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RESEARCH ARTICLE

ASSESSMENT OF SERUM PROTEIN PROFILE IN SICKLE CELL DISEASE

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Abstract

Background: Sickle Cell disease (SCD) is known to be caused by a mutation in the beta-globin gene of the hemoglobin that affects the red blood cells (RBCs) and is passed down through generations. This study was carried out to determine the effect of sickling of RBC on serum protein profile in SCD individuals.

Methods: A case-controlled study was carried out among 80 patients. They were forty-five sickle cell disease individuals (HbSS) attending Children Specialist Hospital Ilorin and thirty-five healthy controls (HbAA). The levels of total protein and albumin were determined spectrophotometrically and serum protein electrophoresis (SPE) was carried out using cellulose acetate electrophoresis.

Results: Significant hypoalbuminemia and hypergammaglobulinemia were observed in SCD groups compared with controls. A higher proportion of the SCD group, 11(25%) had hypergammaglobulinemia and 21(49%) had hypoalbuminemia ($P<0.05$). Plasma protein electrophoresis in SCD patients shows an intense colour at the gamma region which was not seen in control. There was a significant relationship between the patterns of serum protein electrophoresis and the frequency of crisis.

Conclusions: Sickle cell disease crisis and other associated underlying conditions may be prevented early through assessment of serum protein profile, especially SPE. Hypergammaglobulinemia and hypoalbuminemia may be associated with frequent episode of crisis.

Keywords: Serum protein, protein electrophoresis, sickle cell disease, Nigeria.

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INTRODUCTION

Sickle Cell Disease (SCD) is a preventable but irreversible non-communicable, genetically transmitted autosomal recessive blood disorder of significant public health concern in many parts of the world including South America, the Caribbean, Central America, Saudi Arabia, India, East Mediterranean and especially in sub-Saharan Africa.[1] Nigerians continue to bear the greatest burden of sickle cell disease in Sub-Saharan Africa. The three commonest forms of sickle cell disease in sub-Saharan Africa are Sickle cell anemia (HbSS), variant hemoglobin C (HbSC) and Haemoglobin S beta-thalassemia disease (HbSBetaThal) which manifest when there is mutant hemoglobin gene. Sickle cell trait transmitted from both parents leads to aberrant hemoglobin that is less soluble than hemoglobin A and tends to crystallize out, resulting in sickle cell-shaped distortion of the cells, which blocks blood arteries and causes premature death of RBCs. The defective hemoglobin gene is found in 5–7% of the world's population.[2] SCD is the most common type of hemoglobinopathy globally. Sub-Saharan Africa and Asia bear the largest burden of the illness.[3] In certain regions of Sub-Saharan Africa, the prevalence of sickle cell trait ranges from 10 to 45 percent.[4] SCD affects around 2% to 3% of Nigeria's population of over 160 million inhabitants.[5] The high prevalence of SCD in sub-Saharan Africa has been attributed to the survival advantage conferred by the sickle cell trait against *Plasmodium falciparum*. Resistance of individuals with sickle cell trait to *Plasmodium falciparum* creates a selective pressure that has maintained the sickle cell gene within human populations in malaria-endemic regions like sub-Saharan Africa. This phenomenon is termed balanced polymorphism.[6]

SCD is a qualitative hemoglobinopathy caused by a point mutation in the sequence of amino acids on the beta globin chain of the hemoglobin molecule. The sickling mutation changes adenine to thymine on the 17th nucleotide of the beta-globin chain gene (HBB). This inevitably results in the replacement of valine for glutamate on the beta globin chain's sixth amino acid. The abnormal biochemistry of this mutant hemoglobin induces the polymerization of Hb S molecules within the red cells, the so-called sickling. On the sickle hemoglobin, the glutamate protein molecule, which is hydrophilic, polar, and negatively charged, is replaced by a less polar, hydrophobic, neutral amino acid, valine. Under deoxy conditions, the abnormal valine residue causes intra-erythrocytic hydrophobic interaction of sickle hemoglobin tetramers, leading to their precipitation and polymer formation, so-called gelation.[7] Tactoids are formed when all cytosolic hemoglobin molecules precipitate into seven (one inner and six outer) double strands with cross-links. Un-sickling occurs after reoxygenation and the red cell returns to its normal shape. However, repeated sickling and unsickling of the red cell damage the red cell membrane, due to herniation of sickle hemoglobin polymers through the cytoskeleton, thus rendering the red cell permanently sickled. These appear as irreversibly sickled cells in peripheral blood cytology.[8]

