

Immunological and Systemic Inflammation Biomarkers among Saudi Patients with Sickle Cell Anemia in Asymptomatic Steady State Condition

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Abstract

Background and Objective: Sickle cell anemia (SCA) comprises an inherited blood disorder that is life-long and affects many people globally. Despite progress in therapy, SCA is a considerable cause of mortality and morbidity. This study was designed to measure the immunologic parameters and inflammatory cytokines of Saudi patients with sickle cell anemia (SCA) in asymptomatic steady state.

Material and Methods: Fifty asymptomatic sickle cell anemia patients and fifty age- and sex-matched healthy non-sickle cell disease subjects were involved in this study.

Results: The number of white blood cells, neutrophil, lymphocytes, eosinophils, basophils, CD3, CD4 and CD8 count were significantly elevated in stable-state SCA patients when compared with healthy controls. In addition, the mean value of CRP, TNF- α , IL-2, IL-4, IL-6 and IL-8 were significantly elevated in stable-state SCA patients when compared with healthy controls.

Conclusion: High levels of serum cytokines and immune system activation are evident in Saudi SCA patients in asymptomatic steady state.

Keywords: Cytokines; Immune Parameters; Sickle Cell Disease; Stable State

Introduction

Sickle cell anemia (SCA) is a genetic red blood cells (RBCs) disease lead to vaso-occlusion and hemolysis due to abnormal sickle shape and rigid RBCs [1]. Patients with SCA usually suffer from attacks of vaso-occlusive pain, poor quality of life [1,2]. About 275000 individuals suffer of SCA as new cases annually as estimated by WHO [3,4].

Sickle cell anemia (SCA) is an incurable chronic medical problems with homozygous for hemoglobin S (HbS) [5] that induce tissue ischemia and infarction due to vascular occlusion that initiates inflammatory responses [6,7].

Multiple co-morbidities usually associated with SCA as pulmonary hypertension, acute chest syndrome, stroke leg ulcers [8] and spleen infarction especially in subjects living at high altitudes [9]. Cardiac arrest, pulmonary embolism, heart failure, infections, multi-system failure and stroke are the common causes of death among patients with SCA [10-12].

Life-threatening infections due to insufficiency of immune system in patients with SCA is common especially with *Streptococcus pneumoniae* and *Haemophilus influenza* [13]. Therefore, Moreover, crisis in SCA is precipitated by infection [14].

This study was designed to measure the immunologic parameters and inflammatory cytokines of Saudi patients with sickle cell anemia (SCA) in asymptomatic steady state.

Subjects and Methods

Subjects

Fifty sickle cell anemia Saudi patients in stable state that presented at the Hematology Department, King Abdulaziz University Hospital were randomly recruited into the study from the available patients in the Hematology Department out clinic. Cases were selected from patients whose blood samples were submitted to the hematology section for hemoglobin electrophoresis, which was either advised by their treating doctor or was performed to confirm a positive sickling test. All confirmed patients of sickle cell haemoglobinopathy diagnosed by presence of Hemoglobin ‘S’ band on hemoglobin electrophoresis and only homozygous Sickle cell disease patients (patients whose electrophoresis showed presence of Hemoglobin ‘S’ band with or without Hemoglobin ‘F’ band – HbSS genotype) were included in the study. Apparently fifty healthy subjects of both gender and age matched subjects were enrolled and considered as control group. Informed consent was signed by all participants.

Exclusion criteria included sickle cell disease patient with concurrent HIV or overt infection. Also, SCA patient with painful vaso-occlusive crisis (VOC) with musculoskeletal bone.

Methods

Evaluated Parameters

- A. **Flow cytometry analysis:** The human leukocyte differentiation antigens CD3, CD4 and CD8 (Beckman Coulter, Marseille, France) five microliters of appropriate monoclonal antibody was added to fifty µL of a whole blood sample and incubated for 15 minutes at room temperature. The samples were analyzed by flow cytometry using Cytomics FC500 and CXP software (Beckman Coulter) [15].
- B. Analysis of peripheral blood cells: A Beckman Coulter AcT 5diff hematology analyzer was used to apply total and differential peripheral blood cells count analysis [15].
- C. Inflammatory cytokines analysis: Serum IL-2, IL-4, IL-6, IL-8, C-reactive protein (CRP) and tumor necrosis factor alpha (TNF- α) levels were measured with highly sensitive ELISA kits (Quantikine ELISA kits) via R and D Systems Inc., Minneapolis, MN [16].

Statistical Analysis

Independent t-test was used to compare differences between both groups. Statistical analysis of data was performed using SPSS (Chicago, IL, USA) version 17. All data were expressed as the mean ± SD. P < 0.05 indicated statistical significance.

Results

Regarding the demographic variables, both groups were considered homogeneous (Table 1). The mean age of the SCA group was 25.16 ± 7.14 years, where the control group was 24.54 ± 7.63 years. There were no significant differences in weight, height, body mass index (BMI), systolic blood pressure and diastolic blood pressure between both groups.

