Serum Levels of Gamma-interferon and Interleukin-4 in Homozygous Sickle Cell Anaemia Patients

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Authors' contributions

This work was carried out in collaboration between all authors. Authors OTO and WAS designed the study, wrote the protocol and wrote the first draft of the manuscript. Author AAI managed the literature searches and participated in drafting of the manuscript. Authors GAA, POO and AOL participated in design of the study and protocol. Author AFF managed the data analyses and interpretation. All authors read and approved the final manuscript.

Article Information

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ABSTRACT

Background: Impaired leucocytes functions is among mechanisms that have been reported to account for the immunocompromised state of patients with sickle cell disease.
Objective: This study assessed cellular immunity using serum IFN-γ and IL-4 levels in patients with sickle cell anaemia (SCA).
Methods: The study comprised of 40 sickle cell anaemia patients in steady state (asymptomatic for at least 4 weeks) and 40 age and sex-matched healthy HbA control. Serum IFN-γ and IL-4 was determined by Enzyme linked immunosorbent assay (ELISA) as described by the manufacturer of the kit.

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Results: There was a significant increase in the IFN-γ level in sickle cell anaemia patients in steady state (median value 86.1 pg/ml) compared with HbA controls (median value 55.8 pg/ml) (p=0.04). However, there was no significant difference in the median values of IL-4 level between the HbS (homozygous inheritance of sickle gene) patients and the control subjects (IL-4: p=0.42).
Conclusion: High value of IFN- γ may contribute to inflammation and tissue damage in HbS patients, thus worsening morbidity and mortality.

1. INTRODUCTION

Sickle cell anaemia is an autosomal inherited disorder of haemoglobin resulting from the homozygous inheritance of the sickle gene [1]. It has variable clinical expression some of which include recurrent haemolysis, vasoocclusive crises and recurrent infections with their attendant sequalae. Few are discovered only by chance on routine haematological examination for other conditions because they run a mild course [2].

Reports have shown that patients with sickle cell anaemia (HbSS), particularly children, have an increased susceptibility to infection leading to increased mortality [3,4]. Opsonophagocytic defect due to an abnormality of the alternative complement pathway, deficiency of specific circulating antibodies, impaired leucocytes function and loss of both humoral and cell mediated immunity [5,6] are some of the mechanisms that have been reported to account for the immunocompromised state in patients with sickle cell disease.

By secreting cytokines CD4+ T lymphocyte influence the functions of virtually all other cells of the immune system, including other T cells, B cells, macrophages, and natural killer cells [7]. Two functionally distinct subsets of Helper T cells secrete cytokines which promote the activities of T_H1 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. T_H1 cells also secrete IL-3 and GM-CSF to stimulate bone marrow to produce more leukocytes. T_H2 subset of CD4+ cells secretes IL-4, IL-5, IL-6 and IL-10 with IL-4 being the prototype, which stimulate antibody production by B cells [8]. The balance between T_H1 and T_H2 activity may steer the immune response in the direction of cell-mediated or humoral immunity [8]. Nnodim et al. [9] reported a significant higher IFN-γ in SCA patients in Owerri, Nigeria while Musa et al. [10] found no significant difference in SCA patients in Zaria, Nigeria.

The aim of this study is to compare serum IFN-γ and IL- 4 levels between patients with sickle cell anaemia and healthy control subjects as a surrogate for cellular immunity.

2. MATERIALS AND METHODS

The study was carried out at the University College Hospital, Ibadan, Nigeria. The study population comprised of 40 sickle cell anaemia patients in steady state (asymptomatic for at least 4 weeks) and 40 age and sex-matched healthy HbA control. The SS patients were recruited from Hematology Daycare Clinic, they had full clinical examination and were followed up for 1month to ensure that they had no illness. The control subjects were recruited from member of staff.

