Serum magnesium, phosphate and calcium in Nigerian children with sickle cell disease

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Summary

Background: **Biochemical abnormalities have been associated with sickle cell disease. Studies on phosphorus and magnesium in sickle cell disease have been conflicting. There is paucity of information on the role of these ions in the pathogenesis and management of sickle cell disease. This study was set out to determine the serum levels of magnesium, phosphorus and calcium in Nigerian children with sickle cell disease.**

Study design: **A case-control study carried out on children with HbSS genotype (cases) and age-matched controls with HbAA. Serum magnesium, calcium, phosphorus and albumin were measured using colorimetric methods.**

Results: **Eighty-six subjects and forty-five controls were studied. The mean serum magnesium was 0.99mmol/L (0.02) and 0.98mmol/L (0.02) in the cases and controls respectively. The difference was not significant. Mean serum calcium was significantly lower in the cases [2.1mmol/L (0.3)] compared with the controls [2.3mmol/L (0.15)]; p<0.01. Serum phosphorus was significantly higher in the cases than in the controls [2.2mmol/L (0.7) versus (1.5mmol/L (0.6); p<0.001]. There was no statistical difference in the albumin binding of calcium in both groups.**

A positive correlation existed between serum phosphorus and magnesium and also between serum calcium and magnesium in the cases group but no correlation between these parameters and age was found.

Conclusion: **Children with sickle cell anaemia in this study had normo-magnesaemia, hyperphosphataemia and hypocalcaemia. Further studies on changes in intracellular concentrations of these ions in children with sickle cell disease are required. Such findings could be useful in designing better management in individuals with this abnormality.**

Key-words: ***Magnesium, Phosphorus, Calcium, Sickle cell disease.***

Résumé

Introduction: Anormalités biochémiques ont été liées avec la maladie de drépanocytose. Il y a des études qui s'opposent sur le phospore et le magnésium dans la maladie de drépanocytose. Il y a le manque d'information sur le rôle de ces ions dans la pathogenèse et la prise en charge de la maladie de drépanocytose.

Plan d'étude: Une étude de cas de contrôle effectués chez un enfant avec le génotype HbSS (cas) et des contrôles âgés bien assorti avec HbAA. Sérum, magnésium, calcium phosphore et d'albumine ont été mésurés à travers la méthode colorimétrique.

Résultats: Quatre vingt six sujets et quarante cinq contrôles ont été étudies. Le sérum magnesium moyen était 0,99 mmol/L (0,02) chez les cas et les contrôles respectivement. La différence n'etait pas sensible. Sérum calcium moyen était sensiblement inférieur chez les cas (2,1mmol/L (0,3) par rapport aux controles (2,3mmol/L (0,15); P <0,01. Serum phosphore était sensiblement élevé chez les cas plus que chez les contrôles (2,3mmol/L (0,7) contre (1,5mmol/L (0,6); P<0.001. Il n'y avait aucune différence statistique dans l'albumine fixation du calcium dans les deux groupes. Une corrélation positive a eu lieu entre sérum calcium et magnésium dans le groupe des cas mais il n'y avait aucune corrélation entre ces parametres et on avait trouvé l'âge.

Conclusion: Des enfants atteints de la drépanocytose dans cette étude avaient normomagnésaemie, hyperphosphatémie et hypocalcémie. Des études complémentaires sur des changements dans les concentration intracellulaire de cesions chez des enfants atteints de la maladie de drépanocytose étaient exigées. Tels résultats pourraient être nécessaire dans la prise en charge meilleure chez des individuals atteints de cette anormalie.

Introduction

Sickle cell disease was first described by Herrick in 1910[1], and the disease was associated with high morbidity and mortality[2]. Sickle cell disease afflicts about 100 million people worldwide predominantly blacks, Arabs and those of Asian descent[3]. In Nigeria, the incidence of HbSS is about 2%[4,5].

Sickle cell disease in children is associated with higher mortality and morbidity than in adults[6,7].

There is an increasing interest in the role of magnesium in clinical medicine, nutrition and physiology and magnesium has been associated with several clinical disorders[8]. Sickle cell disease has been associated with many intracellular, red blood cell and plasma biochemical abnormalities[9,10]. The few reports on serum magnesium[11,12] and phosphate[13,14] in sickle cell disease have been conflicting. Hypocalcaemia was consistently reported in sickle cell disease and it is believed to be related to the

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sickled red cell membrane permeability; increased red cell Ca^{2+} pump has also been implicated [9,10].

Most of these studies were carried out on adult sickle cell disease population or in older children.

This study sets out to determine the serum magnesium, phosphorus and calcium levels of stable Nigerian paediatric children with sickle cell disease; to find out the likely role of these ions in the pathogenesis of the disease.

