Study of C - reactive protein Levels and Haematological Parameters in Individuals with and without Sickle Cell Anaemia in Abakaliki, Nigeria

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Abstract

BACKGROUND

Sickle cell anaemia (SCA) is a genetic disorder of haemoglobin and chronic inflammatory state. This study aimed to determine and compare C - reactive protein (CRP) levels and blood counts in participants with SCA (HbSS) and control (HbAS and HbAA).

MATERIALS AND METHODS

A cross-sectional comparative study was carried out among steady-state HbSS individuals with age- and sex-matched control from August 2019 to February 2020. Using the method described by Charan and Biswas and sickle cell disease prevalence of 3.5%, a sample size of 47 was calculated. However, 101 participants were recruited using a consecutive sampling technique. An assay of haemoglobin phenotype, CRP level and some haematological parameters, including haemoglobin level, white blood cell count and platelet count, were done. Data analysis was done using SPSS software, version 26.

RESULTS

One hundred and one subjects participated in the study and were made up of 51 (50.5%) males and 50 (49.5%) females with an age range of 1 to 52 years. They comprised 58 HbSS and 43 controls (23 HbAS and 20 HbAA). There was a significant increase in CRP level among HbSS participants (5.4µg/ml ±4.5) compared to those of HbAS (0.02µg/ml ± 0.01) and HbAA (0.02µg/ml ± 0.01) (p = 0.000). Correlation between CRP level and blood counts showed weak positive relationship between CRP and WBC count (r = 0.285, P = 0.07) as well as platelet count (r = 0.156, P = 0.336) but negative correlation between CRP and haemoglobin level (r = - 0.073, P = 0.655).

CONCLUSION

There was a significantly higher CRP level among patients with HbSS in a steady state compared to control (HbAS and HbAA individuals).

Participants with HbSS had a positive but weak relationship between CRP and WBC count, CRP and platelet count and a weak negative correlation between CRP and haemoglobin level. Further studies are required on a broader scale to assess whether CRP level may be used to predict the occurrence of crisis in patients with SCA.

Keywords: *C - reactive protein, Leucocyte, Nigeria, Sickle Cell Anaemia*

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Introduction

Sickle cell disease (SCD) is a group of genetic disorders of haemoglobin due to the inheritance of the sickle globin gene, which results in the production of abnormal haemoglobin (HbS).[1] SCD is caused by a point mutation in a single gene, which results in a mutant β-globin protein (HbS), in which the sixth amino acid is changed from glutamic acid to valine.[1] Homozygous inheritance of HbS is known as sickle cell anaemia (HbSS) and is the most often seen and severest form of SCD. Compound heterozygous inheritance of HbS and other abnormal haemoglobins such as HbSC and HbSβ-that exist but are less common than HbSS.[2] Under a hypoxic condition, there is polymerisation of sickle haemoglobin molecules to form polymers, with resultant damage to the red cell membrane and increased rigidity which is the primary event in the pathogenesis of vaso-occlusive phenomenon with associated pain and is the hallmark of the disease.[3] Despite the limited expression of the mutant protein in SCD, it has multisystem manifestations with damage to every tissue and organ in the body. Patients with SCA usually present with unexplained variable clinical manifestations and disease severity even though they have the same genetic abnormality.[4]

Sickle cell disease (SCD) has been reported as an inflammatory condition in which inflammation is an ongoing process even in a steady state, and oxidative stress is a significant contributory factor.[5,6] Patients with SCA have multiple indicators of an inflammatory response, including elevated C-reactive protein (CRP) levels, white blood cell count, cytokines, activated monocytes, neutrophils, platelets and endothelial cells.[6] C-reactive protein is an acute phase reactant synthesised and secreted by the liver following activation by interleukin-6 (IL-6) from activated monocytes and the liver itself in response to various stimuli.[7] Effects of CRP include: 1. promotion of monocyte chemotaxis to damaged endothelium, 2. the exacerbations of dyserythropoiesis leading to anaemia, 3. alteration of the complement pathway to intensify inflammation, 4. induction of adhesion molecule expression on the endothelial cell and 5. as a positive feedback regulator of inflammatory cytokines, including IL-6.[8] Increased levels of CRP has been previously reported as a general biomarker of inflammation in patients with sickle cell anaemia.[9]

