# C1 Inhibitor, C3 Activator, IgG, IgA, and IgM Titers in Nigerian Sickle Cell Disease Patients with *Plasmodium falciparum*

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## ABSTRACT

**Background:** Sickle cell disease (HbSS) is a major health problem in Nigeria and malaria has been implicated as a leading cause of morbidity/mortality in sickle cell disease patients. Few reasons were put forward to explain the observed morbidity/mortality of HbSS subjects due to *Plasmodium falciparum* (*P. falciparum*) malaria. **Objectives:** To determine the level of immunoglobulin classes (IgM, IgA, and IgG) and regulators of complement system (C1 inhibitor and C3 activator) in Nigerian HbSS patients with and without *P. falciparum* parasitemia. **Methods:** A total of 64 subjects were considered, including 10 HbSS genotypic subjects with *P. falciparum* parasitemia (HbSS+PfM), 18 HbAA genotypic subjects with *P. falciparum* parasitemia (HbAA+PfM), 20 HbSS without *P. falciparum* parasitemia (HbSS-PfM), and 16 HbAA genotypic subjects without *P. falciparum* parasitemia (HbAA-PfM). IgM, IgA, IgG, C1 inhibitor, and C3 activator titers were quantified by single radial immunodiffusion method. **Results:** The mean levels of IgG in HbSS+PfM (2373.90±1772.81mg/dl) and HbAA+PfM (1868.80±0.00mg/dl) were significantly higher compared with HbSS-PfM (644.55±171.15mg/dl) or HbAA-PfM (659.75±158.01mg/dl) patients. HbAA-PfM subjects had the lowest level of IgM (67.27±63.7mg/dl), though no significant difference was observed comparing mean levels of IgM between the four groups. IgA titer was significantly higher in HbSS-PfM patients (249.00±94.8mg/dl) compared with HbAA-PfM ($p<0.05$), HbAA+PfM ($p<0.05$), or HbSS+PfM ($p<0.05$). The mean values of C1 inhibitor were lower in HbSS+PfM and HbAA+PfM compared with HbSS-PfM or HbAA-PfM. However, HbAA+PfM had a significantly lower value of C1 inhibitor compared with HbAA-PfM ($p<0.01$). C3 activator was highest in HbSS-PfM (17.10±7.35mg/dl) and was significantly higher compared with HbSS+PfM ($p<0.05$). **Conclusion:** Increased C1 inhibitor and decreased C3 activator in HbSS+PfM compared with HbAA+PfM shows that deranged regulation of complement factors may be responsible for increased susceptibility of HbSS to *P. falciparum* malaria.

**Keywords: Sickle Cell, Malaria, Nigeria**

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## INTRODUCTION

Sickle cell disease refers to a group of genetic disorders characterized by production of hemoglobin S. The most studied structural modification within hemoglobin concerns hemoglobin S (1). Epidemiological studies performed in regions endemic for malaria show that antibody titers to *P. falciparum* are lower in children with sickle cell trait than in children with genes for haemoglobin A and homozygous SS (2). This observation was supported by Akinyanju (1). Marsh found that sickled haemoglobin (HbS) is protective against malaria but Adeloye et al. and Maharajan et al. reported that HbSS disease patients are at risk of increased morbidity and mortality from malaria (3-5). The reasons suggested by these authors to explain increased morbidity and mortality of HbSS subjects to malaria are based on the nature of sickled RBCs.

Naturally acquired immunity to erythrocytic stages of malaria parasites is multifactorial, involving both antibody and cell mediated effector mechanisms (6). In general, protective antibodies recognize determinants exposed on the surface of the merozoites or parasite-derived molecules that are inserted into the outer membrane of the infected red blood cells (iRBC). Cellular immune responses are induced following phagocytosis of the iRBC with subsequent processing/presentation of malaria peptides to T lymphocytes. Once activated, T lymphocytes provide B-cell help, activate macrophages to phagocytose and kill iRBC.

In HbSS subjects without malaria, abnormal levels of C-reactive protein, serum amyloid-A, orosomucoid, and fibrinogen were reported. Also, random neutrophil migration, chemotactic activity, and lymphocyte transformation index were all defective in individuals with sickle cell disease compared to healthy controls (7). Increased levels of IgG, IgA, IgM (8), reduced C4 (9) and decreased C3 (10) were reported in HbSS having malaria compared with HbAA subjects. However, there was no report on complement regulators in HbSS with or without malaria parasitemia. In this study, the serum levels of complement regulators (C1 inhibitor and C3 activator) and immunoglobulin classes (IgG, IgA and lgM) where determined in HbSS Nigerians with and without malaria parasitemia to provide evidence for increased morbidity/mortality of HbSS subjects from *P. falciparum*.

