ORIGINAL ARTICLE

Increased IgE level in Nigerian sickle cell disease children: Implication for severity of allergic reactions

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ABSTRACT

Background: Susceptibility of sickle cell disease subjects to various infectious agents is on the increase, but information relating SCD to allergic condition is scarce. Hence, assessment of immunoglobulin status in SCD children may provide useful information to improve management of SCD children.

Objectives: To determine the levels of immunoglobulin classes (IgG, IgA, IgM, and IgE) in Nigerian HbSS children below 5 years of age compared with sex- and age-matched HbAA children.

Materials and Methods: Blood samples were collected from a total of 45 children less than 5 years of age who were recruited into the study as follows: 26 HbSS and 19 HbAA subjects for the estimation of serum immunoglobulin levels using enzyme-linked immunosorbent assay (ELISA) techniques and determination of genotype using electrophoresis technique.

Results: IgG concentration was nonsignificantly higher (P = 0.997) in HbSS children (1022.56 ± 148.97 ng/ml) compared to HbAA children (933.68 ± 106.10 ng/ml). IgA (P = 0.906) and IgM (P = 0.986) concentrations were nonsignificantly lower in HbSS children (255.07 ± 133.71 ng/ml) compared to HbAA children. IgE was significantly higher (P = 0.000***) in HbSS (108.67 ± 69.22 IU/ml) compared to HbAA (24.51 ± 17.58 IU/ml) children.

Conclusion: SCD children in steady state have adequate levels of Ig classes. Non-specific elevation of IgE levels may be a factor of inflammatory response in SCD children, and this may be proposed for reduced allergic reaction among SCD children.

Key words: Allergic condition, IgE, inflammation, sickle cell disease

INTRODUCTION

Sickle cell disease (SCD) is a blood disorder in which the red blood cells assume an abnormal rigid sickle shape and undergo sickling when deoxygenated. The condition is inherited as an autosomal recessive condition and is prevalent in the tropics where malaria is endemic.[1] SCD is due to a point mutation in the β-globin gene, resulting in the creation of abnormal hemoglobin (Hb) molecules with a hydrophobic motif that is exposed in its deoxygenated state.[2] The prevalence of healthy carriers of the sickle cell gene (sickle cell trait) ranges between 1% and 40% across Africa.[3] Nigeria has an estimated carrier prevalence of 6-24%.[4] An estimated 150,000 children are born with SCD in Nigeria annually.[5]

Children with SCD are at increased risk for bacteremia that can result in sepsis and death, which is due in large part to the functional asplenia that develops over time in these children. In developed countries and in Africa, the most common organisms involved include *Streptococcus pneumoniae*, *Salmonella species*, and *Haemophilus influenzae*.[6]

The spleen, a major organ of the reticuloendothelial system, is rich in plasma cells, lymphocytes, and macrophages. One of its major roles is the production of antibodies. Asplenia experienced by SCD subjects

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is presumed to cause reduced antibody production in these children. In addition to the production of antibodies, the macrophages of the spleen are involved in the removal of bacteria from the circulation by phagocytosis. Thus, asplenia in SCD subjects decreases the phagocytic potential of SCD subjects, permitting overwhelming bacteremia, septicemia, or meningitis before adequate antibody production could occur.[7]

Extant literature review has shown the relationship between allergic condition (asthma) and SCD. However, it is not yet known if asthma in SCD is a disease resulting from SCD pathophysiology or caused by similar genetic and environmental factors found in typical asthma. The reported prevalence of asthma in children with SCD varies from 2% to approximately 50%.[8] Having asthma increases the risk for developing acute chest syndrome, death, or painful episodes compared to having sickle cell disease without asthma.[8] Asthma in SCD patients is likely a co-morbid condition rather than a disease due to SCD-induced airway inflammation/bronchoconstriction.[9]

This study was designed to determine the levels of immunoglobulin classes (IgG, IgA, IgM, and IgE) in Nigerian HbSS children compared with Nigerian HbAA children. This will provide an insight into the humoral immune status of SCD children and suggest the basis for possible co-morbidity of allergic reactions to SCD.