Tissues are constantly broken down in sickle cell anemia owing to the sickling of red blood cells in the capillaries found in the organs of the body as a result of disturbance in the circulatory system. Following the progression of the disease, some organs are permanently damaged, despite reparative efforts, which sometimes lead to complications such as chronic nephritis, atrophy of

the spleen, or liver damage (in most cases it is enlarged). The liver frequently shows marked congestion, enlargement of the Kupffer cells with erythrophagocytosis, and hemosiderosis of the liver cells. In most cases, the patients suffer to a certain level from malnutrition, a factor that favors reduced albumin levels.[9]

Much data has not been generated on the plasma protein electrophoretic pattern in patients with sickle cell disease and no study has been recorded in Nigeria on the electrophoretic pattern of plasma protein in Sickle cell patients. Thus, the study was designed to determine the pattern of serum protein in individuals with sickle cell disease in Nigeria.

MATERIAL AND METHOD

This study was a case control study conducted within Ilorin, Kwara state, Nigeria for the period of 4 months in Hospitals within Kwara state. Ethical approval was sought and obtained from the Kwara State Ministry of Health Ethical Committee with reference number MOH/KS/EU/777/509. For this study, a total of eighty participants were recruited. Forty-five (45) SCD patients attending any of the Kwara state hospitals and thirty-five

(35) healthy subjects with the genotype of AA were recruited. Individuals who are qualified based on the laid down criteria and volunteer to partake in this study were recruited and individuals with underlying systemic conditions such as liver cirrhosis and nephrotic syndrome were excluded. A semi-structured questionnaire was used to gather relevant information from the participants and their relatives after informed consent had been sought.

Venous blood of 5mls of the sample was collected from the participants using a sterile syringe from the medial cubital vein in the antecubital fossa. Three milliliters of blood was dispensed into a lithium heparinized tube for total protein and albumin estimation while 2mls was dispensed into a serum tube and kept for 1-2 hours to clot and then obtain serum for SPE. Thereafter all samples were centrifuged, serum and plasma were separated accordingly. The plasma sample was used for albumin and total protein estimation which was done with a spectrophotometer using the bromocresol green method and biuret method respectively. SPE was analyzed using the cellulose acetate electrophoresis method.

The results obtained were keyed into Microsoft Excel and imported into IBM SPSS version 25.0 for analysis. The descriptive statistics using frequency and percentage were used to define the demography, hemoglobin genotype and SCD crisis of the study participants. A Student's t-test was used to compare the plasma protein profile between SCD patients and control at $p<0.05$. Chi-square was used to check the association between SPE in SCD and frequency of crisis, and also the association between the pattern of serum protein and study participants at $p<0.05$.

RESULTS

The majority of the participants in both the SCD group (53%) and control (47%) were within 7 to 12 years of age, while most of the participants in the sickle cell disease group were male (56%) and 44% were female. Among the control group, 49% were male and 51% were female. A larger percentage 33 (73%) showed frequent episodes of sickle cell crises among the sickle cell group "Table 1". A higher proportion of the SCD group 11(25%) had hypergammaglobulinemia and 21(49%) had hypoalbuminemia "Table 2".

The protein bands were visualized on an electropherogram using Ponceau S. Albumin, α-1globulin, and β-globulin appeared as homogeneous bands and uniformly stained, while α-2 globulin and γ-globulin bands appeared diffuse. The staining pattern shows that the majority of the participants with SCD exhibited a more intense staining at the gamma region of the electrophoregram than controlled "Figure 1". The staining pattern shows that the control group exhibited a normal pattern of plasma protein electrophoresis "Figure 2".

