ISSN: 2536-7153 SJMLS

Sokoto Journal of Medical Laboratory Science 2024; 9(1): 32 - 39

SJMLS - 9(1) - 003

Evaluation of Some Proinflammatory Cytokine levels in Haemoglobin-S Variants Subjects in Port Harcourt Nigeria.

Ransom Baribefii Jacob[1*], Stella Urekweru Ken-Ezihuo[1], Ebirien-Agana Samuel Bartimaeus[2] and Evelyn Mgbeoma Eze[1]

Faculty of Medical Laboratory Science, Department of Haematology and Transfusion Science, Rivers State University, Port Harcourt, Nigeria [1], Faculty of Medical Laboratory Science, Department of Clinical Chemistry and Molecular Diagnostic, Rivers State University, Port Harcourt, Nigeria [2].

Author for Correspondence: ransom.jacob@ust.edu.ng/08069356274.* DOI: 10.4314/sokjmls.v9i1.3

Abstract

Proinflammatory cytokines as signaling molecules play key roles in vaso-occlusive crisis, inflammation and atherosclerosis and have been implicated in sickle cell disease and anaemia. This cross-sectional study evaluated the level of some proinflammatory cytokines in haemoglobin-S variants steady-state subjects in Port-Harcourt Nigeria. Eighty (80) subjects comprising of haemoglobin-S variants in steady-state and apparently healthy haemoglobin A (HbAA) male and female aged within 5-60years were recruited for the study. Five millilitres (5mls) of venous blood were aseptically collected by venepuncture from each participant into plain tube and analyzed using Elabscience ELISA kits. Data obtained were analyzed using SPSS version 24, and results considered to be significant at $p<0.05$. Results showed that Proinflammatory cytokine in the study are all within detectable normal reference ranges with interleukin 1 (IL-1) values significantly higher in HbSC subjects (13.71±3.82pg/ml), followed by HbSS subjects (10.80±2.48pg/ml), HbAA subjects (6.51±1.20pg/ml) and lowest in HbAS subjects (5.71±0.86pg/ml) ($p<0.0001$). Similar pattern was observed in interleukin 6 (IL-6) values with HbSC subjects (44.45±14.68pg/ml), HbSS subjects (38.20±13.20pg/ml), HbAA subjects (28.95±4.91pg/ml) and HbAS subjects (26.10±4.53pg/ml) respectively ($p<0.0001$) while Tumour Necrosis factor alpha (TNF-α) values were high in HbSS subjects (75.75±25.56pg/ml) and HbSC (71.90±16.30pg/ml) and lowest in HbAA (36.70±22.75pg/ml) and HbAS (33.85±22.39pg/ml) respectively ($p<0.0001$). Comparison of IL-1, IL-6 and TNF-α level based on gender showed no statistically significant difference across the various studied groups ($p>0.05$). This study has revealed that the values of IL-1, IL-6 and TNF-α are all within detectable normal reference ranges in haemoglobin-S variant steady-state subjects although significantly elevated values are seen in HbSC and HbSS subjects. Gender has no effect on the expression of IL-1, IL-6 and TNF-α. Measurement of IL-1, IL-6 and TNF-α level is recommended as an indirect way of investigating/monitoring degree of immune response, inflammation, red cell haemolysis and possibly platelet aggregation in haemoglobin-S variant steady-state subjects.

Keyword: *Proinflammatory Cytokines, Interleukins, Haemoglobin-S Variants, Steady-State, Sickle cell disease.*

Introduction

Proinflammatory cytokines as signaling molecules primarily produced by activated macrophages following vascular injuries play key roles in vaso-occlusive crisis, inflammation and atherosclerosis and have been implicated in sickle cell disease and anaemia.

Haemaglobin-S variants constitute the mutant or abnormal form of haemoglobin-S in a population caused by genetic variations that changes the sequences and number of nucleotides within the globin gene/chain. They include the following: haemoglobin AS (HbAS), haemoglobin SC (HbSC), and haemoglobin SS (HbSS) and are responsible for the occurrence of blood disorders

such as sickle cell disease, sickle cell anaemia and haemolytic anaemias (Erhabor *et al*., 2010; Ademola *et al*., 2019; Christian *et al*., 2020; Jacob *et al*., 2023).

