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Red Blood Cell and Serum Magnesium Levels Among Children and Adolescents With Sickle Cell Anemia

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Abstract

Patients with sickle cell anemia (SCA) can acquire many biochemical abnormalities, including altered magnesium levels. However, the roles of magnesium in the pathogenesis and management of SCA need to be determined. The aim of this work was to evaluate magnesium levels among pediatric patients with SCA in Basra, Iraq. The study employed a case-control design and examined 87 patients with SCA (3–15 years old) who had attended the Basra Center for Hereditary Blood Diseases while in a steady state and 90 apparently healthy control subjects. Complete blood count, red blood cell (RBC), and serum magnesium, calcium, potassium, sodium, zinc, and copper levels were measured in all subjects. The results revealed significantly lower RBC and serum magnesium levels among the patients with SCA (3.62 ± 0.42 and 1.35 ± 0.19 mg/dL, respectively) than those among the control subjects (4.47 ± 0.55 and 1.87 ± 0.27 mg/dL, respectively). In addition, compared to the control subjects, the patients with SCA had significantly lower serum levels of potassium, sodium, and zinc; significantly higher serum levels of copper; and significantly higher Ca/Mg and Na/Mg ratios. Among the SCA patients, the RBC magnesium level was significantly negatively associated with the frequencies of vaso-occlusive crises ($r = -0.423$, $P < 0.001$) and disease-related hospitalization ($r = -0.225$, $P < 0.05$). To conclude, the RBC magnesium level, but not the serum magnesium level, is significantly associated with vaso-occlusive crises and hospitalization. Therefore, screening and management of low RBC magnesium levels in SCA patients are required.

Keywords Magnesium · Sickle cell anemia · Children

Introduction

Sickle hemoglobin (HbS) is known to interact with diverse genes and environmental factors, producing a multisystemic disease with several phenotypes [1].

Sickle cell anemia (SCA) is the most common and most severe variant of the major genotypes of sickle cell disease (SCD): homozygous sickle cell anemia (hemoglobin SS), the sickle thalassemias (Hb $S\beta^o$, and Hb $S\beta^+$), and hemoglobin SC (Hb SC) disease [2].

SCA is an inherited disorder of hemoglobin (Hb) synthesis characterized by life-long severe hemolytic anemia, pain crises, and chronic organ system damage that adversely affects life expectancy [3].

The severity of SCA is related to several variables, including the concentration of HbS in red blood cells (RBCs), the degree of Hb deoxygenation, the intracellular Hb concentration, and the level of fetal hemoglobin (HbF) [4].

The nutritional approach to the management of SCD is one of the most effective protocols adopted in the management of the syndrome. Reliable data show that deficiencies of various nutrients are exacerbated by the sickling process. Some of these nutrients include magnesium (Mg), zinc (Zn), copper (Cu), vitamin A, vitamin C, and vitamin E, and their deficiencies result in many of the pathophysiological complications of SCD [5].

The observed level of magnesium in SCA patients varies. Some studies have reported normal levels [6], while others have found low [5, 7] circulating magnesium levels. However, plasma magnesium levels do not reflect the intracellular magnesium content or the amount of free magnesium, which is important in the context of cellular functions [6–8]. Decreased RBC magnesium levels have been reported despite normal serum magnesium levels due to the increased activity of the Na/Mg exchanger in Mg-loaded sickle erythrocytes [8].

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Low levels of total magnesium in sickled erythrocytes have been associated with increased sickling due to RBC dehydration, leading to increased polymerization [9, 10], which contributes to many clinical complications, such as stroke, leg ulcers, and poor general nutrition [10]. These effects may be due to recurrent bouts of poor health and frequent hospitalization, which affect eating habits and reduce feeding time among SCA patients. Anemia, which is common in SCA patients, leads to increased turnover of hemopoietic cells, a hypermetabolic state, and increased micronutrient and energy demands [11].

Other causes of the low magnesium concentrations in patients with SCA are hypoxia-induced RBC sickling associated with increased membrane permeability and magnesium efflux via increased activity of a putative Na/Mg exchanger [12, 13], glomerular hyperfiltration and excessive urinary excretion of many trace metals due to damaged glomeruli, and loss of trace metal carrier proteins [14].

The role of magnesium in SCD management has been investigated. Previous studies found a decrease in the length of hospital stay among patients given oral or intravenous (IV) Mg [9, 12]. However, more recent studies did not find an effect of IV magnesium sulfate ($MgSO_4$) on hospital length of stay, pain scores, or total cumulative analgesia when used for acute vaso-occlusive crises (VOCs) or on quality of life in children hospitalized for sickle cell pain crisis [15, 16].