SCD is characterised by abnormally high total white blood cell count, secondary to inflammation and hyposplenism.[10] High white blood cell count in patients with SCA may be a prognostic indicator of clinical severity. Some studies have demonstrated an association between CRP and leucocyte count.[11,12] Elevated white blood cell counts correlate with increased pain frequency and other adverse outcomes in patients with SCA.[13] C-reactive protein (CRP) and white blood cell counts as acute-phase reactants have been proven as a significant predictor of inflammation and disease severity in SCA.[8,13]

Previous studies have reported elevated CRP levels in patients with SCA.[14,15] To the best of our knowledge, no published report in the literature evaluated CRP level and its relationship with blood counts among patients with SCA in

our locality. Therefore, this study aimed to determine CRP level in patients with HbSS (SCA) in a steady state and compare it with persons with HbAA and HbAS control and establish the relationship between CRP level and some haematological parameters in patients with SCA.

Materials and Methods

This study was a cross-sectional comparative study involving patients with HbSS in steady state and HbAA controls (confirmed with Haemoglobin electrophoresis in an alkaline medium).

Steady-state was defined as a period without acute illness, pain, and infection for at least four weeks before recruitment and without blood transfusion in the preceding three months.

The study area was Alex Ekwueme Federal University Teaching Hospital, Abakaliki, Nigeria, between August 2019 and February 2020. The study population comprised patients with HbSS in a steady state aged 1–52 years with age-.and sex-matched HbAA control. We recruited patients with confirmed HbSS (SCA) with Haemoglobin electrophoresis or high-performance liquid chromatography, not on hydroxyurea, who had not received blood transfusion in the preceding three months and had not had a febrile illness in the preceding four weeks. Exclusion criteria included failure to give consent, those with febrile illness, a history of blood transfusion in the preceding three months into the study, pregnant women, and those with HIV or other comorbidities.

Using the method described by Charan and Biswas,[16] and the incidence of sickle cell disease of 3.5%,[17] a sample size of 47 was calculated. However, 101 participants were recruited, made up of 58 participants with HbSS in steady state and 43 controls (23 HbAS and 20 HbAA individuals). Patients were recruited consecutively from the sickle cell clinic of Alex Ekwueme Federal University Teaching Hospital, Abakaliki. Age-.and sex-matched controls with HbAA and HbAS were recruited from among children who came for immunisation in the hospital, children who came for a medical certificate of fitness for admission into school, medical students and staff.

We collected data on our participants' socio-demographic characteristics and clinical status using semi-structured interviewer-administered questionnaires and a review of medical records.

During blood sample collection, the skin overlying any prominent vein at the antecubital region or dorsum of the hand was thoroughly cleaned by circular motion methylated spirit and allowed to air dry. Five millilitres of blood was collected using a size 21G needle with an attached syringe. Dry cotton wool was placed at the blood collection site, and gentle pressure was applied until the bleeding stopped. 2.5 ml of blood was dispensed into ethylenediaminetetraacetic acid (EDTA) bottle, and the remaining 2.5 ml of blood was into a plain bottle and allowed to stand for 2 hours at room temperature to clot and subsequently centrifuged at 3000 g for 5 min using the universal benchtop centrifuge model 80-2 (Gallenkomp, England). The serum extracted was transferred to another plain bottle, stored at −20°C freezers until thawed at room temperature, and analysed for CRP.

The serum level of CRP was assayed using commercially available CRP kits (East Wing Diagnostic Limited). This assay was based on the immunoturbidometric method and was performed according to the manufacturer's instructions.

