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ASSESSMENT OF IL-2, IL-10, TNF-α AND SELENIUM LEVELS IN SICKLE CELL ANAEMIA INDIVIDUALS IN THEIR STEADY STATE ATTENDING NAUTH, NNEWI IN SOUTHEASTERN NIGERIA.

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ABSTRACT

Background: *Sickle-cell anemia (SCA) results from a point mutation, in which an adenine nucleobase in the sixth codon of the β-globin gene is replaced by a thymine (GAG → GTG). The immune-inflammatory system is a complex network of cells and humoral elements that includes many cytokines. Cytokines are peptides used by cells for intercellular communication and for controlling the inner environment of the cells in which they operate. They are produced by cell types that have important roles in the immune response, inflammation, haemopoiesis, healing and systemic response to injury.*

Aim*: The aim of this study was to assess serum levels of IL-2, IL-10 &TNF-α in sickle cell disease anemia subjects placed on selenium supplements in their steady state.*

Methods: *This is a cross- sectional study. A total of fifty aged matched participants were recruited for this study. 50 SCA subjects with sickle cell anemia (SS) were placed on selenium supplements 200mcg for 90 days.IL-2,1L-10& and TNF-α were measured using enzyme linked immunosorbent assay (ELISA) method.*

Results: *The result showed that the mean serum level of IL-2, (119.06±21.68), significantly reduced while IL-10 and TNF-α (119.59±19.20 and 16.23±2.83) significantly increased. Significance at (p>0.05) after administration of selenium supplements while compared with the baseline values (125.06±42.68, 116.45±17.30 and 15.96±3.32) respectively.*

Conclusion: *SCA is a well-studied monogenetic disease with an established chronic inflammatory component. IL2 is a pro-inflammatory cytokine while both IL 10 and TNF-α are both anti-inflammatory cytokines. The intake of selenium supplement helped in suppressing the proinflammatory mediator while the protypical anti-inflammatory cytokine stepped their activities.*

INTRODUCTION

Sickle-cell anemia (SCA) results from a point mutation, in which an adenine nucleobase in the sixth codon of the β-globin gene is replaced by a thymine (GAG → GTG) (1). The molecular translation replaces glutamic acid with valine, thereby producing an abnormal form of hemoglobin called hemoglobin S. Although the molecular lesion is limited to a single nucleotide, the SCA gene is pleiotropic and leads to multiple phenotypic expressions. SCA patients may present with various complications, such as recurrent episodes of vaso-occlusion, acute chest syndrome (ACS), stroke, infections, and priapism. These complications vary considerably among patients and over time (5,1). Sickle cell anemia (SCA) is a genetic disorder. That affects hemoglobin production and causes red blood cells to change in shape and breakdown faster than normal (1,2). Sickle cell disease (SCD), is the most common blood disorder in the world. It can cause major problems and longterm disability. Thus, it requires daily care and management. (2) The aim of this study was to evaluate the serum levels of IL-2, IL-10 and TNF-α in HbSS individuals in their steady state, Hb AS and Hb AA.

METHODS

5ml of venous blood sample was collected from all participants who enrolled for the study and dispensed into plain sterile bottle, allowed to clot, centrifuge at 3,000r.p.m for 10 minutes, serum was separated into another plain sterile container, refrigerated at -200 c until assay. A total of one hundred and fifty participants (150) were recruited for this study and classified into three groups according to their genotypes Hb AA, Hb AS and Hb SS (N =50) respectively. IL-2, IL-10 and TNF-α were measured using Enzyme linked immunosorbent assay method (ELISA), while Selenium level was measured using atomic absorption spectrophotometry method (AAS). SPSS version 26 was used for the statistical analysis. Ethical approval was obtained from NAUTH ethics committee. Structured questionnaire was filled by each participant and also obtained their informed consent to be voluntarily part of the study. Those who complied with the inclusion criteria were recruited in the study.

RESULTS

TABLE 1 LEVELS OF IL-2, IL-10, TNF- α and Se AMONG THE THREE GENOTYPES.

Groups (n=150)	IL-2 (pg/l)	IL-10 (pg/l)	TNF-α (pg/ml)	Se(mcg/l)
SS (n= 50)	132.06±7.64	128.79±5.89	16.01±1.87	5.73±1.34
AS (n=50)	123.73±9.82	112.37±3.98	15.95±1.27	6.20±1.58
AA (n=50)	120.55±4.73	113.27±3.40	15.02±1.56	7.25±1.78
F-value	29.869	205.789	5.702	12.141
p-value	0.001*	0.001*	0.004*	0.001*
SS VS AS	0.001*	0.001*	1.000	0.003*
AAVS SS	0.001*	0.001*	0.001*	0.001*
AA VS AS	0.123	0.978	0.012*	0.422

TNF=Tumor Necrosis Factor
IL-2 = Interleukin 2
IL-10 = Interleukin 10
Se = Selenium
SS= Homozygous sickle cell anaemia genotype,
AS= Heterozygous sickle cell carrier genotype,
AA= Normal Healthy individual genotype.
*P < 0.05 level
**p ¿0.005*level*
***p < 0.001 level

The results showed that the mean serum Selenium and TNF-alpha levels were significantly different when compared amongst the groups, consequently, the mean serum IL-2 and IL-10 levels were significantly different when compared amongst the groups (p < 0.05). Interleukin 2 and 10 were significantly higher in SS p=0.001. Furthermore, IL-2, IL-10 and Se were statistically significant in AA vs SS and SS vs AS. However, the mean serum TNF-alpha level was

significantly higher in the SS group than in the AA group and significant in the association between AA vs AS only. A study conducted by Taylor *et al* observed an elevated levels of IL-10 in 13 out of 42 SCD patients and 1 out of 25 controls (22). Meanwhile, in another study IL-10 levels were significantly reduced in SCD patient in crisis when compared to SCD patients in their steady state invariably IL-10 levels was higher in SCD groups in their steady state (18) and this corroborates with the findings in this present study. Elevated level of IL-10 was observed in this study. High circulating levels of IL-10 in healthy state of SCD may represent chronic polyclonal activation of B cells or defective regulation of antibodies. IL-10 is a cytokine with potent anti-inflammatory properties plays a crucial role in limiting host-immune response to pathogen thereby preventing damage to the host and maintaining normal tissue homeostasis.