Blood samples (10 ml) were collected from the participants using the antecubital vein and allowed to clot for 30 minutes before centrifugation for 15 minutes at approximately 1000 x g. Serum was removed and aliquot samples were stored at -20℃ until assay. Serum was analyzed for IFN-γ and IL-4 by Enzyme linked immunosorbent assay (ELISA) using R & D Systems Quantikine immunoassay Human IFN-γ and IL-4 kits respectively. All serum samples were thawed once at the time of assay. Samples were dispensed into 96-well microtiter ELISA plates pre-coated with monoclonal antibodies to human cytokines (IFN-γ and IL-4) and the plates were incubated for 2 hours at room temperature. The plates were washed thereafter three times with wash buffer (phosphate-buffered saline) and incubated for 2 hours room temperature with horseradish peroxidase (HRP)-conjugated anticytokine antibodies that corresponded to each cytokine tested. The bound enzyme was then detected by incubation in the dark with a substrate- hydrogen peroxide and tetramethylbenzidine (TMB) resulting in colour development. The color development was stopped with stop solution (sulfuric acid) and the intensity of the color was detected by measuring the optical density of each well using a microplate reader (Bio-Rad)

set to 450 nm wavelength. A standard curve was created by reducing the data using computer software capable of generating a four parameter logistic (4-PL) curve-fit.

Data was analyzed using Statistical Package for the Social Sciences (SPSS) version 17 by SPSS, Inc. Chicago, IL, USA. The results were expressed as median, ranges and also pictorially presented in Box plots. Statistical significance was calculated using Mann Whiney U test. The level of significance was <0.05.

3. RESULTS

The study comprised of 80 participants (40HbS and 40HbA). The median age of the SCA patients was 25.5 years which was comparable with that of the control with median age of 27 years (p=0.35) as shown in Table 1. The age range was between 16 and 40 years for the patients and 18 to 38 years for the control.

3.1 Comparison of IFN-γ and IL-4 between HbS Patients and Controls

Table 2 shows the serum levels of IFN-γ and IL-4 of the HbS patients and control HbA subjects. HbS patients had significantly higher median IFN-γ level than the control HbA subjects (p=0.04) but IL-4 level did not differ in both groups (p=0.42).

3.2 IFN-γ Profile of the HbS Patients and the Control HbA Subjects by Gender

Fig. 1 shows that the female HbS patients had significantly higher median values of IFN-γ 88.3 pg/ml (4-350 pg/ml) than the control HbA subjects 59.8 pg/ml (0.8-313.4 pg/ml) (*p=0.01*). Male HbS patients had non-significantly higher median IFN-γ 77.6 pg/ml (4.6-199.3 pg/ml) than their male control counterpart 51.5 pg/ml (4.6-160 pg/ml). Female HbS patients had higher median IFN-γ level than male HbS patients but no significant difference (*p=0.82*). The female HbA control had higher median value of IFN-γ than the male HbA control however there was no significant difference (*p=0.54*).

3.3 IFN-γ Profile of the HbS Patients and the Control HbA Subjects by Age Groups

As shown in Table 3, in the age group lesser than 20 years, the serum IFN-γ value was higher among HbS than the control subjects but not statistically significant. In age group 21-30 years IFN-γ value was significantly higher among HbS than the controls (p= 0.02). Overall IFN-γ is significantly higher among HbS compared to HbA controls (p=0.04).

3.4 IL-4 Profile of the HbS Patients and the Control HbA Subjects by Gender

As shown in Fig. 2, male HbS patients had lower median IL-4 value, 61.3 pg/ml (8.3-117.9 pg/ml) than their counterparts in the control group, 67.4 pg/ml (4.6-160 pg/ml) however the difference was not significant (p=0.44). The female HbS patients also had lower median IL-4 value 70.5 pg/ml (3.1-150.4 pg/ml) than their counterparts in the control group, 86 pg/ml (13.8-190.1 pg/ml), the difference was also not significant (p=0.28). Female HbS patients had higher median IL-4 than male HbS patients but no significant difference (p=0.29). The female HbA control had higher median value of IL-4 than the male HbA control however there was no significant difference (p=0.87).

