ASSESSMENT OF PLASMA ALBUMIN, TOTAL PROTEIN AND PROSTAGLANDINS IN SICKLE CELL CHILDREN IN EKITI STATE, NIGERIA.

Oyeyemi A.O[1], Sonuga A.A[2],

1 Department of Biochemistry, Ekiti State University, Ado Ekiti, Nigeria.

2 Department of Science Laboratory Technology, Ekiti State University. Nigeria

ABSTRACT

Background: Sickle cell disease (SCD) is an inherited blood disorder caused by abnormal haemoglobin. Sickle cell disease limits the oxygenating role of haemoglobin, resulting in the damaging or the "sickling" of the red blood cells .This disease continues to be a global health problem that presents major challenges to our health care systems. **Materials and methods:** This study was carried out to determine, the levels of plasma albumin, total protein and prostagladins in children with sickle cell anaemia in Ekiti State, Nigeria. One hundred and twenty (120) children altogether were recruited for this study. They were divided into three groups of forty each, classified as sickle cell (SS), carrier (AS) and normal (AA). The subjects were recruited from Ekiti state teaching hospital, Ado Ekiti, Federal Medical center, Ido Ekiti and some selected hospitals in Ekiti State. Biuret Method, Bromocresol Green (BCG) and Enzyme Linked Immunosorbent Assay(ELISA) methods were used in the determination of Total protein, Albumin and prostaglandins(PGE_1 and PGE_2) respectively. **Results:** The mean albumin, total protein, PGE1 and PGE2 values for the sickle cell children (5.1±1.1g/dL, 9.6±0.9g/dL, 10.04± 0.42ng/ml and 11.31±0.51ng/ml)were significantly higher ($p<0.05$) than non-sickle cell children (4.6±1.0 g/dL, 8.1±0.8g/dL, 0.72±0.05ng/ml and 0.81±0.04ng/ml). **Conclusion:** This study shows that albumin, total protein and prostaglandin concentration may be implicated in children with sickle cell disease but has no implication in children who are sickle cell carrier.

Keywords: Sickle cell anaemia, Albumin, Total protein, Prostaglandins, Biuret Method, Bromocresol Green (BCG) method.

INTRODUCTION:

Sickle cell disease (SCD) is one of the most common genetic diseases worldwide, and predominates in Middle East, Mediterranean regions, Southeast Asia, and sub-Saharan Africa especially Nigeria (Serjeant *et al*, 2001). The prevalence of sickle cell trait ranges between 10 and 45% in various parts of sub-Saharan Africa (WHO 2013 , Serjeant *et al*, 2013). In Nigeria, carrier prevalence is about 20 to 30% (Uzoegwu *et al*,2003). Estimate from a large retrospective study in Benin City, South-South Nigeria revealed an SCD prevalence of 2.39% and a carrier rate of about 23% (Nwogoh *et al*,2012). Sickle cell anaemia results from a point mutation in the genetic code such that a single amino acid (glutamic acid) is replaced by another (valine) in the globin chain of haemoglobin (Hb). This substitution transforms normal adult haemoglobin (HbA) into sickle haemoglobin (HbS). If an individual is homozygous for the sickle cell mutation (HbSS) almost all the haemoglobin is sickle haemoglobin. In the heterozygous individual (HbAS), up to 40% of the haemoglobin is sickle haemoglobin. Under conditions of low oxygen tension HbS molecules undergo aggregation and polymerize and the red cells aquire a ‘sickle’ or ‘holly leaf’ shape. Sickling of red cells leads to two major consequences, a chronic haemolytic anaemia and occlusion of small blood vessels resulting in ischemic tissue damage. Sickle haemoglobin (HbS) results from a substitution of one amino acid (Valine) for another amino acid (Glutamic acid) at position six of the -globin polypeptide chain. This substitution is caused by a single-base mutation in codon 6 within the -globin gene on chromosome 11, where the sequence GAG occurs instead of GTG.

