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Original Article

Serum total Calcium to Magnesium ratio is higher in Sickle cell disease patients with proteinuria than without proteinuria

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Abstract

Intracellular imbalance in the levels of calcium to magnesium ratio could lead to clinical complications in Sickle cell disease patients (SCD). Proteinuria is common in SCD patients especially with increasing age and may affect the intracellular level of calcium to magnesium ratio. This study evaluates the total calcium to magnesium ratio in SCD patients with proteinuria and compared to those without proteinuria. Serum total calcium and magnesium were determined using colorimetric method, while urine protein was assayed using urinalysis dipstick and sulphosalicylic turbidometric method. Out of the 100 SCD patients, urine protein was detected and assayed in 27 while 73 were negative for urinary protein. Total calcium to magnesium ratio and urine protein were significantly higher ($p<0.001$), while magnesium ($p<0.001$) and calcium ($p<0.01$) were significantly lower in SCD patients with proteinuria compared to those without proteinuria. Total calcium to magnesium ratio correlated positively ($r=0.38$; $p<0.05$) with proteinuria in SCD patients. Total calcium to magnesium ratio is higher in SCD patients with proteinuria than without proteinuria. Routine calculation of this ratio could be helpful in assessing the intracellular balance of calcium and magnesium in the management of SCD patients.

1. Introduction

The intracellular balance in Calcium to Magnesium ratio is critical for normal physiological activities of the body because both have opposite functions. Calcium and magnesium are regulated together through dietary intake, negative feedback mechanism, intestinal absorption and renal re-absorption [1]. Calcium is an important essential element which must be balanced by adequate magnesium concentrations otherwise it may cause damage to cells of the body. Low concentrations of magnesium in sickled red blood cells have been associated with increased sickling, polymerization and dehydration [2-3]. The careful control of red blood cell volume in Sickle cell anaemia (SCA) is dependent to some extent on the changing equilibrium between calcium and magnesium, whereas calcium stimulates the movement of potassium and water from the cells through calcium-potassium channel (Gardos channel), magnesium inhibits potassium-chloride co-transport thereby promoting red blood cell hydration [4-5]. Findings in literature regarding the serum levels of calcium, magnesium and calcium to magnesium ratio in SCD patients have not been consistent. We earlier reported low magnesium levels in Sickle cell disease (SCD) patients compared to control subjects with normal haemoglobin [6]. Studies that have reported calcium to magnesium ratio in SCD patients evaluated ionized calcium and magnesium [7]. It was reported that differences in magnesium levels between SCA and HbAA were observed only by measuring ionized magnesium and not total serum magnesium levels. This was due to low correlation between total and ionized magnesium. It was concluded that significant differences between SCD and HbAA would have been missed by measuring total serum magnesium [7]. Because of these observations, this study was designed to measure serum total calcium to magnesium ratio in SCD patients with proteinuria. It may help highlight the impact of proteinuria on the calcium to magnesium ratio and by extension their effect on red blood cell volume in this group of patients. A higher than normal serum ionized calcium to magnesium ratio has been associated with the activation of the Gardos channel leading to cell dehydration, sickled red blood sickling and vaso-occlusive disease [7].

Sickle cell nephropathy (SCN) is common and is a potentially life threatening condition in SCD patients. Functional changes occur in Kidneys of SCD patients and the prevalence increases with increasing ages of patients. Progressive renal failure at older ages is a major complication and cause of morbidity and mortility [8]. Factors that could predict renal failure in SCA patients include hypertension, proteinuria, anaemia, hyposthenuria and haematuria [9-10]. The environmental conditions (hypoxia, acidosis and hypertonicity) of renal medulla promote sickled cell polymerization, sickling and vaso-occlussive crisis [11]. Also the inability of the Kidney of SCD patients to concentrate urine maximally (hyposthenuria) may also contribute to renal abnormalities. This inability to maximally concentrate urine could predispose SCD patients to dehydration, vaso-occlusive crisis and urinary loss of these cations. The objective of this study was to evaluate the levels of total calcium to magnesium ratio in SCD patients with proteinuria.