Cytokines constitute a category of loosed or broad proteins, peptides and/or glycoproteins signaling molecules produced at various immunologic sites by activated macrophages to display specific important role in cell signaling and aid the cell-to-cell message translation in immune response to stimulus by foreign antigens (Venugopal, 2007; Jacob *et al*., 2018a).

Cytokine play a pivotal role in coordination and regulation of immune responses and are named depending on the site from which they are produced, those made by lymphocytes are named as lymphokine, those made by monocytes are called monokines, chemokine (cytokines with chemotactic activities), and interleukin (cytokines made by one leukocyte and acting on other leukocytes (Jacob *et al*., 2018b). Cytokines are released following characteristic vascular endothelium damage with formation of thrombosis at the site underlying intimal hyperplasia as a result of abnormal presence of circulating endothelial cell adhesion molecules, such as intercellular adhesion molecule-1(ICAM-1), vascular cell adhesion molecule-1(VCAM-1), E-selectin in the plasma and increased numbers of circulating endothelial cells in the blood of patients in acute painful crisis (Sippert *et al*., 2017).

The vascular endothelium thus plays a critical role in vaso-occlusion and ischemic organ damage by several mechanisms e.g including adhesion of red blood cell (RBC), white blood cells (WBC) and possibly injury caused by ischemia-reperfusion (Sippert *et al*., 2017; Sundd *et al*., 2019). The damage of the vascular endothelium results in occlusion of microvasculature, infection and possible inflammation and this stimulates the activities of cytokines and acute-phase proteins in patients with haemoglobin variants HbSS or sickle cell anaemia (Pathare *et al*., 2003). Thus, proinflammatory cytokine play role in inflammation/vascular damage and this is mediated in phases involving acute inflammatory response that is characterized by rapid onset and is of short duration (Gaspani *et al*., 2002; Niemand *et al*., 2003).

Sickle cell anaemia (SCA) and Sickle cell disease (SCD) are genetically inherited haemolytic disorder characterized by chronic inflammation and studies of the cytokines and chemokines in sickle cell anaemia patients (Elammary *et al*., 2020). Due to the high level of inflammation in this subjects, they exhibit higher levels of several proinflammatory cytokines, including interleukin-1 beta (IL-1β) (Pathare *et al*., 2003), and these cytokines are regulated by genetic factors such that IL-1β, IL-6, IL-3, tumor necrosis factor α (TNF-α), granulocyte monocyte-colony stimulating factor (GMCSF), endothelin-1, and prostaglandin modify the susceptibility and severity of the disease (Gilli *et al*., 2016; Jacob *et al.,* 2018b).

The sickled red blood cells, as well as leukocytes, platelets and the vascular endothelium constitute elements that are capable of obstructing blood vessels and triggering vaso-occlusive crises. This is achieved as the sickled red cells get haemolyzed either extravascularly or intravascularly (Pitanga *et al*., 2013). Intravascular haemolysis occurs when red blood cells (RBCs) rupture and release free hemoglobin into the plasma. The free haemoglobin thus released has inflammatory and oxidant effects that lead to endothelium dysfunction (Rother *et al*., 2005; Pitanga *et al*., 2013). Other haemolysis products, including heme, reactive oxygen species (ROS) and reactive nitrogen species, are also released into the bloodstream, where they cause increased oxidative stress and decreased plasma levels of the vasodilator, nitric oxide (NO) (Rother *et al*., 2005; Pitanga *et al*., 2013).

Several cytokines, such as interleukin-1 beta (IL-1*β*) and tumor necrosis factor-alpha (TNF-*α*), are associated with the activation of leukocytes, particularly monocytes and neutrophils, in sickle cell anaemia (Levy *et al.,* 2002). Recently, the involvement of several other cytokines, such as IL-18, IL-17, IL-23, IL-12 and IL10, in inflammatory responses in sickle cell anaemia patients has been described (Pitanga *et al*., 2013).

This study is aimed at evaluating the levels of proinflammatory cytokines such as interleukin 1 (IL-1), interleukin 6 (IL-6) and tumour necrosis factor (TNF-α) in haemoglobin-S variant steady-state subjects in Port Harcourt Nigeria.

