BIOCHEMICAL ESTIMATION OF ERYTHROCYTE AND PLASMA ELECTROLYTES, UREA AND CRATININE AS AN INDEX OF KIDNEY FUNCTION OF SICKLE CELL DISEASE PATIENTS IN EDO STATE, NIGERIA.

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ABSTRACT

Erythrocyte sodium (Na^+), potassium (K^+) as well as plasma Na^+, K^+, chloride (Cl^-) bicarbonate (HCO_3^-), urea and creatinine concentrations were determined in forty patients of age range (15 -- 25years) with sickle cell disease (SCD, HbSS), all of whom were asymptomatic and thirteen apparently healthy subjects of same age range, who have no evidence of any haemoglobinopathy. Analysis of the results showed a decreased erythrocyte K^+ (78.13±5.31mmol/L) and increased erythrocyte Na^+ (14.58± 6.73mmol/L) levels in SCD patients compared to the control (HbAA: 79.35±8.25; 11.59±4.04mmol/L respectively). These differences were however not significant ($p \geq 0.05$) but significant when compared to HbAS ($p \leq 0.05$). Plasma K^+ (4.31±0.27mmol/L) increased, while Na^+ (137.40±3.00mmol/L) and HCO_3^- (16.95±2.57mmol/L) concentrations decreased in SCD patients when compared to control (3.80±0.30; 139.94±2.44; 21.88±1.54mmol/L) respectively. These differences were significant ($p \leq 0.05$). However, there was no significant change in the plasma levels of Cl^- in all the groups ($p \geq 0.05$). Plasma, Urea and Creatinine concentrations were also elevated in SCD patients (20.78±2.90; 1.45±0.47mg/dl respectively) than the control subjects (18.47±2.87; 0.92±0.30mg/dl respectively, $p \leq 0.05$).

INTRODUCTION.

Sickle cell disease is the result of a hereditary abnormality in the haemoglobin molecule which indicates structural deformity of the red blood corpuscle. Because of this defect, the life span of the red cell is shortened and a chronic hemolytic anaemia exists in spite of compensatory marrow hyperplasia (Konotey – Ahulu, 1974; Bailas 1999; Ogamdi and Onwe, 2000). Certain abnormalities that occur in sickle cell disease are difficult to relate directly to the presence of haemoglobin – S or the sickling phenomenon. That non – haemoglobin components of the erythrocytes from the patients with sickle cell disease may be altered was suggested by an abnormal concentration or flux of electrolytes in the sickled cell (Harris, 1959).

In many cases of sickle cell disease, the entire kidney is affected and the patient urine is positive for bile, protein and blood (Durosinmi, 1989; Akenzua et al, 1994; Adams et al, 1998). Starting in the first decade of life with progressive damage thereafter (Skler et al, 1990; Ogamdi and Onwe, 2000), sickle cell nephropathy results from acidic and hyperosmolar milieu of the renal medulla which causes the deoxygenated red cells to sickle more readily, resulting in ischaemic damage; renal impairment progressing to focal interstitial nephritis, tubular dysfunction, nephrotic syndrome and papillary necrosis.

The earliest manifestation of sickle cell nephropathy is hyposthenuria, which is reversible with blood transfusion in young children but not in children older than ten years (Aderebigbe et al, 1994; Mcmahon and Mark, 1997; Adams et al, 1998, Wislez et al, 1999).

However, comparative information on the distribution of electrolytes, urea and creatinine in subjects with sickle cell disease is sparse. This study is aimed at establishing the erythrocyte and plasma electrolyte levels in our sickle cell population, as well as to determine the relationship between plasma, urea and creatinine levels and haemoglobin types, and to see if there is any relationship between levels of these parameters and the pathophysiology of sickle cell disease. These assessments are of utmost importance in view of their relationships in the diagnosis and management of some secondary metabolic complications which occur in sickle cell anaemia such as electrolyte imbalance, acidosis, dehydration, pyrexia, and renal dysfunction.

