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The impact of interferon-gamma level on the health status of patients with sickle cell disease in Basrah

W.N. Ibraheim[1], H.A. Jasim[1], A.S. Abdullah[2]

[1]*College of Medicine, University of Basrah, Iraq*
[2]*Al-Sadder Teaching Hospital, Oncology & Hematology Centre, Basrah, Iraq*

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Correspondence:
Hanadi A. Jasim,
College of Medicine,
University of Basrah, Iraq
Address: 61001, Basrah, Iraq
E-mail: medicalresearch11@yahoo.com

Sickle cell disease (SCD) is one of the most prevalent autosomal recessive diseases, characterized by the generation of abnormal hemoglobin S. Our study aimed to assess how the serum level of interferon-gamma affects the health status of patients with SCD in Basrah. A total of 90 participants were enrolled in this study and divided into two main groups: a SCD group and a control group. The SCD group included 30 patients with SCD in steady state and 30 patients with SCD in vaso-occlusive crisis; the control group included 30 age- and sex- matched apparently healthy individuals. Approval was obtained from the Research Ethics Committee of the College of Medicine, University of Basrah before conducting the study. Two milliliters of venous blood were drawn from all the participants, and ELISA tests were utilized to determine the levels of serum interferon-gamma. There was a statistically significant increase in the serum level of interferon-gamma among SCD patients (both in steady state and in crisis) compared to the control group ($p = 0.05$). There were no significant differences in the levels of interferon-gamma between the patients in steady state and during vaso-occlusive crisis ($p > 0.05$). Interferon-gamma may influence the general health of sickle cell patients and contribute to the cause of inflammation, no matter whether the patient is in stable condition or is experiencing a crisis.
Key words: *sickle cell disease, interferon-gamma, vaso-occlusive crisis*

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One of the most prevalent autosomal recessive diseases – sickle cell disease (SCD) – is characterized by the generation of abnormal hemoglobin S and is associated with substantial morbidity and mortality, poor quality of life and short life expectancy. SCD is one of the genetic disorders that affects people worldwide, and the Middle East, Mediterranean areas, Southeast Asia, Saudi Arabia, and sub-Saharan Africa have the highest prevalence rates [1].

Under low oxygen tension, hemoglobin S forms crystals. Deoxygenated sickle hemoglobin polymerizes into long fibers, thereby changing the normal morphology of red blood cells and transforming them into a crescent shape [1]. As a result, the cell is vulnerable to several intracellular and membrane alterations. The ability of red blood cells to regain their original shape and adhere to blood vessel walls is impacted by these alterations [2].

SCD is frequently characterized by painful vaso-occlusive crisis (VOC), recurring infections, and chronic inflammation. Cytokines are a general term for a large group of secreted molecules involved in cell-to-cell signaling during immune responses. Interferon-gamma (IFN-γ) is released from the activated TH1 cells [3]. As a result of cell death caused by ischemia, molecules linked to severe inflammatory damage (DAMPS) are released. These subsequently promote a number of inflammatory pathways. The activation of NK cells, which can cause pneumonitis by producing IFY and IFN-γ-induced chemokines, is one of the results of ischemia perfusion damage. Damage from ischemia-reperfusion injury is caused by calcium overload and ROS production [2].

Severe VOC are caused by complicated, multifaceted mechanisms. Sickle red blood cells play a role in nearly all clinical presentations of SCD and contribute to the initial VOC process. Patients with SCD have higher levels of multiple cytokines during steady state and VOC [4].

Monocytes, polymorphonuclear neutrophils, and platelets are also actively involved in numerous adhesive contacts. Therefore, hemolysis, infections, and clinical and subclinical microcirculation occlusions are important factors stimulating the production of cytokines and acute-phase proteins [5].

In our study, we will measure IFN-γ levels in order to better understand its role in the pathophysiology of sickle cell crisis.

MATERIALS AND METHODS

Study design and setting

From August 2022 to January 2023, 60 SCD (hemoglobin SS) patients (30 patients in steady state (patient stability is generally good) [6] and 30 patients

in VOC) were enrolled in this cross-sectional study. All patients who had been diagnosed and registered at Basrah Center for Hereditary Blood Diseases were examined during their subsequent visits to the clinic, along with 30 apparently healthy controls who were age- and gender-matched to the patients. Approval was obtained from the Research Ethics Committee of the College of Medicine, University of Basrah before conducting the study.

Assessment of serum interferon-gamma levels

Approximately 2 mL of venous blood were collected from each participant into a gel tube and allowed to coagulate. Then, serum was separated by centrifugation at 3000 rpm for 15 minutes and stored in deep freeze (–20°C) for the measurement of INF-γ using a commercially available ELISA kit (KAC1231, the Human INF-γ solid-phase sandwich ELISA kit from Invitrogen). The manufacturer's instructions were followed.

Inclusion criteria:

1) children with SCD confirmed by hemoglobin electrophoresis;

2) children with SCD (hemoglobin SS) in steady state and during VOC;

3) signed Informed consent from the participants' parents.

Exclusion criteria:

1) patients who were hepatitis B or C positive;

2) patients lost to follow-up.

Statistical analysis

The level of serum INF-γ was treated as a non-parametric variable and inter-group comparison was performed using the Kruskal-Wallis test to identify statistically significant differences at $p \leq 0.05$.