Due to the abnormal amino acid in the -globin chain, HbS forms long, insoluble polymers when deoxygenated, and the red blood cells (RBCs) containing HbS become less deformable and form a “sickle” shape. It was previously thought that the clinical consequences were simply due to this abnormal, rigid sickle red blood cell occluding small blood vessels. These relate to concentration of HbS and other haemoglobin variants such as HbF within the cell which reduces its ability to polymerise (Nagel *et al,* 1979), disturbances in the red cell membrane making the cell less responsive to oxidant stress, and altered membrane lipids resulting in increased rigidity of leukocytes and endothelial cells, and platelet aggregation (Noguch *et al,* 1983, Brugnara *et al* 1986), The loss of red blood cell elasticity is central to the pathophysiology of sickle-cell disease. In sickle red blood cells, mutated hemoglobin can polymerize when deprived of oxygen, assembling themselves into long polymer fibers that

push against the membranes of the cells, forcing them out of shape. The stiff, ill-shaped cells can become lodged in small capillaries throughout the body, leading to painful episodes known as sickle cell crisis(Lu Lu *et al*, 2017).These cells fail to return to normal shape when normal oxygen tension is restored. As a consequence, these rigid blood cells are unable to deform as they pass through narrow capillaries, leading to vessel occlusion and ischemia.

Patients with sickle cell anemia/trait may experience hepatic changes which include: changes in the shape of red blood cells favors intravascular hemolysis and in this way impediment of the liver vascular bed, leading to tissue damage. Furthermore, hemolysis induces deposition of bilirubin causing intrahepatic cholestasis and cholelithiasis. The incidence of liver disease in sickle cell disorders is difficult to ascertain despite being a component of the multiorgan failure that occurs in sickle cell disease (Hassell *et al*, 1994) .

Albumin, produced only in the liver, is the major plasma protein that circulates in the bloodstream. Albumin is essential for maintaining the oncotic pressure in the vascular system. A decrease in oncotic pressure due to a low albumin level allows fluid to leak out from the interstitial spaces into the peritoneal cavity, producing ascites. Albumin is also very important in the transportation of many substances such as drugs, lipids, hormones, and toxins that are bound to albumin in the bloodstream. Proteins are linear chains or polymers of amino acids, which are covalently linked by peptide bonds (Rawn,1989). They are large molecules of varying molecular weight ranging from 1 to 1000kda. Protein functions mainly as transporter of substances within the blood circulation and the defense of the body against damage. Proteins in the plasma or serum are readily accessible and can be analyzed directly to produce diagnostic information on disease states in patients (Luzio and Thompson,1990) The measurement of total protein content of plasma may be used to assess degree of hydration of a patient (Baron *et al*,1989). Total serum or plasma proteins in normal healthy adults remains constant with little variation inindividuals over a long period. This variation has been attributed to factors such as diet, immunity, genetic and disease of the liver (Edozien, 1957). Prostaglandins are powerful locally acting vasodilators that inhibit the aggregation of blood platelets. Through their role in vasodilation, prostaglandins are also involved in inflammation. They are synthesized in the walls of blood vessels and serve the physiological function of preventing clot formation, as well as regulating the contraction of smooth muscle tissue.(Nelson *et al*, 2005) . In monocytes and macrophages, PGE_2 inhibits class II (Ia-DR) antigen expression, production of IL-1β, and antigen presentation.(Zlotnick *et al*,1985) .In lymphocytes, PGE_2 impairs IL-2 production by T-helper cells and decreases

response to IL-2.PGE_2production by suppressor macrophages which may downregulate the inflammatory process, thus preventing harmful systemic inflammation. PGE_2levels have been shown to increase significantly during crisis in the some studies and remained elevated after symptoms had resolved.(Evangeline *et al,* 1998)

Abnormal liver function tests are noticed in patients with Sickle Cell Anemia, even in the absence of liver disease, therefore there is a need to investigate the levels of albumin, total protein and prostaglandins in sickle cell children and sickle cell carriers in this study population.

