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PLASMA PROTEIN PROFILE IN CHILDREN WITH HbAS AND HbSS IN UGHELLI GOVERNMENT HOSPITAL, DELTA STATE, NIGERIA

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ABSTRACT

The plasma protein profile in children with HbAA, HbAS and HbSS was studied in this research with the view of knowing the group with higher plasma protein values and ascertaining the effects of crises in plasma protein level in sickle cell condition. In all 300 children between the ages of 5 to 15 were recruited from the sickle cell clinic of the Marierie Memorial Government Hospital, Ughelli, Delta state. The children were studied in seven groups. Plasma total protein was determined by the Biuret method using an Olympus AU400 automated chemistry analyzer while Plasma albumin, globulin, and fibrinogen determination were performed manually using a Sebia K20 electrophoresis chamber with Cellogel strips in Tris-hippurate buffer, pH 8.8 according to the manufacturer's instructions. The results from this study revealed that the HbSS subjects had significantly higher fibrinogen levels (0.71±0.82) than the HbAS subjects (0.42±0.56) and healthy HbAA subjects (0.30±0.57). The mean globulin in the homozygous (HbSS) was significantly greater than the mean values found in normal children ($P< 0.05$). Also, a statistically significant higher mean globulin value was observed in HbAS ($P< 0.05$). The total protein level was also found to be higher in HbSS when compared with that of the control (HbAA) and the carriers (HbSS) at $P<0.05$. The results also showed that when HbSS subjects were in crisis, it was only the fibrinogen level that increased statistically significantly ($P<0.05$). It was concluded from this study that individuals with HbSS and HbAS have higher total plasma protein and globulin and individuals with HbSS have higher fibrinogen concentration than normal healthy individuals with HbAA of same age grade. Crisis was also said to be associated with higher fibrinogen values in HbSS individuals but does not influence the values of globulin, albumin and total plasma protein hence regular estimation of the plasma fibrinogen levels in individuals with sickle cell condition might be a useful indicator of hyper coagulability and early determination of plasma fibrinogen will help in diagnosing hyperfibrinogenaemia earlier and may help to prevent vaso-occlusive crises. It was therefore recommended that the causes of these differences observed be ascertained in further studies.

Keywords: *sickle Cell Disease, Sickle Cell Trait, Plasma Proteins, Albumin, Globulin, Fibrinogen*

INTRODUCTION

Sickle-cell anaemia is a common genetic condition due to a haemoglobin disorder inheritance of mutant haemoglobin genes from both parents. The frequency of the carrier state determines the prevalence of sickle cell anaemia at birth. Nigeria which is by far the most populous country in Africa, has 24% of its population being carriers of the mutant gene and the prevalence of the sickle cell anaemia is about 20 per 1000 births (Savitt and Goldberg ,

1989) .The sickling occurs because of a mutation in the haemoglobin gene. Life expectancy is shortened, with studies reporting an average life expectancy of 42 in Males and 48 in Females (Savitt and Goldberg, 1989). The disease is described as an inherited autosomal recessive disorder characterized primarily by chronic anaemia and periodic episodes of pain. Individuals who possess one copy of the normal beta globin gene (HbA) and one copy of the sickle variant (HbS) are referred to as having the sickle cell trait, but these individuals do not express symptoms of sickle cell disease. Hence, sickle cell traits present with varied problems including increased urinary tract infection in women, gross hematuria, complications of hyphema, splenic infarction with altitude hypoxia or exercise, and life-threatening complications of exercise, exertional heat illness (exertionalrhabdomyolysis, heat stroke, or renal failure) or idiopathic sudden death (Roberts and de Montalembert , 2007; Bardakdjian and Wajcman, 2004; Buseri et al., 2007 and John, 2010). Sickle cell trait is not usually regarded as a disease state because it has complications that are either uncommon or mild. Sickle cell anaemia affects millions throughout the world. It is particularly common among people whose ancestors come from sub-Saharan Africa; Spanish-speaking regions (South America, Cuba, Central America); Saudi Arabia; India; and Mediterranean countries such as Turkey, Greece, and Italy. In the Unites States, it affects around 72,000 people, most of whose ancestors come from Africa. The disease occurs in about 1 in every 500 African-American births and 1 in every 1000 to 1400 Hispanic-American births. About 2 million Americans, or 1 in 12 African Americans, carry the sickle cell trait (Bardakdjian and Wajcman, 2004). Although several hundred HBB gene variants are known, sickle cell anaemia is most commonly caused by the haemoglobin variant HbS. In this variant, the hydrophobic amino acid valine takes the place of hydrophilic glutamic acid at the sixth amino acid position of the HBB polypeptide chain. This substitution creates a hydrophobic spot on the outside of the protein structure that sticks to the hydrophobic region of an adjacent haemoglobin molecule's beta chain.