Subjects and methods

Venous blood samples were obtained into EDTA (ethylene diamine tri-aectic acid) bottles during routine clinical visits. The venous samples were collected during their crises free periods. There were eighty-five paediatric HbSS patients (age range 7months-170months) and forty-five apparently healthy HbAA controls (10months-177months) into tubes without anticoagulants and Ethical approval was given for the study.

The patients were recruited from the outpatient paediatric sickle cell clinics of the Lagos University Teaching Hospital and the Massey Children Hospital in Lagos, Nigeria. The controls were recruited from well baby clinics and from healthy babies attending follow up clinics for minor or acute illnesses. Questionnaires were administered to all the patients after verbal consent was sought from their parents. The blood sample was allowed to clot and retract; serum was separated; haemolysed samples were excluded from the analysis.

Table I Serum magnesium, phosphorus and calcium in Nigerian children with sickle cell disease

Analyte	Controls n=45	Cases n=85	p value
Magnesium (mmol/L)	0.98 (0.02)	0.99(0.02)	0.08
Phosphorus (mmol/L)	1.5 (0.6)	2.2 (0.7)	0.000**
Calcium (mmol/L)	2.3 (0.15)	2.1 (0.3)	0.001*
Albumin(g/L)	38.3 (5.2)	38.9 (7.8)	0.641

**significant at 0.01*
***significant at 0.001*

The haemoglobin genotype was confirmed by cellulose acetate electrophoresis on the haemolysates. Serum was stored at -20°C until analysis that was done in batches at 2-weekly intervals. Magnesium was analysed manually using Calmigite dye method[15], The magnesium kit was produced by Teco Diagnostics (U.S.A.).

The colorimetric method has been found to correlate well with atomic absorption spectroscopy[16,17]. The intra-assay coefficient of variation (CV) of the method was3.1% (n=10), while the day to day (inter-assay) CV was 4.2% (n=20). Calcium, Phosphorus and albumin were analysed colorimetrically using cresolphthalein complexone[18], Molybdate dye[19] and bromocresol green[20] methods

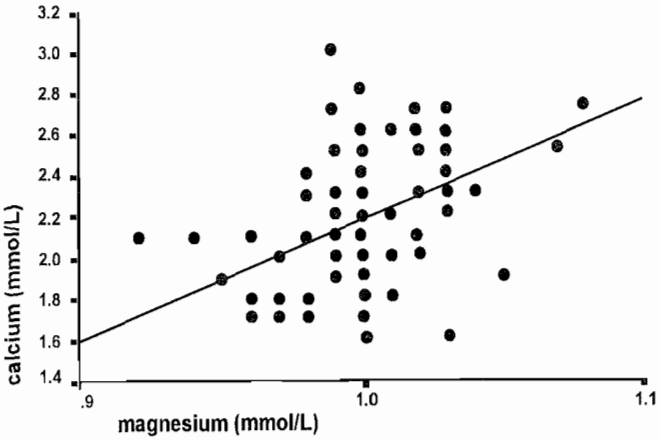

Fig. 1 *Relationship between serum magnesium and calcium in SCD*

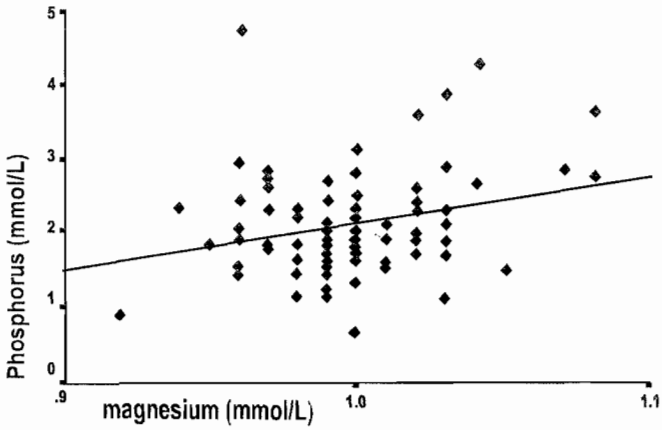

Fig. 2 *Relationship between serum magnesium and Phosphorus in SCD.*

respectively. The kits were produced by Randox Diagnostics (U.K) and all the glassware used for analysis were acid washed. Appropriate standards and controls were used. Albumin assay was carried out because of the binding effect it has on calcium and to a weaker extent on magnesium[8].

Statistical analysis

Statistical analysis was performed with the software package "SPSS Version 10.0". The results are expressed as means and standard deviations. Comparison of means was by the Independent "t" test. The statistical significance was set at $p<0.05$. Pearson product moment correlation was used for the correlation studies.

Results

Results for the patients and controls are presented in table 1. The statistical significance between the mean values for sickle cell disease patients and controls are indicated.