Low levels of IL-10 are associated with enhanced immunopathology response to infection as well as increased risk for development of many autoimmune diseases (8). However, excess inflammation can give rise to systemic metabolic and hemodynamic disturbances harmful to the host. Low level of IL-10 can also enhance inflammatory response to microbial challenge but also lead to development of inflammatory bowel disease (17). An Impaired IL-10 expression or signaling can enhance clearance of pathogen during an acute infection,but also exaggerate inflammatory response resulting in exacerbated immunopathology and tissue damage (10,8) Conversely, some pathogens can harness immunosuppressive capacity of IL-10 leading to persistent infection (25,26).

In a study conducted in Yemen it was observed that the cytokines IL-2, IL-4 and IL-10 were significantly elevated in vaso-occlusive crisis (VOC) than steady state SCA and apparently healthy controls which is not in tandem with the present study. Sickle cell anaemia patients in VOC and those in steady state showed similarly elevated IL-2 levels compared to those of controls. Numerous studies have highlighted many more seemingly contradictory functions of this cytokine with respect to immune enhancing functions, IL-2 has a role in supporting proliferation (11,12,18) and survival (26) of Tcells and differentiation of naïve Tcells into effector and memory cells (10,11,12,14). IL-2 also has anti-inflammatory properties as other proinflammatory cytokines (Bachmann and Kopf, 2002). Vascular endothelial activation, induction of red cell adhesiveness to vascular endothelium, induction of neutrophil adhesiveness to endothelium development of vascular intimal hyperplasia (5). There are no previous data in vivo production of IL-10 in SCD. Abnormal in vivo production of IL-2 may be deleterious to both mediated and humoral responses in SCD with resultant increased risk morbidity.

Elevated level of TNF-α was observed in this study and this corroborated with the findings of other studies (14,15,16 and 17). In SCD inflammation precedes or is associated with pain crises (Solovey *et al.,* 2019) and levels of cytokines such as tumor necrosis alpha increases during inflammation (Francis *et al.,* 2022, Pober *et al.,*2018, Lipowsky *et al.,* 2019). TNF-α produced by activated platelets, monocytes and macrophages. It is an inflammatory cytokine. Experimental evidences show that SCD patients in steady state show a significant endothelial cell activation and cytokine production (3,6,7).

Increased levels of inflammatory cytokines activated endothelial cells of microvascular origin (17) have been found in the blood of SCD patients even during the steady state and this is in tandem with the findings of present study. Serum TNF-α and IL-10 levels are associated with clinical cause of SCA. TNF-α is the main mediator of acute inflammatory response (4). IL-10 acts by inhibiting the proliferation and production of IL-2 and TNF-α Thus, higher serum of TNF-α levels in SCA patients indicate a persisting inflammatory process.

In this present study the TNF-α levels were significantly higher among SCA patients in their steady state compared with HbAS. This finding agrees with other studies that have shown this cytokine to be elevated in adults with SCA in steady state (18, 24 and 27). The increase in TNF-α levels could be due to chronic inflammatory processes even in steady state SCA (22 and27). Some studies showed no statistical difference in the levels of 1L-10 and TNF-α in both test and control (27) Graido-Gonzalez et al 1998 found IL-10 levels in adults with SCA increase during crisis but not steady state. The main effect of IL-10 is to inhibit the synthesis of other cytokine (25 and 26). These researchers observed decreased serum levels of IL-10. Although changes in levels of pro-inflammatory cytokines and anti-inflammatory properties have been previously demonstrated in SCD. (26) The role of cytokines in SCA has not been established yet, (26) but Evangeline *et al* (2006), showed that inflammation appears to play a significant role in vasoocclusive mechanism in SCD by demonstrating elevated levels of TNFα and IL-2. (18) Ischaemic events produced by the occlusion of vessel are stressful and involve intricate interaction between red blood cells, the endothelium and leukocytes. These interactions are known to be regulated by cytokines and adhesion molecules. Several studies have shown increased levels of cytokines in serum even during the steady state of SCA. This study confirms and substantiates the above premise. Low serum selenium levels observed in this study may also suggest that a weakened antioxidant potential may be associated with sickle cell disease patients. This is in line with the study of Nnodim *et al* (2014). Se (0.78 µg/mL) levels among SCA patients, however, were significantly lower than those of the controls (0.84 µg/mL; $P < 0.0001$) and (101.4 ng/mL; $P < 0.0001$), respectively. The findings in this study are also in tandem with a study carried out by Turk *et al* (2019) The findings from this study are in tandem with other researches suggesting that the pathophysiology of sickle cell disease is associated with inflammation and inflammatory responses. Serum TNF-α and IL-10 levels are associated with clinical cause of SCA. TNF-α is the main mediator of acute inflammatory response thus, higher serum of TNF-α levels in SCA patients indicate a persisting inflammatory process.

CONFLICT OF INTEREST

The authors declare no conflict of interest

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