This clumping together (polymerization) of Hb S molecules into rigid fibres causes the "sickling" of red blood cells (Bardakdjian and Wajcman, 2004). Polymerization occurs only after red blood cells have released the oxygen molecules that they carry to various tissues throughout the body. Once red blood cells return to the lungs where haemoglobin can bind oxygen, the long fibres of HbS molecules depolymerise or break apart into single molecules. Cycling between polymerization and depolymerisation causes red blood cell membranes to become rigid. The rigidity of these red blood cells and their distorted shape when they are not carrying oxygen can result in blockage of small blood vessels. This blockage can cause episodes of pain and can damage organs (Buseri et al., 2007). Haemoglobin molecules, which reside in red blood cells, are responsible for carrying oxygen from the lungs to various parts of the body for use in respiration. The HBB gene codes for one of the two types of polypeptide chains found in adult haemoglobin. Normal adult haemoglobin is a tetrameric protein consisting of two alpha chains and two beta chains. HBB codes for the beta chain, which is often referred to as beta globin. Mutant beta globin is responsible for the sickling of red blood cells seen in sickle cell anaemia. In 1949 - Notable physical chemist *Linus Pauling* and associates publish "Sickle Cell Anaemia, a Molecular Disease" in *Science.* This paper

explains how protein electrophoresis was used to show that sickle cell haemoglobin differed in structure from normal haemoglobin. This was the first time that the cause of a disease was linked to a change in protein structure (Roberts and de Montalembert, 2007). For the disease to be expressed, a person must inherit either two copies of Hb S variant or one copy of Hb S and one copy of another variant. Carriers, who have one copy of the normal HBB gene (HbA) and one copy of HbS, are described as having sickle cell trait and do not express disease symptoms (Buseri et al., 2007). The clinical course of sickle cell anaemia does not follow a single pattern; some patients have mild symptoms, and some have very severe symptoms. The basic problem, however, is the same: the sickle-shaped red blood cells tend to get stuck in narrow blood vessels, blocking the flow of blood. These results in conditions such as Hand-foot syndrome**,** Fatigue, paleness, and shortness of breath and others as symptoms of anaemia or a shortage of red blood cells (Buseri et al., 2007). A patient may experience pain wherever sickled blood cells block oxygen flow to tissues. The frequency and amount of pain vary. Some patients have painful episodes (also called crises) less than once a year, and some have as many as 15 or more episodes in a year. Sometimes pain lasts only a few hours; sometimes it lasts several weeks. For especially severe on-going pain, the patient may be hospitalized and treated with painkillers and intravenous fluids. Pain is the principal symptom of sickle cell anaemia in both children and adults (Bardakdjian and Wajcman, 2004).