MATERIALS AND METHODS

Patients

Forty patients (volunteers) with homozygous sickle cell disease attending the Haematology Clinic of the University of Benin Teaching Hospital (UBTH), Benin – City, Edo State of Nigeria were used in this study. All sickle cell disease patients were on routine maintenance drugs (Proguanil tablets, 100mg daily; Folic acid tablets 5mg daily). The ages of these patients ranged from 15 – 25 years. The diagnosis of homozygous sickle cell disease was made clinically and confirmed by standard procedures (Dacie and Lewis, 1975).

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Table 1: ERYTHROCYTE SODIUM AND POTASSIUM LEVELS IN SICKLE CELL PATIENTS

Group	Subjects	N	Na^+ mmol/L	K^+ Mmol/L
A (M+F)	HbAA	17	11.59 ± 4.04	79.35 ± 8.25
	HbSS	40	14.58 ± 6.73^{b}	78.13 ± 5.13^{b}
	HbAS	13	9.69 ± 0.48	80.85 ± 3.78
B (M)	HbAA	11	12.72 ± 4.67	78.27 ± 8.08
	HbSS	21	14.60 ± 6.81^{b}	78.48 ± 5.38
	HbAS	8	9.63 ± 0.52	79.25 ± 3.88
C (F)	HbAA	6	9.05 ± 0.84	81.33 ± 8.94
	HbSS	19	$14.79 \pm 5.34^{a, b}$	77.74 ± 5.34^{b}
	HbAS	5	9.80 ± 0.45	83.40 ± 1.82

Values represent mean ± standard deviation
N: Number of subjects investigated
M: Male
F: Female
a: p≤0.05 Vs Control
b: p≤0.05 Vs HbAS

Table 2: PLASMA ELECTROLYTE LEVELS IN SICKLE CELL PATIENTS

Group	Subjects	N	Na^+ (mmol/L)	K^+ (mmol/L)	Cl^- (mmol/L)	HCO_3^- (mmol/L)
A (M+F)	HbAA	17	139.94 ± 2.44	3.80 ± 0.30	106.71 ± 4.22	21.88 ± 1.54
	HbSS	40	$137.40 \pm 3.00^{a, b}$	$4.31 \pm 0.27^{a, b}$	106.00 ± 4.60	$16.95 \pm 2.57^{a, b}$
	HbAS	13	$135.46 \pm 2.40a$	3.70 ± 0.30	104.46 ± 3.48	20.92 ± 0.95
B (M)	HbAA	11	140.09 ± 2.88	3.80 ± 0.30	106.55 ± 4.39	22.00 ± 1.41
	HbSS	21	138.23 ± 3.00^{b}	$4.38 \pm 0.30^{a, b}$	106.38 ± 4.50	$15.90 \pm 3.69^{a, b}$
	HbAS	8	135.25 ± 2.19	3.71 ± 0.35	103.75 ± 3.28	20.63 ± 0.74
C (F)	HbAA	6	139.67 ± 1.51	3.70 ± 0.26	107.00 ± 4.34	21.67 ± 1.86
	HbSS	19	136.47 ± 2.86^{a}	$4.23 \pm 0.22^{a, b}$	105.58 ± 4.71	17.47 ± 3.31^{a}
	HbAS	5	135.80 ± 2.95	3.68 ± 0.24	105.60 ± 3.85	21.40 ± 1.14

Values represent mean ± Standard deviation
N: Number of subjects investigated
M: Male
F: Female
a: p≤0.05 Vs Control
b: p≤0.05 Vs HbAS

Controls

Thirty subjects (volunteers) were screened and selected as controls and are within the same age range. Determination of haemoglobin types was done by electrophoresis on cellulose acetate strips. Haemolysate was prepared by mixing two drops of saline washed red blood cells with two drops of distilled water. Electrophoresis was performed at 250V, 6mA for 15 minutes in a Tris EDTA/Boric acid buffer pH 8.4. The electrophoretic bands were stained with ponceau S. Standards for HbA and HbS were obtained from Haematology Department, UBTH, Benin – City.

Sample collection

Whole venous blood (5.0ml) from patients and controls was collected in the heparinized plastic specimen containers, shaken by inversion, and centrifuged at 10,000g for 5mins at $4^{o}C$. The supernatant plasma was separated, collected and stored at $4^{o}C$. The samples were brought to physiological temperature in a temperature – regulated water bath before being analyzed.