MATERIALS AND METHOD

Selection Of Subject: This was a cross sectional case control study involving 120 children grouped into 3 groups of 40 subjects each; the groups were having the status of AA, AS, and SS respectively. The participants were recruited from Ekiti State University Teaching, Ado Ekiti State, Federal Medical Centre, Ido Ekiti and . Anthropometric data were obtained from all the subjects following the consent of the parents of the children involved. Ethical clearance were obtained from the institution ethical committee.

Sample Collection

The blood samples used for the test were collected from sickle celled patients(SS) and non-sickle Celled(AA),sickle cell carriers (AS) at Ekiti State University Teaching Hospital, Ado-Ekiti, and Federal Medical center, Ido-Ekiti, Ekiti State, Nigeria .Intravenous blood (5 ml) was collected from the patients using a sterile syringe. Samples obtained for the study were drawn into EDTA bottles, and plasma was separated via centrifugation and stored at -20°C prior to analysis.

Estimation of Albumin

The measurement of serum albumin was based on its quantitative binding to the indicator 3,3,5,5-tetrabromocresol sulphonepthalein (bromocresol green BCG) as described by Cheesbrough (1999).

Estimation Of Total Protein

Total protein was determined by Biuret method as described by Cheesbrough (1999)

Evaluation of Plasma Prostaglandin PGE_1 and PGE_2

This was done by using Enzyme Linked Immunosorbent Assay(ELISA) as described by Van Weemen and Schuurs (1972**).**

Statistical Analysis

The results obtained was grouped and expressed as mean ± Standard Error of Mean (SEM). The data collected was analyzed using one –way Analysis of variance (ANOVA) and Duncan multiple range test to compare the data obtained from the test groups to those of the control (Zar, 1986).

RESULTS

The mean age (years) of the non sickle cell, sickle cell carrier, and sickle cell children were not statistically different (3.70±2.0 , 3.85±2.1 and 3.85±2.1 respectively).The mean weight of non sickle cell and sickle cell carrier children were not statistically different (18.10±1.6 and 17.9±1.8) , but there was a significant reduction in age of the sickle cell children.(13.91±2.0).

There was no statistically difference in the mean concentrations of albumin, total protein,PGE_1 and PGE_2 between non sickle cell and sickle cell carrier(4.6±1.0, 8.1±0.8, 0.72±0.05, 0.81±0.04) versus (4.7±1.1, 8.4±0.9, 0.89±0.04, 0.95±0.05) respectively. A significant increase occurred in concentrations of albumin, total protein , PGE1 and PGE2 in sickle cell children when compared with non sickle cell and sickle cell carrier (3.85±2.1, 5.1±1.1, 9.6±0.9, 10.04±0.42 , 11.31±.51) respectively.

TABLE 1: The mean concentration of Plasma Albumin, Total Protein, Prostaglandins , Age and Weight of non-sickle cell, sickle cell carrier and sickle cell children.

PARAMETER	AA	AS	SS
AGE(years)	**3.70±2.0[a]**	**3.5±2.0[a]**	**3.85±2.1[a]**
WEIGHT (Kg)	**18.10±1.6[a]**	**17.9±1.8[a]**	**13.91±2.0[b]**
ALBUMIN (g/dL)	**4.6±1.0[a]**	**4.7±1.1[a]**	**5.1±1.1[b]**

