ORIGINAL ARTICLE

Selected inflammatory and haemolytic indicators among adolescents living with sickle cell anaemia in a malaria-endemic population

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ABSTRACT

Objectives: Transition between paediatric and adult care represents a critical period in the management of sickle cell anaemia. Both the sickling condition itself and malaria are associated with inflammation, thus, the need to investigate the impact of malaria infection on neutrophilic response, glutathione and bilirubin levels among adolescents with sickle cell anaemia in a malaria-endemic population.

Methods: This study was carried out among 68 steady-state sickle cell anaemia adolescent attending clinic at University of Calabar Teaching Hospital, Calabar-Nigeria. The subjects were asymptomatic for malaria. All tests were carried out by standard methods. Statistical analysis of data was carried out using SPSS 22.0. A p-value of ≤ 0.05 was considered to infer a statistically significant difference.

Results: Leucocyte counts were significantly higher, while neutrophil function rate was lower in sickle cell anaemia subjects compared to control subjects. Bilirubin mean values were also significantly higher while glutathione mean values were lower among subjects living with sickle cell anaemia. These derangements were heightened by malaria infection. Glutathione correlated negatively with total white cell count, neutrophil count and unconjugated bilirubin while a positive relationship was observed between the former and neutrophil function rate.

Conclusion: Asymptomatic malaria infection impacts negatively on immune response among persons living with sickle cell anaemia. This reveals an important intervention target for the transition from paediatric to adult care in the management of sickle cell anaemia in malaria-endemic areas.

Keywords: Sickle cell anaemia, inflammation, leucocytes, haemolysis

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INTRODUCTION

Sickle cell disease is a group of inherited disorders with significant contributions to childhood mortality, especially among persons of African, Middle East and Mediterranean nativity (1,2). Sickle cell anaemia (haemoglobin SS) is the disease variant predominantly found in Nigeria where its persistence is largely driven by inadequate healthcare coverage particularly in rural and sub-urban populations (3). This has been attributed to lack of premarital testing for haemoglobin types among prospective couples which in turn increases the probability of having children with sickle cell anaemia. While access to adequate healthcare greatly improves survival of affected individuals from childhood into adult life, the transition between paediatric care and adult care has been recognized as a critical period in the management of the disorder even in developed countries with improved healthcare (4-6). Thus, life expectancy among sickle cell anaemia patients continues to be a critical area of challenge in the management of the condition particularly among adolescents.

In addition to the known anaemia, platelet involvement and occlusive processes associated with sickling of red blood cells, there also exists overt inflammation alongside chronic haemolysis in sickle cell anaemia (1,7). Inflammation from bacterial infection has been mainly addressed through routine vaccination. Unfortunately, other infections such as that from *Plasmodium falciparum* which causes malaria has no vaccine yet and is endemic in Nigeria where children are particularly vulnerable (8-10). Certain factors have been identified as triggers for the onset of sickle cell-related crisis. These include malaria, exposure to excess cold or heat, physical and emotional stress, dehydration, fever and high altitude. Malaria-triggered crisis is common among sickle cell anaemia subjects in Nigeria (11-13). Sickle cell anaemia and malaria infection are associated with inflammation and independently induce phagocytic response and haemolysis (14-17). Thus, the Nigerian population of children with sickle cell anaemia could be at increased risk of inflammation. The main objective of this study was to investigate the impact of malaria infection on neutrophil count as well as its function rate, glutathione and bilirubin levels among adolescents with sickle cell anaemia in a malaria-endemic population.

METHODS

This study was carried out among 68 steady-state sickle cell anaemia adolescent attending clinic at University of Calabar Teaching Hospital, Calabar-Nigeria. The study subjects included equal numbers of male and female sickle cell anaemia patients with age and gender-matched Hb AA controls. Ethical approval was obtained from The Health and Research Ethics Committee of University of Calabar Teaching Hospital, while informed consent was obtained from each participant and the respective guardian. The subjects were asymptomatic for malaria. Malaria infection was determined by microscopy on direct thick and thin film. The leucocyte counts were carried out by automation using SMART-1 (three-part differentiation) Haematology Analyzer from Kinghawk Technology Co., Ltd, China. This analyser was controlled and calibrated according to manufacturer's instructions to ensure it was fit for use.

Neutrophil function rate was determined using the *Escherichia coli* culture method. In this method, *E coli* was sub-cultured in peptone water from a stock culture isolated in a nutrient agar medium. After incubating blood sample in the *E coli* broth, blood film was made and stained with Leishman stain prior to microscopic examination. Function rate was derived from the number of phagocytized neutrophils per the total number of neutrophils present.

Quantitative determination of glutathione (GSH) was carried out by enzyme-linked immunosorbent assay Method using kits from Bioassay Technology Laboratory, China. Total and conjugated bilirubin were determined using kits from Randox Laboratories Limited, UK.

Unconjugated bilirubin was derived mathematically from Total and conjugated bilirubin values. Statistical analysis of data (Student t-test and Pearson's correlation) was done using SPSS 22.0. A p-value of ≤ 0.05 was considered to infer a statistically significant difference.

RESULTS

Leucocyte counts were significantly higher, while neutrophil function rate was lower in sickle cell anaemia subjects compared to control subjects. Bilirubin mean values were also significantly higher while glutathione mean value was lower among subjects living with sickle cell anaemia as shown in Table 1.

Apart from absolute lymphocyte count, which showed no significant difference, other leucocyte counts were higher in asymptomatic malaria infection. Neutrophil function rate was however further reduced in this group of sickle cell anaemia subjects. There also occurred much higher levels of bilirubin values but lower glutathione level in the same group (Table 2).