Understanding the role of magnesium in overall metabolism and its influence on clinical interventions could provide insight into the management of SCA. Therefore, this study was conducted to determine the serum and RBC magnesium levels in children with SCA in a steady state compared with those in healthy children and to evaluate the associations of magnesium levels with selected minerals and trace elements as well as selected hematological parameters and disease severity.

Subjects and Methods

This case-control study was conducted from March to December 2014 on patients with SCA and age- and gender-matched healthy children and adolescents.

Patients

Children and adolescents with SCA between 3 and 15 years of age who had consulted the Center for Hereditary Blood Diseases at Basra Maternity and Children Hospital were recruited. A total of 87 patients (47 males and 40 females) were included in the study.

A patient in a steady state needed to fulfill the following criteria: no acute painful episodes requiring treatment in the emergency department or in the hospital for at least four consecutive weeks after a previous painful crisis, no blood transfusion during the previous 4 months, and no history of underlying illness, such as infection, during the previous 4 weeks [17, 18].

The severity of SCA was assessed by the frequencies of VOCs, hospital admission, and blood transfusion. Severe disease was defined as frequent VOCs (≥ 3/year), frequent blood transfusions (≥ 3/year), frequent hospitalization (≥ 3 times/year), and an episode of cerebrovascular accident (CVA), acute chest syndrome (ACS), or avascular necrosis of bone (AVN) [19, 20].

Growth parameters and vital signs were measured, and a full physical examination was performed for all patients, including general and systemic evaluations.

Patients treated with medications such as aminoglycosides, furosemide, or thiazides, which may have affected Mg levels during the previous 3 weeks, were excluded [21, 22]. Patients who were currently using Mg-containing drugs, such as tonics; patients who used Zn supplements; and patients with concomitant chronic illnesses other than SCA, such as heart failure, hypertension, diabetes mellitus, and renal problems, were also excluded. The total excluded patients were 19.

Control Group

The children and adolescents in the control group were obtained from primary health care (PHC) centers. Four PHC centers were randomly selected using systematic random sampling from a list of 39 health care centers in the first and second sectors in the Basra city center. The starting point was selected randomly, and the selection proceeded with every 10th center.

A total of 90 (48 males and 42 females) apparently healthy children between 3 and 15 years old who were attending these PHC centers for registration purposes for primary and secondary schools, vaccination, and minor health problems such as cough, cold, or tooth extraction, examination for school and kindergarten registration, dermatological diseases, and other simple health problems were recruited. Children with a concomitant chronic illness or a family history of hemoglobinopathies, or who were using drugs containing Mg or Zn, were excluded from the study.

Official Endorsement

Approval from the Basra Directorate General of Health to conduct the study on patients with SCA registered at the CHBD and healthy children and adolescents at the PHC centers was obtained before study initiation. The study was also approved by the ethical committee of Basra Medical College.

Parental Consent

After the research staff explained the purpose of the study, informed consent was obtained from at least one parent before

study recruitment for all children and adolescents included in the study.

For all participants, weight and height were measured, and body mass index (BMI) was calculated.

Laboratory Procedures

All patients enrolled in the study were diagnosed with SCA by high-performance liquid chromatography (HPLC) using the VARIANT™ β-Thalassemia Short Program, Bio-Rad Laboratories, Hercules, CA, USA.

Venous blood was collected from the patients and controls under standardized conditions. A portion of each blood sample was added to EDTA tubes for hematological analysis, another portion was added to a tube containing heparin for the estimation of intracellular Mg in RBCs, and the remainder was transferred to plain tubes for the rest of the analyses. After sera separation, specimens were either immediately analyzed or stored in freezing conditions until analysis within 2 days.

The Hb level, mean corpuscular volume (MCV), mean corpuscular hemoglobin concentration (MCHC), and total white blood cell (WBC) and platelet counts were measured using the SYSMEX KX-21N Automated Hematological Analyzer.

The serum magnesium (Mg) level was measured using the calmagite colorimetric method (BIOLABO, France). Washed RBCs were used for intracellular measurement of magnesium in RBCs using a procedure similar to that used for the serum magnesium level. Calcium (Ca) was measured with o-cresolphtalein complexone (CPC) (BIOLABO, France), and phosphorous (P) was assayed using a colorimetric method (RANDOX, UK). Potassium (K) was measured by a photometric turbidimetric test (HUMAN, Germany), and sodium (Na) was measured using a colorimetric method (SPECTRUM, Egypt). Zinc (Zn) and copper (Cu) were measured using a spectrophotometric method (colorimetric test with 5-Brom-PAPS and Dibrom-PAESA, respectively) with a kit provided by SPECTRUM (Egypt).