2. Materials and Methods

2.1 Study participants

This is a case-control study of 150 participants made up of 100 SCD patients in steady clinical state and 50 apparently healthy subjects with Hb AA. The study protocol was reviewed and approved by the Ethics committee of the Edo State Ministry of Health, Benin City and all participants gave informed consent before the commencement of study. Demographic data, history of medication and blood transfusion were obtained using structured questionnaires. The participants were who met the selection criteria were consecutively enrolled. The SCD patients were on routine visit to the clinic and not on oral magnesium therapy for any reason. The study was conducted at the Department of Medical Laboratory Science, University of Benin, Benin City from January to December 2014. The control subjects were staff and students of the University of Benin and patients were recruited into the study only once regardless of the number of visits to the clinic.

2.2 Inclusion Criteria

The individuals who made up the study group were confirmed sickle cell disease patients and were homozygote for sickle

cell haemoglobin (HbSS). They were on steady clinical state but presented to the clinic for their routine visits. They had no signs and symptoms of acute illness, such as vaso-occlusive crisis, acute chest syndrome or bacterial infection and were not transfused within the last 4 months before they were recruited into the study.

2.3 Exclusion criteria

Those with acute illness, on magnesium therapy for any reason or had received blood transfusion within the last 4 months were excluded. Subjects with other haemoglobinopathies were also excluded.

2.4 Sample Preparation

Blood samples (3mL) were collected by venepuncture from each subject and dispensed into plain containers. They were allowed to clot at room temperature. These were spun in a bucket centrifuge at a speed of 2500rpm for 10 minutes. The sera were kept frozen at a temperature of -20^{o}C before the analyses were done. The measured parameters were assayed by colorimetric method. Magnesium and calcium were quantitated using reagents kits supplied by Teco diagnostics, Anaheim, USA and Randox Laboratories, UK respectively. Early morning urine specimens were also collected from the subjects. Urine protein was initially assayed using urinalysis dipsticks method while those that were positive for macroalbuminuria were re-evaluated using Sulphosalicylic acid (SSA) colorimetric technique.

2.5 Data Analysis

The mean±standard error of means of the cases and controls were compared using Students t-test. Pearson correlation coefficient was calculated in order to correlate the levels of measured variables with proteinuria. A p-value of 0.05 was considered statistically significant.

3. Results

The results from the study are as presented in tables 1,2 and 3. One hundred adult SCD patients (male:female 55:45) were enrolled in the study. The age of the subjects ranged from 18 to 23 years with a mean of 19.02±0.80. Out of the 100 SCD patients, 27(27%) were positive for proteinuria while 73% were negative for proteinuria. Table 1 shows the levels of serum calcium, magnesium and calcium to magnesium ratio in SCD patients compared with control subjects. Statistically significant increases were observed for urine protein ($p<0.02$), serum calcium ($p<0.002$) and calcium to magnesium ratio ($p<0.02$) while significant decrease ($p<0.001$) in magnesium was observed in SCD patients compared with controls. Table 2 shows the levels of measured parameters in SCD patients with proteinuria and those without proteinuria. The mean age of those with proteinuria was significantly higher ($p<0.02$) than those without proteinuria. Statistically significant increases were observed for urine protein ($p<0.001$) and calcium to magnesium ratio ($p<0.001$), while significant decreases were observed for serum calcium ($p<0.01$) and magnesium ($p<0.001$) in patients with proteinuria compared to those without proteinuria. Table 3 shows that proteinuria correlated negatively with calcium (r-0.26; $p<0.05$), magnesium (r=-0.52; $p<0.01$) and positively with calcium to magnesium ratio (r=0.38; $p<0.05$) in SCD patients with proteinuria.

Table 1: Levels of total serum calcium, magnesium and calcium to magnesium ratio in sickle cell disease patients compared with controls.

Measured variables	SCD patients (Hb SS)n=100	Control subjects (HbAA) n=50	p-value
Age (Years)	19.02±0.80	22.31±1.01	0.001
Urine Proteins(g/L)	0.050.02	00.0	0.02
Serum Calcium(mmol/L)	2.42±0.03	2.32±0.04	0.002
Serum Magnesium(mmol/L)	0.55±0.03	1.09±0.02	0.001
Calcium/magnesium ratio	4.39±0.02	2.13±0.01	0.001

Table 2: Levels of total serum calcium, magnesium and calcium to magnesium ratio in sickle cell disease patients with proteinuria compared to those without proteinuria