RESULTS

We enrolled 60 patients with SCD and 30 apparently healthy subjects (with no history of blood disorder) as a control group. The subjects were from 2 to 15 years of age. The mean age of the patients in steady state and in VOC was 8.05 ± 3.88 years and 8.48 ± 3.79 years, respectively; the mean age of the controls was 8.58 ± 3.82 years. There were no significant differences in the mean age of the cases and the controls ($p > 0.05$). There were 18 (60%) females and 12 (40%) males in the group of SCD patients in steady state, while in the group of SCD patients in VOC, there were 11 (36.7%) females and 19 (63.3%) males (*table 1*).

It was found that the SCD patients, both in crisis and in steady state, had a statistically significant higher level of IFN-γ than the healthy controls ($p < 0.05$) as seen in *table 2* and *table 3*.

IFN-γ levels did not significantly differ between the patients in steady state and in crisis ($p > 0.05$) as in *table 4*.

DISCUSSION

IFN-γ is a lymphokine produced by activated T (and NK) cells. The IFN-gene encodes a 146-amino-acid protein, which is post-translationally processed into two species with different glycosylation patterns, 20 kDa and 25 kDa. Natural IFN-γ is highly basic, pH2-labile, and can aggregate to physiologically active dimers. IFN-γ plays a role in regulating cell proliferation, innate immunity, and adaptive immunity. It is a key macrophage activating factor that also controls the differentiation process of myeloid cells [7].

Our study showed that the SCD patients during VOC and in steady state had statistically higher levels of IFN-γ than the controls ($p < 0.05$). This is in agreement with findings reported by Khalifa et al. [8] and Pathare et al. [5], who proved that in sickle cell patients, elevated levels of IFN-γ may lead to tissue damage and inflammation, thus exacerbating morbidity and mortality.

We found no statistically significant difference in the levels of IFN-γ between stable sickle cell patients

Table 1
Characteristics of the study population

Variables	Patients with SCD in steady state (*n* = 30)	Patients with SCD in crisis (*n* = 30)	Controls (*n* = 30)	*p*-value
Age (mean ± SD), years	8.05 ± 3.88	8.48 ± 3.79	8.58 ± 3.82	0.25
Sex (male/female), *n* (%)	12 (40)/18 (60)	19 (63.3)/ 11 (36.7)	12 (40)/ 18 (60)	0.66

Table 2
The difference between serum levels of IFN-γ in the patients with SCD in steady state and in the control group

Group	Mean ± SE	*p*-value
Patients with SCD in steady state (*n* = 30)	14.7 ± 0.41	0.002
Controls (*n* = 30)	4.61 ± 0.22	

Table 3
The difference between serum levels of IFN-γ in the patients with SCD in crisis and in the control group

Group	Mean ± SE	*p*-value
Patients with SCD in crisis (*n* = 30)	12.46 ± 0.61	0.0001
Controls (*n* = 30)	4.88 ± 0.38	

Table 4
The difference between serum levels of IFN-γ in the patients with SCD in crisis and in steady state

Group	Mean ± SE	*p*-value
Patients with SCD in steady state (*n* = 30)	12.46 ± 0.61	0.15
Patients with SCD in crisis (*n* = 30)	14.79 ± 0.39	

and those in crisis, which may be related to the small sample size. This is also in agreement with the Nnodim et al. [9] and Khalifa et al. [8], who reported a considerable elevation in the serum level of IFN-γ in SCA children, but in contrast to Musa et al. [10] finding that there was no significant change.

Based on immunological data, A. Mahmoud demonstrated a significant rise in IFN-γ in SCD patients in VOC compared to SCD patients at steady state and controls, while similar amounts of IFN-γ were found in the 2 patient groups by Mahmoud [5], Okongwu et al. [1].

A number of studies have shown that serum cytokine levels are elevated even in steady-state SCD. Considerable subclinical microvascular occlusions are thought to occur during steady-state SCD as a result of continuous necrosis and localized tissue ischemia. Increased adhesiveness of sickle reticulocytes and reversibly sickled erythrocytes to the vascular endothelium causes these subclinical micro-infarctions [3].

The regulation of lymphocyte effector mechanisms by local patterns of cytokine and hormone production is now well understood. Th1 cells, mainly producing such cytokines as IFN-γ, tumor necrosis factor-β, and IL-2, may promote the production of IgG2a opsonizing and complement-fixing antibodies, activate macrophages, and cause delayed-type hypersensitivity and antibody-dependent cell-mediated cytotoxicity [5].

IL-12 binds to IL-12 receptors on the surface of T cells and induces the secretion of IFN-γ. Repeated infections associated with non-pathogenic mycobacteria, are common in children with defects in genes encoding IL-12, IL-12 receptor, or IFN-γ receptor [2].

Lastly, our results demonstrated a statistically significant increase in the level of IFN-γ in the SCD patients both in steady state and in VOC in comparison to the healthy controls. In sickle cell patients, either in stable condition or during VOC, IFN-γ may contribute to the etiology of inflammation.

CONCLUSION

IFN-γ may influence the general health of sickle cell patients and contribute to the cause of inflammation, no matter whether a patient is in a stable condition or is experiencing a VOC.

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CONFLICT OF INTEREST
The authors declare that there is no conflict of interest.

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