Erythrocyte harvest

The erythrocytes were harvested at 3000g and washed thrice in physiological saline until a clear solution was obtained. The harvested erythrocytes were then haemolyzed in distilled water (0.04ml of cell: 25ml of distilled water).

Estimations

Blood and plasma electrolytes were determined as follows: Sodium and Potassium concentrations were estimated using Corning 400 flame photometer (Tietz, 1989). Chloride concentration was determined using a

Table 3: PLASMA CONCENTRATION OF UREA AND
CREATININE IN SICKLE CELL PATIENTS

GROUP	SUBJECT	N	UREA (mg/dl)	CREATININE (mg/dl)
A (M + F)	HbAA	17	18.47 ± 2.89	0.92 ± 0.30
	HbSS	40	20.78 ± 2.90[a]	1.45 ± 0.47[a, b]
	HbAS	13	19.08 ± 2.14	1.00 ± 0.28
B (M)	HbAA	11	18.27 ± 2.83	1.00 ± 0.33
	HbSS	21	22.24 ± 2.76[a, b]	1.75 ± 0.20[a, b]
	HbAS	8	19.13 ± 2.36	1.09 ± 0.31
C (F)	HbAA	6	18.83 ± 3.19	0.76 ± 0.19
	HbSS	19	19.16 ± 2.14	1.12 ± 0.46
	HbAS	5	19.00 ± 2.00	0.88 ± 0.19

Values represent mean ± Standard deviation
N: Number of subjects investigated
M: Male
F: Female
a: $p \leq 0.05$ Vs Control
b: $p \leq 0.05$ Vs HbAS

Jenway chloride meter while bicarbonate level was assayed using Van Slyke (1921) procedure.

Plasma urea was determined using the diacetyl monoxime method of Ormsby (1942) and plasma creatinine was measured using Bonsnes and Taussky (1954) method employing standard diagnostic kits (Randox Laboratories Ltd., UK). All reagents used were of analytical grade. Statistical analysis was by student's t – test.

RESULTS

The variations in the mean erythrocyte Na^+ and K^+ levels are as presented in Table 1. Patients with sickle cell disease (SCD, HbSS) showed higher Na^+ values (14.58 ± 6.73mmol/L) than the control group (11.59± 4.04mmol/L) but was not significant. However, when compared with HbAS there was a significant increase in the Na^+ levels ($p \leq 0.05$). The K^+ values on the other hand were significantly lower in HbSS (78.13 ± 5.31mmol/l.) than in HbAS (80.85 ± 3.78mmol/L), but when compared to the control (HbAA, 79.35 ± 8.25mmol/L) it was not significant ($p \geq 0.05$). However, in males, erythrocyte Na^+ levels of SCD patients was higher than that of HbAS ($p \leq 0.05$) while in females Na^+ and K^+ levels of HbSS were higher than HbAS ($p \leq 0.05$).

Table 2 shows the plasma electrolyte levels of SCD patients. Results obtained indicate that patients with SCD had significantly lower levels of Na^+ (137.40 ± 3.00mmol/L) when compared to the control (139.94 ± 2.44mmol/L, $p \leq 0.05$). However, when compared with HbAS (135.46 ± 2.40mmol/L), there was a significant increase ($p \leq 0.05$). The potassium values on the other hand, were significantly higher in SCD patients (4.31± 0.27mmol/L) than in both control (3.80 ± 0.30mmol/L) and HbAS (3.70 ± 0.30mmol/L, $p \leq 0.05$). However, there was no significant change in the plasma levels of chloride in all the groups ($p \geq 0.05$). The bicarbonate levels were significantly lower in SCD patients (16.95 ± 2.57mmol/L) than in both the controls (21.88 ± 1.54mmol/L) and HbAS (20.92 ± 0.95mmol/L, $p \leq 0.05$).

Plasma concentrations of urea and creatinine of SCD patients are presented in Table 3. SCD patients had significantly raised levels of both urea (20.78 ± 2.90mg/dl) and creatinine (1.45 ± 0.47mg/dl) when compared to the control (18.47 ± 2.87, 0.92 ± 0.30mg/dl, $p \leq 0.05$). However, the increased level of urea observed in SCD patients did not vary significantly with HbAS. Furthermore, male SCD patients seem to have higher urea and creatinine levels than their female counterparts ($p \leq 0.05$).

