

Original Article

Complement Levels in Nigeria Patients with Sickle Cell Anaemia in the Asymptomatic State

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ABSTRACT

Objectives: Significantly low serum levels of complement components have been reported in patients with Sickle cell anaemia (SCA) which may result in a high susceptibility to infections. However some studies have reported a normal or high complement level. The aim of our study was to assess some haematological parameters and Complement levels in adult patients with steady state SCA in Zaria, North western Nigeria. This will serve as a guide towards infection surveillance in the quest to reduce significant morbidity and mortality in our setting.

Methods: A cross sectional study of 40 patients with SCA in steady state and 40 healthy (non-matched) non-SCA controls at the Haematology clinic of Ahmadu Bello University Teaching Hospital Zaria, was carried out over a 6 month period in 2016. Institutional ethical approval and informed written consent were obtained. Venous blood was analyzed for haematological parameters by an automated method and complement levels; alternative pathway was assessed by measuring C3, classical pathway by C4 and Terminal pathways by C5 levels using ELISA technique. Data was analyzed with statistical package for social science (SPSS) software version 20.0(2001) and a p value of ≤ 0.05 was considered significant.

Results: The age range of patients with SCA and controls were 18 to 46 years and 18 to 48 years respectively and these were not significantly different $p > 0.05$. The median levels of C3 (245 $\mu\text{g/ml}$), C4 (245 $\mu\text{g/ml}$) and C5 (40 $\mu\text{g/ml}$) were within normal range in the study subjects but significantly higher ($p < 0.05$), than those of the controls C3 (165 $\mu\text{g/ml}$), C4 (125 $\mu\text{g/ml}$) and C5 (35 $\mu\text{g/ml}$). Haematological parameters showed no correlation with the levels of Complement components studied. **Conclusion:** Although Complement components C3, C4, and C5 levels in patients with SCA are significantly high, the infectious susceptibility may be due to other immunological abnormalities.

KEYWORDS: Complement C3, C4, and C5, infection surveillance, sickle cell anemia

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INTRODUCTION

Homozygous sickle cell disease (SCD) and sickle cell anemia (SCA) affect about 2% of Nigerians at birth.^[1] The World Health Organization estimated that about 20–25 million individuals worldwide have SCA; 12–15 million in sub-Saharan Africa,^[2,3] and in Nigeria, >150,000 children are born with the disease annually and 4 million people are afflicted.^[3-5] The clinical manifestation of this disease is mainly due to the repeated vaso-occlusion, chronic intravascular

hemolysis, microvascular ischemia, and organ damage.^[5] In the absence of intervention, bacterial infection is the leading cause of morbidity and mortality in individuals with SCA^[6] due to a weakened immune function. This may be as a result of impaired splenic function,

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autosplenectomy,^[7] micronutrient deficiency, and poor complement activation and/or low complement levels. Complement are serum glycoproteins secreted mostly by hepatocytes^[8] either constitutively or in response to noxious stimuli.^[8,9] The major functions of the complement system include lysis of infectious organisms, activation of inflammation, opsonization, and immune clearance. The levels of complement may thus influence infection susceptibility.

Tamouza *et al.*,^[10] in their study, reported that major infections occur when the spleen is still partially functional and some increased risk persists despite modern prophylactic measures, suggesting additional immune deficits are present. Subsequently, Anyaegbu *et al.*^[11] also showed an inverse correlation between complement activity and the number of crises suffered by patients with SCA. The findings of Salawu *et al.*,^[12] in Ile-Ife, Nigeria, and Donadi *et al.*,^[13] in Brazil, both reported significantly high serum levels of C3, C4, and C5 in asymptomatic steady-state patients with SCD compared to controls. However, in contrast to these studies, Wilson *et al.*,^[14] in Jamaica, and Dieye *et al.*^[15] reported that concentrations of C3 and hemolytic factors B and D were significantly lower in asymptomatic patients with SCD than in controls.

