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Serum Biochemical Biomarkers of Bone, Liver and Kidney Integrity in Sickle Cell Disease

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Abstract: **Sickle cell disease is often associated with multiple organ damage. Biomarkers help in identifying the extent of organ damage; hence this study was aimed at determining the level of key biochemical biomarkers in Sickle cell disease (SCD) with the view of establishing a test panel for use in assessing SCD and correlating these with severity of the SCD.**
The comparative evaluation of both sickle cell patients (HbSS), and normal control HbAA was carried out, their samples were analyzed using Access biochemistry auto- analyzer (KENZA 240 TX) with Bio-labo diagnostics kits.
Result showed calcium was significantly lower, (P<0.05) Alkaline Phosphatase (ALP), Uric Acid, Liver transaminases, and Lactate dehydrogenase were significantly higher their P value being less than 0.05.
From this study it could be concluded that sickle cell patients are susceptible to bone, and kidney damages, hence it's recommended that routine sickle cell disease examinations should include the organ specific biochemical test as prognostic markers for the severity of the SCD during and after the crisis.

Keywords: **Sickle cell disease (SCD), Serum Biochemical Biomarkers of Bone, Liver and Kidney Intergrity.**

1. INTRODUCTION

Sickle cell disease (SCD) or sickle cell anemia (SCA) refers to a group of disorders caused by inheritance of a pair of abnormal hemoglobin genes including sickle cell gene, that can affect the skeletal system owing to accelerated hematopoeisis and bone infarction ((Meremiku, 2008; Nelson, et al., 2003). This abnormality is as a result of substitution of a single nucleotide from thymine to adenine (G**A**G→G**T**G) in the β- chain of hemoglobin resulting in amino acid valine instead of glutamic acid (Rees et al., 2010). Unlike normal red cells which are usually smooth and donut-shaped, sickled red cells cannot squeeze through small blood vessels. Instead, they get hooked and cause blockages in arterioles thereby depriving organs and tissues of oxygenated blood. This process produces periodic episodes of pain and ultimately damages tissues and vital organs including liver, spleen, kidney, heart and bones (NIH, 2004).

Sickle cell disease is highly prevalent in Africans, Arabs and those of Asian descent (Oheme-Frempong et al., 1994). The incidence of the disease in Nigeria is about 2% (Ohaeri and Shokunbi ,2001) and also affects an estimated 1 - 2% (120,000) of newborns in Africa annually. The sickle cell gene was observed to be most common in mosquito endemic areas (Meremiku, 2008).

Sickle cell disease has long been associated with biochemical abnormalities,(Oladipo,et al., 2005). Which is accompanied with Bone changes, but the pathogenesis is not fully understood , (Nouraie,et al .,2011). Osteoporosis and Osteomalacia are common conditions in SCD. Studies by Allon, (1990) have shown that Mineral salt such as calcium and inorganic phosphate are critical in bone formation and metabolism. The role of calcium supplementation to restore bone health in SCD has not been well documented (Adewoye,et al.,2008). Hypocalceamia in SCD is reported to be related to sickled red cell membrane permeability (Nduke and Ekeke, 1987), and increase in red cell calcium pump (Nduka, et al., 1995).The level of alkaline phosphatase indicates severity of bone damage and is a useful guide of progress in the management of bone pains in sickle cell anemia (Afonja and Boyd ,1986). Phosphate levels in SCD are significantly higher than found in normal patients and this could be attributed to increased release of phosphate from cell in chronic hemolytic state

(Oladipo ,et al., 2005).The level of phosphate could be used as a marker for predicting frequency of crisis (Smith ,et al.,1981;Al-harbi ,et al.,1999). Alkaline phosphatase levels is a marker for the severity of bone damage in SCD and has been used to monitor progress in bone pain management in sickle cell anemia (SCA), (Afonja and Boyd ,1986).

Sickle cell disease has also been associated with liver damages (Pandey,et al.,2012) and determination of liver enzymes in SCD will further elucidate the problem sickle cell disease poses on the liver. Factors like intra-hepatic sinusoidal sickling, billirubin gallstones, transfusion hepatitis further complicates hepatic dysfunction in SCD,(Schubert,1986;Kakarala, and Lindberg, 2004;Beutler,1999). Compromised hepatic integrity is associated with increased Aspartate aminotransferase levels, (Pandey, et al., 2012). As a result of intravascular hemolysis, SCD patients have been reported to have a high AST level (Nsiah, et al., 2011). Lactatate dehydrogenase (LDH) exists as an iso-enzyme and it is found in all tissues. Athough it is total LDH that is measured in clinical practice, it has been observed that other LDH isoenzymes found in brain, liver and muscle are reduced in SCD (Kato et al., 2006; O'Driscoll et al., 2008). It is elevated further during vasocclussive crisis (VOCs) (Karayalain,et al.,1981). According to Ballas and Marcolina (2006), increase in LDH may not entirely be as a result of hyper- hemolysis but also due to increase tissue damages during VOCS. Intravascular hemolysis has been implicated as the dominant source of Increase of LDH levels in sickle cell disease (Adhikary, et al., 1986).