Each biochemical test was run in duplicate, and the procedure was followed as described by the manufacturer. Quality control sera from Randox, UK were included in each assay batch for all analytes. The inter-assay coefficients of variation (CVs) for Mg, Ca, Na, K, P, Zn, and Cu were 2.2, 3.1, 2.8, 3.5, 3.9, 2.5, and 3.1%, respectively.

The normal values of the biochemical variables were determined according to levels measured in the healthy children and adolescents recruited in this study and are presented as the means ± standard deviations (SDs) in Table 1. The Ca/Mg and Na/Mg ratios were calculated for each subject by dividing the serum calcium and sodium levels by the serum Mg level, respectively. All biochemical test results are stratified as low (defined as a level > 2 SD below the mean), normal (a level within 2 SD of the mean), or high (a level > 2 SD above the mean).

Statistical analyses were performed using Statistical Package for the Social Sciences (SPSS) software version 17 (IBM, Chicago, IL, USA). The data are expressed as the means ± SDs. Comparisons of proportions in crosstabs were performed using the chi-squared test when each cell in a 2 × 2 table had an expected frequency of 5 or more, or using Fisher's exact test when one or more of the cells had an expected frequency of less than 5.

The *t* test was used for quantitative comparisons between two means of different samples. One-way analysis of variance (ANOVA) was used for quantitative comparisons of more than two means of different samples. A bivariate Pearson correlation analysis was used to assess the correlations between the variables. Correlation coefficients measured the strength and direction of the linear relationship between two variables. For all tests, a P value of < 0.05 was considered statistically significant.

Table 1 Reference values of the biochemical parameters from the control children recruited in this study compared with international reference values

Biochemical parameters	Control group in this study		International reference range [23]
	Mean ± SD	Normal range	
Red blood cell (RBC)			
Magnesium (mg/dL)	4.47 ± 0.55	3.37–5.57	4.0–6.4
Serum			
Magnesium (mg/dL)	1.87 ± 0.27	1.33–2.41	1.7–2.61
Calcium (mg/dL)	9.72 ± 0.98	7.76–11.68	8.6–10.6
Potassium (mEq/L)	4.14 ± 0.32	3.50–4.78	3.4–4.7
Sodium (mEq/L)	141.73 ± 3.88	133.97–149.49	135–147
Phosphorus (mmol/L)	1.13 ± 0.14	0.85–1.41	1.03–1.87
Zinc (μg/dL)	90.34 ± 16.38	57.58–123.10	70–120
Copper (μg/dL)	109.66 ± 24.42	60.82–158.50	80–160

Ref. 23 = Lo SF (2016). Reference intervals for laboratory tests and procedures, Chapter 727. In: Kliegman RM, Stanton BF, St Geme III JW, Schor NF (eds.). Nelson Textbook of Pediatrics, 20th ed. Philadelphia PA: Elsevier Inc., pp. 3464–3473

Table 2 Selected biochemical parameters of the patients with SCA and the participant in the control group

Parameter	SCA ($N = 87$), mean ± SD	Control ($N = 90$), mean ± SD	P value[a]
Red blood cell (RBC)			
Magnesium (mg/dL)	3.62 ± 0.42	4.47 ± 0.55	< 0.001
Serum			
Magnesium (mg/dL)	1.35 ± 0.19	1.87 ± 0.27	< 0.001
Calcium (mg/dL)	9.42 ± 1.13	9.72 ± 0.98	0.060
Potassium (mmol/L)	3.74 ± 0.42	4.14 ± 0.32	< 0.001
Sodium (mmol/L)	137.55 ± 15.34	141.73 ± 3.88	0.013
Phosphorus (mmol/L)	1.21 ± 0.17	1.13 ± 0.14	0.001
Zinc (μg/dL)	67.25 ± 17.78	90.34 ± 16.38	< 0.001
Copper (μg/dL)	142.35 ± 49.92	109.66 ± 24.42	< 0.001
Ratios			
Ca/Mg	7.12 ± 1.37	5.28 ± 0.97	< 0.001
Na/Mg	105 ± 16.4	77.5 ± 11.3	< 0.001

[a] An independent t test was used

Results

The total number of children and adolescents enrolled in this study was 177, including 87 patients with SCA (47 females and 40 males) and 90 subjects in the control group (48 females and 42 males). The mean (± SD) age was 7.65 ± 3.27 years for the patients with SCA and 7.83 ± 3.28 for the control subjects ($P > 0.05$).