Materials and Methods

Study Design and Population

This cross-sectional study was conducted amongst eighty (80) subjects made up of haemoglobin SS (HbSS), haemoglobin SC (HbSC) in steady-state, haemoglobin AS (HbAS) and apparently healthy haemoglobin A (HbAA) male and female within the ages of 5-60 years of age attending tertiary hospitals within Port Harcourt Rivers State Nigeria. The medical history as well as the biodata, socio-demographic and lifestyle of all subjects in the study was obtained with the use of a well-structured questionnaire.

Ethical Clearance/Informed consent

Formal approval was obtained from the Rivers State Ministry of Health Port Harcourt and verbal and/or written consent obtained from each participant.

Blood Sample Collection and Processing

Five millilitres (5mls) of venous blood were aseptically collected through venepuncture from each participant into a plain tube. The blood sample was transported to the laboratory at room temperature and stored at 4^0C prior to analysis using human interleukin 1, 6 and Tumour necrosis factor alpha Elabscience ELISA kits respectively.

Determination of Interleukin 1, 6 and TNF-α using Elabscience ELISA kit as described by Elabscience Biotech Co., Ltd, China.

Samples were assayed using Elabscience ELISA kits that utilize the Sandwich-ELISA as method. The ELISA-micro plates provided with the kit are pre-coated with antibody specific to human interleukin 1, 6 and TNF-α respectively and when the standard samples are added to the micro-ELISA plate wells in combination with the specific antibody, and a biotinylated detection antibody specific for interleukin 1, 6 and TNF-α respectively and Avidin-Horseradish Peroxidase (HRP) conjugate are added to each microplate well successively and incubated; then followed by the washing away of free components, and the addition of the substrate solution that causes the Avidin-HRP conjugate to appear blue in colour. The series of enzyme-substrate reactions taking place is stopped by the addition of stop solution with end product resulting in a yellow colouration. The optical density (OD) is then measured spectrophotometrically at a wavelength of 450nm. The optical density (OD) value obtained from the reading is directly proportional to the concentration of Human interleukin 1, 6 and TNF-α in the sample respectively.

Statistical Analysis

Statistical package for social science (SPSS) version 24 was used for data analysis. Descriptive statistical tools such as mean and standard deviation (SD) was used. Analysis of variance (ANOVA) was used to compare means of more than two groups for inferential evaluation, with Turkey's multiple comparison test to check for mean difference between multiple groups. Student t-test was used for comparison of means.

Results

Social Demographic Data of studied population

A total of eighty (80) subjects consisting of 37 (46.25%) females and 43 (53.75%) males of which 20 (25%) haemoglobin AA (HbAA) (nine (9) males and eleven (11) females); 20 (25%) Haemoglobin AS (HbAS) (five (5) males and fifteen (15) females); 20 (25%) Haemoglobin SS (HbSS) (ten (10) male and females each), and 20 (25%) haemoglobin SC (HbSC) (thirteen (13) males and seven (7) females) all age between 5-60 years of age as shown in Table 1.

Table 1: Social Demographic Data of studied population

Study Groups	Parameters					
	HbAA	HbAS	HbSS	HbSC	Number(n)	Frequency (%)
Age (years)						
5-19	-	1	9	16	26	32.5
20-34	15	17	11	4	47	58.75
35-49	4	1	-	-	5	6.25
50-64	1	1	-	-	2	2.5
Total	20	20	20	20	80	100
Gender						
Male	9	5	10	13	37	46.25
Female	11	15	10	7	43	53.75
Total	20	20	20	20	80	100

Key: HbAA=haemoglobin genotype AA, HbAS=haemoglobin genotype AS, HbSS= haemoglobin genotype SS and HbSC=Haemoglobin genotype SC, n=number of subjects.

Comparative Results of Pro-Inflammatory Cytokines (IL-1, IL-6, TNF-α) in study population.
Table 2 shows the comparison of the mean±SD of the various proinflammatory cytokines measured in the study. Interleukin 1, 6 and TNF-α values were all within normal detectable reference values. However, significantly elevated values were seen in HbSC and HbSS compared to the HbAA and HbAS ($p<0.0001$).