Uric acid is an end product of nucleoprotein metabolism excreted by the kidney. Increase in uric acid occurs as a result of decreased renal clearance or increased nucleoprotein synthesis. Hyperuriceamia leads to gouty arthritis and other recognized complications. Erythrocytes having heamoglobin S do have a shorter life span. During erythropoeisis there is an increased synthesis of nucleic acid consequently increased red cell destruction which will lead to increased nucleic acid degradation, ultimately leading to increased uric acid concentrations, (Reynolds, 1983).

Biomarkers help in identifying extent of organ damage associated with sickle cell complications. The roles these analytes play in the management of SCD, and the role of minerals in restoring bone health in SCD is scarce. Hence the study was aimed to determine the level of key biochemical biomarkers of organ integrity in SCD with the view of correlating them with severity of SCD and help to establish a test panel of biomarkers for organ integrity.

2. MATERIALS AND METHODS

Subjects:

A total of fifty nine (59) sickle cell patients attending the sickle cell clinic, Murtala Mohammed hospital were selected for this study. They included 20 female and 39 male adult subjects. Age-sex matched of thirty seven apparently healthy hemoglobin AA individuals (17 males and 10 females) were assessed to compare biochemical biomarkers. Ethical clearance for the study was obtained from hospital Ethics Committee, while informed consent was obtained from the subjects.

About 5ml of venous blood sample was collected from patients during their crisis period into lithium heparin container which was centrifuged at 3000 RPM and separated.

Methods:

All the biochemical investigations was done by random Access biochemistry auto- analyser (KENZA 240 TX) using Bio-labo diagnostics kits.

Statistical Analysis:

Student t-test used to compare the two means was performed with software package for social sciences (SPSS) version 16.0. Results were expressed as mean ± standard deviation and $P < 0.05$ was considered significant.

3. RESULT

Results of the different organ biochemical markers comparison of HBSS patients and HBAA control is shown in Table 1. Bone integrity related biomarkers; calcium was significantly low in HBSS patients ($P<0.05$) while their inorganic phosphate and Alkaline Phosphatase level was significantly high when compared to HBAA patients ($P<0.05$). Liver integrity biomarkers ALT and AST were significantly increased in HBSS patients ($P<0.05$,)howbeit within the normal range, while no significant change was observed in serum albumin of HBSS patients when compared to the HBAA patients ($P>0.05$). Renal integrity related biomarker, uric acid showed a significant elevation in HBSS patients ($P<0.05$) when compared to the HBAA controls.

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TABLE 1: Serum Bone, Liver and Kidney biochemical biomarkers level in sickle cell patients

Biochemical Biomarkers	Mean ±SD		P value
	HBSS N=59	HBAA N=37	
Bone Biomarkers			
Calcuim (mmol/L)	**1.60 ±0.37**	**2.22 ±0.20**	**0.001**
Inorganic Phosphate (mmol/L)	**2.60 ±0.54**	**1.22 ±0.37**	**0.001**
Alkaline Phosphatase (U/L)	**255.25 ±187.46**	**155.64 ±36.26**	**0.002**
Liver Biomarkers			
Asparatate aminotransferase (U/L)	**50.33 ±24.96**	**23.13 ±9.55**	**0.001**
Alanine aminotransferase (U/L)	**28.79 ±14.84**	**20.18 ±18.08**	**0.013**
Lactate Dehydrogenase (total) (U/L)	**586.22 ±239.22**	**131.64 ±47.33**	**0.001**
Albumin (g/dl)	**38.11 ±4.89**	**35.78 ±6.39**	**0.046**
Kidney Biomarker			
Uric Acid (µmol/L)	**441.69 ±166.44**	**289.59 ±91.18**	**0.001**

P value less than 0.05 ($P<0.05$) is significant

4. DISCUSSION

Hypocalcaemia has been reported in SCD, (Nduke and Ekeke, 1987). Abnormal calcium homeostasis has been implicated in the pathogenesis of sickle cell (Nduka,et al.,1995;Litosch and Kwang,1980;Mohamed,et al.,1993).The Significantly lower calcium levels in sicklers compared to controls ($P<0.05$) observed in this study, agrees with the findings of Pandey, et al.,(2012). Reasons that could be advanced for these findings may include; An increased Ca2+-Mg2+ ATpase activity prevalent in SCD (Nduka, et al., (1995);Litosch and Kwang,(1980);Luthra and Sears,(1982). Reduced Calcium absorption from the intestinal tract and impaired Vitamin D Synthesis which is also prevalent in SCD (Mohammed,et al.,1993). In SCD the membrane of the red cell are abnormal ,as a result of deoxygenation this results in elevation of cation permeability into the cell leading to an accumulation of calcium within the red blood cell sometimes up to 10 fold higher than that found in normal patients which consequently exacerbates red cell destruction (Luthra and Sears ,(1982);Rhoda,et al.,(1990);Gibson, et al.,(1998). This could also be attributable to high binding affinity of red blood cell to calcium as a result of altered red cell membrane (Litosch and Kwang,1980).

