SERUM CALCIUM, INORGANIC PHOSPHATE AND SOME HAEMATOLOGICAL PARAMETERS IN SICKLE CELL DISEASE IN ENUGU METROPOLIS

By

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SUMMARY

Objectives: Sickle cell disease has long been associated with bone deformities and pain. Mineral salts such as calcium and inorganic phosphate are critical in bone formation and metabolism. This investigation was designed to study the serum concentration of these minerals as well as some haematological parameters in persons who suffer from sickle cell disease.

Methods: Forty five patients who have sickle cell disease (HbSS) attending the sickle cell clinic of the University of Nigeria Teaching Hospital, Enugu, were recruited for the study after obtaining informed consent. Twenty healthy persons (HbAA) served as controls. Serum calcium level was determined by EDTA titration, inorganic phosphate by spectrophotometric method of Goldberg and the Haematological parameters by Bain method.

Results: The age range of both test subjects and controls was 3 to 26 years. There were no significant differences in calcium and inorganic phosphate levels of test and control subjects ($p>0.05$). There were however, significant differences when values of haematological parameters were compared in tests and control subjects ($p<0.05$).

Conclusion: The results suggest that serum calcium and phosphate levels may not be affected significantly in sickle cell disease.

Key words: Calcium, phosphate, sickle cell disease, haematological parameters

INTRODUCTION

Calcium is one of the essential minerals of the human tissue. It plays vital roles in blood coagulation, muscle contraction, enzymatic biocatalysis, hormonal actions and membrane functions. The primary dietary sources of calcium are milk and diary products[1].

Inorganic phosphate is an important metabolite formed as an obligate product in carbohydrate metabolism. It also occurs in biological tissues and as component of coenzymes, phosphoproteins, nucleotides and phospholipids. In energy metabolism, it functions as activator and allosteric effector[2, 3].

Calcium and phosphate share close biochemical relationships, which link their metabolic fate together. They form the mineral matrix of bones and teeth and contribute to the physical strength of these structures. The deficiency of calcium and phosphorous is associated with such disorders as rickets, osteomalacia and osteoporosis.

Haematological parameters such a packed cell volume (PCV), haemoglobin (Hb) and reticulocyte count (retics) are non-specific indicators of effective erythropoesis and erythrocyte functional viability. They are affected to different degrees in some haematological/metabolic diseases and hence have become immensely useful in diagnosis, prognostication and monitoring of treatment[4, 5].

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Sickle cell disease is a genetic disorder arising from substitution of glutamic acid with valine at position 6 of the B-chain of the primary structure of haemoglobin. The explanation for the pin-point mutation is not yet clear but the change in amino acid sequence of the B-chain has caused significant changes in the stereochemistry of the pigment which has led to shortened life-span of erythrocytes with sickle haemoglobin[6,7] (HbS). It is only when the HbS occurs in the homozygous state or when it combines with other haemoglobin variants that the disorder is expressed. Epidemiological reports indicate that the frequency is higher in Mediterranean countries, African Americans and Africans[8].

In view of the close association of sickle cell disease with bone pain, skeletal deformities, leg ulcers and the active role of calcium/phosphate metabolism in skeleton integrity, it becomes necessary to investigate serum calcium and inorganic phosphate concentrations in persons known to have sickle cell disease in Enugu metropolis. Some haematological parameters, which are relevant to erythropoesis, are also studied to furnish more data on the pathological features of the disorder. It is hope that the results would be useful in the management of sickle cell disease.

MATERIALS AND METHODS

Forty five persons (24 males and 21 females) known to have sickle cell disease, of age range 3 – 26 years and attending the sickle cell disease clinics of the University of Nigeria Teaching Hospital Enugu were recruited for the study. Informed consent was obtained from these persons. Twenty apparently healthy persons of the same age range who were known to have haemoglobin genotype HBAA served as controls.

Blood samples were collected from both tests and control subjects and dispensed into EDTA and plain specimen bottles. The anti-coagulated blood was used for the determination of PCV, Hb, retics count and blood film picture study[9] while serum was used for calcium and inorganic phosphate determination[10, 11].

Serum calcium was determined by the EDTA titration method; inorganic phosphate by the spectrophotometric method of Goldberg and the haematological parameters by the Bain method.

The mean values were analyzed statistically using the Student t-test.