Endemic infections such as pneumonia, urinary tract infection, and enteric infections are common in patients with SCA. If complement levels are low, this may be responsible for the increased frequency of sickle cell crisis and increased morbidity and mortality in these patients. Despite the fact that patients with SCA have an identical genotype, the severity of the disease can be extremely variable. The sickle mutation has been described on five different haplotypes in different geographical areas with different clinical expression. This has led to phenotype diversity observed in these patients for prognostic purposes, accurate diagnosis, and thus planning for the best-tailored treatment. At Ahmadu Bello University Teaching Hospital, Zaria, Northwestern Nigeria, no studies have been done on the complement components in patients with SCA. Therefore, assessment of the complement levels in these patients will guide in infection surveillance while appraising the management of these patients in our environment. The objective of this study is to determine, compare, and review some hematological parameters and the levels of complement components C3, C4, and C5 in adult patients with SCA in steady asymptomatic state.

MATERIALS AND METHODS

The study was approved by the Ethical and Scientific Committee of Ahmadu Bello University, Zaria, Nigeria.

The research participants were adult Nigerians, 18 years and above of both genders who volunteered and provided written informed consent. Patients were recruited from the hematology clinic. Exclusion criteria included individuals unable to understand the investigational nature of the study or to give informed consent. Pregnant women and patients with chronic debilitating illnesses (tuberculosis, HIV, diabetes, and malignancies) were also excluded from the study.

A total of 40 patients with SCA and 40 controls (prospective blood donors with low number of female's limits full sex-match) were recruited into this study. Participants underwent complete history taking and physical examination as well as laboratory investigations. Venous blood was collected into an ethylenediaminetetraacetic acid bottle, complete blood count determined using multiparameter hematology analyzer XT-2000i, and erythrocyte sedimentation rate (ESR) using Westgren Method, and the serum was analyzed for complement assay, while the red cells was utilized for alkaline electrophoresis.

On electrophoresis, all patients with SCA showed no hemoglobin A (but showed elevated levels of hemoglobin S [HbS]) consistent with HbSS phenotype, while the control group showed no HbS, consistent with hemoglobin AA (HbAA).

Specific Enzyme-Linked Immunosorbent Assay kits Wkea (www.wkeamedsupplies.com) were used for human complement C3, C4, and C5 assay (double-antibody technique-quantitative method), according to manufacturer's procedure using the stored serum. The optical density was determined using a microplate reader (Bio-Rad) set at 450 nm wavelength.

Data were analyzed using Statistical Package for the Social Sciences software version 20.0 (2011, Armonk, NY: IBM Corp). The serum complement component C3, C4, and C5 concentrations of both the patients and controls were compared using Student's *t*-test or Mann-Whitney U-test as appropriate and tested for differences between the mean or median values. The outcomes with $P \leq 0.05$ were considered to be statistically significant.

RESULTS

A total of 40 consecutive steady-state patients with SCA and 40 healthy nonmatched controls were studied in Zaria, Nigeria. However, only 38 patients with SCA and 37 controls could be analyzed for complements' C3, C4, and C5 levels after data cleaning. The age range of the patients was 18–46 years, whereas the controls were between 20 and 48 years, although the mean age of the patients (26.50 ± 6.79 years) was significantly lower

Table 1: Demographic and hematological parameters

Variable	SCA (n=40)	Control (n=40)	P
Age (years), mean±SD	26.50±6.79	30.90±6.55	0.004
Female (%), mean±SD	25 (62.5)	10 (25)	
Hematocrit (%), mean±SD	23.8±4.56	34.70±3.63	<0.0001
Platelets (×10 ⁹ /L), mean±SD	445.60±158.38	274.3±66.01	<0.0001
Neutrophils (%), mean±SD	55.30±11.06	51.80±13.18	0.202
Lymphocytes (%), mean±SD	42.90±11.03	45.90±13.72	0.288
Total WBC (×10 ⁹ /L), median (range)	8.995 (7.3-10.0)	5.2 (4.6-6.6)	<0.0001
Reticulocytes count (%), median (range)	6.65 (5.4-8.5)	0.35 (0.2-0.6)	<0.0001
Monocytes (%), median (range)	1.0 (0.0-1.8)	0.0 (0.0-1.0)	0.067
Eosinophils (%), median (range)	1.0 (0.0-1.8)	1.0 (0.0-2.0)	0.239
ESR (mm/h), median (range)	10.5 (8.0-15)	6.0 (4.0-9.5)	<0.0001