In the comparison of serum protein profiles between SCD patients and the control there is no significant difference observed in the total protein, albumin, globulin and albumin-globulin ratio (A/G ($P>0.05$) "Table 3". In the association between the pattern of SPE and frequency of crisis, SCD patient with regular episodes of crisis had significant hypergammaglobulinemia (20%) and hypoalbuminemia compared with their counterparts with occasional episodes of crisis ($P<0.05$) "Table 4".

DISCUSSION

SCD is a genetically inherited disorder resulting from structural hemoglobinopathy that manifests as chronic congenital hemolytic anemia. The prevalence of sickle cell disease is highest in tropical Africa and indeed, the country with the highest burden is Nigeria where the trait occurs in 25–30% and sickle cell anemia occurs in approximately 2% of all births.[5] The most common features of SCD are chronic hemolytic anemia and recurrent vaso-occlusion. The latter is responsible for the painful crises that characterize the disease.

In this study 25 SCD participants were male and 20 participants were female. This shows that SCD affects both genders equally Usman, *et al.* [10] also reported that SCD was equally distributed among both genders. Participants with frequent crises were more than those with occasional crises, similar result was reported by Borhade and Kondamudi.[11] Painful episodes was the main clinical characteristics of SCD which often requires hospitalization.

In this study, a higher proportion of participants in the SCD group were hyperagammaglobulinemia and hypoalbuminemia.

This is similar to the study conducted by Adu *et al.*[12] which reported hypergammaglobulemia in SCD patients. Plasma protein electrophoresis pattern with Intense staining at the gamma band (Hypergammaglobulinemia) was observed in this study, similar finding has also been reported in another chronic ailment such as pulmonary TB[13,14] and HIV infection[15,16] It has been established that patients with chronic inflammation usually have higher levels of serum γ-globulin, which is part of the complex immunological response of body tissues to injurious stimuli.[13] The reason for the increase in the gamma globulin level is due to the stimulation of the humoral mechanism that occurs in SCD patients.

This study observed significantly high hypoalbuminemia (47%) among SCD groups compared to the control group (11%). Moreover, the pattern of alpha-1, alpha-2 and beta sub-fractions were similar for both cases and controls on the serum electrophoresis. This is similar to the study conducted by Adu *et al.* [12] which reported a similar pattern of alpha-1, alpha-2 and beta sub-fractions in SCD patients and AA patients. It is noteworthy that some participants in the control group showed abnormal electrophoretic patterns, such as mild diffuse rise in the γ-band, which was evident in 7.6% of un-infected control, with normal albumin band. This is probably due to genetic predisposition and another intrinsic factor that can influence the gamma globulin pattern.

The serum protein profile shows no significant differences between sickle cell and control. The results of the total proteins assay revealed that there were no significant differences between SCD and control. Changes in serum protein/protein losses usually correspond with a reduction in the levels of albumin, less being influenced by the decrease or increase in globulins[17] However, the serum albumin was significantly lower in SCD participants compared with the control group. This is similar to the finding of a study reported by Ugonabo *et al.*[18] which reported lower serum albumin for the same age group compared to the control. The low level of albumin might be due to malnutrition, an increase in the utilization of albumin and also the inability of the liver to synthesize albumin effectively. It has been noted that in SCD individuals, the presence of HbS in red blood cells initiates many harmful pathways that lead to vaso-occlusion, hemolytic anemia and down-stream reduced nitric oxide (NO) bioavailability, and increased inflammation, oxidative stress and platelet aggregation resulting into hypoalbuminuria. In addition, SCD patients are at a higher risk of endothelial damage [19] and have a range of comorbidities, including liver and renal disease,that could affect their serum albumin.[19]

The globulin values for the control group were not significantly lower when compared with the SCD group. The finding could be attributed to an increase in gamma globulin. An increase in globulin level could be due to increased gamma fraction. This is similar to the study conducted by Ugonabo *et al.*[18] which reported an increase in plasma globulin levels in SCD patients. The increase in globulin level causes a shift in the A/G ratio in SCD patients in this study. The results obtained showed a greater mean A/G in control subjects (1.07 ± 0.30) when compared to cases. This is similar to the study conducted by Ugonabo *et al.* [18] which reported a decrease in A/G ratio in SCD patients compared to control. This suggests that a low level of plasma albumin and an increased plasma globulin level result in a decrease in the A/G ratio. The A/G ratio is a non-specific marker of disease, it is usually employed to determine the causes of changes in serum proteins.[20]

This study also reported that a higher proportion of SCD patients (33%) experiencing a regular episode of crisis have hypoalbuminemia. This might be due to the influence of hypoalbuminemia on the pathophysiology of SCD. A low level of albumin serves as a risk factor for crisis in SCD patients.