RESULTS

The mean values and standard deviation (SD) obtained for the test subjects and controls are as in table 1.

Table 1
Mean Values Of Minerals And Haematological Indices In Patients With Sickle Cell Disease.

Parameter	Mean Value in Test Subjects (HbSS)	Mean Value in Controls (HbAA)
Calcium (mmol/L)	2.47 ± 0.17	2.58 ± 0.12
Phosphate (mmol/L)	1.37 ± 0.17	1.35 ± 0.18
PCV (L/L)	0.22 ± 0.02	0.38 ± 0.03
Hb (g/dl)	7.5 ± 1.10	12.8 ± 1.30
Retics (%)	4.5 ± 1.00	0.65 ± 0.22

There were no significant differences in the calcium and phosphate levels of the test subjects and controls ($p>0.05$). There were, however significant differences in the values of the haematological parameters of the test and control subjects ($P<0.05$).

The blood film in the test subjects showed moderate to marked anisocytosis, poikilocytosis, hypochromia, polychromasia and normoblastosis in the red cells. The controls showed normocytic and normochromic blood picture.

The leucocytes and platelets showed normal number, morphology and distribution in about 95% of the test subjects while 5% showed moderate leucocytosis and neutrophilia.

DISCUSSION

Abnormality of calcium and phosphorous metabolism has long been associated with such bone deformities as rickets, osteomalacia and osteoporosis. The normal serum levels of these

minerals recorded in the test subjects of this study indicated physiologic control in sickle cell disease patients.

It has been reported that erythrocyte calcium is not adversely affected in many diseases[12]. This report is in agreement with the results of this present study. The inference from this is that the activity of the parathyroid hormone, which regulates calcium metabolism in addition to vitamin D, is unaffected in sickle cell disease.

However, bone deformities, leg ulcers and bone pains are reported features of sickle cell disease[13]. It may be reasoned from this study that the bone pathology may not be a result of abnormal serum calcium or phosphate levels, though both are known to be involved in the maintenance of the physical strength of bone. It may therefore be that the normal serum levels of these minerals in sickle cell disease patients is due to bone resorption processes while the consequent demineralization of the bones is responsible for the bone pathology.

Calcium is critical in erythrocyte membrane integrity and cytoskeleton. Although the primary lesion of sickled erythrocyte is in the haemoglobin molecule, the rigidity of the cell, particularly in the deoxygenated state, constitute a metabolic stress for the membrane. The cation pump of erythrocyte which regulates influx and efflux of ions across concentration gradient is regulated by calcium ATPase complex. It is probable that the activity of calcium ion in the membrane pump is effective. It is reported that normal erythrocytes have low calcium due to low membrane permeability for the cation and an active extrusion of the cation from the cells into the external medium against a concentration grdient[14]. Calcium ions have also been suggested to enter HbS erythrocytes faster and hence have a higher concentration in such cells than in normal erythrocytes[15].

There is paucity of information on inorganic phosphate metabolism in sickle cell disorder. However, normal phosphate concentration has been reported in sickle cell disease patients[16, 17]. This also agrees with the results of the present study. The biochemical relationship between calcium and phosphate metabolism may link the two minerals in this study also. Phosphate moieties act as allosteric effectors of haemoglobin function which facilitates haem-haem interaction (positive co-operativity) and oxygen affinity[18].

The haematological values that were determined (PCV, Hb, retics and blood film picture) showed the same features that have been documented for sickle cell disorder[19, 20]. The results indicate reduced red cell mass and hypochromia in spite of erythropoesis as indicated by polychromasia, reticulocytosis and normoblastosis. The leucocytosis and neutrophilia, which were observed in blood films of some patients, suggested underlying bacterial infections.

Although sickle cell pathology may affect all the formed elements of the blood, leucocytosis is primarily observed when there is septicaemia. Thrombopoesis did not appear to be adversely affected in all patients studied. This suggests normal platelet production. Although intravascular thrombus formation has been reported in sickle cell anaemia, the normal distribution, number and morphology revealed in the blood picture do not suggest adverse effect on platelets. The results of the haematological values obtained in this study indicate that sickle cell pathology affects these indices almost equally.

CONCLUSION

It can be concluded that calcium/inorganic phosphate metabolism is not adversely affected in sickle cell disease. The sequestration of these minerals from the bone into the blood, to maintain normal blood levels, may be responsible for the bone pathology in sickle cell disease.

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