Pearson's correlations shown in Table 3 indicate that glutathione correlated negatively with total white cell count, neutrophil count and unconjugated bilirubin while a positive relationship was observed between the former and neutrophil function rate.

Table 1. Selected indicators of inflammation and haemolysis among adolescents living with sickle cell anaemia .

Parameters	Hb AA Subjects (n=68)	Hb SS Subjects (n=68)	p-value
WBC ($\times 10^9$/l)	5.24 ± 1.26	11.44 ± 2.53	0.001
LYMPH ($\times 10^9$/l)	2.59 ± 0.57	4.28 ± 1.28	0.001
MXD ($\times 10^9$/l)	0.40 ± 0.18	1.42 ± 0.32	0.001
NEUT ($\times 10^9$/l)	2.24 ± 1.09	5.73 ± 2.32	0.001
NFR (%)	36.01 ± 4.44	25.84 ± 4.38	0.001
TB (µmol/l)	16.52 ± 2.68	34.36 ± 7.63	0.001
CB (µmol/l)	8.27 ± 1.64	15.19± 4.94	0.001
UB (µmol/l)	8.25 ± 1.99	19.17± 4.74	0.001
GSH (µg/l)	3.99 ± 1.88	1.45 ± 0.35	0.001

WBC = White blood cell, LYMPH = Lymphocyte, MXD = Mixed cell, NEUT = Neutrophil, NFR = Neutrophil function rate, TB = Total bilirubin, CB = Conjugated bilirubin, UB = Unconjugated bilirubin, GSH = Glutathione

Table 2. Selected indicators of inflammation and haemolysis among adolescents living with sickle cell anaemia and infected with malaria parasite.

Parameters	Hb SS subjects with no malaria infection (n=33)	Hb SS subjects with asymptomatic malaria infection (n=35)	p-value
WBC ($\times 10^9$/l)	9.93 ± 1.57	12.86 ± 2.45	0.001
LYMPH ($\times 10^9$/l)	4.57 ± 1.31	4.01 ± 1.21	0.073
MXD ($\times 10^9$/l)	1.30 ± 0.35	1.53 ± 0.26	0.003
NEUT ($\times 10^9$/l)	4.06 ± 0.90	7.30 ± 2.15	0.001
NFR (%)	28.18 ± 3.42	23.63 ± 4.04	0.001
TB (µmol/l)	30.20 ± 5.04	38.28 ± 7.63	0.001
CB (µmol/l)	13.41 ± 3.96	16.87± 3.23	0.003
UB (µmol/l)	16.80 ± 3.32	21.41± 4.83	0.001
GSH (µg/ml)	1.67 ± 0.34	1.24 ± 0.21	0.001

WBC = White blood cell, LYMPH = Lymphocyte, MXD = Mixed cell, NEUT = Neutrophil, NFR = Neutrophil function rate, TB = Total bilirubin, CB = Conjugated bilirubin, UB = Unconjugated bilirubin, GSH = Glutathione

Table 3. Pearson's correlation values for subjects with sickle cell anaemia

Parameters	Pearson's correlation (r)	p-value
Glutathione and total white cell count	-0.695	0.001
Glutathione and neutrophil count	-0.614	0.001
Glutathione and neutrophil function rate	0.380	0.001
Glutathione and unconjugated bilirubin	-0.352	0.003

DISCUSSION AND CONCLUSIONS

The present study on selected inflammatory and haemolytic indicators in subjects living with sickle cell anaemia in a malaria -endemic population was carried out among affected adolescents between 12-18 years of age who were attending Haematology Clinic at University of Calabar Teaching Hospital in Calabar, Nigeria. This study has revealed the impact of malaria infection on neutrophilic response in sickle cell anaemia. There is heightened neutrophilia with reduced function rate among malaria-infected sickle cell anaemia subjects. The contributions of white blood cells to the pathophysiology of sickle cell anaemia remains a subject of interest. Adhesive properties of these cells to the endothelium of the microcirculation have been understood to play important role in sickle cell crisis (19,20). Associations between polymorphonuclear leucocytosis and increased rate of early death, acute chest syndrome and stroke have been reported (21,22). Elevation of neutrophil concentration in the blood of patients with sickle cell anaemia has been attributed to mechanisms such as accelerated release of neutrophils from the bone marrow, decrease in the rate at which neutrophils leave the blood as well as demarginating of intravascular neutrophils (23).

Chronic haemolysis associated with sickle cell anaemia was evident in the study as unconjugated bilirubin values were on the high side. Malaria infection apparently contributed to the haemolytic episodes as much higher mean value was recorded in this category. A reversed pattern of finding was, however, recorded for glutathione level. Chronic haemolysis potentiates generation of reactive oxygen species, reduced antioxidant capacity and ultimately increased oxidative stress (24,25).

Glutathione depletion observed in this study correlated with increasing unconjugated bilirubin value and absolute neutrophil count but decreasing neutrophil function rate. This study has shown that reduced levels of glutathione and high bilirubin concentrations are markers of oxidative stress. This observation is consistent with the findings previously reported from the studied population (25).

Lowered immunity remains a challenge for sickle cell anaemia subjects living in malaria-endemic areas. This reveals an important intervention target for the transition from paediatric to adult care in the management of sickle cell anaemia.

The present study had the limitation of not following up the subjects. Further studies may adopt a longitudinal study approach and monitor these markers from childhood to adult life.

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