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ORIGINAL RESEARCH

Serum Interleukin-6 Levels as a Biomarker for the Clinical Severity of Sickle Cell Disease

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ABSTRACT

Background: Sickle cell disease (SCD) is a common hemoglobinopathy associated with high morbidity and mortality. While interleukin-6 (IL-6) contributes to elevated acute-phase protein levels in steady-state SCD, its relationship with disease severity remains unclear. This study investigated IL-6 as an inflammatory biomarker and its association with the clinical severity of SCD. **Materials and Methods:** This observational, cross-sectional study was conducted at the Department of Medicine, Srirama Chandra Bhanja Medical College and Hospital, Cuttack, from August 2023 to August 2024. Patients with homozygous sickle cell disease (HbSS), confirmed by hemoglobin (Hb) electrophoresis and in steady state, were included.Disease severity was classified according to van den Tweel et al.,and serum IL-6 levelswere measured using the Elecsys® IL-6 immunoassay kit. **Result:** Ninety-four patients were included in the study. Based on disease severity, patients were categorized into mild (n=53), moderate (n=18), and severe (n=23) groups. The mean age of the patients in the mild, moderate, and severe groups was 29.68 years, 27.83 years, and 31.57 years, respectively. There were no significant differences in mean Hb (P=0.43), total leukocyte count (P=0.19), hematocrit (P=0.80), mean corpuscular volume (P=0.31), or mean corpuscular hemoglobin (P=0.39) across groups. However, the mean (SD) serum IL-6 level was significantly higher in the severe group [109.86 (90.53) pg/ml] compared to the moderate [81.52 (60.94) pg/ml] and mild [48.75 (33.61) pg/ml] groups (P<0.001). **Conclusion:** Serum IL-6 levels could serve as a useful marker for assessing the clinical severity of SCD, with higher IL-6 levels correlating with greater disease severity.

Keywords: clinical severity, HbSS, IL-6, steady state, vaso-occlusive crisis.

INTRODUCTION

Sickle cell disease (SCD), the most prevalent monogenic disorder, is a significant public health issue in developing countries,owingto its high morbidity and mortality rates. Hemolysis and the reduced oxygen affinity of sickle hemoglobin(HbS) in individuals withSCD (genotype SS) cause moderate anemia.[1]Vaso-occlusive crisis (VOC) is a common complication of SCD, where repeated episodes of vaso-occlusion lead to organ damage and increase the risk of early mortality.[2,3]The clinical severity of sickle cell anemia(SCA) is highly variable,influenced partly by inherited modifying factors, such as the presence of fetal hemoglobin (HbF), and partly by socioeconomic conditions and environmental factors affecting general health.[4]

In SCD, abnormal hemoglobin (Hb) polymerizes when deoxygenated, forming sickle-shaped erythrocytes.[5,6] This abnormal polymerization, along with the adhesion of sickled red blood cells and leukocytes to the vascular endothelium, causes vascular obstruction, tissue ischemia, and reperfusion injury, triggering an intense inflammatory response.[6]

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Interleukin-6 (IL-6) is an immune proteinproduced in response toboth acute or chronic inflammation.[7] There areincreased serum levels of acute-phase proteins in patientswith SCD during the steady state of the disease,possibly due to vascular obstruction triggering an inflammatory response.[8] Cytokines, especially IL-6, appear responsible for theseelevated acute-phase protein levels in the steady state of SCD.[8]In individuals with homozygous SCD (HbSS), serum IL-6 levels are assessed during the steady (asymptomatic) state.[9]

While elevated IL-6 levels are known to affect both humoral and cell-mediated immune functions in SCD, increasing the risk of morbidity,[9]the relationship between IL-6 levels and the clinical severity of SCD remains insufficiently explored. Investigating IL-6 as a potential biomarker could provide valuable insights into its role in disease progression and help manage patients based on disease severity.

Therefore, the present study investigated the level of the inflammatory biomarker IL-6 and its association with the clinical severity of SCD.

MATERIAL AND METHODS

Study design

This observational, cross-sectional study was conducted at the Department of Medicine, Srirama Chandra Bhanja Medical College and Hospital, Cuttack, from August 2023 to August 2024. The studywas approved by the 52nd Institutional Ethics Committee (Approval number: 1491/16.08.2023). Written informed consent was obtained from all participants prior to the commencement of the study.

Inclusion criteria

Patients with HbSS,confirmed by Hb electrophoresis, who were in a steady state, were included in the study.

Exclusion criteria

Exclusion criteria included pregnancy, hydroxyurea treatment, other sickle cell syndromes (e.g., HbS-β thalassemia, SE, SC, SD), recent blood transfusion (within the last 3 months), or a VOC attack in the past month. Moreover, patients enrolled in special programs or trials that could affect their clinical or hematological status were also excluded.