CONCLUSION

SCD crisis and other associated underlying conditions may not be obvious during early stages. Therefore, assessment of serum protein profile may serve as an early indication of overt pathological conditions. This study documented hypoalbulminemia, decreased A/G ratio and hypergammaglobulinemia in the SCD group compared to the control group. These findings might be related to vaso-occlusion, hemolytic anemia, increased inflammation, oxidative stress and platelet aggregation which take place in SCD individuals and these are also associated with the severity of crisis in SCD individuals.

Declarations

Ethical approval

The ethical approval was obtained from the Kwara State Ministry of Health with reference number MOH/KS/EU/777/509

Consent to participate

The participants in this study gave consent to the research work and publication

Consent for publication

Not applicable

Availability of data and materials

The datasets generated and analyzed during the current study are included in this article

Competing interest

All authors declare no conflict of interest

Funding

Not applicable

Authors' contribution

MI & HAY conceptualized the idea; MI, HAY, WOG & TJO were involved in writing the original draft to final editing, Methodology, data presentation and discussion of findings; AZL, GOA, KAO, AOB, WOG, ANP, SIE, AN, MI were involved in editing and review

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Not applicable

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ANNEX

TABLE 1: Demography, hemoglobin genotype and sickle cell disease crisis of the study participants.

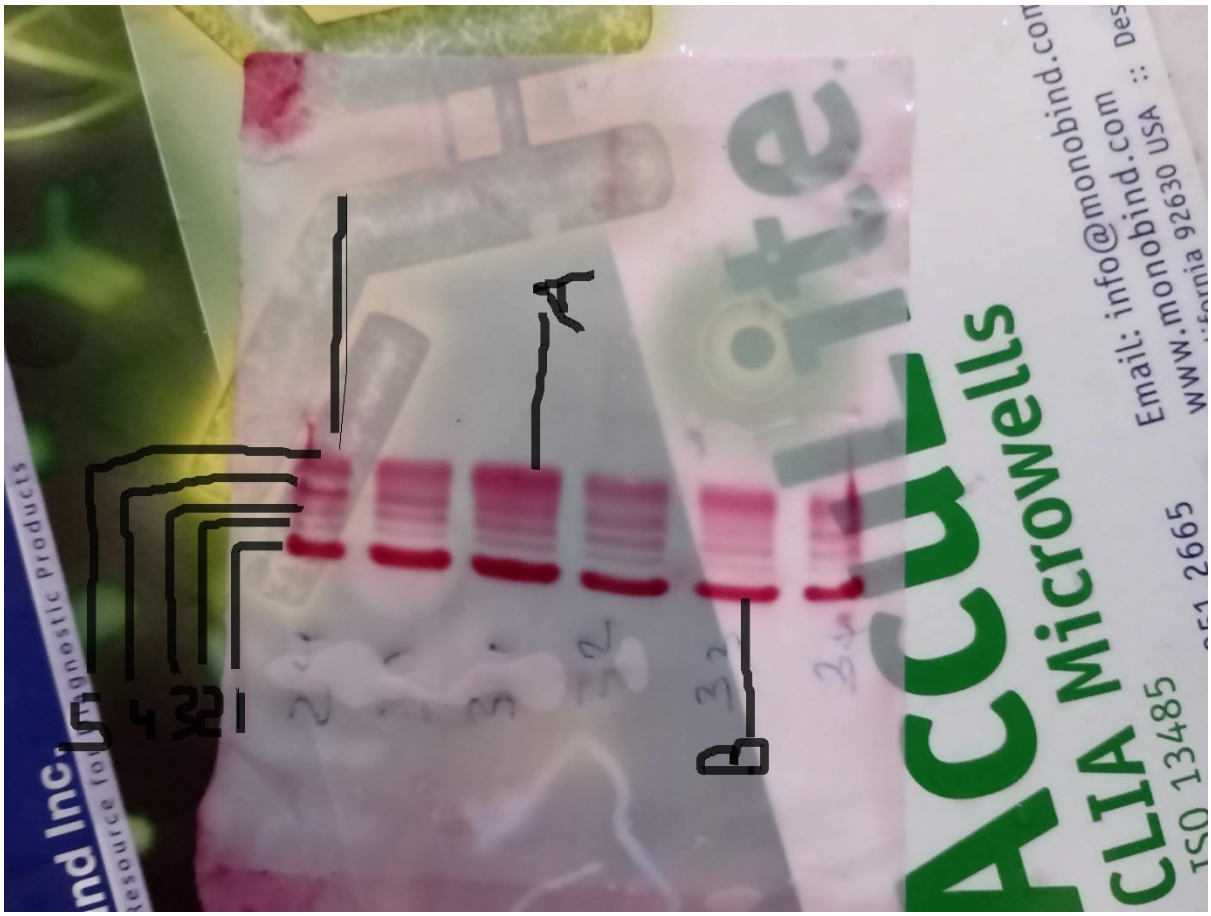
1. Characters		**2. SCD 3. n (45)**	**4. Control 5. n (35)**
6. Age (years)	7. 2 – 6	8. 21 (47%)	9. 12 (34%)
10.	11. 7 – 12	12. 24 (53%)	13. 23 (66%)
14. Gender	15. Male	16. 25 (56%)	17. 17 (59)
18.	19. Female	20. 20 (44%)	21. 18 (51)
22. Hb genotype	23.	24. SS	25. AA
26. SCD crisis	27. Frequent	28. 33 (73%)	29. NA
30.	31. Not frequent	32. 12 (27%)	33. NA

