to each well and the blanks left empty and incubated for 90 minutes at 37^{0}C. The solution was removed; 100ml biotinylated detection antibody specific to the analyte was added to it and incubated for 60 minutes as 37^{o}C. The solution was then aspirated, the wells was washed thrice and 100ml horseradish peroxidase conjugate was added and the mixture left for 30 minutes at 37^{o}C, after which the solution was aspirated and washed five times. Substrate reagent (90ml) was added and the mixture incubated for 15 minutes at 37^{o}C, and finally 100µl of stop solution was added. The optical density (OD) of the blank well was set at zero. The absorbance OD of each well was read at 450nm using a microplate reader. Optical density values were proportional to the concentration of measured analyte.

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

Hematopoietic cytokines of SCA revealed non-significant increase ($p > 0.05$) in erythropoietin (EPO) during crisis (15.32+6.85iµ/l) and in interleukin-6 (IL-6) during crisis (13.2+0.5pg/ml) and during steady state (11.87+0.91pg/ml) compared to controls EPO (12.85+3.60iµ/l) and IL-6 (10.63+0.45pg/ml). The anti-inflammatory cytokines revealed significant decrease ($p < 0.05$) in IL-1 during crisis (5.6+0.82pg/ml) and during steady state (4.75+0.39pg/ml) compared to controls IL-1 (3.5+0.53pg/ml) and IL-10 (8.5+0.45pg/ml). Pro-inflammatory cytokines revealed significant increase ($p < 0.05$) in tumor necrosis factor-alpha (TNF-α) during crisis (15.17+0.91pg/ml) and during steady state (11.63+0.45pg/ml) in IL-3 during crisis (20.92+8.92pg/ml) and during steady state (15.70+3.61pg/ml).

Parameter	Crisis State	Steady State	Control
EPO (iµ/l)	15.32+6.85	13.05+3.60	12.85+3.60
IL-3 (pg/ml)	20.92+6.92	15.70+3.61	10.2+0.4*

Key: EPO – erythropoietin, IL-3 – Interleukin-3,*Significant at <0.05

Table 1: Hematopoietic Cytokine Profile of SCA during Crisis and Steady State.

Parameter	Crisis State	Steady State	Control
TNF-α (PG/Ml)	15.17+0.91	11.63+0.45	7.5+0.6*
IL-6 (pg/ml)	13.2+0.5	11.87+0.91	10.63+0.45

Key: TNF-α – Tumor necrosis factor-alpha, Interleukin-6,*Significant at p<0.05.

Table 2: Pro-inflammatory Cytokine Profile of SCA during Crisis and Steady State.

Parameter	Crisis State	Steady State	Control
IL-1(pg/ml)	5.6+0.82	6.2+0.44	3.5+0.53
IL-10 (pg/ml)	3.92+0.78	4.75+0.39	8.5+0.45*

Key: TNF-α – Tumor necrosis factor-alpha, Interleukin-6,*Significant at p<0.05

Table 3: Anti-inflammatory Cytokine Profile of SCA during Crisis and Steady State.

Discussion

Many researchers have reported cytokine alterations in sickle cell disease [10]. In the present study, we recorded

significant increase in the levels of the pro-inflammatory cytokines IL-6 and TNF-α in sickle cell anemia patients during vasoclusive crisis. This is similar to other studies which reported significant increase in IL-6 during vasoclusive crisis [10,14,15]. The reported increase in TNF-α is also similar to other studies who had reported significant increase in TNF-α during vasoclusive crisis in sickle cell patients [16]. Cytokines have been linked to several possible mechanisms of vasoclusion in sickle cell anemia such as vascular endothelial activation, red cell adhesion to vascular endothelium, neutrophil adhesion to vascular endotheillium, development of vascular intimal hyperplasia, platelet activation, endothelin-1 synthesis and endothelial apoptosis dysregulation [8]. 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 findings of the present study provide scientific data for cytokine alterations during vasoclusive crisis in sickle cell anemia which underscores the diagnostic and prognostic importance of cytokine profile for the management of patients.

References

1. Siransy Lk, Dasse RS, Adou H, Koualou P, Kouamenan S, et al. (2023) Are IL-1 family cytokines important in management of sickle cell disease in Sub-Saharan African patients? Frontiers in Immunology 14: 95405.

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(1): 82-97.

3. Abubakar Y, Ahmad HR, Faruk JA (2019) Hematological parameters of children with sickle cell anemia in steady and crisis state in Zaria. Annals of Tropical Pathology 10(2): 122-125.

4. Luciano MMP, Xerez Albuquergue CCM (2023) Interleukin-6 gene polymorphisms influencing in hematological indices from sickle cell anemia patients. Brazillian Journal of Development 9(2): 6581-6594.

5. Alaka AA, Alaka OO, Lyanda AA (2023) Nitric oxide and zinc levels in sickle cell hemoglobinopathies: a relationship with the markers of disease severity. Pomeranian Journal of Life Science 69(1): 6-12.

6. Cardoso EC, Silva-Neto PV, Hounkpe BW, Chenou F, Albuquerque XMCC, et al. (2023) changes in heme levels during acute vaso-oclusiva crisis in sickle cell anemia. Hematology/Oncology and Stem cell Therapy 16(2): 124-132.

7. Sesti-Costa R, Costa FF, Conran N (2023) Role of macrophages in sickle cell disease erythrophafocytosis and erythropoiesis. International Journal of Molecular Sciences 24(7): 6333.

8. Lugos MD, Okoh JB, Pilot UY, Vwamdem 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): 1-5.

9. Ogbuabor AO, Arji NG, Ngwu CU (2022) Knowledge and determinants of hepatitis B virus testing and vaccinaton status among sickle cell disease patients. International Journal of Pathogen Research 11(2): 1-6.

10. Arishi WA, Alhadrami HA, Zourob M (2021) Techniques for the detection of sickle cell disease; a review. Micromachine 12(5): 519.

11. Ogbuabor AO, Chineke AJ, Ufelle SA (2022) Significance of some hematological parameters in type 2 diabetic patients in the Enugu State University of Science and Technology Teaching Hospital, Enugu State, Nigeria. International Journal of Research and Reports in Hematology 5(2): 237-242.

12. Abbas M (2014) Haematological parameters in Sudanese children with sickle cell disease. American Journal of Research communication 2(2): 20-32.

13. Mustafa AEM, Abdlgadir O, Elsheikh NMHN (2018) A clinical and hematological study on the sickle cell anemia among children in EY Obeid Hospital, Sudan. International Journal of Medical Research and Health Sciences 7(11): 66-71.

14. Manitelamio LCPC, Mokono OS, Mbani JC, Atipo-Tsiba OG, Niama FR, et al. (2021) Haematimetric parameters in sickle cell patients in a critical and interietal period at the National Sickle Cell Reference Center in Brazzaville, Republic fo the Congo. International Journal of Science and Research 10(4): 891-893.

15. Obeagu EI, Obeagu GU (2023) Evaluation of hematological parameters of sickle cell anemia patients with osteomyelitis in a tertiary hospital in Enugu, Nigeria. Journal of Clinical and Laboratory Research 6(1): 1-3.