The patients with SCA had significantly lower growth parameters than the control subjects, including weight (22.55 ± 7.86 vs. 28.25 ± 8.59 kg), height (118.13 ± 19.45 vs. 125.80 ± 14.78 cm), and BMI (15.44 ± 2.23 vs. 17.51 ± 2.21) ($P < 0.05$). In addition, the patients with SCA had significantly lower Hb levels (8.07 ± 1.06 vs. 11.57 ± 1.46 g/dL) and significantly higher WBC (10.66 ± 6.75 vs. 6.92 ± 1.49 × 10^9/L) and platelet counts (349.33 ± 171.58 vs. 289.25 ± 60.81) than the control subjects ($P < 0.05$).

The biochemical analysis demonstrated that RBC magnesium and serum magnesium, potassium, sodium, and zinc levels were significantly lower among children and adolescents with SCA than those among the participants in the control group ($P < 0.05$). However, for serum phosphorus and copper levels, the Ca/Mg and Na/Mg ratios were significantly higher in the patient group, but serum calcium was not significantly different between the groups ($P > 0.05$; Table 2).

No significant difference in serum magnesium levels among different age groups or genders was observed ($P > 0.05$). Additionally, RBC magnesium levels were significantly lower among patients with ≥ 3 vaso-occlusive crises/year ($P < 0.05$), while RBC magnesium levels were not significantly associated with other indicators of clinical severity ($P > 0.05$). In addition, serum magnesium levels were not significantly different between patients with severe disease and those with milder disease (Table 3).

The frequencies of low, normal, and high serum and RBC magnesium levels were assessed in both groups of children and adolescents; 45 (51.7%) of the patients with SCA had low serum magnesium levels, and 22 (25.3%) had low RBC magnesium levels. Meanwhile, none of the control subjects had

Table 3 Serum and RBC magnesium level with respect to the disease severity among patients with SCA

Variables	Severity		Serum magnesium (mg/dL)	P value	RBC magnesium (mg/dL)	P value
	Per year	No	Mean ± SD		Mean ± SD	
Vaso-occlusive crises	< 3	46	1.35 ± 0.20	0.897	3.71 ± 0.36	0.024
	≥ 3	41	1.34 ± 0.19		3.51 ± 0.44	
Blood transfusions	< 3	72	1.35 ± 0.14	0.695	3.62 ± 0.40	0.649
	≥ 3	15	1.33 ± 0.22		3.57 ± 0.48	
Hospitalizations	< 3	71	1.34 ± 0.20	0.513	3.62 ± 0.41	0.796
	≥ 3	16	1.38 ± 0.17		3.59 ± 0.45	
Associated disease						
Acute chest syndrome	Yes	76	1.39 ± 0.16	0.486	3.70 ± 0.37	0.475
	No	11	1.34 ± 0.20		3.60 ± 0.42	

An independent t test was used

low serum or RBC magnesium levels, seven (7.8%) had high serum magnesium levels, and one (1.1%) had high RBC magnesium levels. None of the patients with SCA had high serum or RBC magnesium levels.

The bivariate Pearson correlation analysis was performed to study the potential associations of different factors such as age, sex, height, weight, body mass index, frequency of vaso-occlusive crisis, blood transfusions per year, Hb, white blood cell count, and biochemical parameters including sodium, potassium, zinc, copper, calcium, and phosphorous with serum and RBC magnesium levels among patients with SCA. The analysis revealed that only the RBC magnesium level had a significant negative association with the frequency of VOCs and that serum magnesium was negatively and significantly associated with the Ca/Mg and Na/Mg ratios (Figs. 1, 2, 3, and 4).

Discussion

Our study is the first to investigate the levels of serum and RBC magnesium (Mg) in SCA children and their healthy counterparts living in Basra, Iraq, a region known for its high prevalence of sickle cell diseases, thalassemia, and other hemoglobinopathy. It is a preliminary case-control study designed to evaluate the levels of both serum and RBC magnesium in pediatric patients with SCA and the associations of these levels with other minerals and trace elements as well as clinical variables.

We noticed that the mean values for the biochemical parameters from our healthy control subjects are lower than the international reference values [23], which may be due to dietary, geographical, environmental, and genetic variations as well as different methods of measurement. Therefore, to eliminate any bias due to such variations in the current work, the mean values and the calculated acceptable range for the estimated biochemical variables (RBC magnesium and serum magnesium, calcium, sodium, potassium, zinc, and copper) obtained from our recruited healthy control subjects were used for comparison with the same biochemical variables from patients with SCA.