Eye problems: The retina, the "film" at the back of the eye that receives and processes visual images, can deteriorate when it does not get enough nourishment from circulating red blood cells. Damage to the retina can be serious enough to cause blindness (Bardakdjian and Wajcman, 2004). Yellowing of skin and eyes are signs of jaundice, resulting from rapid breakdown of red blood cells. The underlying problem involves haemoglobin, a component of red blood cells. Haemoglobin molecules in each red blood cell carry oxygen from the lungs to body organs and tissues and bring carbon dioxide back to the lungs. In sickle cell anaemia, the haemoglobin is defective. After haemoglobin molecules give up their oxygen, some may cluster together and form long, rod-like structures. These structures cause red blood cells to become stiff and assume a sickle shape (Baltimore, 2001). Unlike normal red cells, which are usually smooth and donut-shaped, sickled red cells cannot squeeze through small blood vessels. Instead, they stack up and cause blockages that deprive organs and tissues of oxygen-carrying blood. This process produces periodic episodes of pain and ultimately can damage tissues and vital organs and lead to other serious medical problems. Normal red blood cells live about 120 days in the bloodstream, but sickle red cells die after about 10 to 20 days. Because they cannot be replaced fast enough, the blood is chronically short of red blood cells, a condition called anaemia (Bardakdjian and Wajcman, 2004).

Sickle cell trait is not usually regarded as a disease state because it has complications that are either uncommon or mild. Nevertheless, under unusual circumstances, serious morbidity or mortality can result from complications related to polymerization of deoxy-haemoglobin S. A previous study was earlier conducted to study Sickle cell traits and it revealed that there was enhanced lipid per oxidation along with imbalance in the pro-oxidant and antioxidant

status in patients with sickle cell anaemia. Moreover, sickle cell traits present with varied problems including increased urinary tract infection in women, gross haematuria, complications of hyphema, splenic infarction with altitude hypoxia or exercise, life-threatening complications of exercise etc. Renal medullary carcinoma in the young as well as disease from autosomal dominant polycystic kidney disease is other well-known occurrences in sickle cell traits. In view of the above facts, this article aims to also investigate plasma protein profile in sickle cell disease and the trait (John, 2010). Findings have also revealed Plasma Proteins as Sources of Amino Acids for the Tissues**.** When the tissues become depleted of proteins, the plasma proteins can act as a source of rapid replacement (Guyton and Hall, 2011). Indeed, whole plasma proteins can be imbibed totally by tissue macrophages through the process of pinocytosis; once in these cells, they are split into amino acids that are transported back into the blood and used quantities too small to supply the body's needs. This second group of amino acids that cannot be synthesized is called the *essential amino acids.* Use of the word "essential" does not mean that the other 10 "nonessential" amino acids are not required for the formation of proteins, but only that the others are *not essential in the diet* because they can be synthesized in the body. Synthesis of the nonessential amino acids depends mainly on the formation of appropriate a-keto acids. Thus Plasma proteins function as a labile protein storage medium and represent a readily available source of amino acids whenever a particular tissue requires them (Guyton and Hall, 2011).

The changes seen in the serum protein values in the past studies, although not specific for the diagnosis of disease, have invariably yielded extremely valuable information regarding clinical conditions. Previous information on plasma protein values in American Negroes with the homozygous disease were based on few samples (Edozien, 1957, 1961; Edozien et al., 1960) and their results still do not involve carriers of the disease (HbAS). Some were case reports, and the information gathered regarding the serum proteins suffered from the very limited number of patients investigated. It must also be stated that most of their values were compared with values commonly found in a normal population and none of them involved this region of interest in their studies. In this study, however, the protein pattern of sickle cell (HbSS) subjects and carriers (HbAS) were compared with that of normal children of identical age range. A study of the protein pattern in a large number of sickle cell anaemia patients is, therefore, considered necessary in the hope that this would provide additional information that would help in the management of sickle cell condition. Low albumin/globulin ratio have been said to be a characteristic of the protein pattern in the normal African (Edozien, 1957, 1961; Edozien et al.1960). They further reported that changes seen in the serum protein values, although not specific for the diagnosis of disease, have invariably yielded extremely valuable information regarding clinical conditions. This work aims at evaluating the plasma protein profile of children with HbAS and HbSS against that of normal children with HbAA and finding out the possible effects of crisis on the concentration of plasma protein in subjects with HbSS

MATERIALS AND METHODS

Sample Size

The sample size was determined by the Krejcie & Morgan methods (Krejcie and Morgan, 1970). In all 300 children between the ages of 5-15 were selected for the research. The children were recruited from the sickle cell clinic of Marierie Memorial Government Hospital, Ughelli between November, 2011 and May, 2012. They comprise of 100 children with genotype HbAS, 100 children with genotype HbSS and 100 children with normal haemoglobin genotype HbAA.

