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Role of Interferon Gamma in the Pathogenesis of Sickle Cell Crisis

Ahmed Mahmoud Khalifa[1], Bashair Mater Albeladi[2], Hajar Abdulmalik Amin[2], Nida Mahmoud Johari[2], Rawan Sultan Alosaimi[2], Wafa Ahmad Hamdi[2], Amal Fathy Gharib[2], Howaida Mahmoud Hagag[2], Asmaa Hassan Farghly[2], Khadiga Ahmed Ismail[2,3*]

[1]Forensic and Toxicology Department, Faculty of Medicine, Ain-Shams University, Cairo, Egypt.

[2]Medical Laboratory Department, Faculty of Applied Medical Science, Taif University, Taif, Saudi Arabia.

[3]Parasitology Department, Faculty of Medicine, Ain-Shams University, Cairo, Egypt.

Abstract

Sickle cell disease (SCD) is considered as one of the major public health disease worldwide. The high burden of the disease in Saudi Arabia and Egypt need more effort to control. Current evidence suggests that available care is suboptimal. Different types of crisis are considered significant complication of SCD, the pathogenesis of SCD is mainly due to the production of pro-inflammatory cytokines. As a result of the association of chronic inflammation with crisis onset and the role of IFN gamma as regulatory cytokines, we tested the association of level of IFN gamma with pathogenesis of sickle cell crisis. Study subjects comprised of 70 volunteers (42 (60%) males and 28 (40%)) and among 70 subjects, 50 were sickle cell patients [34 (48.6%) with stable condition, 16 (22.9%) with hemolytic crisis] and 20 (28.6%) healthy normal persons (Control group). Serum IFN gamma concentrations were determined by ELISA. It was found from the results that IFN gamma levels were significantly increased in both stable condition and hemolytic crisis patients in comparison to healthy normal control. There is a statistically significant increase in the level of interferon gamma in SCD patients with stable condition and in crisis in comparison to healthy control. Interferon gamma has a role in pathogenesis of inflammation in sickle cell individuals in the steady state or in crisis in comparison to healthy normal control.

Key words: Sickle cell disease, Crisis, Interferon gamma.

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Correspondence to: Ismail KA, Forensic and Toxicology Department, Faculty of Medicine, Ain-Shams University, Cairo, Egypt.
Email: khadigaahmed68@yahoo.com

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1. Introduction

Sickle cell disease (SCD) is one of the commonest autosomal recessive disorders associated with production of abnormal hemoglobin S and is associated with high morbidity and mortality. It is one of the genetic diseases in the world and its highest prevalence occurs in Middle East, Mediterranean regions, Southeast Asia, Saudi Arabia and sub-Saharan Africa [1-2]. Around 5-7% of the world population carries the gene of abnormal haemoglobin [3-4]. Sub-Saharan Africa and Asia population suffer a lot from this haemoglobinopathy which consider the greatest burden in these areas [5].

Prevalence in Saudi Arabia is patchy and probably underestimated, the prevalence of SCD in Saudi Arabia varies significantly in different parts of the country and Eastern province is considered with the highest prevalence, after that the southwestern provinces. The reported prevalence for sickle-cell trait ranges from 2% to 27% and up to 2.6% will have SCD in some areas. Clinical and hematological variability exists in SCD in Saudi Arabia with two major phenotypes: a mild phenotype and a severe phenotype [6].

SCD occurs as a result of point mutation which leads to a qualitative haemoglobinopathy resulting from a structural change in the sequence of amino acids on the beta globin chain of the haemoglobin molecule. There is a single base change from adenine to thymine on the 17^{th} nucleotide of the beta globin chain gene (HBB) which causes a sickling mutation. The abnormal biochemistry of this mutant haemoglobin induces polymerization of HbS molecules within the red cells due to substitution glutamate by valine which causes sickling. On the sickle haemoglobin, the hydrophilic, polar, and negatively charged glutamate protein molecule, is replaced by a less polar, hydrophobic, neutral amino acid, valine. Under reduce oxygen tension conditions, the abnormal valine residue causes intraerythrocytic hydrophobic interaction of sickle haemoglobin tetramers, leading to their precipitation and polymer formation [7]. Individuals with Sickle Cell Anemia (SCA), the homozygous (HbSS) sickle cell disease (SCD), are at an increased risk of invasive bacterial infections [8]. Currently, however, little has been done to characterize T and B lymphocyte phenotype, function and contribution to chronic inflammatory diseases in SCA. Limited studies done indicate that abnormalities in both T and B cells occur in SCA [9].