Table 2: Comparative Results of Pro-Inflammatory Cytokines (IL-1, IL-6, TNF-α) in study population.

Parameters	Study Groups						
	HbAA(n=20)	HbAS (n=20)	HbSS (n=20)	HbSC (n=20)	P-value	F-value	Remarks
IL-1(pg/ml)	6.51±1.20 [a]	5.72±0.86 [a]	10.80±2.48 [b]	13.71±3.82 [c]	<0.0001	49.159	S
IL-6(pg/ml)	28.95±4.91 [a]	26.10±4.53 [a]	38.20±13.20 [b]	44.45±14.68 [b]	<0.0001	13.140	S
TNF-a (pg/ml)	36.70±22.75 [a]	33.85±22.39 [a]	75.75±25.56 [b]	71.90±16.30 [b]	<0.0001	20.605	S

Key: *Values in the same row with different superscripts (a, b, c) differ significantly when compared at $p<0.05$. S=Significant, NS= Not Significant, HbAA=haemoglobin genotype AA, HbAS=haemoglobin genotype AS, HbSS= haemoglobin genotype SS and HbSC=Haemoglobin genotype SC. IL-1= Interleukin 1, IL-6= Interleukin 6, TNF-α =Tumour necrosis factor alpha.*

Comparative Results of Pro-Inflammatory Cytokines (IL-1, IL-6, TNF-α) Parameters in Studied Population Based on Gender.
Table 3 describe the comparison of measured parameters in the study population based on gender (male and female) and showed that there is no statistically significant difference across the groups

when compared although values of interleukin 1 (IL-1) and 6 (IL-6) were higher in females compared to males and tumour necrosis factor alpha (TNF-α) values were higher in males compared to females ($p>0.05$) in HbAA and HbAS and in the reversed order interleukin 6 (IL-6) in HbSS and interleukin 1 (IL-1) in HbSC ($p>0.05$) respectively.

Table 3: Comparative Results of Pro-Inflammatory Cytokines (IL-1, IL-6, TNF-α) Parameters in Studied Population Based on Gender.

		Haemoglobin AA			
Parameters	**Male (n=11)**	**Female (n=9)**	**P value**	**F value**	**Remarks**
IL-1 (pg/ml)	6.23±0.92	6.74±1,38	0.366	0.928	NS
IL-6 (pg/ml)	27.44±4.09	30.18±5.36	0.224	1.258	NS
TNF-α (pg/ml)	39.00±24.42	34.81±22.31	0.694	0.400	NS
		Haemoglobin AS			
Parameters	**Male (n=5)**	**Female (n=15)**	**P value**	**F value**	**Remarks**
IL-1 (pg/ml)	5.43±0.84	5.81±0.87	0.409	0.845	NS
IL-6 (pg/ml)	25.20±3.70	26.40±0.87	0.621	0.503	NS
TNF-α (pg/ml)	42.40±25.2	31.00±21.5	0.338	0.985	NS
		Haemoglobin SS			
Parameters	**Male (n=10)**	**Female (n=10)**	**P value**	**F value**	**Remarks**
IL-1 (pg/ml)	9.90±2.64	11.70±2.05	0.107	1.699	NS
IL-6 (pg/ml)	42.30±9.53	34.10±15.48	0.171	1.426	NS
TNF-α (pg/ml)	83.80±23.05	67.70±26.53	0.165	1.448	NS
		Haemoglobin SC			
Parameters	**Male (n=13)**	**Female (n=7)**	**P value**	**F value**	**Remarks**
IL-1 (pg/ml)	14.08±3.77	13.02±4.10	0.570	0.579	NS
IL-6 (pg/ml)	45.69±15.78	42.14±13.22	0.617	0.506	NS
TNF-α (pg/ml)	69.38±10.26	76.57±24.31	0.361	0.938	NS

Key: *S=Significant when compared at $p<0.05$, NS= Not Significant when compared at $p<0.05$, HbAA=haemoglobin genotype AA, HbAS=haemoglobin genotype AS, HbSS=haemoglobin genotype SS, HbSC=haemoglobin genotype SC, IL-1= Interleukin 1, IL-6= Interleukin 6, TNF-α =Tumour necrosis factor alpha.*