Parameters	SCD patients with proteinuria n=27	SCD patients without proteinuria n=73	p-value
Age(years)	18.81±1.50	15.02±1.60	0.02
Urine protein(g/L)	0.22±0.02	0.00	0.001
Serum calcium(mmol/L)	2.25±0.03	2.34±0.04	0.01
Serum magnesium(mmol/L)	0.49±0.05	0.92±0.03	0.001
Calcium/magnesium ratio	4.59±0.04	2.23±0.02	0.001

Table 3: Correlation of proteinuria with measured variables in SCD patients

Parameters	R-value	P-value
Macroalbuminuria and serum calcium	-0.26	p>0.05
Macroalbuminuria and serum magnesium	-0.52	0.01
Macroalbuminuria and calcium/magnesium ratio	0.38	0.05

4. Discussion

The aim of this study was to evaluate the impact of proteinuria on the levels of calcium, magnesium and calcium to magnesium ratio. The data presented showed that urine protein, serum calcium and calcium to magnesium ratio were significantly higher while serum magnesium was significantly lower in SCD patients compared with control subjects. The SCD patients with proteinuria had significantly higher ($p<0.001$) levels of urine protein and calcium to magnesium ratio, decrease levels of serum calcium ($p<0.01$) and magnesium ($p<0.001$) compared to those without proteinuria. Increased serum calcium to magnesium ratio is an indication of intracellular imbalance in calcium and magnesium levels. Increased serum calcium and low magnesium levels promote or activate Gardos channel and de-activation of potassium-chloride co-transport mechanism, thereby leading to cellular dehydration [7].

The decrease magnesium levels as observed in this study are consistent with previous studies [2,6,12]. Our observation did not agree with others who reported decreased levels of ionized magnesium and not total magnesium. They reported significantly decrease in total magnesium levels in SCA patients compared with Caucasians and not between SCA and AA groups of African Americans. Differences in magnesium levels between SCA and AA control group were only observed by measuring serum ionized magnesium and not total magnesium levels [7]. Measurements of calcium to magnesium ratio in several disease conditions have yielded conflicting findings, examples include Diabetes mellitus and cardiovascular diseases [13-16]. Low plasma magnesium level but increased red blood cells magnesium was reported in children with SCD [17]. On the contrary, a higher serum magnesium level in SCD patients than controls was observed in another study which was

attributed to chronic haemolytic states in the SCD patients [18]. No significant difference between serum magnesium levels in Nigerian SCD patients compared with HbAA controls was also reported [19].

The calcium to magnesium ratio observed in this study between SCD patients and controls (4.39±0.02 vs 2.13±0.01) was higher than 2.42±0.213 vs 2.09±0.16 and 1.955±0.10 reported for SCA patients, African American controls and Caucasians respectively using ionized calcium and magnesium [7]. The high calcium to magnesium ratio was thought to result in activation of the Gardos channel, sickled red blood cell sickling, dehydration, vaso-occlusive crisis. These complications often lead to proteinuria which is a manifestation of glomerular injury in SCD patients. Some of the factors that may predict renal insufficiency in SCD patients such as hypertension, anaemia and haematuria may be exacerbated by higher calcium to magnesium ratio. A sustained imbalance in the calcium to magnesium ratio over a period of time may results in excited firing state of biochemistry of the cells [20]. In SCD patients (who are constantly under stress condition), muscle, nerves, hormone secreting cells and inflammatory response may go into an overreaction mode [20]. In addition, calcium levels may get excessively high due to low magnesium which will further activate potassium and water loss (cellular dehydration). The significant decreases in serum calcium and magnesium levels and higher calcium to magnesium ratio observed in SCD patients with proteinuria may be as a result of urinary loss of calcium and magnesium.

In this study, total instead of ionized calcium and magnesium levels were determined because the former are readily available in our setting. Because these essential elements regulate electrolytes and water movement between intra and extracellular spaces their measurements could be used in the management of these patients [21-23]. The ratio is of importance in assessing the potential dehydration status of SCA patients with proteinuria. Increased potassium loss and cellular dehydration seen in these patients may be promoted by high calcium and low magnesium levels.

5. Conclusion

Total serum calcium to magnesium ratio is higher in SCD patients with proteinuria than those with proteinuria. The ratio could be routinely calculated to assess the imbalance of these essential elements.

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