Location

This research was carried out at the Department of Human Physiology, Delta State University, Abraka, Nigeria. The blood Samples were collected from children who came for treatment and check-up at the Mariere Memorial Government Hospital Ughelli, the laboratory analysis were also done at the Laboratory and Haematology Section of the Mariere Memorial Government Hospital, Ughelli, Delta State.

Subjects Inclusion/ Exclusion Criteria

- The subjects must not have taken any drug that can affect the measurement of serum proteins include chlorpromazine, corticosteroids, isoniazid, neomycin, phenacemide, salicylates, sulfonamides, and tolbutamide.
- Subjects with chronic renal failure and Hypertension were removed from the selection
- The subjects were made to fast for 3- 4 hours before blood sample was collected.

Research Design

The subjects were grouped into seven (A, B, C, D, E, F and G). Group A consist of 100 subjects with genotype HbAA, Group B consist of 100 subjects with genotype HbAS, Group C consist of 100 subjects with genotype HbSS which were not in crisis Group A served as the control group while group B and C served as experimental groups). Furthermore, forty subjects were selected from group C to form group D and E. Group D (control group) consist of 20 HbSS subjects not in crisis and Group E consist of 20 HbSS subjects in crisis. Ten (10) subjects from Group E were regrouped into group F and G as follow; Group F was made up of ten HbSS subjects from group E studied when they were in the period of crisis while Group G consisted of the same ten subjects in group F studied after their period of crisis.

Sample Analysis

Genotypes of subjects were determined by means of haemoglobin electrophoresis on cellulose acetate by the method of Dacie and Lewis, (1968) as described in Baker et al., (2007) and Fischbach et al., (2009). Other investigations done along with the electrophoresis to support the diagnosis of the sickle cell disease included blood film examination and sickling test which were determined by the methods of Baker et al., (2007), Abayomi, (2007) and Fischbach et al., (2009). Plasma total protein was determined by the Biuret method using an Olympus AU400 automated chemistry analyzer as described below according to the

manufacture instructions. Plasma albumin, globulin, and fibrinogen determination were performed manually using a Sebia K20 electrophoresis chamber (Sebia Hispania, Barcelona, Spain) with Cellogel strips (Cellogel, Milano, Italy) in Tris-hippurate buffer, pH 8.8 (Cellogel) according to the manufacturer's instructions. For statistical data comparisons, data were evaluated by one way ANOVA and Student *t*-Test. All values are given as mean ± SD with n values indicating the number of subjects analyzed. $P<0.05$ are considered significant. For Ethical Recommendation**,** Approval was sought and obtained from the Research and Ethical committee, College of Health Sciences, Delta State University Abraka. Approval was also obtained from the Grant Committee of the Mariere Memorial Government Hospital, Ughelli. Formal consent was also obtained from subjects and their parents.

RESULTS

The results obtained are presented in tables 1, 2 and 3 as MEAN ± SD. $P<0.05$

Table 1: Shows the values of Fibrinogen, Albumin, Globulins and Total Protein.

Parameters	HbAA (Control)	HbAS (Carriers)	HbSS (Sicklers)
Fibrinogen	0.30±0.57	0.42±0.56	0.71±0.82*
Albumin	4.50±0.59	4.39±0.66	4.40±0.71
Globulins	3.10±0.33	3.40±0.89*	3.60±0.78*
Total Protein	7.60±0.72	7.79±0.92*	8.00±0.99*

Values are Mean±SD, n=100. The superscript represent significant differences of test in comparison with respective controls ($P<0.05$).