Magnesium plays a vital role in several cellular functions through its action as a cofactor in many enzymatic reactions and on the movement of ions across cellular membranes [22, 24]. We found that both serum and RBC magnesium levels were significantly lower in the patients with SCA than those in the control subjects. Among the SCA patients, a significant negative association between RBC magnesium and the frequency of VOCs was observed. Significant negative associations between serum magnesium and the Ca/Mg and Na/Mg ratios were identified. The lower serum magnesium level among the patients compared to that among the control subjects is consistent with the findings of many researchers [5, 11, 24], but not with other reports [6, 25]. Similarly, the lower RBC magnesium level in the patients with SCA than that in the control subjects is consistent with several previous reports [8, 24, 26]. Low levels of magnesium in SCD patients may result from excessive urinary excretion, hypermetabolic rate, and increases in micronutrient and energy demand because of the increased turnover of hemopoietic cells due to chronic hemolysis, or perhaps diminished dietary consumption [5, 8, 11]. In addition, hypomagnesemia may result

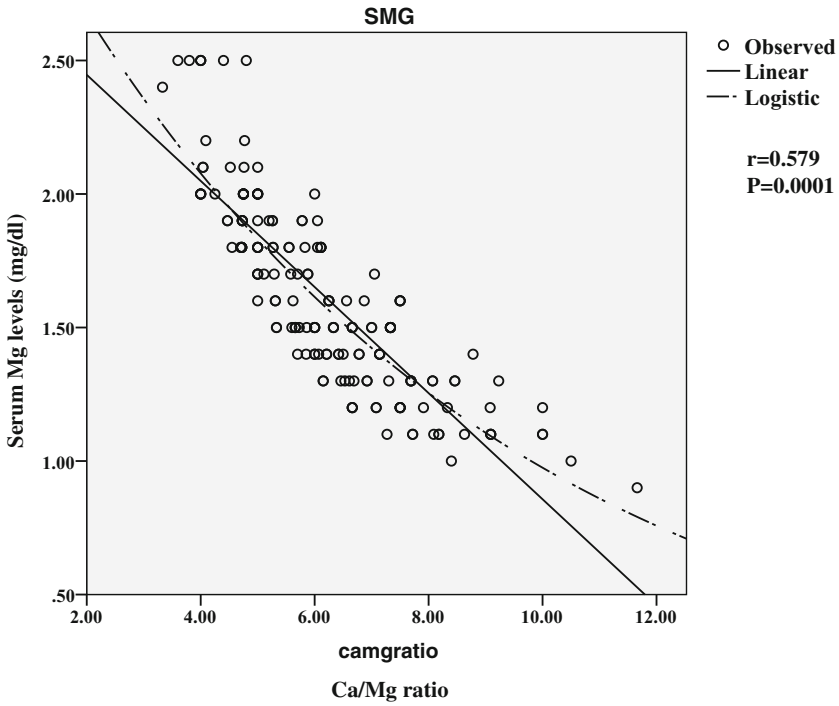

Fig. 1 Correlation between serum Mg levels (mg/dl) and Ca/Mg ratio in patients with sickle cell anemia (SCA). Pearson correlation coefficient (r) = 0.579, P = 0.0001

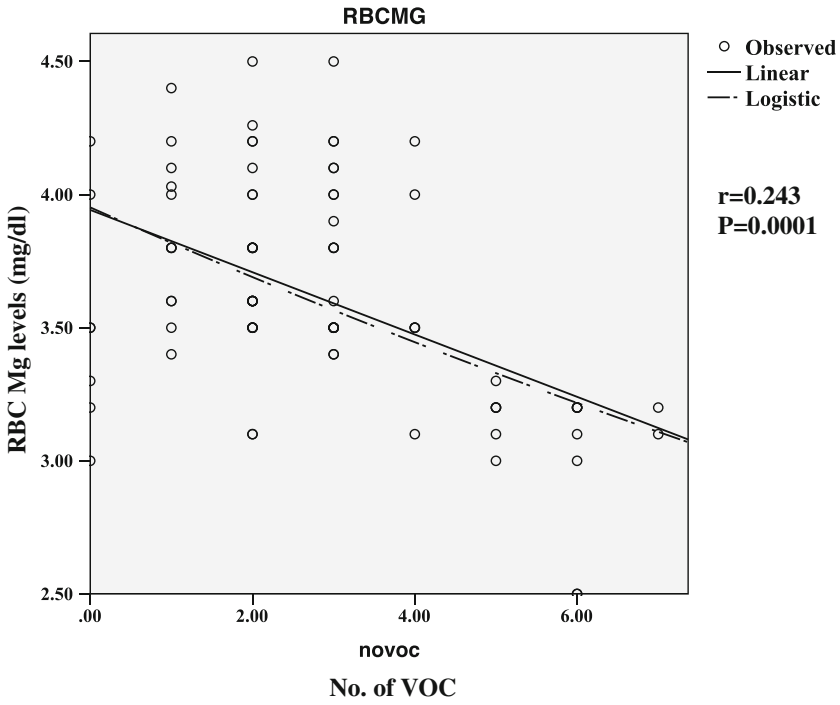

Fig. 2 Correlation between RBC Mg levels (mg/dl) and number of vaso-occlusive crises (VOCs) in patients with sickle cell anemia (SCA). Pearson correlation coefficient (r) = 0.243, P = 0.0001

from pathological changes in the cell membrane and from the use of medications [27].

