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Estimation of Serum Interleukin-6 level as a Useful Marker for Clinical Severity of Sickle Cell Disease among Sudanese Patients

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Abstract

Background: *Sickle cell disease (SCD) is an inherited disease characterized by a complex and varied physiopathology and exhibits wide clinical diversity. SCA's variability and pose significant management challenges to all medical staff*
Objectives: *We aimed in this study to correlate between serum interleukin-6 level and the clinical severity events of SCD in Sudanese Patients*
Method: *Case control study conducted on 133 patients with Sickle cell disease were collected and level of serum Il-6 were determined by ELISA and compared to 93appearantly healthy individuals as Control group.*
Result: *There was significant association between elevation in serum IL-6 and development of complications in SCD, Chi squared (χ^2) = 0.000 (P < 0.05).*
Conclusion: *These findings indicated that serum level of IL-6 may contribute to susceptibility to many crisis and may use as useful marker for early broadcasting tool for SCD crisis.*

Keywords: *Sickle Cell Disease, Severity, Serum IL-6, SCD Marker*

Introduction

Sickle cell anaemia (genotype SS) causes moderate anaemia resulting both from haemolysis and from the reduced oxygen affinity of haemoglobin S.[1,2] The main clinical disability arises from repeated episodes of vascular occlusion by sickled red cells resulting in acute crises and eventually in end-organ damage.[3,4]

The clinical severity of sickle cell anaemia is extremely variable. This is partly due to the effects of inherited modifying factors, such as interaction with thalassemia or increase synthesis of Hb F and partly to socioeconomic conditions and other factors that influence general health.[5] Interleukin-6 (IL-6) is an endogenous chemical which is active in inflammation. Besides being an immune protein, it is also a pyrogenic, and is responsible for fever in autoimmune, infectious or non-infectious disease[6.] It is produced in the body, wherever there is inflammation, either acute or chronic. Interleukin-6 is implicated in a host of chronic disease conditions associated with inflammation, significantly increased values of IL-6 and IL-2 were found[7]. Studies has demonstrated increased serum levels of some of the acute phase proteins in patients during the steady state of sickle cell disease. This may be a result of a subclinical vaso-occlusion which in turn leads to a covert inflammatory response. Cytokines, and in particular IL-6, produced after this response, seem to be responsible for the high levels of acute phase proteins in the steady state of this disease.[8,9] In patients with childhood sickle cell disease (SCD) serum interleukin-6 (IL-6) levels were measured during the steady (healthy) state of disease. Results revealed significantly higher circulating levels of IL-6 in the SCD patients (60 +/- 7 pg/ml) compared with the healthy controls (12 +/- 5 pg/ml). The impact of high circulating levels of IL-6 may be deleterious to humoral and cell-mediated immune functions in SCD, with resultant heightened risk for morbidity.[10, 11]

Material and Methods

This is a descriptive analytical case control facility based study, conducted at Dr. Gaafar Ibn-Auf Paediatric Tertiary Hospital, as a part of previous work started from March /2016 up to April /2017. The local ethics committee and ministry of health approved the research conducted in accordance with WMA Declaration of Helsinki (2008).

Subject

Study population was 133 patients with Sickle cell disease regardless to age, gender and ethnic group. Clinical data was collected by a questionnaire, a recording form and an authorized clinician who carry out the clinical examination. After informed consent, a venous blood sample (2.5ml) was collected into EDTA container (whole blood) for CBC using Sysmex (KX 21N) and other 2.5ml of venous blood was collected into plain container for serum IL6 level detection using ELISA (STAT FAX-2600) and IL6 kits (Bio legend IL6 human cytokine ELISA).

Interleukin-6 Assay

Human interleukin-6 enzyme-linked immunosorbent assay (ELISA; STAT FAX-2600) was utilized to measure serum IL-6 levels. In summary of method, the captured antibody was coated to the plate, and the standards were reconstituted as directed. After adding plate reagent to each well, 50 pi standards or serum samples in duplicate was added and the plate was incubated for 1 h at 37°C. Unbound material was then removed by aspiration and washing. Next conjugate reagent was added and the plate was incubated for 30 minutes. After incubation, unbound labeled antibody in the conjugate reagent was then removed by aspiration and washing. The bound IL-6 was quantified by an enzymatic reaction, resulting in a detectable color change, using the ELISA reader (Thermo Lab System, Finland), set at 450 nm. To estimate the amount of IL-6 in the serum, a standard curve was constructed utilizing the known standard concentrations. Concentrations of samples were determined by referring to the standard curve and expressed as pg/ml. The detection limit was 5 pg/ml.

Statistical analysis

Statistical methods Concentrations are expressed as mean titers (M ± Standard error of the mean. Statistical analysis of the mean titers of the serum IL-6 levels was performed utilizing an independent t test with significant P.value (>0.05).