Blood in an EDTA bottle was used to analyse Haemoglobin phenotypes using Haemoglobin electrophoresis by cellulose acetate method in an alkaline medium and for the analysis of full blood count using haematology

analyser BC-2800 (manufactured by Shenzhen Mindray Bio-Medical Electronic Co. Ltd, Germany).

Data collected were analysed with SPSS software version 26 (SPSS Chicago Inc., IL, USA). Descriptive statistics were used to compute percentages, proportions, means and standard deviation. Analysis of variance was used to compare means. Pearson's correlation tests were used to determine the relationship between variables. Statistical significance was established when probability, $P < 0.05$.

Ethical considerations

Ethical approval for this study was obtained from the Research and Ethics Committee of Alex Ekwueme Federal University Teaching Hospital, Abakaliki, with reference number 29/11/2016 – 02/10/2020. Consent was obtained from every adult participant before being recruited into the study. For minors, patient assent, where appropriate, was obtained in addition to parental permission.

Results

One hundred and one participants were recruited for the study, with 51 (50.5%) males and 50 (49.5%) females, with an age range of 1 to 52 years. They comprised 58 SCA (HbSS) patients and 43 sex-.and age-matched HbAA and HbAS controls (20 HbAA and 23 HbAS individuals). The mean age of HbSS individuals was 11 ± 6.3 years, while that of control was 11 ± 3.4 years and 11 ±0.2 respectively [Table 1].

Patients with HbSS had mean CRP level of 5.4ug/ml ±4.5 which was significantly higher than the mean CRP level of HbAA (0.02ug/ml ±0.01) and HbAS individuals (0.02ug/ml ±0.01) (P = 0.000). Similarly, patients with HbSS had significantly higher white blood cell (WBC) count and platelet count compared to controls (HbAA and HbAS individuals) (p <0.05). On the contrary, patients with HbSS had significantly lower Hb levels compared to HbAA and HbAS control (P <0.05) [Table 2].

Table 1:
Socio-Demographic Features of the Participants

Parameter	HbSS, n (%)	HbAS, n (%)	HbAA, n (%)
Mean age (years)	11± 6.3	11± 3.4	11 ±0.2
Gender			
Male	27 (49.1%)	10 (43.5%)	10 (50.0%)
Female	30 (51.9%)	13 (56.5%)	10 (50.0%)
Total	57 (100%)	23 (100%)	20 (100%)

HbAA = Haemoglobin AA, HbAS = Haemoglobin AS, HbSS = Haemoglobin SS

Table 2:
C - reactive protein and Haematological Parameters among Individuals with HbSS, HbAA and HbAS

Parameter	Mean	±SD		P-value
	HbSS	HbAA	HbAS	
C-reactive protein (µg/ml)	5.4 ±4.5	0.02 ±0.01	0.02 ±0.1	0.000
WBC count (x10^9/l)	11.6 ±7.0	4.3 ±2.1	3.4 ±5.0	0.000
Haemoglobin level (g/dl)	8.1 ±0.8	13.1 ±0.3	12.6 ±2.4	0.000
Platelet count (x10^9/l)	312 ±101	204 ±82	201 ±92	0.000

SD = Standard deviation, HbSS = Haemoglobin SS, HbAS = Haemoglobin AS, HbAA = Haemoglobin AA, WBC = White Blood Cell

The correlation between serum CRP level and blood counts showed a positive relationship between CRP level and WBC count, though not significant (r = 0.286, P = 0.07). Similarly, there was weak positive relationship between CRP level and platelet count, though not significant (r = 0.156, P = 0.336) [Figure 2]. There was a negative correlation between CRP and haemoglobin levels among patients with SCA, though not significant (r = - 0.073, P = 0.655).