MATERIALS AND METHODS

Before commencement of the study, ethical approval was obtained from Lagos State University Teaching Hospital Ethical Committee.

A total of consecutive 45 participants were recruited from Lagos State University Teaching Hospital, Nigeria as follows: 26 SCD patients and 19 healthy sex-and age-matched HbAA control subjects. Children older than 5 years of age and children known to have had immunoglobulin infusion therapy in the last 6 months prior to recruitment were excluded.

A short structured questionnaire that was designed to obtain information from the participants about age, sex, history of previous immunoglobulin infusion, co-morbid medical conditions, and drug history was administered. Only those subjects whose parents consented to participate were recruited. The questionnaire was pilot tested and applied by a pediatrician in local language when necessary. For illiterate parent/guardian, the consent form was countersigned by a witness.

Two milliliters of blood sample was collected on enrollment into two sample bottles: 1 ml was collected into an ethylenediaminetetraacetic acid (EDTA) bottle for determination of Hb genotype and another 1 ml was collected into a plain bottle for immunoglobulin quantitation. Serum immunoglobulins (IgA, IgG, IgE, and IgM) were measured in the sera using enzyme-linked immunosorbent assay (ELISA) method as described by the manufacturers of the kits. The genotype was determined using the electrophoresis technique.

Serum Analysis for Immunoglobulin Classes

ELISA was used to determine the concentrations of serum IgG, IgA, IgM (Immunology Consultant Laboratory, USA), and IgE (Leinco Technologies, 410Axminister Drive St. Louis, Missouri 63026, Washington, USA). The assay was carried out following manufacturers' instructions.

Principle of ELISA assay

In this assay, immunoglobulin present in the sample reacts with the anti-Ig antibody adsorbed to the surface of solid-phase polystyrene microtitre wells. After the removal of unbound proteins by washing, the anti-Ig antibodies conjugated with horseradish peroxidase (HRP) form complexes with the previously bound immunoglobulin following the addition of a chromogenic substrate, 3,3,5,5-tetramethylbenzidine (TMB). The absorbance at 450 nm is a measure of the concentration of the immunoglobulin in the test sample.

Procedure of ELISA

The procedure for the determination of immunoglobulin concentrations in the serum was the same for all immunoglobulin classes, but the incubation periods were different.

Standard samples were prepared from the human Ig calibrator by serial dilution of the calibrator with the diluent concentrate as instructed by the manufacturer. One hundred microliters of the standards and sera samples was pipetted into microwells already coated with anti-Ig antibodies and incubated at room temperature for 60 min; the plate was covered during incubation. Following incubation, the wells were aspirated of their contents and completely filled with appropriate wash solution. The plate was washed three times.

One hundred microliters of appropriately diluted enzyme–antibody conjugate was pipetted into each well and the plate was incubated at room temperature for 20 min in a dark chamber. After incubation, another process of washing was performed and 100 µl of TMB substrate solution was added into each well. This was followed by incubation for 10 min after which 100 µl of stop solution was added to each well. The absorbance (at 450 nm) was determined using ELISA reader.

Using the absorbance values, a standard curve was constructed and the value for the concentration of immunoglobulin in each well was extrapolated from the standard curve.

Statistical Analysis

Data obtained were analyzed using SPSS version 17 statistical software. The values for each of the groups were presented as mean and standard deviation. Analysis of variance (ANOVA) was used to determine the level of significance within each group, while Student's *t*-test was used to determine significant difference between groups. Values of $P \leq 0.05$ were regarded as statistically significant.

RESULTS

Table 1 shows the demographic features of the subjects.

Serum IgG level was nonsignificantly higher in HbSS children compared with HbAA children, whereas serum IgA and IgM levels were nonsignificantly lower in HbSS children compared to HbAA children. IgE was significantly higher in HbSS children compared to HbAA children [Table 2].

DISCUSSION

SCD patients are prone to frequent and sometimes fatal infection as a result of some abnormality of the immune system.[10] The present study suggests that allergic conditions may not be severe in children with SCD.