Discussion

The mean±SD values of interleukin 1 (IL-1), IL-6 and tumour necrosis factor alpha (TNF-α) in this study were higher in HbSS and HbSC subjects compared to the HbAA and HbAS subjects ($p<0.0001$) meaning that subjects with haemoglobin SC and SS variants have higher expression of interleukin 1, 6 and TNF-α compared to the HbAA and HbAS variants in the study population. By implication, it means that subjects with the HbSC and HbSS variants are more prone to inflammatory stimulation, activation and expression than subjects with HbAA and HbAS haemoglobin variants.

The elevated levels of interleukin 1, interleukin 6 and tumour necrosis factor alpha **(TNF-α)** in HbSS and HbSC will not be unconnected with the frequent intravascular or extravascular haemolysis that occurs in this set of subjects due to the defective nature of their red blood cells. The destruction of the red blood cell can result in

the release of free haemoglobin into the plasma. The free haemoglobin released has the ability to stimulate inflammatory and oxidant effects that lead to endothelium dysfunction attracting the release of cytokines and thus the higher values in the HbSS and HbSC subjects. Such is not the case with HbAA and HbAS.

Furthermore, subjects with HbSS and HbSC are prone to infections that are capable of stimulating vigorous immune responses in cell. These responses can activate rolling of leukocytes and inflammatory actions that triggers the release of cytokines thus the higher values in cytokines interleukin 1, interleukin 6 and tumour necrosis factor alpha **(TNF-α)** in the HbSC and HbSS subjects and not HbAS and HbAA subjects in the study.

Findings in this study is in line with the observation of Zhang and Jianxiong (2007) on cytokine levels in sickle cell subjects. Also, in line with the reports (Pathare *et al*., 2003; Gilli *et al*.,2016; Elammary *et al.*,2020) who observed that sickle cell disease subjects exhibits higher levels of several proinflammatory cytokines, including interleukin-1 beta (IL1β), IL-6, IL-3, tumour necrosis factor α (TNF-α), granulocyte monocyte-colony stimulating factor (GM-CSF), endothelin-1, and prostaglandin E2 and further suggested that these cytokines are regulated by genetic factors that modify the susceptibility and severity of the disease.

Similarly, finding in this study is also in tandem with previous reports (Asare *et al*. 2010; Qari *et al*., 2012) who indicated an increase in IL-1a and IL-1b among patients with sickle cell disease and asserted that the increased IL-1a and IL-1b are triggers for inflammatory reactions leading to primary leukocyte recruitment, endothelial cell activation and production of other many inflammatory mediators, in particular IL- 8. Previous reports (Gillis *et al.,* 2016; Iughetti *et al.,*2016) showed that an increased production of inflammatory cytokines could contribute to the pathophysiology of sickle cell disease and can be responsible for the damage to the different organs and the occurrence of common cyclic events in sickle cell patients.

Furthermore, values in this study are higher for IL-1, TNF-α and lower for IL-6 and as such not in agreement with the study of Siransy *et al*. (2023) who in their study found increase level of interleukin 1, 6 and TNF-α in sickle cell disease (SCD) subjects in crisis state compared to those in steady state and concluded that an insightful understanding of the correlation between inflammatory mediators such as cytokines, and stroke or other organ damage and may provide new biomarkers or therapeutic approaches for sickle cell disease management.

Also, observation in this study is at deviant from the findings of Pitanga *et al.* (2016) who found low IL-1b (9.12 ± 4.0pg/ml) levels both in normal children and in steady state sickle cell disease patients. Values in this study is higher because the subjects are mainly adults with fully developed immune system as compared to the children population in the study of Pitanga *et al*. (2016).

Finding in this study showed that the levels of interleukin 1 was highest in the HbSC subjects compared to the HbSS subjects ($p<0.0001$). Similar pattern was observed in interleukin 6 (IL-6) and TNF-α with higher values in HbSC when compared with HbSS, though there was no statistically significant difference ($p>0.05$). Furthermore, a comparison of mean±SD values of interleukin 1, 6 and TNF-α in HbAA and HbAS shows no statistically significant difference in the study subjects ($p>0.05$) meaning that there is less inflammatory activities in this set of subjects. This could be attributed to the normal red cell shaped of the blood in these subjects thus there is less destruction of red cells and release of free haemoglobin capable of initiating the stimulation of immune response and leukocytes recruitment or rolling.