TABLE 2: Abnormal pattern of serum protein electrophoresis in control and sickle cell disease participants.

Pattern	Control	SCD	χ2 value	P value
Hypergammaglobulinemia	5 (14%)	11 (25%)	1.27*	<0.05
Hypogammaglobulinemia	0	0	NA	NA
Hypoalbuminemia	4 (11%)	21 (47%)	1.034*	<0.05
Hyperbetaglobulinemia	8 (23%)	5 (11%)	5.03	>0.05
Hypoalphaglobulinemia	1 (3%)	5 (11%)	5.34	>0.05

Values are expressed in (%). The calculated Chi-square values were compared with the critical χ2 statistic value for p = 0.05 (95% confidence level). NA = Not applicable. *significant difference

FIGURE 1. Electrophoregram showing different protein bands, albumin, α1, α2, β and Y globulins.



1: Albumin

2: Alpha-1 globulin

3: Alpha-2 globulin

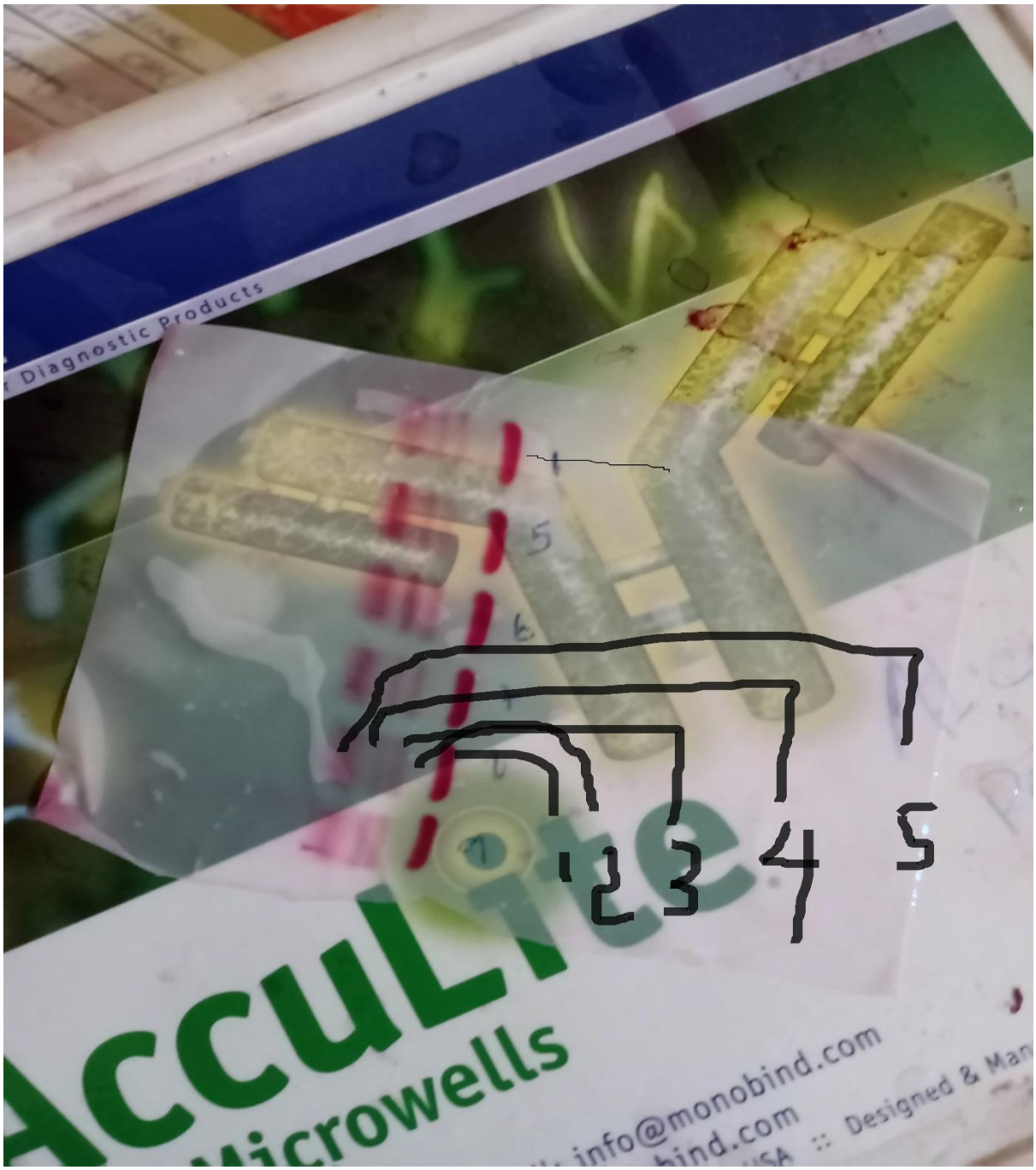
4 Beta globulin

5 Gamma globulin

A: Hypergammaglobulinemia

B: hypoalbuminemia

FIGURE 2. Shows a normal pattern of electrophoresis in healthy individuals.



1: Albumin 2: Alpha-1 globulin 3: Alpha-2 globulin 4: Beta globulin 5: Gamma globulins

