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Serum zinc and magnesium levels in steady state sickle cell children with or without anaemia attending Jos University Teaching Hospital, North-Central Nigeria

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ABSTRACT

Background : Sickle cell anaemia (SCA) is a public health problem. Zinc and Magnesium are essential metal antioxidants important in protection of erythrocytes membrane from oxidative stress; a trigger of vaso- occlusive crisis.

Aim: This study aims to evaluate the serum levels of zinc and magnesium in steady state sickle cell children with or without anaemia.

Settings and Design: Comparative cross-sectional study. Forty children aged 1-15 years with sickle cell disease (SCD) in steady state with or without anaemia and 40 age- and- sex matched HbAA counterparts (non-SCD) were consecutively recruited for the study.

Materials and Methods: Each forty SCD and non-SCD children were included in the study. Haemoglobin variants were analysed using haemoglobin electrophoresis. Packed cell volume was determined using haematocrit centrifuge and reader. Serum zinc and magnesium were assayed colorimetrically. Data were analysed using SPSS version 26. Student unpaired t- test was employed to assess the significance of the differences. p-value <0.05 was considered significant.

Results : Serum magnesium unlike zinc was significantly ($p < 0.05$) higher in non-SCD group (2.18mg/dl) compared to the SCD group (2.09mg/dl). There was no significant ($p > 0.05$) effect of SCD and anaemia on serum zinc.

Conclusion : This study has shown that serum Mg was significantly lower in children with SCD compared to non-SCD counterpart.

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1. Introduction

Sickle cell disease (SCD) is an inherited red blood cell disorder of public health importance with high mortality and morbidity.¹ SCD affects 20–25 million people globally, of which 12–15 million live in Africa.² Nigeria currently has the highest burden of sickle cell disease in the whole world with an estimated 150,000 affected children born

every year.² Sickle cell anaemia (SCA) which is the most common form of SCD is characterized by chronic hemolysis and painful vaso-occlusion, resulting in organ dysfunction.³ Red blood cells have a rich oxygen supply and are densely packed with redox active haemoglobin residues. Overwhelming production of free radicals can lead to destruction of the cells, which can lead to anaemia and even death.⁴ Antioxidants such as zinc and magnesium are important in reducing the level and detrimental effects of

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reactive species.

Zinc and magnesium are metallo-nutrients. They are important in red blood cell maintenance, body growth and development.⁵ Zinc is involved in several forms of cellular metabolism and plays roles in immune function, wound healing, protein DNA synthesis as well as cell division.⁶ Zinc possesses antioxidant and antimicrobial properties.⁶ Magnesium modulates the movement of ions across cellular membranes and is crucial in erythrocyte volume control, which prevents dehydration and destruction in sickle cell pathology.⁷

Magnesium deficiency has been associated with sickling, increased polymerization and vaso-occlusion (VOC) in sickle cell due to cell dehydration. The K-Cl cotransport plays a very important role in sickle cell dehydration and is inhibited by significant increase levels of magnesium.^{3,8} This study aimed to determine the serum Zn and Mg levels in children with SCD. Knowledge gained from this study, could add to the pool of knowledge or serve as template for supplementation study.

2. Aim

To determine the serum level of zinc and magnesium in steady state SCD children with or without anaemia.

3. Objectives

To determine the serum level of zinc and magnesium in steady state SCD children with or without anaemia and HbAA (non-SCD). To relate this to age, gender, anemic status, socioeconomic status, nutritional status, and to analyze the relationship between anaemia, SCD and serum zinc and magnesium levels.

4. Materials and Methods

This hospital-based comparative cross-sectional study was carried out in the Department of Paediatrics Research Laboratory, University of Jos/Jos University Teaching Hospital.

Known SCD children aged 1 to 15 years attending sickle cell clinic of Jos University Teaching Hospital (JUTH) and age and sex matched HbAA (non-SCD) children were recruited consecutively for this study from March to May 2023. JUTH is a tertiary health institution in North Central Zone of Nigeria and also serves as a referral centre for neighbouring States such as Bauchi, Gombe, Nasarawa, Taraba, Adamawa and part of Kaduna State. Jos is the capital city of Plateau State⁹ in North-Central Nigeria. Consent was obtained from the parents of all the participants while those older than 7 years gave their assent before the commencement of the study. Structured questionnaire was used to collect data on nutritional habits of the children and parents' socio-economic status.