Among individuals with SCA, the observed immunological abnormalities vary depending on severity of disease and type of the crisis. Unlike individuals with severe disease, those with mild SCA may have normal immunological parameters, with the exception of serum opsonization activity which is often impaired in severe as well as mild disease [10]. This variability is consistent with the considerable heterogeneity in the genetic makeup of individuals with SCA that impacts disease severity [11]. The aim of this study therefore is to estimate the level of IFN gamma in order to shed light on its role in the pathogenesis of sickle cell crisis.

2. Experimental

2.1. Subjects

The study was conducted on 50 SCD patients and 20 healthy subjects called as control. Blood samples were collected from different specialized hospitals, Taif, KSA and the Medical Genetics Unit, Pediatric Hospital, Ain Shams University Hospital, Cairo, Egypt, from January 2017 to January 2018. The diagnosis of SCD was established by Giemsa stain thin blood film, hemoglobin electrophoresis, high performance liquid

chromatography (HPLC) and sickling test. Five milliliters of blood were taken under sterile conditions. The sera were then separated and stored at -20ºC until the analysis for serum INF-γ levels determination

2.2. Methods

2.2.1. Detection of serum INF-γ levels

All serum samples were tested for serum INF-γ level using commercially available Enzyme Linked Immunosorbent Assay (ELISA) kit Bio (Cat#: KAC1231) Invitrogen™, 50 µl of Serum samples, controls and standards, then added 50 µl of anti-IFN-γ HRP conjugate into all the wells and then incubated at room temperature for 2 h on a horizontal shaker set at 700±100 rpm. Thereafter, solution was decanted from the wells and wells washed for 4 times. 200 µL of freshly prepared Chromogen Solution was added to each well and incubated the plate for 15 min at room temperature on a horizontal shaker, speed was set at 700±100 rpm followed by 50 µL of Stop Solution was added into each well. Plate was read within 30 min after adding the Stop Solution and the concentration was calculated on the standard curve (in pg/mL).

2.3. Statistical analysis

Collected data were coded, tabulated and introduced to a Personal Computer (PC) using the Statistical Package for Social Science (SPSS) for windows version 19. The level of serum INF-γ in the studied groups was expressed as non parametric variable and was compared between groups using Kruskal-Wallis test to clarify statistically significant differences at $p<0.05$.

3. Results

Our study demonstrated that 42 (60%) males, 28 (40%) females with total subjects of 70 and among 70 subjects, 34 (48.6%) were in stable condition, 16 (22.9%) were in hemolytic crisis and 20 (28.6%) were normal healthy control subjects. The age of subjects ranged from 0.5-42 years old. It was found that there is a statistically significant high level of IFN- γ in sickle cell disease patients and sickle cell trait patients in comparison to healthy control and also there is a statistically significant high level of IFN- γ in patients with sickle cell crisis and in sickle cell stable patients in comparison to healthy control.

Table 1. Clinical characteristics of sample.

Clinical Characteristics	SCD in Steady State (N=34)	SCD in Haemolytic Crises (N=16)	Control Group (N=20)
Gender (Male:Female)	20:14	10:6	12:8
Age in years	0.5-41 years	23-42 years	1-40 years

Table 2. Level of interferon gamma in different conditions.

Parameters	Test Statistic ±S.E*	Significance	95% Confidence Interval	
			Lower Bound	Upper Bound
Stable Vs control	8.063 ± 2	0.001[a]	3.2017	12.9253
Crisis Vs control	8.852 ± 2.4	0.001[b]	3.0658	14.6392
Stable Vs crisis	0.788 ± 2.1	0.931[c]	6.0194	4.4415

[a]Significant increase in interferon gamma level in stable condition in comparison to control ($p<0.05$).

[b]Significant increase in interferon gamma level in crisis condition in comparison to control ($p<0.05$).

[c]Non significant difference in the level of interferon gamma between stable and crisis conditions ($p>0.05$).

*SE: Standard Error.

Table 3. Level of interferon gamma in different types.

Parameters	Test Statistic ±S.E*	Significance	95% Confidence Interval	
			Lower Bound	Upper Bound
Disease Vs control	9.182 ± 2.5	0.001[a]	3.1777	15.1880
Trait Vs control	7.978 ± 2	0.001[b]	3.1728	12.7850
Disease Vs trait	1.203 ± 2.2	0.856[c]	4.2239	6.6319

[a]Significant increase in interferon gamma level in sickle cell disease in comparison to control ($p<0.05$).