## SUBJECTS AND METHODS

**Subjects.** A total of sixty-four subjects were recruited to the study. Informed consent was obtained from them before sample collection and the need for the study was explained in local language when necessary. The subjects were divided into four groups viz: 10 HbSS genotypic subjects with *P. falciparum* parasitemia (HbSS+PfM), 18 HbAA genotypic subjects with *P. falciparum* parasitemia (HbAA+PfM), 20 HbSS genotypic subjects without *P. falciparum* parasitemia (HbSS-PfM) and 16 HbAA genotypic subjects without *P. falciparum* parasitemia (HbAA-PfM).

**Methods.** Five millilitres of venous blood were collected from each subject into a plane bottle for the measurement of IgG, IgA, IgM, C1 inhibitor and C3 activator by radial immunodiffusion. Briefly, 3% noble agar was prepared in phosphate buffered saline (PBS, pH 7.2). One millilitre each of the appropriate anti-sera (anti-human immunoglobulin classes and complement regulators) was mixed with noble agar. The agar-antiserum mixture was allowed to set. Different concentrations (25% - 200%) of the

standard serum were prepared in PBS and added to the punched agar. The plates for IgG, C1 inhibitor, and C3 activator were incubated for 4 hours in a humid chamber, while those for IgA and IgM were incubated for 18 hours at $37^0$C.

The standard curves for various immunoglobulin classes and complement regulators were plotted on a semi-log graph paper and the concentrations of the test and control samples were extrapolated from the standard curves.

**Statistical Analysis.** Data were presented as mean ± standard deviation. Student t-test was used to test the significant of differences between the mean values. The probability value (P) less than 0.05 was considered significant.

## RESULTS

As indicated from Table 1, HbSS+PfM had the highest mean value of IgG (2373.90 ± 1772mg/dl) while HbSS-PfM had the lowest mean value of IgG (644.55 ± 171.15mg/dl). However, HbSS+PfM had significantly higher values compared with HbAA-PfM ($p<0.01$) or HbSS-PfM ($p<0.01$) but not significantly higher compared with HbAA+PfM ($p>0.10$). Also, the mean IgG was significantly higher when comparing HbAA+PfM with HbAA-PfM ($p<0.01$) or HbSS-PfM ($p< 0.00$). The mean value for HbAA-PfM was not significantly higher compared with mean value for HbSS-PfM ($p > 0.10$).

**Table 1. Mean (±S.D) titer (mg/dL) of serum immunoglobulin classes in HbAA or HbSS subjects with or without *P. falciparum* malaria parasitemia**

| | IgG | IgA | IgM |
|---|---|---|---|
| HbSS+PfM | 2373.90±1772.81 | 159.80±45.4 | 69.13±31.79 |
| HbAA+PfM | 1868.00±100 | 155.00±68.56 | 102.67±53.19 |
| HbSS-PfM | 644.55±171.15 | 249.00±94.86 | 93.35±90 |
| HbAA-PfM | 659.75±158.01 | 170.24±54.90 | 67.29±63.76 |

HbSS-PfM had the highest mean value of IgA (249.00 ± 94.86 mg/dl) and HbAA+PfM had the lowest mean value for IgA (155.00 ± 68.56mg/dl). There was no significant difference in the mean value of IgA in HbAA-PfM compared with HbSS+PfM ($p>0.10$) or HbAA+PfM ($p>0.10$). Moreover, no significant increase was observed between the IgA value of HbSS+PfM and HbAA+PfM ($p>0.10$). However, mean IgA level in HbSS-PfM was significantly higher compared with HbAA-PfM ($p<0.05$), HbAA+PfM ($p<0.05$) or HbSS+PfM ($p< 0.05$).

Regarding IgM, no significant differences were obtained when comparing the mean values of the four groups.

The highest mean value of C1 inhibitor was obtained for HbAA-PfM while HbAA+PfM had the lowest mean value. The mean level of C1 inhibitor in HbSS+PfM was not significantly lower than the mean value of C1 inhibitor in HbAA-PfM ($p>0.05$) or HbSS-PfM ($p>0.10$) and not significantly higher compared with the mean value in HbAA+PfM ($p>0.1$). The mean value of C3 activator for HbSS-PfM was the highest while HbSS+PfM had the lowest value. The mean value of C3 activator in HbSS-PfM was significantly higher compared with the mean value of C3 activator in HbSS+PfM ($p<0.05$). (Table 2)