The results show that there was no significant difference in the serum magnesium levels in both patients (0.99mmol/L(0.02)) and controls (0.98mmol/L(0.2)). The mean serum calcium was significantly lower in the sickle cell disease patients (2.1mmol/L(0.3)) compared to the controls (2.3mmol/L(0.15)), $p<0.01$ while the mean serum inorganic

phosphorus level was significantly higher in the sickle cell disease patients (2.2mmol/L(0.7)) than in the controls (1.5mmol/L(0.6)), p<0.001. There was no significant difference between the serum albumin in patients and controls (p>0.05).

In the children with sickle cell disease, there was a significant positive correlation between serum magnesium and calcium(r=0.472,p<0.001); magnesium and phosphorus (r=0.278,p<0.01) and albumin and calcium (r=0.290, p<0.01). This is shown in figures 1 and 2.

In the controls, there was also a positive correlation between serum magnesium and phosphorus (r=0.297,p<0.01) and serum calcium and albumin (r=0.807,p<0.001), but not between serum calcium and magnesium (r=0.237,p>0.1).

There was no correlation between all parameters and age at diagnosis of sickle cell disease.

Discussion

Magnesium is a ubiquitous element in nature and in the human body and is the second most abundant intracellular cation[21]; it is known to participate in over 300 enzymatic reactions in the body[22].

Magnesium deficiency has been associated with several disorders including sickle cell disease[11]. The use of magnesium salts to stabilize the red cell membrane and to prevent dehydration of the membrane has been advocated[23]. The K-Cl co-transporter is a major determinant of sickle cell dehydration and is inhibited by increasing erythrocyte magnesium content[23]. However there are conflicting reports as to the status of magnesium in sickle cell disease. Olukoga[11] reported a low plasma magnesium level in sickle cell disease and in a separate study[24] reported a negative correlation between the low erythrocyte level that was found and the plasma magnesium levels in sickle cell disease. Prasad[25] also reported a low plasma magnesium level but an increased erythrocyte magnesium level in sickle cell disease. Another study[12] reported a higher serum magnesium level in sickle cell disease patients than in controls, this was suggested to be due to the chronic haemolytic states in the patients. In this study, there is no significant difference between the serum magnesium levels in patients and controls, this is similar to findings in a study by De Franceschi[26] *et al* who also found no significant difference between the plasma magnesium in patients with sickle cell disease and normal controls.

Abnormal calcium homeostasis has been implicated in the pathogenesis of sickle cell disease and hypocalcaemia has been reported [9,10,27,28]. The lower calcium level in the sicklers compared to controls in this study agrees with the findings in previous studies [9,10,27,28], and some of the reasons given for the hypocalcaemia in sickle cell disease include an increased Ca^{2+}-Mg^{2+}ATPase activity[9,10,29], reduced calcium absorption from the intestinal tract and impaired vitamin D synthesis[28].

Red cell membrane abnormalities with increased permeability to calcium and subsequent accumulation of calcium in red blood cells of patients with sickle cell disease have been reported[29]; it was suggested that there may be an alteration in the calcium binding properties of the red cell membrane of sickled cells because there is a high affinity binding in sickled cells compared to normal red cells[10]. These factors are likely contributors to diminished calcium levels in the serum.

Raised serum phosphorus was observed in these children with sickle cell disease, contrary to findings in an earlier study by Al-Harbi[14] *et al*, who found a lower serum phosphate in children with sickle cell disease due to a lower renal phosphate re-absorption as a result of increased parathyroid hormone secretion. Smith[13] *et al* however reported a significantly higher serum phosphate in the sickle cell disease patients compared to the controls and this was thought to be due to the increased tubular re-absorption of phosphates in these patients, higher serum phosphate was also related to increased urinary sodium clearance. The parathyroid hormone (PTH) level in the study was however normal despite the hyper-phosphataemia suggesting that other factors are responsible for the hyper-phosphataemia apart from PTH anomalies[13]. In our study, there was a positive correlation between serum phosphorus and magnesium, probably related to the renal handling of both ions. Further studies are needed to elucidate this relationship. Another possible reason for the hyper-phosphataemia could be the release of phosphate from the cells in the chronic haemolytic states. Serum phosphate was suggested to be a marker for predicting frequency of crises in sickle cell disease and a positive correlation was established between serum phosphate levels and frequency of crisis[13,14]. Ekeke *et al*[30] also suggested that reduction in serum phosphate levels with an extract of Cajanus Cajan beans in sickle cell disease brought about an improvement in erythrocyte function.

Magnesium salts are currently used in clinical practice to reduce erythrocyte dehydration in sickle cell disease[26,31,32]. Decreased magnesium erythrocyte levels have been reported despite normal plasma magnesium levels[26], this should serve as a basis for further studies on the handling of magnesium ion across cell membranes. Renal handling of these ions in sickle cell disease may also need to be studied in order to elucidate the correlations observed in this study.

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