The results obtained in table 1 above showed that the HbSS patients had significantly higher fibrinogen levels (0.71±0.82*) than the HbAS subjects (0.42±0.56) and healthy HbAA subjects (0.30±0.57) which served as control at ($P < 0.05$). This increase may be due to vaso-occlusive crisis experienced by the HbSS subjects. There was no statistically significant change observed in mean albumin. This is an indication that blood colloid osmotic pressure for HbSS is not affected by the sickling condition. The mean globulin in the homozygous sickle cell subject (HbSS) was significantly greater than the mean values found in normal children ($P< 0.05$). Also, a statistically significant higher mean globulin value was observed in HbAS ($P< 0.05$). The total protein level was also found to be higher in HbSS when compared with that of the control (HbAA) and the carriers (HbSS) at $P<0.05$.

Table 2: Shows the values of Fibrinogen, Albumin, Globulins and Total Protein in and not in crisis.

Parameters	NOT IN CRISIS (n= 20)	IN CRISIS (n=20)
Fibrinogen	0.36±0.28	0.49±0.16*
Albumin	4.29±0.41	4.30±0.50

Globulins	3.60±0.63	3.92±0.57
Total Protein	7.89±0.74	8.22±0.80

Values are Mean ±SD. The superscript represent significant differences of test in comparison with respective controls (P<0.05).

The results from Table 2 showed that when subjects were in crisis, it was only the fibrinogen that was statistically significant at (P<0.05), though the values for albumin, globulins and total protein were increased during crisis when compared with those that were not in crisis, the increase was not statistically significant at (P<0.05).

Table 3: Shows the values of Fibrinogen, Albumin, Globulins and Total Protein during and after crisis.

Parameters	**DURING CRISIS (n= 10)**	**AFTER CRISIS (n=10)**
Fibrinogen	0.49±0.16*	0.25±0.22
Albumin	4.30±0.50	4.35±0.44
Globulins	3.92±0.57	3.42±0.69
Total Protein	8.22±0.80	7.70±0.74

Values are Mean ±SD. The superscript represent significant differences of test in comparison with respective controls (P<0.05).

The results from Table 3 showed that during crisis the level of fibrinogens in the HbSS were very high but after the crisis the value reduced. This was statistically significant. Though albumin increased slightly (4.35±0.44), it was not statistically significant. Globulin and total proteins also reduced after crisis which was not statistically significant. The implication of this is that the blood transfused during crisis had helped to stabilize the subjects under study.

DISCUSSION

Sickle-cell anaemia is a common genetic condition due to a haemoglobin disorder inheritance of mutant haemoglobin genes from both parents. The frequency of the carrier state determines the prevalence of sickle cell anaemia at birth. Nigeria which is by far the most populous country in Africa has 24% of its population being carriers of the mutant gene and the prevalence of the sickle cell anaemia is about 20 per 1000 births (Savitt and Goldberg, 1989). Plasma proteins are Component of the plasma and plasma is that clear straw coloured (i.e. pale yellow) supernatant component of a centrifuged blood that constitutes about 55 per cent of the blood volume (Ezeillo, 2011). Essentially all the albumin and fibrinogen of the plasma proteins, as well as 50 to 80 per cent of the globulins, are formed in the liver. The remainders of the globulins are formed almost entirely in the lymphoid tissues. The rate of plasma protein formation by the liver can be extremely high, as much as 30 g/day. Certain disease conditions cause rapid loss of plasma proteins. The rapid production of plasma proteins by the liver is valuable in preventing death in such states. In *cirrhosis of the liver,* large amounts of fibrous tissue develop among the liver parenchyma cells, causing a