**Table 2. Mean (±S.D) titer (mg/dL) of serum C1 inhibitor and C3 activator in HbAA or HbSS subjects with or without *P. falciparum* malaria parasitemia**

| | C1 inhibitor | C3 activator |
|---|---|---|
| HbSS+PfM | 21.60±11.03 | 9.60±5.80 |
| HbAA+PfM | 14.00±4.90 | 11.25±5.95 |
| HbSS-PfM | 27.60±19.89 | 17.10±7.35 |
| HbAA-PfM | 28.80±9.94 | 14.00±1.03 |

## DISCUSSION

The serum level of IgG in HbSS-PfM subjects in this study was slightly lower than that of HbAA-PfM. Other investigators have reported a reduced serum IgG in HbSS-malaria compared with HbAA without malaria (11). However, Adekile et al. observed increased serum IgG in HbSS subjects compared with HbAA ones (12). Reduced IgG observed in HbSS-PfM suggests that perhaps at the time of the investigation, the subjects were not harbouring clinical doses of infectious agents to trigger over-production of IgG. Significantly elevated levels of IgG in HbAA+PfM subjects compared with HbAA-PfM individuals are in line with previous studies (10, 13).

Immunoglobulin M was elevated in HbAA+PfM or HbSS+PfM subjects compared with HbAA-PfM group. This finding is similar to the finding of others [8, 10]. IgM was reported to be positively related to active transmission of malaria [8]. The increase in serum IgG and IgM observed in HbAA or HbSS with malaria may be due to the production of IgG and IgM as a result of polyclonal activation of B-cells by malaria antigen. Another evidence supporting increased serum IgG and IgM in HbAA+PfM or HbSS+PfM subjects is RBC rosette formation and sequestration (14). Erythrocytes rosetting is a strategy used by the parasite to remain sequestered in the microvasculature so as to avoid destruction in the spleen and liver. However, this causes obstruction to the blood flow in micro capillaries causing endothelial cell damage. In response to endothelial cell damage, cytokines such as IL-1, IL-6 and TNF are produced. IL-1 is known to stimulate T and B cells while IL-6 differentiates B cells into antibody forming cells.

The reason for a slightly higher level of IgG in HbSS+ PfM subjects compared with HbAA+PfM individuals could be a result of higher *Plasmodium* density caused by incomplete clearance of parasite from the blood or maybe a result of more sequestration ability of *Plasmodium*-infected sickled RBCs . However, higher IgM in HbAA+PfM subjects compared with HbSS- PfM patients as seen in this study may suggest that the HbAA+PfM patients experience more severe *Plasmodium* infection since IgM is associated with rosetting and pathogenicity. Infection is positively correlated with the development of a diverse IgG repertoire especially IgG reactive to *Plasmodium* infected red blood cells (15).

The mean serum IgA in HbSS-malaria was significantly higher compared with HbAA-PfM subjects consistent with other studies (11, 12). The normal level of IgA was reported in another study (16). HbSS+ malaria has a reduced mean serum IgA when compared with HbSS-PfM. Odegbemi et al. observed that HbSS+ malaria patients had the highest values of the 3 classes of immunoglobulin (IgM, IgA and IgG). In addition, mean serum IgA was reduced in subjects with HbAA genotype + malaria (13). This is consistent with the study of Marwah et al. while Fribourg-Blanc et al. showed a normal mean serum IgA in HbAA + malaria patients compared with HbAA-PfM (17, 18). The mean serum IgA observed in this study may be associated with IL-5 (a Th-2 cytokine)

which favors IgA production by B-cells. In malaria, the protection against the early stage of malaria is associated with Th-1 cytokines while Th-2 cytokines are increased in sickle cell disease. This might explain raised IgA in HbSS with or without malaria parasitemia.

There was no significant difference between the serum levels of C1 inhibitor in HbSS-PfM and HbAA-PfM subjects in this study. This observation is comparable with a previous study which reported normal level of C4 in HbSS suggesting that the classical pathway was neither activated nor inhibited in HbSS. In HbAA+PfM patients, the lowest mean serum C1 inhibitor value was obtained. The value was significantly lower compared with HbAA-PfM subjects (11).

This agrees with previous findings where the lower level of C4 was related to its consumption as a result of activated classical pathway by malaria antigen (9). HbSS+PfM subjects also had a low level of C1 inhibitor though not significant when compared with HbAA-PfM.

The highest value of C3 activator was observed in HbSS- PfM subjects though not significant when compared with HbAA-PfM individuals. This result indicates a higher activation of the alternative pathway of complement system in HbSS-PfM than in HbAA-PfM, which may result in low C3. Dieye et al. observed reduced C3 and activation of alternative pathway in HbSS subjects without malaria parasitemia (11). The alternative pathway is activated by immunoglobulin A.

HbAA+PfM and HbSS+PfM both showed reduced mean values of C3 activator, compared with HbAA-PfM suggesting reduced activation of alternative pathway.