A comparison of interleukin 1, 6 and TNF-α in Haemoglobin-S steady state subjects based on gender shows no significant difference ($p>0.05$) implying that gender has no effects on the expression of these cytokine in the study subjects.

Conclusion

This study has revealed that the values of IL-1, IL-6 and TNF-α in haemoglobin-S variant

steady-state subjects are all within detectable normal reference ranges although significantly elevated values are seen in HbSC and HbSS subjects. Also, gender has no effect on the expression of IL-1, IL-6 and TNF-α in haemoglobin-S variant steady-state subjects. The elevated level of cytokines in the HbSS and HbSC sponsors inflammation, vaso-occlusion and atherosclerosis in these subjects.

Recommendation

Measurement of proinflammatory cytokines such as IL-1, IL-6 and **TNF-α** level in haemoglobin-S variant steady state subjects is recommended as a way of investigating and monitoring the degree of immune response, inflammation, red cell haemolysis and possibly platelet aggregation in haemoglobin-S variants subjects.

References

Ademola, A., Olusogo, B. and Oladapo A. (2019). Hemoglobin phenotypes in Nigeria: Data from a National Reference Laboratory. *American Journal of Clinical Pathology*; **152(1)**: 115–116.

Asare, K., Gee, B.E., Stiles, J.K., Wilson, N.O., Driss, A., Quarshie, A., Adams, R.J., Kutlar, A. and Hibbert, J.M. (2010). Plasma interleukin-1beta concentration is associated with stroke in sickle cell disease. *Cytokine*; **49(1)**: 39-44.

Christian, S.G., Jacob, R.B. and Adedeji, M.R. (2020). Evaluation of Haemo-rheological Variability of Haemoglobin Genotypes in Descent of Rumuche Community, Emohua Local Government Area, Rivers state Nigeria. *Nigerian Biomedical Science Journal*; **17(2)**: 6-11.

Erhabor, O., Adias, T.C., Jeremiah, Z.A. and Hart, M.L. (2010). Abnormal hemoglobin variants, ABO, and Rhesus blood group distribution among students in the Niger Delta of Nigeria. *Pathology and Laboratory Medicine International*; **2**:41–46.

Elammary, Y., Sewelam, N., Al-Wakeel, H., El-Ghamrawy, M. & Zayed, S. (2020). Interleukin-1β and interleukin-6 gene polymorphisms in Egyptian sickle cell disease patients. *Egyptian Pediatric Association Gazette*; **68**:14-21.

Gaspani, L., Bianchi, M., Limiroli, E., Panerai, A.E. and Sacerdote, P. (2002). The analgesic drug tramadol prevents the effect of surgery on natural killer cell activity and metastatic colonization in rats. *Journal of Neuroimmunology*; **129(1-2)**:18-24.

Gilli, S.C.O., Pericole, F.V., Benites, B.D., Sippert, E.A., Castilho, L.M., Addas, C. M., et al (2016) Cytokine polymorphisms in sickle cell disease and the relationship with cytokine expression. *Experimental Hematology*; **44(7)**:583-589.

Iughetti, L., Bigi, E.and Venturelli, D. (2016). Novel insights in the management of sickle cell disease in childhood. *World Journal of Clinical Pediatrics*; **5(1)**: 25-34.

Jacob, R.B., Ihua, N., Eze, E.M. and Jeremiah, Z.A. (2018a). Does Single/Combined Administration of Tramadol/ViagraHave Reversal Effects on Haematological and Anti-Inflammatory Cytokines in Male Albino Rats? *Asian Hematology Research Journal*; **1(3)**:1-8.

Jacob, R.B., Eze, E.M. and Jeremiah, Z.A. (2018b). Haematological and cytokine parameters in Male Albino rats administered orally with Single/Combined Doses of Tramadol/Viagra. *European Journal of Biomedical and Pharmaceutical Science*; **5(9)**:102-107.