A significant negative association was found between RBC magnesium levels and the frequency of VOCs. However, no significant association was found between serum magnesium levels and the frequency of VOCs. Nnodim et al. in Nigeria also reported that patients with VOCs have significantly reduced serum magnesium levels [28]. Magnesium may contribute to vasodilation through endothelium-dependent release of NO and inhibition of calcium in smooth muscle. It may also decrease vaso-occlusion by slowing clotting time. In addition, magnesium has been shown to improve RBC hydration; thus, low magnesium levels increase the complications of SCA, including the frequency of VOCs [9, 15, 29].

In addition to low magnesium, in our SCA patients, the levels of some minerals including sodium and potassium

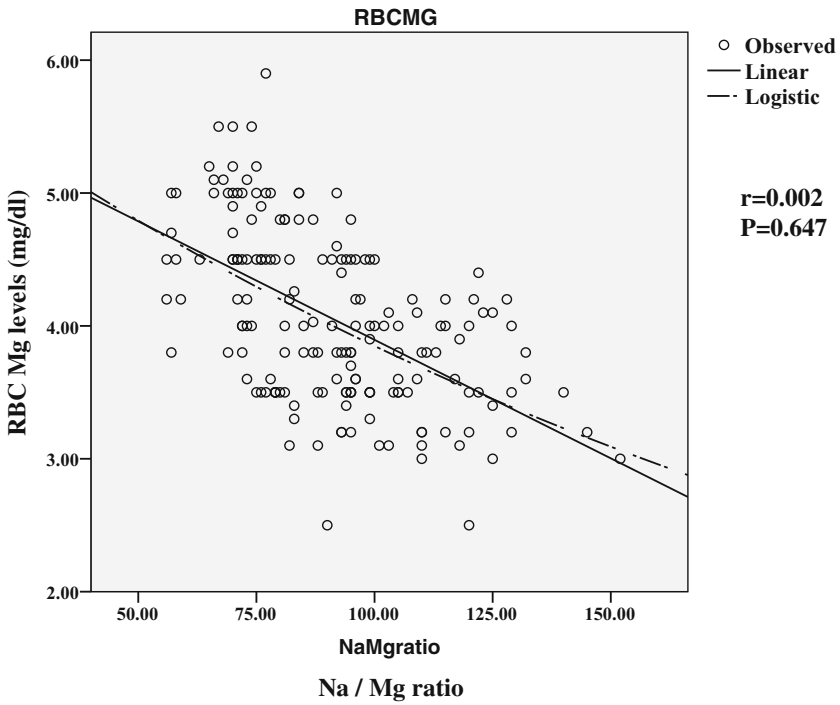

Fig. 3 Correlation between RBC Mg levels (mg/dl) and Na/Mg ratio in patients with sickle cell anemia (SCA). Pearson correlation coefficient (r) = 0.002, P = 0.647

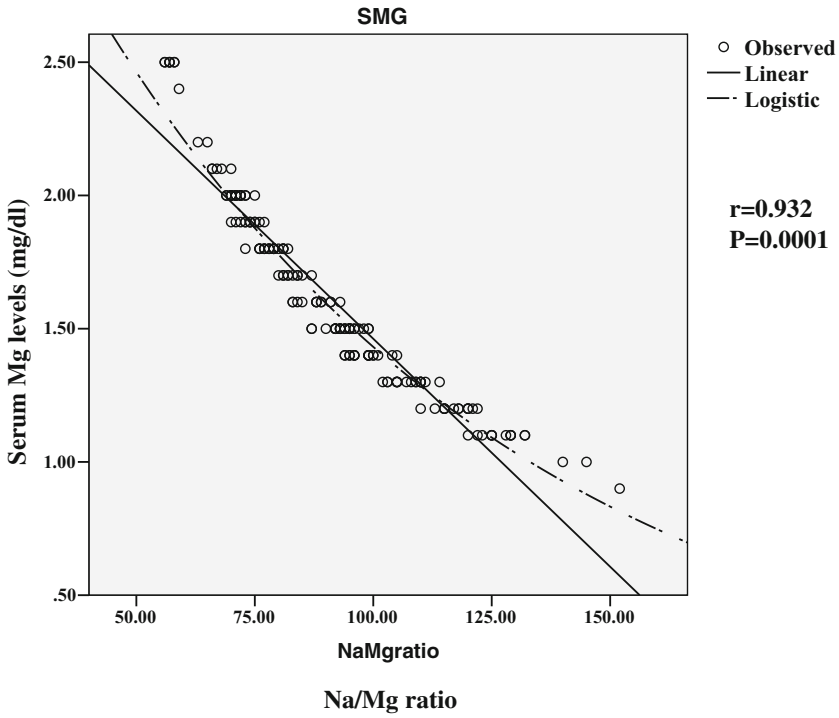

Fig. 4 Correlation between serum Mg levels (mg/dl) and Na/Mg ratio in patients with sickle cell anemia (SCA). Pearson correlation coefficient (r) = 0.932, P = 0.0001