Serum phosphate level in this study was found to be significantly higher ($P< 0.05$) than found in normal subjects, this confirms with an earlier study by Smith, et al.,(1981),which is attributable to increased tubular reabsorbtion of phosphates and an increased urinary sodium clearance,(Oladipo,et al.,2005).

Sickle cell disease is associated with bone destruction, which precipitates increased Alkaline phosphatase levels (ALP) (Pandey,et al .,2012). Higher ALP are associated with painful vaso-oclussive crisis (Mohammed,et al .,1993; Kotila,et al.,2005). A significantly higher ALP serum level was found in the Sickling patient from this study when compared to normal subjects ($P < 0.05$), which confirms observation reported by Pandey,et al (2012).This observation may be because ALP are found in cell surface of most tissues , and more concentrated in erythrocytes than in serum and during vaso-occlussive crisis hyper hemolysis occurs thus elevating ALP in serum. These findings shows that bone integrity is affected in sickle cell patients hence need for proper monitoring during management.

Aspartate and alanine transaminases (AST and ALT) are found within the cells and their elevation in SCD may be traceable to hyper-hemolysis that occurs in vaso-occlusive crisis. Some studies have linked SCD to liver damage

(Chukwu,et al.,2012),but in this study, though a significantly higher serum values of these enzymes were observed when compared with normal subjects but were within the higher region of normal range, more so the AST value was more elevated compared to a slight increase in ALT which is more liver specific, an observation confirmed by Kotila, et al.,(2005), who reported a minimal elevation of transaminases. This minimal elevation in the transaminases could be due to tissue damages prevalent in SCD and increased lyses occasioned by the hemolytic condition. Furthermore the liver helps in the synthesis of protein, If the integrity of liver was affected there will be a reduction in the level of protein which was not the case in this study where there was no significant change in the value of albumin of the sickling patients (38.11±4.89) when compared to the mean serum values in control subjects (35.78±6.39).

In the case of Uric acid levels in SCD, several studies have been reported which have documented hyper-uricemia in SCD (Reynolds ,1983;Gold ,et al.,1968; Ball and Sorrenson ,1970 ;Morgan ,et al .,1984 ;El-Hazimi et al .,1989).The increased UA production is associated with increased marrow activity, increased nucleic acid turnover (Reynold ,1983),decreased excretion of UA as a result impaired tubular function (Reynold ,1983 ;El-Hazimi ,et al., 1989). In this study an increased serum uric acid levels was observed which was significant $P < 0.05$) when compared with controls. Interestingly divergent views have been advanced as to whether the observable hyper-uriceamia in SCD is detrimental to their overall health or beneficial on the long term basis. As reported by Reynolds, 1983;Gold ,et al.,1968;El-Hazimi ,et al.,1983), a sustained hyperuricemia in SCD leads to the development of gouty Arthritis and Renal function disorder. But in contrast, as reported by Bruce, et al.,(1980), gouty arthritis has been a rare occurrence in SCD in the sense that despite the high incidence of hyper uricemia it is rarely accompanied by expected gouty arthritis ,but this may be because there is a limitation with the diagnosis of gout using hyperuricemia .Others have advanced an anti-sickling role for uric acid in SCD (Ekeke and Nduka,1987), with the view that hyperuricemia was rather compensatory because it guards against sickling of red blood cell in SCD. Suffice to say that in this study despite the hyperuricemia observed gouty arthritis was not diagnosed but this could suggest renal insufficiency.

It has been demonstrated by Neely et al., (1969) that LDH in SCD was elevated and increases during vaso-occlussive crises and is related to plasma heamoglobin levels. Intravascular hemolysis has been fingered as the principal source of elevated LDH (Naumam, et al., 1971; Neely,et al.,1969).

Lactate dehydrogenase from this study was found to increase significantly ($P< 0.05$) compared to control subjects, this agrees with previous studies Gregory et al., (2005). There is a strong association between LDH and other markers normally elevated in hemolysis (AST) , although the correlation was weak with ALT which is a highly specific marker for hepatocellular damage.

Conclusively, from this study it can be observed that sickle cell patients are susceptible to bone, and kidney damage hence it's recommended that routine sickle cell disease examinations should include the organ specific biochemical test as prognostic markers for the severity of the SCD during and after the crisis.

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