DISCUSSION/CONCLUSION

Patients with homozygous SCD usually suffer from intermittent painful crises characterized by musculoskeletal or abdominal pain attributed to microvascular occlusion by sickled erythrocytes (Tomer et al, 2001).

The results presented in this study indicate that patients with SCD show an increased erythrocyte Na^+ concentration and a decreased erythrocyte K^+ concentration when compared to the control. The electrolyte distribution in the red cells of patients with homozygous SCD differs from normal cells. Erickson et al (1937) reported approximately 10% less K^+ in sickled cells than in normal red cells. Tosteson et al (1952) showed that when sickle cell anaemia cells pass from the oxygenated to the non – oxygenated stated, the cells lose approximately 23mEq of K^+ and gain 13mEq of Na^+ per litre. This difference was related to the mechanical process of sickling rather than to anoxia per se. It was also ascribed to a partially ineffective Na^+, K^+ – ATPase pump and to influence of hypoxia on red blood cell membrane permeability to Na^+ and K^+ (Gladner and Nathan, 1978).

Increase in total erythrocyte membrane ATPase activity may also increase the severity of sickle cell anaemia and this is thought to be mediated through its own catalytic action in the accumulation of various ions for example, H^+ and Ca^{2+} as well as by depletion of the ATP stores of the cells which probably promotes sickling and gelation of sickle cells (Eluwa et al, 1987).

The intracellular gelation that causes red cell sickling lead to functional changes in the red cell membrane which contribute to the rigidity of the membrane and thus lead to increased ion flux across the membrane. Repeated episodes of sickling and unsickling

cause a net efflux of ions out of the cell, accompanied by a loss of water and therefore, an increase in haemoglobin concentration enhanced by a concomitant loss of membrane mass which in turn promote intracellular gelation even at fairly high oxygen saturation (Masys et al, 1974).

Reticulocytes show a ten – fold increase in K^+ leakage during hypoxia resulting in cell shrinkage and rigidity (Mantzev et al, 1971). This sequence may have a role in the pathogenesis of sickle cell crises. Thus, this could explain the higher plasma levels of K^+ in SCD patients obtained in this study. The altered membrane permeability to Na^+ and K^+ would therefore lead to a corresponding change in the extracellular cation concentration which results in an increased plasma level of K^+ and a decreased Na^+. This corroborates earlier studies of Famodu et al, (1992). It is possible therefore that the percentage of the sickled cells in the blood at any given time may determine the degree of extracellular cation concentration.

When male and female patients were compared, sex does not seem to significantly affect the pattern of distribution of Na^+ and K^+ in the erythrocytes of SCD patients. The decreased level of HCO_3^- in SCD patients is consistent with the fact that one of the secondary manifestations of sickle cell anaemia is metabolic acidosis resulting in a significant reduction in plasma HCO_3^-, partial pressure of carbon dioxide and pH of venous blood. A reduction in the plasma HCO_3^- concentration is usually compensated for by a corresponding increase in the Cl^- concentration, but results obtained in the present study showed no significant changes in the plasma levels of chloride in SCD patients. It is possible that electrochemical neutrality is being maintained in these patients by other anions such as acetoacetate and lactate. Measurement of the levels of these anions is going on in our laboratories to delineate the actual mode of maintenance of acid base balance in SCD patients.

The electrolyte imbalance observed in SCD patients in this study reflects some form of renal dysfunction. This is supported by the observed increased urea and creatinine levels in the SCD patients. There was a 13% increase in the level of urea and a 58% increase in the level of creatinine in patients with SCD when compared to the control (Table 3). It is interesting to note that the increases in the concentrations of urea and creatinine were significant in male patients only.

Although the variance in severity of sickle cell disease observed in different patients is attributable to the co-existence of various Hb genotypes in the same individual such as S – thalassemia, haemoglobin – C, SC etc, and other genetic determinants, Fetal Hb (HbF) levels have also been implicated as a mediating factor in the incidence and severity of the crises of sickle cell anaemia (Eluwa et al., 1987). The quest for a specific remedy that will compensate for the fundamental defect of sickle cell anaemia is the prime goal of most research in the prevalent regions.

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