and trace elements including zinc were significantly decreased, while phosphorous and copper levels were significantly increased; however, no significant difference in calcium levels was found compared with the control subjects. These minerals and trace elements are all important in red blood cell maintenance, body growth, and development. Therefore, they should be maintained at normal concentrations within the body; however, they are distributed unequally either intracellularly or extracellularly. Therefore, their maintenance and a balanced water content in cells are vital for proper cellular and biochemical functioning.

In sickle cell disease, dehydration is an important factor, leading to increased sickle hemoglobin polymerization during deoxygenation and decreasing the life span of RBCs [30].

Dehydration in sickle cell disease is caused by the loss of potassium, chloride, and water from erythrocytes via stimulation of two vital pathways: the K-Cl cotransporter and the Ca-activated K^+ channel or the Gardos channel [13, 26, 30]. Dehydration is also associated with a decreased magnesium content in RBCs, which is most likely due to abnormally elevated red cell membrane permeability to cation such as magnesium, calcium, potassium, and sodium during deoxygenation episodes [31] and to abnormal stimulation of the Na-Mg exchange transporter [12, 13, 26]. A low magnesium RBC content has also been found to cause increased potassium loss by activating the K-Cl cotransporter, while an increased RBC magnesium content would prevent the loss of potassium by inhibiting the K-Cl cotransporter [32], indicating that the magnesium level can modulate K efflux and decrease dehydration and sickling events. Magnesium supplementation in SCA patients has been shown to have a beneficial effect on the course of the disease and contributed to improved RBC deformability [18]. These findings are consistent with those of Pandey et al. [33], and could be related to the primary role of magnesium in preventing RBC dehydration through the regulation of cellular cation transporters, such as the potassium-chloride (K-Cl) cotransporter and calcium and potassium channels [10, 13, 24, 32].

In our work, both sodium and potassium levels were significantly lower in the SCA patients than in the control group participants. This finding is consistent with the findings from a study by Gupta et al [31]. However, others have reported higher serum potassium and lower sodium levels among SCA patients than among control subjects [33, 34]. These changes in potassium and sodium levels in SCA could be due to reduced fluid intake, accelerated influx and outflow of sodium and potassium, increased insensible loss, and the high incidence of hyposthenuria [31]. They could also be attributed to the low magnesium levels observed in our work and the significant negative association of serum magnesium with the Na/Mg ratio. The Na-K pump in erythrocytes, which is activated by magnesium ions, has a magnified role in the pathophysiology of sickle cell erythrocytes through its regulation and control of sodium and potassium ion passage across the cellular membrane [35]. It has been suggested that Na–K pump inhibition may be useful in therapy [33].

In this study, no correlation was observed between zinc and magnesium; however, they shared similar actions as antisickling agents, reducing sickle cell hemoglobin polymerization progression by 89.69 and 48.40%, respectively [36].

Zinc has an essential role in numerous biochemical pathways, and zinc's action in the blood mainly involves antagonizing calcium's action on the RBC membrane and stabilizing the erythrocyte, thereby preventing hemolysis [37, 38]. It also acts as an antioxidant [37, 38]. Since SCA is associated with high oxidative stress [39], a low zinc level could lead to an increased level of oxidizing agents that could cause damage to the permeability of the cellular membrane and affect ion movement [37]. It has also been suggested that zinc and magnesium improved the Fe^{2+}/Fe^{3+} ratio of Hb S molecules by 16.21 and 2.63%, respectively, which would increase the oxygen affinity of sickled cells by modifying their operational states, thereby returning them to their original biconcave shape [36, 37]. The finding of low zinc levels in the patients with SCA compared with those in the control subjects is consistent with the findings of other researchers from different countries [40–42]. This finding could be attributable to inadequate intake of trace elements because of chronic pain, reduced appetite, and hemolysis in addition to increased demand and increased urinary excretion, leading to lower zinc levels in patients with SCA. The current study did not demonstrate a significant association between serum or RBC magnesium levels and zinc levels. Zinc deficiency in SCD has been shown to be correlated with disease severity, and zinc supplementation can improve the RBC membrane status and improve red cell dehydration by antagonizing intracellular calcium [43].