4.1. Exclusion criteria

Patients who were transfusion dependent and those who received transfusion within the last three months or had intercurrent illness such as infection, inflammation during the previous 4 weeks prior to the study (non-steady state).¹⁰ Those on Zn or Mg containing supplements and those unwilling to participate in the study.

5. Sample Collection and Laboratory Analysis

3mls of venous blood were collected from each subject, 2mls were dispensed into plain tubes, allowed to clot, retract and was centrifuged at 3000rpm for 5 minutes, separated, and labeled appropriately. The serum was stored at 4°C until analysis of Zn and Mg were done using Mispa excel chemistry analyser (version 2.1e lite, by Agappe diagnostics Ltd) using the method of 2-(5-Brom-2-pyridylazo)-5-(N-Propyl-N-sulfopropylamino)-phenol (5-Br-PAPS) as described by Johnson and Eliasson (1987)¹¹ and xylydyl blue method as described by Mann and Yoe (1956)¹² respectively. Capillary blood was collected using capillary tube and filled up-to 2/3 for PCV determination using hematocrit machine and reader. Haemoglobin genotype was by preparing haemolysate and subjecting it on cellulose acetate paper for alkaline haemoglobin electrophoresis.¹³

5.1. Statistical analysis

Data obtained were analysed using the International Business Machine Statistical Package for Social Sciences (IBM SPSS) version 26 and presented as Mean \pm SD. Student unpaired t- test was employed to assess the significance of the differences between two groups. P-value <0.05 was considered statistically significant.

6. Results

6.1. Mean serum Zn and Mg levels of SCD and non-SCD children by age and gender

Forty SCD and 40 non-SCD children participated in this study. Most of the SCD (50%) and non-SCD (42.5%) children were aged 11 to 15 years. Serum Zn and Mg were non-significantly ($p>0.05$) higher in children aged 1 to 5 years (118.8ug/dl & 2.20mg/dl) compared to the other age groups of 6 to 10 years (92.0 & 2.03) and 11 to 15 years (94.7ug/dl & 2.06) respectively. The reverse was observed in non-SCD children as both parameters were non-significantly ($p>0.05$) lower in children aged 1 to 5 years (Table1).

55% of the SCD children were males and 52.5% of the non-SCD children were females. Serum Zn and Mg were non-significantly ($p>0.05$) higher in female SCD (98.2ug/dl for Zn & 2.10mg/dl for Mg) and female non-SCD children (99.6ug/dl & 2.20mg/dl). On the overall, serum Zn was non-significantly ($p>0.05$) higher in non-SCD (97.6ug/dl)

compared to SCD (96.8µg/dl). Serum Mg was significantly ($p<0.05$) higher in non-SCD (2.18mg/dl) compared to SCD children (2.09mg/dl) (Table 1).

6.2. Deficiency distribution of serum Zn and Mg in SCD and non-SCD children by age and gender

Three (7.5%) of the SCD children were deficient in Zn and Mg while 5% and 2.5% of the non-SCD were deficient in both respectively. Deficiency was not observed in children aged 11 to 15 years. Higher rate of deficiency was among children aged 1 to 5 years. Two (66.67%) of the

SCD males and 33.33% females were Zn and Mg deficient respectively. None of the non-SCD males were Zn or Mg deficient (Table 2).

6.3. Mean PCV level and distribution of anaemic and non-anaemic SCD and non-SCD children by gender

PCV was significantly ($p<0.05$) lower in SCD (24%) compared to the non-SCD children (44%). Thirty-nine (39/40; 97.5%) of SCD children were anaemic and 56.4% were males. Ten (25%) of non-SCD were anaemic and 6/10 (60%) were females. Fifteen (15/30) of the non-SCD females were non-anaemic (Table 3).

6.4. Mean serum Zn and Mg levels in anaemic and non-anaemic SCD and non-SCD children

There was no significant ($p>0.05$) effect of SCD and anaemia on serum Zn and Mg levels.