Data collection

Baseline complete blood count (CBC) and high-performance liquid chromatography (HPLC)Hbvalues were obtained from patient records. All patients were evaluated for clinical phenotypes, including pain, anemia, jaundice, pneumonia, stroke, and osteonecrosis. Severity scores were based on a combination of anemia, complications, total leukocyte count (TLC), and transfusion scores, following the criteria outlined by van den Tweel et al. [10]

Interleukin-6 Assay

About 5ml of blood was collected from each patient. A 2.5 ml venous blood sample was collected into an ethylenediamine tetraacetic acid (EDTA) container (whole blood) for CBC, and another 2.5 ml of venous blood was collected into a plain container for serum IL-6 level detection using the Elecsys® IL-6 immunoassay kit. The assay, based on the sandwich principle, had a total duration of 18 minutes, and included two incubation steps. During the first incubation, the sample was mixed with a biotinylated monoclonal IL-6-specific antibody. In the second incubation, a monoclonal IL-6-specific antibody labeled with a ruthenium complex [Tris(2,2'-bipyridyl)ruthenium(II)-complex (Ru(bpy)2/3+)] and streptavidin-coated microparticles were added. These antibodies formed a sandwich complex with the antigen in the sample. The reaction mixture was then aspirated into the measuring cell, where the microparticles were magnetically captured onto the electrode surface. Unbound substances were removed with a buffer solution. A voltage was applied to the electrode, inducing chemiluminescent emission, which was detected by a photomultiplier.

Results were determined using a calibration curve, which was generated specifically for the instrument via 2-point calibration and a master curve provided by the reagent barcode or e-barcode. Results were reported in pg/ml, with the normal range being <7 pg/ml.

Statistical analysis

The statistical analysis was performed using the Statistical Package for the Social Sciences (SPSS). Descriptive analysis was used to present the study outcomes. Continuous variables were described as mean and standard deviation (SD), whereas categorical variables were expressed as numbers and percentages. The mean serum IL-6 titers were statistically analyzed using the ANOVA test. A P value <0.05 was considered statistically significant.

RESULTS

A total of 94 patients with HbSS were included in the study.Based on the severity of SCD, patients were categorized into mild, moderate, and severe groups. Fifty-three patients werein the mild group, while 18 and 23 patients were in the moderate and severe groups, respectively.

The comparison of demographic and hematological variables according to patient severity is presented in Table 1. The mean (SD) age of the patients in the mild, moderate, and severe groups was 29.68 (9.99) years, 27.83 (10.31) years, and 31.57 (9.49) years, respectively. More than half of the patients in the mild group were female (54.7%), whereas 69.6% of patients in the severe group were male. However, both males and females were evenly distributed in the moderate group.The mean Hb levels were comparable across the three groups (P=0.43). Similarly, total

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leukocyte count (TLC, P=0.19), hematocrit (P=0.80), mean corpuscular volume (MCV, P=0.31), and mean corpuscular hemoglobin (MCH, P=0.39) were comparable between all groups.

The serum level of IL-6 showed significant differences between the mild, moderate, and severe patient categories,as shown in Figure1. The mean (SD) serum level of IL-6 was significantly higher in the severe group [109.86 (90.53) pg/ml] compared to the moderate [81.52 (60.94) pg/ml] and mild [48.75 (33.61) pg/ml] groups (P<0.001).

Table 1: Demographic and hematological variables of patients

Parameters	Mild group (N=53)	Moderate group (N=18)	Severe group (N=23)	P value
Age (years)	29.68 (9.99)	27.83 (10.31)	31.57 (9.49)	0.49
Sex, n (%)				
Male	24 (45.3)	9 (50.0)	16 (69.6)	0.15
Female	29 (54.7)	9 (50.0)	7 (30.4)	
Hemoglobin	7.92(1.64)	7.43 (1.99)	7.43 (1.99)	0.43
TLC	11518.81 (2942.54)	12004.44 (3612.42)	12935.39 (2893.06)	0.19
Hematocrit	24.68 (4.06)	24.43 (5.47)	23.96 (3.93)	0.80
MCV	80.37 (9.95)	79.31 (15.95)	76.24 (6.73)	0.31
MCH	25.41 (3.38)	26.42 (3.60)	25.04 (2.84)	0.39
Data presented as mean (SD), unless otherwise specified. MCH, mean corpuscular hemoglobin; MCV, mean corpuscular volume; SD, standard deviation; TLC, total leukocyte count.				