Table 1. Demographic characteristics of the HbS and HbA subjects

Variables	HbS n=40 No (%)	HbA n=40 No (%)	Total n (%)	*p* value
Age (years)				
<20	13 (32.5)	5 (12.5)	18 (22.5)	
21-30	18 (45)	22 (55)	40 (50)	0.35
31-40	9 (22.5)	13 (32.5)	22 (27.5)	
Sex				
Male	18 (45)	15 (37.5)	33 (41.3)	
Female	22 (55)	25 (62.5)	47 (58.7)	0.50

Table 2. Comparison of IFN-γ and IL-4 between HbS patients and controls

Variables	HbS patient n=40	Control n=40	Z	*p* value*
	Median	**Median**		
IFN-γ(pg/ml)	86.1	55.8	1.98	0.04
IL-4(pg/ml)	67.4	76.7	0.81	0.42

Mann- Whitney U test

3.5 IL-4 Profile of the HbS Patients and the Control HbA Subjects by Age Groups

As shown in Table 4 among the HbS patients in the age group lesser than 20 years, IL-4 was lower than the control subjects. These value statistically differed from each other (*p*=0.03). In age group 21-30 years, IL-4 in the HbS patients was higher than that in the control subjects but the difference was not statistically different from each other (*p*=0.69). Among the HbS in the age group 31-40 years, IL-4 was lower than the control subjects. These values were not statistically different from each other (*p*=0.41). Overall, the total IL-4 value did not differ between HbS and HbA controls (*p*=0.42).

4. DISCUSSION

Acute or chronic inflammation can contribute to morbidity and mortality in patients with sickle cell anaemia. In this study, IFN-γ was significantly higher among HbS patients than control but this was not affected by gender. This difference might be accounted for by subclinical chronic inflammatory state in HbS patients. This is in agreement with previous studies. [11,12,13] but at variance with that of Taylor et al[14] who reported no significant difference in the IFN-γ level between HbS and HbA individuals. The difference observed in this study might be due to different ELISA kit used. It is noteworthy that the difference in IFN-gamma levels between controls and SCA subjects was found only for the 21-30 year-old groups. Further larger studies might be able to shed light on the age-dependency. This may indicate an increase predisposition to inflammation by this age group. IFN-γ level was found to be significantly higher in female HbS patients as compared to their female HbA counterparts. However, IFN-γ value was not affected by gender status both in HbS patients and control population. This was in agreement with Raghupathy et al. [15] who found that there was no gender bias on the IFN-γ level. The increase in IFN-γ level in female HbS patients in this study may be due to the disease couple with hormonal factor in line with Karpuzoglu-Sahin et al. [16] who found that estrogen increases the production of IFN-γ.
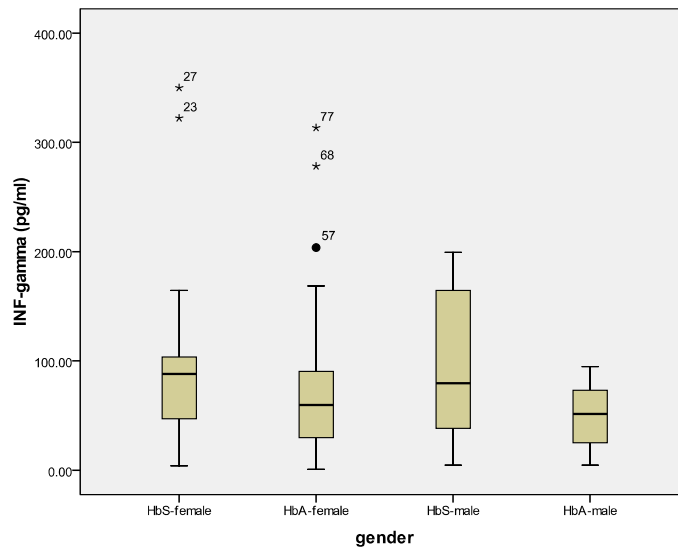

Fig. 1. IFN-γ profile of the HbS patients and the control HbA subjects HbS patients and the control HbA subjects by gender

("" - outliers)*

Table 3. IFN-γ profile of the HbS patients and the control HbA subjects by age

Variable age (years)	n(%)	HbS median Pg/ml	n(%)	HbA median Pg/ml	Z	*p*-value
<20	13 (32.5)	99.1	5 (12.5)	90.4	0.20	0.84
21-30	18 (45)	88.3	22 (55)	55.8	2.40	0.02
31-40	9 (22.5)	38.5	13 (32.5)	34.2	1.54	0.12
Total	40	86.1	40	55.8	1.98	0.04

Table 4. IL-4 profile of the HbS patients and the control HbA subjects by age groups