reduction in their ability to synthesize plasma proteins. This leads to decreased plasma colloid osmotic pressure, which causes generalized oedema (Guyton and Hall, 2011). In this research, the plasma protein profile of sickle cell anaemic subjects and the plasma protein profile of carrier of the disorder were evaluated against that of normal HbAA subjects. The result from this study reveals that the HbSS subjects had significantly ($p<0.05$) higher fibrinogen levels (0.71±0.82) than the HbAS subjects (0.42±0.56) and healthy HbAA subjects (0.30±0.57) which served as control as shown in Table 4.1. This increase may be due to vaso-occlusive crisis experienced by the HbSS subjects. There was no statistically significant change observed in mean albumin. This is an indication that blood colloid osmotic pressure for HbSS is not affected by the sickling condition and that there is normal functions of albumin in the three groups. The mean globulin in the homozygous sickle cell subjects (HbSS) was significantly greater than the mean values found in normal children ($P< 0.05$). This finding is in line with Isichei (1979) in his study that showed plasma globulin is higher in children with sickle cell disease. This hyperfibrinogenaemia noticed during sickle-cell crisis in this study can be a contributing factor to red cell slugging and increased whole-blood viscosity at the onset of crisis which could lead to hyper coagulability and also lead to vaso-occlusive crisis. This therefore shows that estimation of the plasma fibrinogen levels in sickle cell disease patients might be a useful indicator of hyper coagulability, while early diagnosis may help to prevent vaso-occlusive crises in these patients as previously explained by Richardson *et al.*, (1976). There was no statistically significant change observed in mean albumin during crisis. This is an indication that blood colloid osmotic pressure for HbSS is not affected by the sickling condition (Guyton and Hall, 2011). Even when studied after crisis, the difference in albumin noticed was not statistically significant as shown in Table 2 and 3. This result supports the earlier assertion by Edozie, (1960) that plasma albumin level is not affected by sickle cell disease.

The results from Table 4.2 showed that when subjects were in crisis, it was only the fibrinogen that was statistically significant ($p<0.05$), though the values for albumin, globulins and total protein were increased during crisis when compared with those that were not in crisis, the increase was not statistically significant ($P<0.05$). The results from Table 3 showed that during crisis the level of fibrinogens in the HbSS were very high but after the crisis the value reduced. This was statistically significant, though albumin level (4.35±0.44) also increased, but the increase was not statistically significant. Therefore this study suggest that fibrinogen and albumin produced by the liver increase during anaemic conditions a fact also supported by Bogoch *et al.* (1955) in a study showing increase in diseases or damages in the liver during anaemic crisis and Rosalki and Mcintyre (1999) who claimed changes in the level of fibrinogen and albumin during liver damages. Globulin and total proteins also reduced after crisis but these reduction was not statistically significant ($p<0.05$). The implication of this is that the blood transfused during crisis had helped to stabilize the subjects under study. This is also in accordance to findings from Isichei (1979). From the study, total protein level was also found to be significantly ($p<0.05$) higher in HbSS and (HbAS) when compared with that of the control the (HbAA). This higher total plasma protein resulted from the higher globulin values recorded in the study.This finding also agreed with Isichei (1979) who

reported that children with sickle cell disease of different age groups had a higher total serum protein values.

RESEARCH FINDINGS

The plasma protein profile of the subjects used in this study has shown that:

- The albumin level is almost the same in individuals with HbSS, HbAS and normal healthy individuals with HbAA.
- The globulin values in HbAS and HbSS were significantly greater than that in normal children of the same age group, showing that the globulin fraction is largely accountable for the high total protein noticed.
- Individuals with HbSS and HbAS are also associated with higher total plasma protein values than HbAA individuals. This increase in total protein was as a result of the increase in globulin concentration
- Also painful crisis is associated with a further increase in plasma fibrinogen level in sickle cell disease but does not affect the concentration of globulin and albumin and total plasma protein

CONCLUSION AND RECOMMENDATION

In conclusion, the result from this study showed that higher plasma protein is associated with sickle cell disease and that the total plasma protein and globulin levels in both the homozygous and heterozygous sickle cell is higher than that of normal healthy individuals with HbAA of same age grade. The globulin fraction is largely accountable for the high total protein observed in this study. Painful crisis was also discovered in this study to be associated with increase in plasma fibrinogens in HbSS and so, estimation of the plasma fibrinogen levels in sickle cell disease patients might be a useful indicator of hypercoagulability and early determination of plasma fibrinogen will help in diagnosing hyperfibrinogenaemia earlier and may help to prevent vaso-occlusive crises in sickle cell disease patients. We therefore recommend that the cause of these differences be ascertained in further studies and that a regular determination of plasma fibrinogen should always be done in individual with HbSS to avoid vaso-occlusion.

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