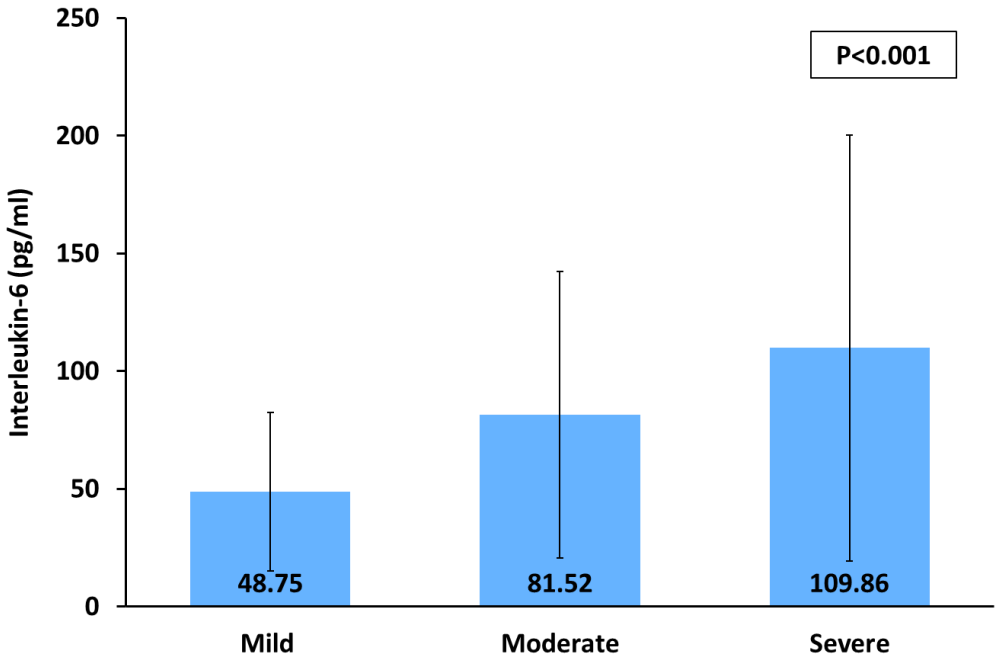

Figure 1: Serum levels of interleukin-6 according to patient severity

DISCUSSION

Sickle cell disease, in its homozygous form, is often associated with increased morbidity and early mortality. This hemoglobinopathy can present with a range of symptoms commonly observed in immunodeficiency disorders.Changes in gene expression and the generation of numerous pro-inflammatory and anti-inflammatory mediators have been associated with SCA.[11]Serum levels of proinflammatory cytokines, such as IL-6 and IL-8, are elevated in patients with SCA (both in steady state and crisis).[12]

The present study comparedthe demographic and hematological variables of patients based on the severity ofSCD. No significant differences were observed in age, sex distribution, Hb, TLC, hematocrit, MCV, and MCH across the severity groups. Similarly, a study conducted in Sudan assessed the severity of SCA in children based on clinico-pathological parameters. None of the parameters mentioned—age, sex, hematocrit, MCV, and MCH—were shown to statistically influence the disease severity score.[13]In contrast, the study by Bhaskar et al. reported slightly higher baseline Hb and hematocrit levels compared to the present study, with

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statistically significant differences observed between mild, moderate, and severe severity groups.[14]

In this study, serum IL-6 levels were significantly higher in severe cases compared to those with moderate and mild SCD. These findings were consistent with a study by Alaaeddin M et al., where IL-6 levels in patients with severe SCD were significantly higher than in those with moderate or mild disease (380 pg/ml vs 69 pg/ml vs 35 pg/ml; P<0.001).[15]Another distinct study, unlike the objective of the present study, indicated that IL-6 levels [71 (40) pg/ml vs. 20 (7) pg/ml; P<0.05] were significantly higher in children with asymptomatic HbSS than in controls.[16] Moreover, OlensckiGilli SC et al. reported significantly greater gene expression of IL-6 in patients with SCDcompared to controls (P=0.03).[17]

Previous studies have documented higher levels of IL-6 protein in SCD, with some reporting a distinct and statistically significant increase in IL-6 levels in patients during either a painful crisis or steady state.[18] On the other hand, another study showed that IL-6 levels were elevated in children and adolescents with SCA, both during steady state and crisis, with a more pronounced increase observed during crisis.[12]

Interleukin-6 plays a variety of roles, including stimulating the hepatic acute-phase response to infection and injury, as well as promoting the differentiation and activation of macrophages, T cells, and B cells. It is also implicated in the pathogenesis of several diseases.[17] However, in SCD, there is a significant association between elevated serum IL-6 levels and the development of complications.[15] Moreover, increased circulating IL-6 levels in otherwise healthy SCD patients may impact both cell-mediated immunity and humoral immune functions. These elevated IL-6 levels in the steady state of SCD may reflect chronic polyclonal activation of B cells and/or impaired regulation of antibody production. The abnormal in vivo production of this type 2 cytokine can be detrimental to both cell-mediated immunity and humoral immune functions in SCD, leading to an increased risk of morbidity.[9] Therefore, estimating serum IL-6 levels could serve as a valuable marker for understanding the severity of SCD and for the early detection and treatment of SCD-related complications. Moreover, serum IL-6 levels may contribute to susceptibility to crisis and could be used as a useful marker for early identification of SCD crisis.

Limitations

The study had several limitations. The small sample size may affect the generalizability of the results. The same inflammatory marker was not evaluated in patients with SCD in crisis. Additionally, only IL-6 was measured, while other inflammatory cytokines were not studied, limiting the scope of the findings.

CONCLUSION

In conclusion, higher IL-6 levels were associated with greater disease severity in SCD. Therefore, measuring serum IL-6 levels could serve as a useful marker for assessing the clinical severity of SCD.

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