[b]Significant increase in interferon gamma level in sickle cell trait in comparison to control ($p<0.05$).

[c]Non significant difference in the level of interferon gamma between sickle cell disease and sickle cell trait ($p>0.05$).

*SE: Standard Error.

4. Discussion

Sickle cell anemia is an autosomal inherited disorder of hemoglobin resulting from the homozygous inheritance of the sickle gene. Sickle cell disease (SCD) is caused by a single nucleotide mutation of b-globin which causes polymerization of the abnormal hemoglobin (Hb) upon deoxygenation and dramatic alteration in the shape and surface properties of red blood cells (RBCs). Through interactions with multiple blood and immune cell populations, sickle RBCs promote inflammation, obstruct the vasculature and injure the endothelium, leading to many manifestations that affect most important organs. Patients with sickle cell anemia (HbSS), especially children, have an increased susceptibility to infection leading to increased mortality [20]. SCD crisis are responsible for high morbidity and early mortality. The initial event of life-threatening complications is pain crisis or vaso-occlusive crisis. The pathophysiological basis of these illnesses is end-organ damage due to ischemia and infarction associated with the effects of hemolysis that result from red blood cell sickling. These pathological changes can occur acutely and lead to a various clinical manifestations [21].

Sickle cell disease SCD is the most common form of haemoglobinopathy worldwide. The SCD is most common among people from Africa, India, the Caribbean, the Middle East, and the Mediterranean. However, statistical data about the prevalence of SCD in the Arab world is patchy. The first documentation of abnormal HbS in the Middle Eastern countries recorded from Egypt. More than 200000 infants are born with SCD in Africa every year, while in the United States, about 72000 people affected with SCD. Sickle cell disease is also associated with significant mortality. The increased mortality rate occurs among children between 1 and 3 years of age and adolescents younger than 20 year. However, recently, the mortality rate decreased due to early diagnosis via newborn screening program, better medical care, and education of family members [22]. Approximately 2-27% of the Saudi population is SCD trait carriers and the Saudi Premarital Screening Program estimates that 0.26% of the adult population is trait carriers and that 4.2% of the population is affected by SCD [23].

IFN-γ is cytokine representing TH1/TH2 types, that producing by secreting cytokines CD4+ T lymphocyte influences the functions of virtually all other cells of the immune system, including other T cells, B cells, macrophages, and natural killer cells. Two functionally distinct subsets of Helper T cells secrete cytokines which promote the activities of TH1 subset of CD4+ cells producing IL-2, IFN-γ and TNF-β with IFN- γ thus ultimately activate T cell and macrophages to stimulate cellular immunity and inflammation [24]. The high level of IFN- γ

may cause inflammation and tissue damage in sickle cell patients, thus worsening morbidity and mortality. IFN-γ was significantly higher among in sickle cell patients in comparison to healthy control as shown in table 1 although it is of non-significant difference in its level between stable sickle cell patients and sickle cell patients in crisis this may be due to small number of patients in crisis in comparison to stable patients and it also agreed with the study of Nnodimteam [25] who reported a significant higher IFN-γ in SCA patients while Musa team[26] found no significant difference in SCA patients .

Also, there is high level of IFN- γ which is statistically significant higher among in HbSS patients and sickle cell trait patients in comparison to healthy control as shown in table 2 although it is of non-significant difference in its level between HbSS patients and sickle cell trait patients this may be due to small number of HbSS patients in comparison to sickle cell trait patients.

5. Conclusion

The results showed statistically significant increase in the level of interferon gamma in SCD patients with stable and crisis conditions in comparison to healthy control. Interferon gamma may play a role in pathogenesis of inflammation in sickle cell individuals in the steady state or in crisis.

6. Ethical Consideration

Ethical approval for this study was obtained from the Ethics Review Committee of the College of Applied Medical Sciences at Al-Taif University and Ethics Review Committee of Faculty of Medicine Ain Shams University (RAC #207008). Moreover, all patients included in this study were informed of the study objectives and a written signed consent was taken from each one of them or from first degree relative.

7. Conflicts of Interest

The author(s) report(s) no conflict(s) of interest(s). The author along are responsible for content and writing of the paper.

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