	Group (A)	Group (B)	Significance
Age (year)	25.16 ± 7.14	24.54 ± 7.63	P > 0.05
Weight (kg)	35.75 ± 11.83	29.11 ± 13.72	P > 0.05
Height (cm)	146.19 ± 16.67	143.21 ± 15.84	P > 0.05
BMI (kg/m ²)	18.13 ± 4.16	16.85 ± 3.98	P > 0.05
Systolic blood pressure (mm Hg)	115.41 ± 11.52	116.87 ± 10.31	P > 0.05
Diastolic blood pressure (mmHg)	76.23 ± 6.64	77.15 ± 6.22	P > 0.05

Table 1: Comparison of demographic variables between both groups.

BMI: Body Mass Index

The number of white blood cells, neutrophil, lymphocytes, eosinophils, basophils, CD3, CD4 and CD8 count were significantly elevated in stable-state SCA patients when compared with control group (Table 2). In addition, the mean value of CRP, TNF- α , IL-2, IL-4, IL-6 and IL-8 were significantly elevated in stable-state SCA patients when compared with normal controls (Table 3).

	Group (A)	Group (B)
White blood cells count (10^9 /liter)	9.67 \pm 3.83*	6.41 \pm 3.16
Neutrophil count (% of white blood cells)	55.25 \pm 6.42*	47.31 \pm 5.94
Lymphocytes (% of white blood cells)	39.12 \pm 13.17*	48.32 \pm 10.87
Eosinophils (% of white blood cells)	3.63 \pm 2.32*	3.16 \pm 2.15
Basophils (% of white blood cells)	2.31 \pm 1.17*	2.88 \pm 1.13
RBC ($\times 10^{12}$ /liter)	2.8 \pm 0.5*	4.4 \pm 0.3
CD3 count (10^9 /L)	1.82 \pm 0.97*	1.43 \pm 0.87
CD4 count (10^9 /L)	1.37 \pm 0.63*	1.08 \pm 0.61
CD8 count (10^9 /L)	0.84 \pm 0.32*	0.57 \pm 0.21

Table 2: Mean value and significance of white blood cells, neutrophil, lymphocytes, eosinophils, basophils, CD3, CD4 and CD8 count of group (A) and group (B).

(* indicates a significant difference between the two groups, $P < 0.05$).

	Group (A)	Group (B)
CRP(mg/dl)	13.24 \pm 3.86*	9.89 \pm 3.17
TNF- α (pg/mL)	5.73 \pm 1.52*	4.16 \pm 1.31
IL-2 (pg/mL)	6.42 \pm 2.35*	4.22 \pm 1.98
IL-4(pg/mL)	4.53 \pm 1.61*	3.21 \pm 1.42
IL-6 (pg/mL)	6.75 \pm 2.19*	5.18 \pm 1.83
IL-8 (pg/mL)	15.32 \pm 4.25*	11.48 \pm 3.37

Table 3: Mean value and significance of CRP, TNF- α , IL-2, IL-4, IL-6 and IL-8 of group (A) and group (B).

CRP: C - reactive protein; IL-2: Interleukin-2; IL-4: Interleukin-4; IL-6: Interleukin-6; TNF- α : tumor necrosis factor - alpha; IL-8: Interleukin-8; (* indicates a significant difference between the two groups, $P < 0.05$).

Discussion

Sickle cell anemia is now the world’s most common genetic defects. About 5% of the population carry a haemoglobinopathy trait worldwide, there are about 300,000 born annually worldwide with hemoglobin disorder [17]. Despite the significant increase in research and number of published articles on SCA and its complications remain elusive [18]. Many authors stated that SCA is well recognized as a chronic inflammatory disease [19-21] as C-reactive protein (CRP) and other cytokines are elevated in steady state SCD compared with normal subjects [22]. In addition, researches on animals and human being with SCA proved elevation in inflammatory cytokines [23-27]. However, the results of our study confirm elevation of inflammatory cytokines among SCA patients, our findings agreed with many previous studies.

The possible mechanism that makes inflammatory cytokines increased in patients with SCA which include very short half-life and lyse of sickle red cells (RBC) and increased protein synthesis and catabolism [28,29]. However, systemic inflammation is induced with chronic hemolysis even among steady state SCA [23], In addition transient vaso-occlusive events and subclinical vascular endothelial injury [30].

Moreover, enhanced adhesiveness of sickle reticulocytes and reversibly sickled erythrocytes to the vascular endothelium play a role in increased level of inflammatory cytokines in patients with SCA [31,32].

Concerning immune system parameters, results of the present study confirms immune system activation among SCA patients, our findings agreed with many previous studies. Buisson and colleagues and Hyacinth and colleagues proved that patients with SCA suffer from malnutrition that adversely affect growth and delay musculoskeletal development [33,34]. However, insufficient performance of immune system, endothelial activation and increased inflammatory cytokines are usually associated with malnutrition [35-39]. While, Duits and colleagues stated that elevated inflammatory cytokines enhance chemotactic stimuli that result in elevated neutrophil percentages in steady state SCA patients [40].

The present study has points of strengths and limitations. The major strength point is the randomization nature of this study, as subjects were selected randomly out of the available subjects. In the other hand, the small sample size is the limitations in the present study. Finally, the results of the present study concluded that levels of serum cytokines and immune system activation are evident in Saudi SCA patients in asymptomatic steady state. Moreover, more researches are needed to measure the impact of many life style modifications on modulation of inflammatory cytokines and immune system activation in SCA patients.

Conclusion

Within the limits of the present study, it was concluded that SCA increase the levels of serum cytokines and immune system activation are evident in Saudi SCA patients in asymptomatic steady state.

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