ORIGINAL ARTICLE

Cytokine Expression in Homozygous Sickle Cell Anaemia

Nnodim Johnkennedy[1], Meludu Samuel C[2], Dioka CE[3], Martin Ifeanyichukwu[4], UkaibeNkiru[2], Ihim Augustine[4]*

[1]Department of Medical Laboratory Science, Faculty of Health Science, Imo State University Owerri, Imo State, Nigeria, [2]Department of Human Biochemistry,[3]Department of Chemical Pathology,[4]Department of Medical Laboratory Science, College of Medicine and Health Science, Nnamdi Azikiwe University Nnewi Campus PMB-5001 Nnewi, Anambra State, Nigeria

Abstract:

Background: Sickle cell anaemia is an inherited disease in which the red blood cells become rigid and sticky, and change from being disc-shaped to being crescent-shaped. The change in shape is due to the presence of an abnormal form of haemoglobin. This results in severe pain and damage to some organs. *Aim and Objective:* The study was carried out to determine the levels of cytokine in sickle cell anemia. *Material and Methods*: Thirty confirmed sickle cell patients in steady state (HbSS-SS) and thirty persons with normal haemoglobin (HbAA) as well as sixteen sickle cell disease in crises (HbSS-cr) between the ages of 15 to 30 years were selected in this study. Cytokines including interleukin 1 beta (IL- 1β), interleukin 2 (IL-2), interleukin (IL-6), tumour necrosis factor alpha (TNF-α), and interferon gamma (IFN- λ) were measured by commercially available ELISA kits. *Results*: The results obtained showed that the levels of TNF-α and IL-6 in sickle cell anaemia patients in crisis were significantly elevated when compared with sickle cell in steady state ($P<0.05$). Similarly, the levels of IL-1β, IL-6, and IFN- λ were significantly increased in sickle cell anaemia stable state when compared to HbAA subjects ($P<0.05$). *Conclusion*: This may probably implies that cytokine imbalance is implicated in the pathogenesis of sickle cell crisis. Also, cytokines could be used as an inflammatory marker as well as related marker in disease severity and hence therapeutic intervention.

Keywords: Cytokines, Inflammation, Sickle Cell Anaemia

Introduction:

Sickle cell anaemia is a genetic disease characterized by chronic haemolysis, infections, and recurrent occlusion of microcirculation [1]. This results in painful crisis and chronic organ damage [2]. Hence, occlusions of the microcirculation, infections and haemolysis are important factors that stimulate the production of cytokines and acute-phase proteins. Cytokines are category of small proteins that are necessary in cell signaling [3]. They are released by cells and affect the behavior of other cells, and sometimes the releasing cell itself. Cytokines comprises chemokines, interferons, interleukins, lymphokines and tumour necrosis factor [4]. It is of important to note that a classification that proves more useful in clinical and experimental practice outside of structural biology divides immunological cytokines into those that enhance cellular immune responses, type 1 (interferon gamma, tumour necrotic factor alpha etc.), and type 2 (interleukin-4, interleukin-10, interleukin-13 etc.), which favour antibody responses. It is synthesized by different range of cells, including immune cells like macrophages, B lymphocytes, T lymphocytes and mast cells [5]. They act via receptors, and are especially valuable in the immune system. Cytokines modulate the balance between humoral and cell-based immune responses. Also, they regulate the maturation, growth, and responsiveness of particular cell

populations. Some cytokines are important in enhancing or inhibiting the action of other cytokines in complex ways [6].

Several inflammatory cytokines are induced by oxidative stress especially in sickle cell anaemia. Each cytokine has a matching cell-surface receptor. Subsequent cascades of intracellular signalling then alter cell functions [7]. This includes the up regulation and /or down regulation of several genes and their transcription factors. It then results in the synthesis of other cytokines, an elevation in the number of surface receptors for other molecules, or the suppression of their own effect by feedback inhibition [8].

