


Immuno-Haematological Characteristics of Nigerian Sickle Cell Disease Patients in Asymptomatic Steady State

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ABSTRACT

Aim: The aim of this study is to investigate some immuno-haematological characteristics of Nigerian sickle cell disease (SCD) patients in asymptomatic steady state.

Material and Methods: Thirty asymptomatic SCD patients and 30 apparently healthy age- and sex-matched non-sickle cell disease individuals were investigated. The packed cell volume, white blood cells and differentials, and platelet counts were done on automated blood cells counter, while the ESR was determined by Westergren's technique. C3 activator, C1-INH, IgA, IgG and IgM were estimated by the single radial immuno-diffusion method.

Results: The SCD patients had elevated ESR and a significantly higher total leukocyte count compared to the controls ($t= 5.22$, $p= 0.000$). A positive correlation was found between ESR and C3 activator ($r= +0.449$, $p= 0.047$), and between ESR and serum IgM levels ($r= +0.531$, $p= 0.016$). Serum levels of IgA and C3 activator were significantly higher in SCD subjects (IgA: $t= 2.47$, $p= 0.019$; C3 activator: $t= 2.79$, $p= 0.009$), while the levels of C1-INH and IgM, though higher in SCD subjects, were not significant.

Conclusions: It could be concluded from this study that immune dysfunction are evident in Nigerian SCD patients.

Keywords: Immuno-haematological parameters, sickle cell disease, asymptomatic steady state, Nigeria.

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INTRODUCTION

Sickle cell disease (SCD) is an inherited disorder of haemoglobin that results in chronic haemolysis. Affected individuals are prone to frequent and sometimes severe infections. Several factors predisposing SCD individual to infections have been reported, and included abnormalities of opsonins and antibody production, defects in the alternate complement pathway, leukocyte functions, and cell-mediated immunity (1,2). The spleen which is uniquely positioned to bring circulating antigen into close contact with antigen presenting cells of the reticuloendothelial system in the spleen is also lost early in life as a result of fibrosis resulting from recurrent vaso-occlusive crisis leading to defective function of the organ.

Significantly low serum levels of complement components C3 and C4 have been reported in SCD patients by several investigators (3-5) which are responsible for the low opsonization and chemotactic functions and which explains the high susceptibility of these patients to infections. Chudwin et al. (6) in their study found increased alternative pathway activation in sera from patients with SCD, an indication of impaired host defense in them. The increased activation has been attributed to factors such as dense irreversibly sickle cells, membrane spicules, vesicles derived from such cells, or membrane phospholipids (7, 8). Complement component C1 esterase inhibitor (C1-INH) is an inhibitory protein in the classical pathway of complement system through the inhibition of C1r and C1s. This protein (C1-INH) also interact with C3b to inhibit binding of factor B (C3 activator) to C3b to form C3bBb (alternate pathway C3 convertase) in the alternate pathway.

Immunoglobulins are serum proteins that help fight infections and also involved in the activation of the complement system. Several investigators have reported varying serum concentrations for these immunoglobulins in steady state asymptomatic SCD patients. Dieye et al. (4) and Natta and Outschoorn (9) reported high levels of IgA and IgG, but reduced and normal serum IgM levels in their separate studies, respectively. While Adeodu et al (10) reported significantly high serum IgA and IgM; Mohamed et al (11) did not find any significant difference in the complement and immunoglobulin levels in their SCD subjects. The objective of this study is to investigate some of the immuno-haematological features of Nigerian SCD patients in asymptomatic steady state.

MATERIAL and METHODS

Study Population

Sickle cell disease patients in steady state that presented at the Haematology and Paediatric clinics of the Obafemi Awolowo University Teaching Hospitals Complex (OAUTHC), Ile-Ife, between November, 2003 and March, 2005 were recruited into the study. Apparently healthy age- and sex-matched non-sickle cell disease patients were enrolled as controls. Each patient was assessed clinically to confirm steady state. Some demographic data (age, gender, weight and height) of both the subjects and controls were also documented.

Laboratory Studies

Blood samples were taken, in appropriate bottles, for blood counts (packed cell volume, white blood cells and differentials, platelets, erythrocyte sedimentation rate), serum immunoglobulins (IgG, IgM, and IgA) and complement proteins (C1 esterase inhibitor and C3 activator). Haematological parameters were estimated within six hours of sample collection while serum immunoglobulins and complement protein samples were stored at temperature of -20°C and estimated in batches.