SCA=Sickle cell anemia; SD=Standard deviation; WBC=White blood cell; ESR=Erythrocyte sedimentation rate

Table 2: Serum levels of complement C3, C4, and C5

Complement components (µg/ml)	Median (range)		P
	SCA (n=38)	Controls (n=37)	
C3	245 (215-327.5)	165 (160-192.5)	<0.0001
C4	245 (185-347.5)	125 (120-160.0)	<0.0001
C5	40 (32-50.0)	35 (30-40.0)	0.047

Mann-Whitney-u test - Mean Rank [Patients, Control, C3 (52.92, 22.68), C4 (51.49, 24.15), C5 (42.82, 33.05)]. SCA=Sickle cell anemia

Table 3: Correlation of Haematological parameters and complement C3, C4 and C5 levels

Haematological parameters	SCA n=38 complements						Controls n=37 complements					
	C3		C4		C5		C3		C4		C5	
	r	P	r	P	r	p	r	P	r	P	r	P
Haematocrit	-0.14	0.403	-0.132	0.431	-0.309	0.059	-0.103	0.542	-0.126	0.457	0.026	0.877
WBC	-0.146	0.382	-0.119	0.475	0.125	0.456	-0.074	0.661	-0.193	0.252	0.015	0.928
Platelet	-0.270	0.101	0.014	0.934	-0.001	0.995	-0.08	0.639	0.252	-0.009	0.261	0.119
Reticulocytes	0.261	0.113	0.046	0.782	-0.087	0.602	-0.03	0.858	0.025	0.883	-0.23	0.171
Neutrophils	0.146	0.380	0.066	0.692	-0.125	0.456	-0.043	0.801	-0.069	0.686	0.098	0.564
Lymphocytes	-0.111	0.508	-0.045	0.787	0.132	0.429	-0.013	0.94	-0.014	0.937	0.132	0.435
Monocytes	-0.422	0.008	-0.247	0.135	-0.164	0.325	0.222	0.186	0.136	0.421	0.200	0.236
Eosinophils	-0.11	0.509	0.005	0.976	-0.089	0.594	-0.068	0.688	-0.039	0.821	0.073	0.667
ESR	0.120	0.473	0.171	0.305	0.007	0.968	-0.106	0.531	-0.233	0.164	0.134	0.430

r=coefficient of correlation, P=P value, WBC=Total white cell count, ESR=Erythrocyte Sedimentation rate

than that of the control 30.95 ± 6.55 years $P = 0.004$. Of the 40 patients with SCA, 25 (62.5%) were females, while out of the 40 controls, only 10 (25%) were females [Table 1]. Patients with SCA (study subjects) had significantly lower hematocrit (23.8% ± 4.56%) compared with 34.7% ± 3.63% ($P < 0.0001$) in the controls (HbAA) [Table 1]. In addition, the platelet count of the patients was significantly higher than that of the control [Table 1]. Although the median total white blood cell and reticulocyte counts and ESR of the patients with SCA were significantly higher ($P < 0.0001$) than that of the control [Table 1], there was no significant difference in their differential counts (neutrophils, lymphocytes, monocytes, and eosinophils) [Table 1].

The serum levels of complement components C3, C4, and C5 of the patients and controls were relatively

within the normal range though they were significantly higher than that of the controls ($P < 0.05$) [Table 2].

The analysis of complement components C3, C4, and C5 showed a variably weak and insignificant correlation with hematological parameters except for monocytes which showed a significant negative correlation ($P = 0.008$) [Table 3].