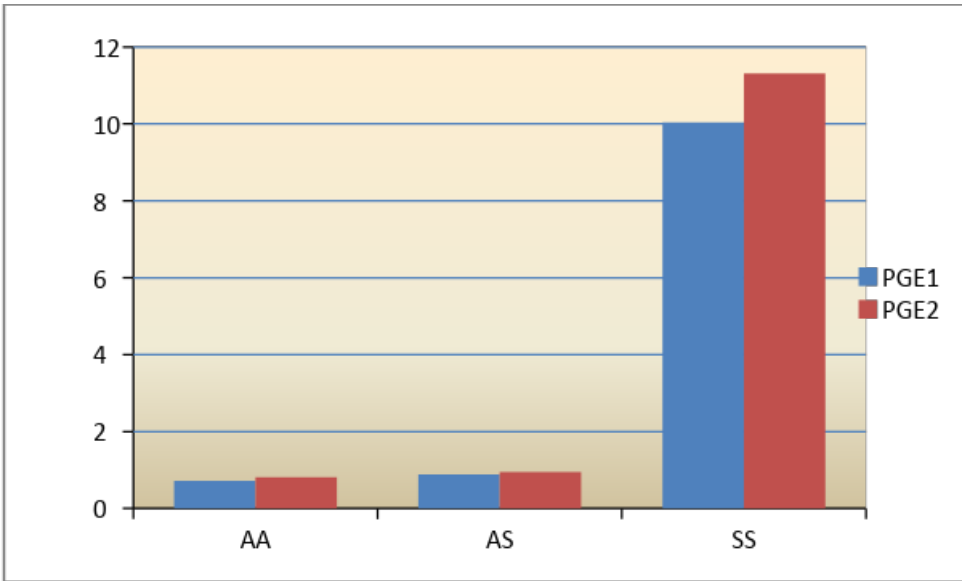
TOTAL PROTEIN (g/dL)	**8.1±0.8[a]**	**8.4±0.9[a]**	**9.6±0.9[b]**
PGE_1(ng/ml)	**0.72±0.05 [a]**	**0.89±0.04 [a]**	**10.04±0.42 [b]**
PGE_2 (ng/ml)	**0.81±0.04 a**	**0.95±0.05 [a]**	**11.31±.51[b]**

AA: non-sickle cell children, AS: sickle cell carrier children, SS: sickle celled children.

Values of the same subscript within the same column are not statistically different at ($P>0.05$) between the control and case group, while values with different subscripts are significantly different at ($P<0.05$).

Results are expressed as mean and standard error of mean..

Figure 1: Concentrations of prostaglandin(PGE_1,PGE_2) in non-sickle cell, sickle cell carrier and sickle cell children.



DISCUSSION

The result of this study shows that the mean serum albumin value was statistically higher in sickle cell children when compared with non-sickle cell children. This is in agreement with the work of Famodu *et al*., 1987 and Isichei *et al* 1979.).

Also, the mean value of total protein in children with sickle cell patients was significantly higher when compared with non-sickle cell children, this is in accordance with the works of Adu *et al* 2012 and Edozien *et al*, 1960. Sickle cell patients exhibit hyperproteineamia which might be as a result of hyperglobulineamia (Isichei *et al*,1979). This hyperglobulinaemia observed by Johnson and his co-workers in sickle cell patients ,might be due to increased gamma (γ) globulin fraction arising from the extent of antigenic stimulation coming from the environment (Johnson *et al*, 1999). Another study attributed this hyperproteineamia to increased destruction of erythrocytes during sickling. (Adenaike *et al*.1998).

The marked increase in PGE1 and PGE2 levels observed in sickle cell patients from the result of this study, might be due to the down regulatory effects of prostaglandin E2 on immune cell function which may contribute to the increased susceptibility to infection observed in children with sickle cell disease.(Evangeline *et al*,2018). PGE2 downregulates inflammatory response by increasing intracellular cyclic AMP levels in immunohematologic cells (Tripp *et al*, 1986)

In addition, there was no significant difference between the mean age value of non-sickle cell children and sickle cell children. This shows that the age may not be a factor in the development of sickle cell disease in children. The significant difference observed between the mean weight value of non-sickle cell children and sickle cell children could be due to the loss of weight in children with sickle cell disease, and this is in accordance with literature.

CONCLUSION

This study shows that albumin, total protein and prostaglandins are implicated in children with sickle cell disease, but do not show any implication in children who are sickle cell carrier and non-sickle cell children. Hence albumin, total protein and prostagladins concentration may be used as diagnostic tools for sickle cell disease, and monitoring their concentrations via the intake of drugs and adequate food may be of help in the management of the disease.

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