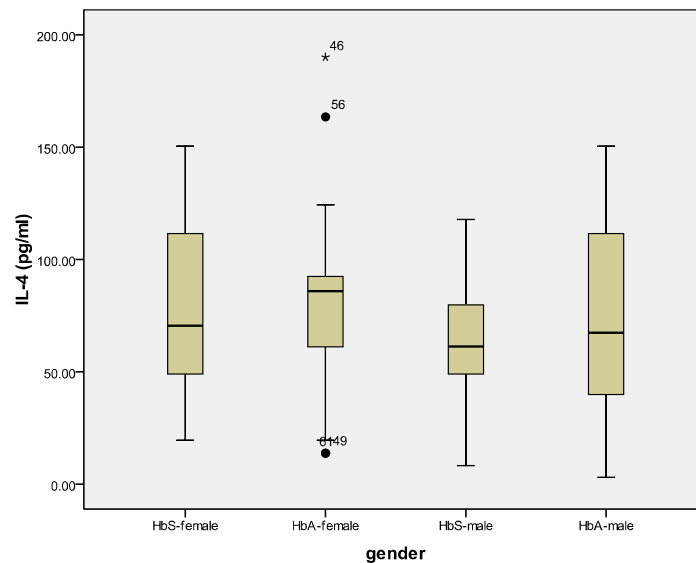| Variable age (years)	n (%)	HbS median (Pg/ml)	n(%)	HbA median (Pg/ml)	z	*p*-value
<20	13 (32.5)	67.4	5 (12.5)	111.5	0.20	0.03
21-30	18 (45)	73.6	22 (55)	61.2	0.40	0.69
31-40	9 (22.5)	61.2	13 (32.5)	79.8	0.82	0.41
Total	40	67.4	40	76.7	0.81	0.49

Fig. 2. Interleukin-4 values of the HbS patients versus controls (HbA subjects) by gender

Miller et al. [17] found that the synthesis of HbF was significantly downregulated by IFN-γ. HbF has been identified as a major factor influencing survival of the female HbS patients compared to the male HbS counterparts with amelioration of clinical severity and improvement in survival. The rise in IFN-γ level and degree of negative influence on the production of HbF has to be further evaluated in HbS patients. Marcal et al. [18] recorded significantly higher IFN-γ in HbS patients and implied that this may contribute to inflammation and tissue damage in these patients. A combination of increased inflammation and possible downregulation of HbF in our patients may combine to increase morbidity.

HbS patients in this study had comparable IL-4 values with the HbA individuals. This is in agreement with Pathare et al. [11] and Musa et al. [10] who found that there was no significant difference in the levels of IL-4 level in HbS and HbA individuals in Oman and Nigerian population respectively. Taylor et al. [19] found significantly higher level of IL-4 in HbS patients than the HbA control subjects in the U S A. This difference observed in this study might be due to difference in geographical location or population. The lack of difference in the levels of immunoglobulin classes between HbS patients and HbA controls, observed by Olaniyi et al. [20] could be explained by lack of increase in IL-4, which is important to antibody production. IL-4 was not affected by

gender in this study and this is in agreement with Raghupathy et al. [15].

5. CONCLUSION

This study showed that the IFN-γ level in HbS patients was significantly higher than in HbA individuals. There was no significant difference in IL-4 in HbS patients and HbA controls.

The high value of IFN- γ may contribute to inflammation and tissue damage in HbS patients, thus worsening morbidity and mortality. Anti-inflammatory medications such as low dose aspirin and Gamma Linoleic acid may have a future role in the management of sickle cell disease patients. It is hereby suggested that IFN-γ may be further explore as an index for assessing severity of sickle cell disease.

6. LIMITATION OF THE STUDY

Only 2 cytokines, IFN-γ and IL-4 representing TH1/TH2 types were considered in this study as examples of pro- and anti-inflammatory cytokines. A larger sample and addition of other cytokines could have enhanced our findings.

CONSENT

Informed written consent was obtained from all the participants.

ETHICAL APPROVAL

Ethical approval was obtained from the University of Ibadan/ University College Hospital (UI/UCH) ethics committee (UI/EC/09/0128).

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COMPETING INTERESTS

Authors have declared that no competing interests exist.

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