TABLE 3: plasma protein profile of sickle cell disease and control

Parameters	Control	SCD	P value
Total Protein (g/L)	72.91± 9.01	70.05 ±8.67	0.157
Albumin (g/L)	40.15 ± 2.76	33.71 ± 4.29	0.131
Globulin (g/L)	37.63 ± 8.99	36.34 ± 9.35	0.536
A/G	1.07 ± 0.30	0.93 ± 0.34	0.958

The values are mean ± SD, and p-values were determined by Student's t-test as appropriate $p < 0.05$ was considered significantly different, *****when there is an intergroup significant difference.

TABLE 4: pattern of serum protein electrophoresis in sickle cell disease patients with frequent episodes of crisis

	Occasional	Regular	$\chi 2$ value	P value
Hypergammaglobulinemia	2 (4%)	9 (20%)	2.652*	<0.05
Hypogammaglobulin	0	0	NA	NA
Hypoalbuminemia	6 (13%)	15 (33%)	0.687*	<0.05
Hyperbetaglobulinemia	3 (6%)	2 (4%)	7.034	>0.05
Hypoalphaglobulinemia	2 (4%)	3 (4%)	8.74	>0.05

Values are found (%). The calculated Chi-square values were compared with the critical $\chi 2$ statistic value for $p = 0.05$ (95% confidence level).NA = Not applicable. *significant difference