Results

All medical and clinical data of the subjects were documented before IL-6 level estimation. The mean age of each group was included in the range 1 to 37 years; SCD duration was about 7.2 years as a mean. Different clinical severity states compared hematological values as showed in **(Table 1).** Serum level of IL-6 show high diversity between Patients' and healthy individual's results as control sample for serum IL-6 levels as showed in **figure1.**

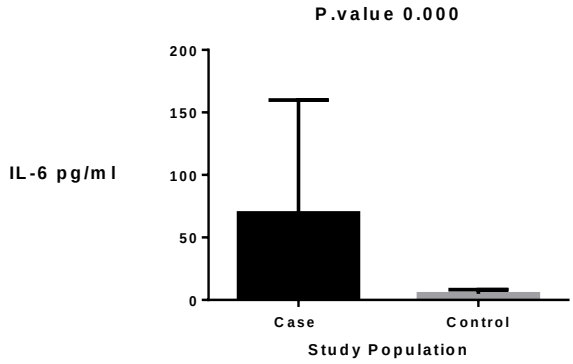

Figure 1: A case and control IL-6 serum level estimation by ELISA technique, as described in Materials and Methods. The ranges of individual levels were as follows: SCD (35-300 pg/ml) and normal controls (5-23 pg/ml)

Table 1: Clinical Severity of Sickle Cell Disease among study population

Clinical Data		Severity of Disease			Total	P.value
		Mild	Moderate	Severe		
Gender	Female	7	23	46	**76**	0.456
	Male	7	12	38	**57**	
Age (mean) years		7.2	8.2	6.8	**7.2**	**0.001**
Stroke		2	18	67	**87**	**0.000**
Sequestration/vasoocclusion		4	21	81	**106**	**0.000**
Hemolytic Crisis		14	35	84	**133**	*a
Leg ulcer		0	1	3	**4**	0.768
Musclo-skeletal pain		9	35	79	**123**	**0.000**
Acute Chest Syndrome		8	23	76	**107**	**0.001**

*Bolded values indicate statistical significance.*a No statistics are computed because Hemolytic Crisis is a constant

Table 2: Relationship between laboratory findings and disease severity

Laboratory parameters	Mild disease N=14 (mean)	Moderate disease N=35 (mean)	Severe disease N=84 (mean)	P
Il-6 level (pg/ml)	**35**	**69**	**380**	**0.000**
TWBCs ($\times10^9$/L)	12.2	13.8	15.1	**0.007**
Neutrophils ($\times10^9$/L)	6.8	7	7.5	**0.000**
Lymphocyte($\times10^9$/L)	5.9	5.7	5.1	**0.001**
RBCs ($\times10^{12}$/L)	2.6	2.7	2.3	**0.045**
Haemoglobin (g/dL)	7.0	8.2	7.3	**0.000**
Haematocrit (%)	22.7	22.9	21.3	**0.034**
MCV(fL)	89	86.3	94	**0.017**
MCH(pg)	32.4	30.7	34.3	**0.000**
MCHC(g/dL)	35.8	35.8	36.5	**0.001**
Platelet($\times10^9$/L)	572.6	450	525	**0.044**

N: Number of cases, TWBCs, total white blood cells count; RBCs, red blood cells count; MCV, mean cell volume; MCH, mean cell haemoglobin; MCHC, mean cell haemoglobin concentration; **Bolded values** indicate statistical significance.

Discussion

SCD, particularly in the homozygous state, may be characterized by significant morbidity and early mortality. Individuals affected by this hemoglobinopathy exhibit many of the manifestations associated with immunodeficiency disorders. The data reported are representative of one phase of a comprehensive evaluation of SCD as a model for immunosuppression. Interleukin-6 is a multifunctional regulatory cytokine that contributes to the immune response, acute-phase reactions, and hematopoiesis. Upon stimulation with antigen, B cells proliferate and differentiate to antibody-forming plasma cells under the influence of various cytokines.[12] IL-6 acts on activated and proliferating B cells and induces their final maturation into antibody- forming plasma cells. Abnormal IL-6 production may play a significant role in a number of diseases, such as autoimmune diseases, chronic inflammation, and lymphoid malignancies, that may be associated with certain viruses.[13] Recent examples characterize cytokines involved in type 1 and type 2 immune responses.[14,15] In the type 1 pattern, IL-2 and interferon-γ (IFN) are autocrine growth factors. IL-2 facilitates helper T cells in their IFN-γ-mediated activation of macrophages (cell-mediated immunity). In type 2, IL-4 and IL-6 are autocrine growth factors promoting suppressor T cells in tilting the immune response toward humoral immunity. IL-6 is considered a type 2 cytokine and inherently suppresses cell-mediated immunity (CMI). Shearer and associates at the National Cancer Institute have shown that a strong CMI response with a type 1 cytokine profile is a better protector against human immunodeficiency virus infection than are strong antibody response and type 2 cytokine profile.15 Elevated circulating levels of IL-6 in healthy SCD patients may actually affect both CMI and humoral functions. In our study we found that IL-6 is highly elevated in proportion to the different severity states of SCD, compared with control group.

Conclusion

In summary, the levels of IL-6 in the different clinical states of SCD may represent contribution in exacerbation of severity of SCD, with resultant increased risk crisis and eventually increase in mortality and morbidity rates.

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