The packed cell volume, white cells and platelet counts were done on automated counter (ADVIA-60 Bayer Corporation, New York, United States of America) and ESR was determined by Westergren's technique (12). C3 activator, C1-INH, IgA, IgG and IgM were estimated by the single radial immuno-diffusion method of Salimonu et al (13), using monospecific antisera (Dade Behring Marburg GmbH, Marburg, Germany).

Statistical Analysis

Data are presented as means and standard deviations (means±SD). Student's t-test was used to test the significance of differences between mean values. Spearman's correlation coefficient and multiple regression analysis were computed where necessary. Probability (p) value greater than 0.05 was considered insignificant. SPSS (SPSS inc., release 11.0.1; 15 November 2001) statistical software was used for all statistical analyses.

RESULTS

Demographic Variables

A total of sixty subjects were investigated. Their clinico-pathologic characteristics are as documented

Table 1. Mean \pm SD of demographic and laboratory parameters of both SCD patients and non-SCD subjects.

Parameter	S	C	t	p
Age (yr)	15.9 \pm 7.5	13.6 \pm 9.8	1.02	0.312
Height (cm)	144.7 \pm 25.1	135.7 \pm 36.9	1.10	0.274
Weight (kg)	38.8 \pm 16.2	39.5 \pm 23.9	0.13	0.895
PCV (%)	22.2 \pm 4.7	38.5 \pm 5.5	12.34	0.000
ESR (mm/hr)	36 \pm 34.7	27.9 \pm 22.7	1.19	0.239
Platelet ($\times 10^9/L$)	197517 \pm 82706	208033 \pm 38107	0.63	0.530
WBC ($\times 10^9/L$)	11716 \pm 4594	6543 \pm 2886	5.22	0.000
Neut ($\times 10^9/L$)	44.9 \pm 17.4	43.1 \pm 11.4	0.47	0.637
IgG (mg/dl)	644.6 \pm 171.2	660.6 \pm 169.7	0.27	0.790
IgM (mg/dl)	93.4 \pm 100.7	71.8 \pm 69.4	0.69	0.493
IgA (mg/dl)	249 \pm 94.9	179.9 \pm 51.9	2.47	0.019
C1 INH (mg/dl)	27.0 \pm 20.2	25.8 \pm 6.70	0.21	0.838
C3 act (mg/dl)	17.1 \pm 7.4	10.6 \pm 4.90	2.79	0.009

S= subject with sickle cell disease, C= control, PCV= packed cell volume, WBC= white blood cell, Neut= neutrophils, ESR= erythrocyte sedimentation rate in the first hour, Ig= immunoglobulin (A, G, M), C1 INH= C1 esterase inhibitor, C3 act= C3 activator, kg= kilogramme, cm= centimeter, yr= year, %= percentage, mm/hr= millimeters in the first hour, mg/dl= milligram per deciliter.

on Table 1. They are made up of thirty SCD subjects in steady state and 30 age- and sex-matched apparently healthy control subjects. Of the thirty SCD subjects, 12 were males while the rest 18 were females, with a male to female ratio of 1: 1.5. The thirty age- and sex-matched non-SCD disease patients were made up of 14 males and 16 females, with a male to female ratio of approximately 1:1. In all, more females were investigated, with a male to female ratio of approximately 1: 1.03. This is not statistically significant. The mean age of the SCD group was 15.9 \pm 7.5 years, while that of the control group was 13.6 \pm 9.8 years. There was no significant difference in their mean ages, weights and heights.

Laboratory Characteristics

Expectedly, the haematocrit was significantly lower in SCD subjects (t= 12.34, p= 0.000) compared to the control subjects, while the white cells count was significantly higher in SCD subjects (t= 5.22, p= 0.000). However, unexpectedly the mean ESR value in the SCD patients was insignificantly higher compared with the control subjects, while the mean platelet count was slightly lower in the SCD subjects, though this is not significant. Serum levels of IgA and C3 activator were significantly higher in SCD subjects (IgA: t= 2.47, p= 0.019; C3 activator: t= 2.79, p= 0.009). Serum C1 INH and IgM levels though higher in SCD

subjects, were not significantly elevated, while serum IgG was slightly higher in the controls.