The effect of a particular cytokine on a given cell depends on the cytokine, its extracellular abundance, the presence and abundance of the complementary receptor on the cell surface, as well as downstream signals activated by receptor binding. These factors can vary by cell type. Cytokines are characterized by considerable redundancy, in which many cytokines appear to share similar functions. It appears that cytokines binding to antibodies have a stronger immune effect than the cytokine alone. Cytokines are necessary in fighting off infections and in other immune responses [9]. But, they can become dysregulated and pathological in inflammation, trauma, and sickle cell anaemia [10]. The aim of this study was to evaluate the levels of cytokines in sickle cell anaemia.

Material and Methods:

Thirty HbSS diagnosed by haemoglobin electrophoresis (15 males and 15 females) aged 10 to 30 years were selected for the study while 16 (8 males and 8 females) were in crisis. These patients were attending General Hospital Owerri. Thirty HbAA normal subjects (15 males and 15 females) were used as control.

Blood sample:

In all subjects, 5 ml of venous blood was collected into a non anti coagulated tubes. The samples were spun in a Wisterfuge centrifuge (model 684) at 1000g for 10 minutes and the serum collected into bijou bottle. Informed consent of the participants was obtained and was conducted in line with the ethical approval of the hospital.

Biochemical assay:

The cytokines: interleukin 1 beta (IL-1β), interleukin 2 (IL-2), interleukin (IL-6),tumour necrosis factor alpha (TNF-α), and interferon gamma (IFN- λ) were measured by enzyme linked immunoabsorbent assay (ELIZA) using standard commercial kits (Biosource International Inc. Camarillo CA)

Statistical analysis:

The results were expressed as mean ± standard deviation. Statistical significance was calculated using Student t-test. The level of significance was calculated at $P<0.05$.

Results:

Table 1: Cytokine Levels in HbAA, Homozygoussickle Cell Anaemia in Steady State and Crisis

Parameters	HbAA	HbSS	HbSS-crisis
IL-1β (pg/mL)	29.6 ± 16.08	41.3 ± 19.2*	42.1 ± 20.4*
IL-2 (pg/mL)	62.91 ± 7.2	66.2 ± 8.6*	66.1 ± 10.2*
IL-6 (pg/mL)	92.34 ± 20.6	128.69 ± 26.4*	169.1±55.4**
IFN- λ (pg/mL)	71.1 ± 23.8	93.8 ± 31.6*	96.1±25.8**
TNF-α (pg/mL)	67.2±14.6	70.9±13.8*	74.5±13.4**

**Significantly different from control at P<0.05, **Significantly different from HbSS at P<0.05.*

Discussion:

Sickle cell anaemia is a chronic, incurable condition presenting mainly as anemia in people homozygous for haemoglobin S (HbS). This abnormal haemoglobin is resulting from the replacement of glutamic acid at position 6 of the globin chain by valine [1, 2]. This is responsible for erythrocyte distortion and fragility in these patients, as well as for thrombosis, fever, splenomegaly, joint pain, lethargy, and weakness. Sickle cell crises refer to the sudden attacks of pain, at various levels of severity, that occur during the lifetime of the patient with sickle cell disease. Sickle cell anaemia in other word is an inherited disorder characterized by homozygosis for HbS and a number of cytokines have been implicated in disease severity [11]. The understanding of the roles of various cytokines in the pathology of sickle cell anaemia is essential for the development of effective therapies for this disease.