Mean serum Zn was higher in anaemic SCD ($97.5\pm 19.30\mu\text{g/dl}$) and anaemic non-SCD children ($101.8\pm 21.70\mu\text{g/dl}$) compared to the non-anaemic SCD ($69.6\pm 0.00\mu\text{g/dl}$) and non-anaemic non-SCD children ($96.2\pm 20.30\mu\text{g/dl}$) respectively. Mg was higher in non-anaemic SCD children ($2.14\pm 0.00\text{mg/dl}$) compared to the anaemic $2.08\pm 0.19\text{mg/dl}$. Mg was higher in anaemic non-SCD children ($2.22\pm 0.26\text{mg/dl}$) compared to the non-anaemic counterpart ($2.17\pm 0.20\text{mg/dl}$) (Table 4).

6.5. Mean of serum Zn and Mg levels by parent's educational and occupation status

Table 5 Represents the mean of Zn and Mg levels by parent's socio-economic status.

Mean of serum Zn and Mg levels by parent's educational and occupation status

6.6. Mean serum Mg and Zn levels based on most frequently consumed food

Table 6 shows the mean serum Mg and Zn levels based on most frequently consumed diet. Three food groups were tested in this study which include: Beef, pap, and custard; Fish, rice, and egg; and Milk and garri, at daily, weekly

and monthly intervals. It was observed that serum Mg level decreased progressively following daily consumption of the listed diets in the order given (2.14mg/dl, 2.13mg/dl, and 2.11mg/dl). There was a relative stability in the levels of serum Zn and Mg by the rate of consumption of the listed group food.

7. Discussion

This study aimed at determining the serum levels of zinc and magnesium in SCD and HbAA children with or without anaemia. Zinc binds with haemoglobin, and increases haemoglobin oxygen affinity. This may be of benefit in inhibiting sickling, provided that high enough levels of red cell zinc can be obtained.⁸ From this study, Zn was non-significantly lower in children with SCD compare with the non-SCD. This may be because all the SCD patients in this study were in steady state and possibly due to higher demand for Zn in SCD to offset higher propensity for rise in reactive species.⁸ When at sufficient levels in the body, zinc is able to reduce the risk of vaso-occlusive crisis (VOC) in sickle cell anemia due to its antioxidant effects. As a result, when levels decrease, patients may experience complications, such as increased VOC. Kudirat et al., (2019)¹⁴ in Kano, North -Western and Ofakunrin et al. (2018)¹⁵ in Jos, North central Nigeria reported that zinc was significantly lower in SCD patients compared with healthy controls. The discrepancy in the degree of significance obtained in this study when compared with the cited reports may be due to differences in sample size, method of analysis and state of health of the SCD children. In the current study, an autoanalyzer¹⁶ was used while in the cited studies atomic absorption spectrophotometer was used.

Conversely, magnesium modulates the movement of ions across cellular membranes and is crucial in erythrocyte volume control, which prevents dehydration and destruction in sickle cell pathology.⁷ The K-Cl cotransport plays a very important role in sickle cell dehydration and is inhibited by a significant increase in the levels of magnesium.³ From this study, serum Mg was significantly (<0.05) lower in SCD children compared to the controls. This may be due to the system's increased utilization of Mg to inhibit the K-CL co-transport in order to prevent dehydration and the consequent VOC.⁷ Low blood Mg level is detrimental to the patient because magnesium deficiency has been associated with sickling, increased polymerization, and VOC in sickle cell due to cell dehydration. Thus, increased intake of Mg-containing foods may be necessary in these patients and consequently, a further study to ascertain the effect of Mg supplementation in children with SCD may be necessary. The result of this study agrees with the reports of Charles et al., (2019)³ and Kudirat, et al., (2019).¹⁴

In addition, age and gender are important influencers of biochemical processes in the body.¹⁷ However, from this study, it was observed that there was no significant

Table 1: Mean serum Zn and Mg levels of SCD and non-SCD children by age and gender

No. (%)	SCD					No. (%)	non-SCD				
	Mean (µg/dl)	p-value	Mean (mg/dl)	p-value	Mean (µg/dl)		p-value	Mean (mg/dl)	p-value		
Age(year)	1-5	5(12.5)	118.8	0.405	2.20	0.766	7(17.5)	94.7	0.547	2.12	0.676
	6-10	15(37.5)	92.0		2.05		16(40.0)	99.8		2.22	
	11-15	20(50.0)	94.7		2.06		17(42.5)	95.1		2.17	
Gender	Male	22(55.0)	98.2	0.771	2.10	0.678	19(47.5)	99.6	0.874	2.20	0.345
	Female	18(45.0)	95.1		2.07		21(52.5)	95.8		2.17	
Overall		40(100)	96.8		2.09	0.859	40	97.6		2.18	0.033
							(100)				