In this study, HbSS patients had nonsignificantly increased IgG level compared to HbAA patients. Although Arinola *et al*.[11] observed reduced IgG level in adult HbSS compared to adult HbAA, our result of IgG level agrees with those of Ballas *et al*.[12] and Adekile *et al*.[13] The slightly increased IgG level could be the result of continuous but mild reticuloendothelial stimulation by chronic extravascular hemolysis in SCD children.

IgM was slightly reduced in HbSS children compared to HbAA children considered for this study. This could be due to the functional asplenia which is yet to become severe in HbSS children. Hyposplenic subjects were reported to be unable to switch from IgM to IgG class of antibody in response to some forms of antigenic challenge.[14]

No significant difference was observed in the serum mean value of IgA when HbSS and HbAA children were compared. This is consistent with the results of a study carried out by Mohapatra *et al*.[15] However, an increased IgA level was reported in another study by Dieye *et al*.[16]

There were no previous documented reports on the levels of serum IgE in Nigerian HbSS children. In the present study, the mean serum level of IgE was significantly higher in HbSS children when compared to HbAA children. This could be as a result of inflammatory reaction in the SCD subjects. Inflammation has been reported to be a secondary response to cholecystitis and cholelithiasis in SCD.[17] Hemolysis and release of molecules associated to Hb catabolism (such as free Hb, iron, and heme) generate oxidant environment with production of reactive oxygen and nitrogen species that lead to inflammation in SCD.[18]

Previous studies assessed inflammation in adult SCD patients using C-reactive protein.[19-21] Implication of increased IgE levels in SCD children should not be underestimated. This may be suggested to lead to hypothetical reduction of allergic conditions in SCD subjects. Increased IgE in SCD subjects may prevent the binding and cross-linking of allergen-specific IgE on the mast cell, which will prevent the release of vasoactive amines after mast cell degranulation and mast cell membrane permeability.

The clinical basis for diagnosis of allergic conditions in children and adults with SCD is not well defined. Thus, many SCD subjects do not undergo evaluation for

Table 1: Demographic features of the subjects

	SCD	Control
Gender (%)	Male (35.56) Female (22.22)	Male (24.44) Female (17.78)
Mean age Age range	26.73±9.47 months 10-48 months	15.00±10.18 months 7-48 months
Malaria parasitemia	Negative	Negative
Allergy (skin reaction)	1	None
Parents' level of education	Post primary school	Post primary school
Occupation	Non-civil servant	Non-civil servant

SCD=Sickle cell disease

Table 2: Serum concentrations (Mean±SD) of immunoglobulin classes in HbAA children and HbSS children

	n	IgG (ng/ml)	IgA (ng/ml)	IgM (ng/ml)	IgE (IU/ml)
HbAA	19	933.68±106.10	299.85±158.20	48.18±5.60	24.51±17.58
HbSS	26	1022.56±148.97	255.07±133.71	43.70±3.65	108.67±69.22
P values		0.997	0.906	0.986	0.000***

HbAA=Heamoglobin AA; HbSS=Heamoglobin SS; SD=Standard deviation; ***=Show significant difference

allergic conditions. Based on the significantly increased level of IgE in the SCD children of our study, we suggest that diagnosis of allergic conditions be carried out in SCD children. This proposition is supported by the fact that allergic conditions and SCD are both inflammatory diseases.[22] Moreover, markers of inflammation are known to be related to SCD severity.[23] But reports on the association between SCD and allergic conditions are scarce.

Prevalence and severity of allergic conditions in SCD subjects need further investigation which should include skin prick test to environmental allergens, broncho-provocation testing, a thorough personal and family history taking, pulmonary function testing.

Serum IgE level increases in atopic disease and parasitic infections.[24-26] Though subjects were not screened for stool/urine helminthes, those with malaria parasitemia were excluded.

The major limitation of our study is its small sample size which was because only those children whose parents/guardians gave consent were recruited for the study. Also, the parameters of allergic test should be increased as suggested above.

CONCLUSION

Serum levels of Ig classes are adequate in steady-state SCD children. Increased level of serum IgE is an indication of inflammation and may be suggested to lead to reduction in allergic conditions in SCD children. Thus, it is advised that SCD subjects be screened for allergic conditions during management.

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