In this study, it was observed that cytokines in sickle cell disease subjects were significantly increased when compared with HbAA. Specifically, the level of serum IL-1β and IL-2 in sickle cell patients were significantly elevated when compared with the control ($P< 0.05$). Many cytokines, such as interleukin-1 beta and tumour necrosis factor-alpha are linked with the activation of leukocytes, especially monocytes and neutrophils, in sickle cell anaemia. The activation of cells and the release of cytokines stimulate the NF-κB transcription factor pathway, which controls the synthesis of interleukin-4, interleukin-6 and interleukin-8 [13]. The significantly increased level of IL-1β is consistent with the work of Musa *et al* [8]. Other studies have shown elevated levels of cytokines in serum during the steady state of sickle cell anaemia [5]. This increased IL-1β could be due to significant subclinical microvascular occlusions in steady state [14]. These could be induced by the enhanced adhesiveness of sickle reticulocytes and reversibly sickled erythrocytes to the vascular endothelium. The degree of stimulation and production of cytokines is not high enough to trigger clinically evident vasoocclusions in the steady state. Also, IL-2 affects lymphocytes, facilitating the synthesis of other cytokines from T cells, promoting NK cell and B-cell growth, and enhancing the responsiveness of immature bone marrow cells to other cytokines. The fact that IL-2 levels were higher in sickle cell anaemia patients in steady state suggests some degree of activation-induced cytokinemia, in keeping with the ischemic in? ammatory pro? le in sickle cell disease [15].

This could be linked to subclinical micro-infarctions induced by enhanced adhesiveness of sickle reticulocytes and reversibly sickled erythrocytes to the vascular endothelium. It could be associated with the numerous infections that are endemic in this area. Such infections include malaria, intestinal parasitic helminth infections, and *Salmonella typhi* infections. However, this balance can be very easily shifted and additional small insults could be enough to result in crisis [8]. This study supports the above premise. It was observed that cytokines, IL-6, IFN-λ and TNF-α were significantly elevated in SCD patients in crisis when compared to HbAA and HbSS steady state. TNF-α and IL-1β impair blood flow and impede recovery from ischemic episodes by increasing adhesion of sickle RBC to endothelium [15]. The interleukin-6 cytokine supports a variety of cellular functions, including differentiation, maturation, proliferation and survival [16]. IFN-γ is a glycoprotein produced by CD4+ and CD8+ T cells after activation by natural killer (NK) cells. The immunoregulatory functions of IFN-γ are diverse. It includes the activation of mononuclear phagocytes, the upregulation of class I molecules of the major histocompatibility complex, the stimulation of NK cell cytolytic activity and the activation of neutrophils. In this study the mean serum level of IFN-γ was higher in SCA patients during the steady state and during crisis [17].

Furthermore, tumour necrotic factor alpha was significantly increased in sickle cell steady state and crisis. TNF-α is named for its ability to stimulate tumour necrosis and regression *in vivo*. It is necessary to note that biological responses to

TNF-α are mediated by two groups of receptors, TNFR55 and TNFR 75, which are present on the membrane of several types of cells, excluding RBCs. TNF-α stimulates increased expression of adhesion molecules on endothelial cells, contributing to leukocyte adhesion. Both cytokines stimulate RBC adhesion to endothelial cells. Likewise, the cytokines enhances neutrophil degranulation, capillary leak and vasoconstriction. TNF-α inhibits cell proliferation and induces cell death [10]. It contributes to the vascular inflammatory state that is present in sickle cell anaemia. This is in line with several studies that patients display higher levels of a number of cytokine [18]. Hence cytokine imbalance is implicated in the pathogenesis of sickle cell crisis. Also, cytokines could be used as an inflammatory marker as well as related marker in disease severity.

References:

1. Nnodim JK, Meludu SC, Dioka CE, Ihim A, Onyemailoh OB and Obi PC. Antioxidant vitamins and glycated haemoglobin status in sickle cell anaemia. *Al Ameen J Med Sci* 2014; 7(2):175-177.
2. Nnodim JK, Meludu SC, Dioka CE, Onah C, Ihim A, Atuegbu C. Trace elements deficiency in patients with homozygous sickle cell disease. *British Journal of Medicine & Medical Research* 2014; 4(21):3879-3888.3
3. Cannon JG. Inflammatory Cytokines in Nonpathological States. *News Physiol Sci* 2000; 15: 298-303.
4. Veiga PC, Schroth RJ, Guedes R, Freire SM, Nogueira-Filho G. Serum cytokine profile among Brazilian children of African descent with periodontal inflammation and sickle cell anaemia. *Archives Oral Biology* 2013; 58: 505-510.
5. Pathare A, Al Kindi S, Alnaqdy AA, Daar S, Knox-Macaulay and Dennison D. Cytokine Profile of Sickle Cell Disease in Oman. *American Journal of Hematology* 2004; 77:323-328.
6. Makis AC, Hatzimichael EC, Mavridis A, Bourantas KL. Alpha-2-macroglobulin and interleukin-6 levels in steady-state sickle cell disease patients. *Acta Haematol* 2000; 104:164–168.
7. David F, Farley J, Huang H, Lavoie JP, and Laverty S. Cytokine and chemokine gene expression of IL-1beta stimulated equine articular chondrocytes. *Vet Surg* 2007; 36(3): 221-227.
8. Musa OP, Onyemelukwe GC, Hambolu JO, Mamman AI, Isa AH. Pattern of serum cytokine expression and t-cell subsets in sickle cell disease patients in vaso-occlusive crisis. *Clinical and Vaccine Immunology* 2010; 17(4):602-604.
9. Chokkalingam V, Tel J, Wimmers F, Liu X, Semenov S, Thiele J, Figdor CG, Huck WTS. Probing cellular heterogeneity in cytokine-secreting immune cells using droplet-based microfluidics.*Lab on a Chip* 2013; (24): 4740–4744.
10. Makis AC, Hatzimichael EC, Bourantes KL. The role of cytokines in sickle cell disease. *Ann Hematol* 2000; 79:407–413
11. Belcher JD, Marker PH, Weber JP, Hebbel RP, Vercellotti GM. Activated monocytes in sickle cell disease: potential role in the activation of vascular endothelium and vasoocclusion, *Blood* 2000; 96: 2451–2459.
12. Bourantas KL, Dalekos GN, Makis A, Chaidos A, Tsiara S, Mavrides A. Cytokines and acute-phase proteins during the steady state of sickle cell disease. *Eur J Hematol* 1998; 61:49-54.
13. Raghupathy R, Haider MZ, Azizieh F, Abdelsalam A, d'Sousa TM, Adekile AD. Th1 and Th2 cytokine profiles in sickle cell disease. *Acta Haematol*2000 103:197-199.
14. Carpenter LR, Moy JN, Roebuck KA; Moy; Roebuck. Respiratory syncytial virus and TNF alpha induction of chemokine gene expression involves differential activation of Rel A and NF-kappa B1. *BMC Infect Dis* 2002; 2: 5
15. Tian B, Nowak DE, Brasier AR, Nowak Brasier. A TNF-induced gene expression program under oscillatory NF-kappa B control. *BMC Genomics* 2005; 6: 137.
16. Dinarello CA. Proinflammatory cytokines. *Chest* 2000; 118 (2): 503–8.
17. Asare K, Gee BE, Stiles JK, Wilson, NO, Driss A, Quarshie A, Adams RJ, Kutlar A, Hibbert JM. Plasma interleukin-1beta concentration is associated with stroke in sickle cell disease. *Cytokine* 2010; 49: 39-44.
18. Pitanga TN, Wendell V, Bruno AVC, Magda OS, Cynara GB, Elisângela VA, Marilda SG. Cytokine profiles in sickle cell anemia: Pathways to be unravelled. *Advances in Bioscience and Biotechnology* 2013; 4: 6-12.
19. Ikram N, Hassan K and Tufail S. Cytokines. *International Journal of Pathology* 2004; 2, 47-58.

[*] ***Author for Correspondence:*** *Dr. Nnodim Johnkennedy, Department of Medical Laboratory Science, Faculty of Health Science, Imo State University Owerri, Imo State, Nigeria, Tel No: +238034237000, E-mail: johnkennedy23@yahoo.com*