The ESR and PCV, as expected, showed significant negative correlation in both groups (S: r= -0.364, p= 0.048; C: r= -0.438, p= 0.016). In the SCD patients, a strong positive correlation was found between ESR and C3 activator (r= +0.449, p= 0.047), and between ESR and serum IgM levels (r= +0.531, p= 0.016). In the controls, the ESR showed significant positive correlation with white cells (r= +0.433, p= 0.017), while a significant negative correlation was also found between the C3 activator and IgA (r= -0.643, p= 0.018).

DISCUSSION

Sickle cell disease patients are prone to frequent and sometimes fatal infections as a result of some abnormalities of the immune system, including those of the spleen, complement proteins, immunoglobulins, leukocyte functions and cellular immunity. This study was designed to investigate some aspects of immuno-haematological characteristics of Nigerian SCD patients in steady asymptomatic state. Complement component C3 activator, also known as alternative pathway factor B, is a regulatory protein which combines with unstable C3b to form the more stable C3bBb that is capable of activating more C3 in the alternative pathway. Activation of the alternative

pathway of complement is associated with a decrease in the serum level of C3 activator. In this study, significantly high serum levels of C3 activator was found in SCD patients compared to the controls. This is similar to the findings of Donadi et al. (14) in Brazil who reported elevated factor B and C3 complement component in asymptomatic SCD patients and those of Rao (15) that reported elevated C3b and factor P, all indicating a defective alternative pathway of complement activation SCD patients. Complement component C1 esterase inhibitor (C1-INH) is an important regulator of many plasma mediator pathways, including the complement system. Jiang et al. (16) in their study showed that C1-INH inhibits alternative pathway activation by inhibiting the activities of factor B and the cleavage of C3 indirectly through C3b and that the removal of C1-INH restored remarkably the activities of the pathway. Our study showed higher serum C1-INH in the SCD patients compared to the controls, which could also suggest a defective alternative complement pathway in Nigerian SCD patients. Immunoglobulin A (IgA) was found to be significantly higher in our cohort of SCD patients similar to reports by other investigators (5, 10, 14). High levels of IgA are associated with hepatic dysfunction, cholelithiasis, haemolysis and post-splenectomy state, all of which are features not uncommonly seen in SCD patients and which could not be ruled out as a cause of the high IgA seen our in patients.

Erythrocyte sedimentation rate (ESR) is an old and widely used laboratory indicator of inflammation. The rate of red cell sedimentation is dependent on several factors including plasma proteins such as fibrinogen, alpha-2 macroglobulin and immunoglobulins (17). In SCD, because of the abnormal red cell shape, red cell rouleaux formation is less thereby reducing erythrocyte sedimentation. The higher ESR found in our SCD subjects could probably result of anaemia and higher serum proteins including immunoglobulins and complement proteins, which can also influence red cell aggregation and which are found at higher concentrations in them. It is also noteworthy that ESR correlated positively with IgM but not IgG or IgA in this study. Immunoglobulin M is a pentamer with ten potential combining sites, thereby making it a better agglutinator of red cells to enhance sedimentation. In a study of correlates of inflammation in patients with rheumatoid arthritis, Verma et al. (18) similarly found IgM to correlate more with disease activity than IgG or IgA. The significant positive correlations found between ESR and C3 activator is a

confirmation of it being classified as a positive acute phase protein (19). In the control subjects, the positive correlation between white cell count and ESR is a further confirmation that leukocytosis is one of the systemic responses to inflammation. It is of interest to note the strong negative correlation between IgA and C3 activator in the control subjects. Aggregated IgA is an activator of the alternative pathway, while the C3 activator is a regulator protein in the same pathway. Decreased values of C3 activator are found when the pathway is activated, and which probably explains the negative relationship between the two proteins.

High platelet count is the usual finding in most patients with SCD in asymptomatic steady state, except in crisis situation such as vaso occlusive crisis (20). The mean platelet count in our SCD patients was lower than the control, though the difference is not significant. Studies have shown that minor episodes of microvascular occlusion do occur in the so called asymptomatic steady state (21) which though insufficient to cause the overt painful crisis, but may consume some platelets. This phenomenon could probably explain the lower platelet count in our SCD patients.

It could be concluded from this study that complement dysfunction and abnormality of immunoglobulins are evident in Nigerian SCD patients and that in the so called asymptomatic steady state; inflammatory processes are ongoing as earlier reported and as shown by increase in some markers of inflammation assessed in these patients.

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