Discussion

This study found that CPR levels were significantly higher among sickle cell anaemia patients than those with HbAS and HbAA. This is similar to Okocha *et al*.,[14] and Hedo *et al*.,15, who also reported significantly higher CRP levels among individuals with HbSS. C-reactive protein production is part of the acute phase response to inflammation. Therefore, we are not surprised that its level is significantly higher in HbSS individuals, as SCD has been associated with chronic inflammatory state. CRP has been used to predict prognosis in patients with some chronic disease conditions as a marker of inflammation.[18] Therefore, finding higher CRP levels among HbSS than in HbAA and HbAS individuals suggests a covert inflammatory response, although the HbSS participants were in a steady state. It has been shown that patients with sickle cell anaemia have moderately elevated CRP levels in the steady state, which increases further during crisis.[19] Previous studies have reported that SCA patients who have elevated CRP levels during the prodromal phase of illness ultimately developed vaso-occlusive crisis within one week on the onset of rising in CRP level from the baseline.[20]

This study also found that individuals with HbSS have significantly higher WBC counts than those with HbAA and HbAS. This is in agreement with previous studies, which also reported significantly higher WBC count in patients with HbSS even in a steady state.[10, 21] White blood cell count contributes to the pathogenesis of SCA by adhering to blood vessel walls and stimulating the vascular endothelial cells to increase its expression of ligands for adhesion molecules on blood cells with consequent obstruction of the lumen of a blood vessel, causing tissue damage and inflammatory reaction. This may explain the finding of this study, where WBC count was found to have a positive correlation with CRP level, though not significant. A study by Jowaireia Gaber *et al*. [22] reported a strong association between elevated white blood cell count and increased C-reactive protein level. However, their study was conducted on patients with SCA in vaso-occlusive crises.

The HbSS group had significantly lower haemoglobin levels than the control group. Previous studies have also reported significantly lower haemoglobin levels among patients with HbSS compared to those with HbAA and HbAS.[10,21] There was also a negative correlation between CRP and haemoglobin levels, though not significant. Huang *et al*., [23] also reported an inverse relationship between CRP and haemoglobin levels. Similarly, Santos-Silva *et al*.,[24] reported a significant inverse relationship between CRP and haemoglobin levels. They also reported CRP/ haemoglobin ratio as a predictor of hospitalisation in 80% of cases. These findings suggest that underline haemolytic activity may be associated with inflammation. It has also been reported that CRP levels correlate with anaemia. The high level is associated with lower haemoglobin level and lower response to oral iron therapy, suggesting that elevated CRP may play an essential role in anaemia of chronic disorders.[25]

Platelet count was found to be significantly higher in patients with HbSS compared with HbAA and HbAS individuals, as has also been reported by previous studies.[10,21] C-

reactive protein was found to correlate positively with platelet count, though not significant. Sherkatolabbasieh *et al*., in their study, also reported a corresponding rise in platelet count to elevated CRP levels among children with inflammatory and infectious diseases.[26] Similarly, another study by Nasri *et al*. reported a positive correlation between CRP and platelet count.[27] Platelet has also been observed to rise as a response to inflammation and may explain the increased platelet count with a corresponding rise in CRP level.

Limitations of the study

The study was not done during crisis to determine whether CPR can be used as an early prediction of vaso-occlusive crisis, which might benefit disease management. Secondly, other acute-phase reactants were not studied.

Conclusion

There was a significantly higher CRP level among patients with HbSS in a steady state compared to control (HbAS and HbAA individuals). Participants with HbSS have a positive relationship between CRP and WBC count, platelet count, though not significant, and a negative relationship between CRP and haemoglobin level, though not significant. Further studies are required on a broader scale to assess whether CRP level may be used to predict the occurrence of crisis in patients with SCA.

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Authors' contribution

UNI, NEO and UCN conceived the study and did sample analysis. UGC and EVN did the data collection. A.C. and UNI did the statistical analysis. UGK contributed to the data interpretation and writing of the manuscript. OBN, IRC, MJA and OHC reviewed the manuscript. All authors had final responsibility for the decision to submit the manuscript for publication.

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