Jacob, R.B., Ken-Ezihuo, S.U., Bartimaeus, E.S. and Eze, E.M. (2023). P-selectin level in haemoglobin-S variant steady-state subjects in Port Harcourt Nigeria. *Hematology and Transfusion International Journal*; **11(5)**:110-113.

Levy, D.E. and Darnell Jr., J.E. (2002) Stats: Transcriptional control and biological impact. Nature Reviews. *Molecular Cell Biology*; **3:** 651-662.

Niemand, C., Nimmesgern, A., Haan, S., Fischer, P., Schaper, F., Rossaint, R., Heinrich, P.C. and Müller-Newen, G (2003). Activation of STAT3 by IL-6 and IL-10 in primary human macrophages is differentially modulated by suppressor of cytokine signaling 3. *Journal of Immunology*; **170(6)**:3263-3272.

Pathare, A., Salam, A. K., Shahina, D. and David, D. (2003). Cytokines in sickle cell disease. *Haematology*; **8(5)**: 329-337.

Pitanga, T. N., Vilas-Boas, W., Cerqueira, B. A. V., Seixas, M. O., Barbosa, C.G., Adorno, E. V. and Goncalves, M. S. (2013). Cytokine profiles in sickle cell anemia: Pathways to be Unraveled. *Advances in Bioscience and Biotechnology*; **4**:6-12.

Pitanga, T.N., Oliveira, R.R., Zanette, D.L., Guarda, C.C., Santiago, R.P., Santana, S.S., Nascimento, V.M.L., Lima, J.B., Carvalho, G.Q., Maffili, V.V., Carvalho, M.O.S., Alcântara, L.C.J., Borges, V.M. and Goncalves, M.S. (2016). Sickle red cells as danger signals on proinflammatory gene expression, leukotriene B4 and interleukin-1 beta production in peripheral blood mononuclear cell. *Cytokine*; **83**:75-84.

Qari, M.H., Dier, U. and Mousa, S.A. (2012). Biomarkers of Inflammation, Growth Factor, and Coagulation Activation in Patients with Sickle Cell Disease. *Clinical and Applied Thrombosis/Hemostasis*; **18(2)**:195-200.

Rother, R.P., Bell, L., Hillmen, P. and Gladwin, M.T. (2005). The clinical sequelae of intravascular hemolysis and extracellular plasma hemoglobin: A novel mechanism of human disease. *Journal of American Medical Association*; **293:** 1653-1662.

Sippert, E.A., Visentainer, J.E., Alves, H.V., Rodrigues, C., Gilli, S.C., Addas-Carvalho, M., Saad, S.T., Costa, F.F. and Castilho, L. (2017). Red blood cell alloimmunization in patients with sickle cell disease: correlation with HLA and cytokine gene polymorphisms. *Transfusion*; **57(2)**:379-389.

Siransy, L. K., Dasse, R. S., Adou, H., Kouacou, P., Kouamenan, S., Sekongo, Y., Yeboah, R., Memel, C., Assi-Sahoin, A., Moussa, S.Y., Oura, D. and Seri. J. (2023). Are IL-1 family cytokines important in management of sickle cell disease in Sub-Saharan Africa patients? *Frontier Immunology*; **14**: 954054.

Sundd, P., Gladwin, M.T. and Novelli, E.M. (2019). Pathophysiology of Sickle Cell Disease. *Annual Review of Pathology*; **14**: 263-292.

Venugopal, J. (2007). Cytokines. Fundamentals of Immunology 1st edition, New Delhi JayPee Brothers Medical Publishers Ltd.

Zhang, J. & Jianxiong, A. (2007). Cytokine, inflammation and pain. *International Anesthesiology Clinic*; **45(2)**:27–37.

Citation: Ransom Baribefii Jacob, Stella Urekweru Ken-Ezihuo, Ebirien-Agana Samuel Bartimaeus and Evelyn Mgbeoma Eze. Evaluation of Some Proinflammatory Cytokine levels in Haemoglobin-S Variants Subjects in Port Harcourt Nigeria. ***Sokoto Journal of Medical Laboratory Science*; 9(1): 32 - 39.** DOI: 10.4314/sokjmls.v9i1.3