The higher copper levels in patients with SCA than in control group subjects reflect the findings of other studies [5, 36]. However, there was no significant association between copper and either RBC or serum magnesium. The clinical significance of the elevation in plasma copper levels is unclear, but it has been reported to occur in the event of decreased plasma zinc levels or as a result of increased oxidative stress and low antioxidant status, which triggers the release of copper from the tissues into the blood to promote tissue repair [36].

Magnesium is a known modulator of calcium, competing with calcium for entry into cells [24]. Both these bivalent cations are important and required for various physiological and biochemical purposes at the organ, tissue, and cellular levels, and for that reason, their concentration have to be kept nearly constant [44]. This implies that slight changes in the extracellular values of calcium and magnesium must be identified to permit the proper modification by the homeostatic systems. A balanced level of calcium and magnesium is controlled and maintained by the combined action of intestinal absorption, excretion through the kidneys, and interchange with bone [45]. Small changes in the magnesium availability within the cell may therefore cause disturbed calcium signaling or calcium toxicity, and as a result, the Ca/Mg ratio is considered to be of high significance for the activity of Ca2_-ATPases and other Ca2_ transporting proteins [44, 45].

Calcium levels were not significantly different between the patients with SCA and the participants in the control group. This finding is consistent with those in a report by Judd et al. [46]. The current study did not demonstrate significant correlations between serum calcium levels and RBC or serum magnesium levels. However, a significantly increased Ca/Mg ratio was found in the patients with SCA compared to that in the participants in the control group, and this ratio was negatively associated with serum magnesium levels. Zehtabchi et al in the USA reported a similar finding [7].

In addition, it was mentioned that a regular estimation of the Ca/Mg ratio would be useful in calculating the intracellular balance of magnesium and calcium in the management of patients with SCA [47].

K^+ efflux and cell dehydration are stimulated by high calcium levels (Gardos channel activation), while low magnesium levels (disinhibition of K-Cl cotransport) will prevent potassium loss and correct cell hydration. A higher-than-normal Ca/Mg ratio could lead to activation of the Gardos channel, leading to cell dehydration, and may also play a direct vascular role in vaso-occlusion in SCA [12, 13, 30, 32]. Recently, inhibition of this channel was suggested as a reasonable therapeutic approach.

However, this strategy is questionable since the effectiveness of Gardos channel inhibitors depends on RBC membrane destruction triggered by a series of sickling and unsickling events in red blood cells during polymerization and depolymerization of Hb S [48]. Nevertheless, new therapeutic approaches are warranted to reduce painful crises and hospitalization since significant positive associations were found between these events and both serum and RBC magnesium levels in our work; reducing these events may improve the health of people with SCA.

This study has many limitations. First, we did not examine associations between magnesium levels and other markers of disease severity, such as fetal hemoglobin levels or haplotypes. Second, we assessed magnesium only during steady state conditions; we did not assess it during VOC or other disease-related complications. Third, we did not measure free magnesium or free calcium levels in the serum or erythrocytes. Finally, the concentrations of intracellular or erythrocyte calcium, sodium, potassium, and zinc were not estimated. Otherwise, our results are similar to those reported in the literature.

Conclusions

From this study, we can conclude that although both serum and RBC magnesium levels are low in pediatric patients with SCA while in a steady state, RBC magnesium levels represent a more reliable marker of SCD-related events than serum magnesium levels, highlighting the importance of assessing RBC magnesium levels in these patients, especially during vaso-occlusive events. Nevertheless, new therapeutic approaches are warranted to reduce painful

crises and hospitalization and may improve the health and quality of life of people with SCA. On the other hand, serum magnesium levels were significantly and negatively correlated with the Ca/Mg and Na/Mg ratios, implying important roles for magnesium in cellular metabolism and the interactions between these ions. Therefore, future research regarding identification and characterization of the roles of magnesium at the molecular level or mechanisms related to the metabolism and transport of magnesium is required to increase our understanding of its interaction with other ions and provide new insight into management strategies for people with SCA or other related diseases.

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Authors' Contributions OA, MK, and LM designed and planned the study. OA collected the data and the samples, and LM performed the lab investigations. All authors contributed to the writing of the manuscript and have read and approved the final manuscript.

Compliance with Ethical Standards

Conflict of Interest The authors declare that they have no conflict of interest.

Ethical Approval All procedures in studies involving human participants were performed in accordance with the ethical standards of the institutional and/or national research committee and with the 1964 Helsinki declaration and its later amendments or comparable ethical standards.

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