In conclusion, this study shows that the level(s) of different immunoglobulin classes (IgA, IgG and IgM) and complement regulators (C1 inhibitor and C3 activator) were similar in HbAA-PfM and HbSS-PfM subjects implying that Nigerian HbSS subjects without malaria crisis had normal spleen function and adequate humoral immunity.

However, reduced value of C1 inhibitor in HbAA+PfM compared with HbSS+ PfM implies that classical pathway of complement system is activated more efficiently in HbAA+PfM than in HbSS+PfM subjects. Finally, increased level of C1 inhibitor and reduced level of C3 activator in HbSS+PfM compared with HbAA+PfM individuals may explain the predisposition of Nigerian HbSS subjects to *P. falciparum* malaria.

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## REFERENCES

1 Akinyanju O. Issues in the Management and Control of Sickle Cell Disorder**.** Archives of Ibadan Medicine. 2001; 2: 237-41.
2 Cornille-Brogger R, Fleming AF, Kagan I, Matsushima T, Molineaux L. Abnormal haemoglobins in the Sudan savanna of Nigeria. II. Immunological response to malaria in normals and subjects with sickle cell trait. Ann Trop Med Parasitol. 1979 ;73:173-83.
3 Marsh K. Malaria--a neglected disease? Parasitology. 1992;104 :53-69.
4 Adeloye A, Odeku EL. The nervous system in sickle cell disease. Afr J Med Sci. 1970 ;1: 33-48.
5 Maharajan R, Fleming AF, Egler LJ. Pattern of infections among patients with sickle-cell anaemia requiring hospital admissions. *Nig J Paediatr* 1983;10: 13-7.
6 Miller LH, Baruch DI, Marsh K, Doumbo OK. The pathogenic basis of malaria. Nature. 2002 ;415:673-9.
7 De Cock KM, Hodgen AN, Jupp RA, Slavin B, Siongok TK, Rees PH et al. Immunoglobin M and malarial antibody levels in hyper-reactive malarial splenomegaly.J Trop Med Hyg. 1986 ;89:119-21.
8 Biswas S, Saxena QB, Roy A. The natural occurrence of circulating antibodies in populations of endemic malarious areas.Indian J Malariol. 1990 ;27:139-48.

9 Neva FA, Howard WA, Glew RH, Krotoski WA, Gam AA, Collins WE et al. Relationship of serum complement levels to events of the malarial paroxysm.J Clin Invest. 1974; 54:451-60.
10 Jhaveri KN, Ghosh K, Mohanty D, Parmar BD, Surati RR, Camoens HM et al. Autoantibodies, immunoglobulins, complement and circulating immune complexes in acute malaria.Natl Med J India. 1997 ;10:5-7.
11 Dieye TN, Ndiaye O, Ndiaye AB, Thiam D, Fall-Seck K, Diop S et al. Complement and serum immunoglobulins in homozygous and heterozygous sickle cell anemia in Senegal Dakar. Dakar Med. 1999; 44:175-9.
12 Adekile AD, Haider MZ ,Serebour F, Al-Zaabi K, Tuli M. Serum Immunoglobulins and IgG Subclasses in SS Patients with Saudi Arabia/India Haplotype. Med Principle Pract .1999; 8 :183 - 8.
13 Odegbemi JO, Williams AI. Immunoglobulin levels in malaria infected Nigerians with and without abnormal Haemoglobin. Afr J Med Med Sci. 1995 ;24:21-5.
14 Rowe A, Obeiro J, Newbold CI, Marsh K. Plasmodium falciparum rosetting is associated with malaria severity in Kenya. Infect Immun. 1995 ;63:2323-6.
15 Bull PC, Marsh K. The role of antibodies to Plasmodium falciparum-infected-erythrocyte surface antigens in naturally acquired immunity to malaria.Trend Microbiol. 2002.10: 55-8.
16 Mohapatra BN, Dash BP, Kar BC. Serum immunoglobulins in sickle cell disease. J Assoc Physicians India. 1993; 41:418-9.
17 Marwah GS, Arora S, Prabhakar H. Haemagglutination antibody titres & immunoglobulin levels in cases of Plasmodium infection.Indian J Med Res. 1989; 89:326-9.
18 Fribourg-Blanc A, Druilhe P, Brasseur P, Rhodes-Feuillette A, Ballet JJ, Tharavanij S. Immunological evaluation of cell-mediated and humoral immunity in Thai patients with cerebral and non cerebral Plasmodium falciparum malaria: II. Evolution of serum levels of immunoglobulins, anti malarial antibodies, complement fractions and alpha interferon. Southeast Asian J Trop Med Public Health. 1985; 16: 307-13.