SCD= sickle cell disease; non-SCD = non-sickle cell disease

Table 2: Deficiency distribution of serum Zn and Mg in SCD and non-SCD children by age and gender

	SCD Zn No. (%)	Mg No. (%)	Non-Zn No. (%)	SCD Mg No. (%)
Deficiency by age (year)				
1-5	2(66.67)	1(33.33)	2(100.00)	1(100.00)
6-10	1(33.33)	2(66.67)	0(0.00)	0(0.00)
11-15	0(0.00)	0(0.00)	0(0.00)	0(0.00)
Total (%)	3(100.00)	3(100.00)	2(100.00)	1(100.00)
Deficiency by gender				
Male	2(66.67)	2(66.67)	0(0.00)	0(0.00)
Female	1(33.33)	1(33.33)	2(100.00)	1(100.00)
Total (%)	3(100.00)	3(100.00)	2(100.00)	1(100.00)

SCD= sickle cell disease; non-SCD = non-sickle cell disease

Table 3: Mean PCV level and distribution of anaemic and non-anaemic SCD and non-SCD children by gender

	SCD	NON-SCD	
Mean PCV level p-value	24%	44%	0.001
Total No. anaemic	39 (97.50%)	10 (25.00%)	
Total No. non-anaemic	1 (2.50%)	30(75.00%)	
Overall total	40 (100.00%)	40 (100.00)	
Anaemia by Gender			
No. of anaemic females	17(43.6%)	6 (60%)	
No. of anaemic males	22(56.40%)	4(40%)	
Overall total	39 (100.00%)	10 (100.00%)	
No. of non-anaemic females	1 (2.56%)	15(50%)	
No. of non-anaemic males	0 (0.0%)	15(50%)	
Overall total	1 (2.56%)	30 (100.00%)	

SCD= sickle cell disease; non-SCD = non-sickle cell disease

Table 4: Serum Mean Mg and Zn levels in anaemic and non-anaemic SCD and non-SCD children

	SCD		Non- SCD	
	Non anaemic Mean±SD	Anaemic Mean±SD	Non- anaemic Mean±SD	Anaemic Mean±SD
Zn(µg/dl)	69.6±0.0	97.5±19.30	96.2±20.30	101.8±21.70
Mg(mg/dl)	2.14±0.0	2.08±.19	2.17±.20	2.22±.26

p -value 0.618 and 0.303 for Mg and Zn respectively

Table 5: Serum Zn and Mg increased non-significantly ($p>0.05$) with rise in socio-economic status of the parents.

No. (%)			Zn		Mg	
			Mean ($\mu\text{g/dl}$)	p- value	Mean(mg/dl)	p- value
Mother's Education	None	1(1.3)	93.8	0.377	1.70	0.232
	Primary	18(22.5)	97.3			
	Secondary	35(43.8)	98.4			
	Tertiary	25(31.3)	118.2			
	Other	1(1.3)	117.6			
Mother's occupation	Unemployed	12(15.0)	92.5	0.312	2.10	0.487
	Unskilled worker	11(13.8)	96.1			
	Skilled worker	32(40.0)	99.0			
	Professional	25(31.3)	103.9			
Father's Education	None	0(0.0)	.	0.655	.	0.223
	Primary	5(6.3)	95.7			
	Secondary	32(40.0)	97.6			
	Tertiary	42(52.5)	104.4			
	Other	1(1.3)	98.9			
Father's Occupation	Unemployed	1(1.3)	96.1	0.454	1.70	0.566
	Unskilled worker	2(2.5)	85.9			
	Skilled worker	36(45.0)	98.6			
	Professional	41(51.2)	118.2			