DISCUSSION

Patients with SCA are often prone to infections; this may be 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 the levels of complements in adult Nigerian patients with SCA in asymptomatic state.

In this study, the significantly high serum levels of complement components C3 and C4 found in patients with SCA compared to the controls are similar to the findings of Al-Awamy *et al.* in the Eastern Province of Saudi Arabia,^[16] Salawu *et al.*, in Ile-Ife, Nigeria,^[12] Donadi *et al.*^[13] in Brazil who reported elevated factor B and C3 complement component in asymptomatic patients with SCD, and those of Chudwin *et al.*,^[17] who reported elevated C3b and factor P, all indicating a defective alternative pathway of complement activation in these patients. Abnormalities of the immune system have been reported, particularly loss of splenic function which is designed to bring circulating antigen into close contact with the reticuloendothelial system, as such the high levels of C3 and C4 in the SCA groups may be due to the chronic antigenic exposure in our environment. Furthermore, most components are acute phase reactants, and their concentration increases in states of infection, trauma, and injury which may be ongoing at subclinical and microvascular levels.

However, in contrast to this study, Sisson *et al.*,^[18] Wilson *et al.*,^[19] in Jamaica, and Dieye *et al.*^[15] reported that concentrations of C3 and Hemolytic factors B and D were significantly lower in asymptomatic patients with SCD than in controls. Whereas Dieye *et al.*^[15] reported normal concentrations of C4, in Ghana, and Akanmori *et al.*^[20] reported significantly lower levels of complements C3 and C5 which may account for the increased susceptibility to infections. However, Mohamed *et al.*,^[21] in Sudan, showed no alteration or involvement of the complement system or immunoglobulin levels which were not associated with an increased susceptibility to infections in patients with SCA.

The significantly lower hematocrit levels in these patients relatively to the control (hemoglobin phenotype AA) observed in this study is similar to previous reports, and this is as a result of the chronic hemolysis and higher susceptibility to infections.^[12,22] Expectedly, the white cell count was significantly higher in patients with SCA buttressing earlier findings, that this is due to chronic pain resulting in the redistribution of leukocytes between the marginating and circulating pools of leukocytes.^[12,22] In this study, we also observed a significantly higher platelet count in patients with SCA compared to the apparently healthy controls, which is the expected finding in most asymptomatic SCD patients^[22] except in crisis situation such as vaso-occlusive crisis.^[23] However, this is contradictory to the findings of Salawu *et al.*,^[12] who reported lower but statistically nonsignificant mean platelet counts in asymptomatic patients with SCD. Minor episodes of microvascular occlusion (thus consuming some platelets) do occur in the so-called asymptomatic

steady state but may be insufficient to cause the overt painful crisis.^[24] The higher ESR found in this study agrees with the previous reports,^[12,25] and this finding could be as a result of anemia and higher concentrations of serum proteins including immunoglobulin's, fibrinogen, alpha-2 macroglobulin, and complements proteins, which could influence red cell aggregation.^[26]

Platelets are known to function in association with complement to activate complement and vice versa. However, there was no statistically significant correlation between C3, C4, and C5 with platelet counts in this study. The correlation between C3, C4, C5, and other hematological parameters also showed no linear relationship with all the parameters employed. ESR in SCA participants showed a moderate relationship which is consistent with the work of Salawu *et al.*,^[12] and Gabay and Kushner,^[27] where ESR and C3 activator correlated positively, being a strong evidence that C3 is a positive acute phase protein. However, C5 had an inverse moderate relationship with ESR among SCA participants in this study too.

CONCLUSION

This study shows that although complement components are acute phase proteins, there is an increase in C3, C4, and C5 levels in patients with asymptomatic state SCA, indicating lack of immune abnormalities; however, the activity of these complements, immunoglobulin levels, as well as other external factors need to be considered in a future study in order to explain the infectious susceptibility in these patients.

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Conflicts of interest

There are no conflicts of interest.

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