Table 6: Mean serum Mg and Zn levels based on most frequently consumed diet

		Mg		Zn	
		Mean \pm SD (mg/dl)		Mean \pm SD ($\mu\text{g/dl}$)	
Beef, pap & custard	Daily	2.14 \pm 0.19		91.5 \pm 21.80	
	Weekly	2.15 \pm 0.21		100.2 \pm 18.90	
	Monthly	2.00 \pm 0.22		95.0 \pm 17.60	
	Not at all	.		.	
Fish ,rice & egg	Daily	2.13 \pm 0.20		97.5 \pm 20.90	
	Weekly	2.14 \pm 0.21		97.2 \pm 18.90	
	Monthly	2.13 \pm 0.21		96.4 \pm 23.30	
	Not at all	.		.	
Milk & garri	Daily	2.11 \pm 0.22		95.5 \pm 20.70	
	Weekly	2.15 \pm 0.21		96.8 \pm 19.00	
	Monthly	2.17 \pm 0.16		105.0 \pm 20.7	
	Not at all	.		.	

difference in Zn and Mg levels of the children with SCD and non-SCD by age and gender. This agrees with the report of Kudirat, et al. (2019).¹⁴ It implies that Zn or Mg does not decrease or increase in SCD or non-SCD children because of their ages or gender, though it was observed that the rate of deficiency in these parameters decreased with increase in age.

Also, the mean hematocrit level was observed to be significantly lower in the children with sickle cell disease compared to children without sickle cell disease (Table 3) without any significant influence of age and gender. Haemoglobin, is an iron-containing protein in blood that transports oxygen to tissue. The difference in the mean hematocrit levels observed may be a result of chronic hemolysis, low erythropoietin response, and shortened red cell survival. Patients with SCD generally have a background rate of red cell sickling, which drastically

shortens the life span of red cells leading to a chronic haemolytic anaemia and jaundice even in a steady state.¹⁸ Our finding agrees with the report by Erhabor et al. (2019).¹⁸ SCD anaemia is one of the most common forms of SCD. From this study, almost all the SCD children were anaemic (39/40) compared to the non-SCD (10/40). This surprisingly, did not have significant ($p>0.05$) effect on serum Zn and Mg levels. This may be due to differences in redistribution of these minerals in the face of inflammatory and anaemic conditions. Also, ten out of the 40 non-SCD children were found to be anaemic. This implies that 25% of the non-SCD children were anaemic. This calls for a great need for more impactful policies on the health and nutritional wellbeing of Nigerian children to be enacted so as not to make the achievement of Sustainable Development Goal-2 (SDG-2) which aim to eradicate the global burden of malnutrition¹⁹ a mere dream.

Furthermore, serum zinc and magnesium concentrations were found to increase with a rise in parents' educational and employment status, though this was not significant. The result obtained in this study suggests that parental/guardian's socioeconomic status influences the nutritional status of a child. This is similar to the finding by Duke (2006).²⁰ Nutrition is a critical part of health and development. Better nutrition is related to improved infant, child, and maternal health, and stronger immune systems.²¹ From this study, poor growth and under nutrition were more common in children with SCD, this was assessed by the high rate of low Hb and anaemia among SCD children compared to non-SCD.¹⁴ In addition, there was a relative stability in the levels of serum Zn and Mg by the rate of consumption of the listed grouped staple foods (beef, pap, and custard; fish, rice, and egg; and milk and garri). This may suggest that the natural foods were not able to supply the needed quantity of these minerals. Thus, there may be a need for a supplementation study.

8. Conclusions

This study has shown that serum Mg was significantly lower in children with SCD. This may suggest a need to assess the level of this mineral in the treatment of SCD children. There may also be a need for a well-controlled supplementation study for a comprehensive report on the importance of supplementing these patients with Mg.

9. Ethical Approval

Ethical clearance was obtained from the Ethics Committee of JUTH, Plateau State, Nigeria, with reference number JUTH/DCS/IREC/127/XXXI/436 under JUTH Health Research Ethics Committee number NHREC/JUTH05/1022.

10. Authors' Contribution

OCA conceived, designed, supervised, involved in the statistical analysis/interpretation of result and reviewed the manuscript, DJ carried out the sample collection, laboratory analysis and drafted the manuscript, DF ran the statistical analysis and interpretation of the result; OAO participated in sample collection, supervision and review of the manuscript.

11. Source of Funding

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12. Declaration of Conflict of Interests

The authors declare that there was no conflict of interest.

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