Pediatric Hematology Oncology Journal 4 (2019) 17–22

Contents lists available at ScienceDirect

Pediatric Hematology Oncology Journal

journal homepage: https://www.elsevier.com/journals/pediatric-hematology-oncology-journal/

Plasma level of antioxidants is related to frequency of vaso-occlusive crises in children with sickle cell anaemia in steady state in Nigeria

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ARTICLE INFO

Article history:
Received 25 November 2018
Received in revised form
26 March 2019
Accepted 26 March 2019
Available online 27 March 2019

Keywords:
Antioxidants
Reactive oxygen species
Sickle cell anaemia
Vaso-occlusive crises

ABSTRACT

Introduction: Sickle cell anaemia (SCA) is associated with deficiency of plasma antioxidants. Frequency of vaso-occlusive crises (VOC) is an index of disease severity. This study determined the relationship between plasma antioxidants in children with SCA and frequency of VOC.
Method: It was a cross-sectional comparative study comprising 80 children each with SCA and HbAA. Those with SCA were grouped into three based on frequency of pain episodes. Antioxidant trace elements (zinc, copper, selenium, and manganese), vitamins A, C and E and antioxidant enzymes (superoxide dismutase and glutathione dismutase) were quantified using atomic absorption spectrophotometry, HPLC and ELISA respectively.
Results: Children with SCA had lower plasma concentrations of zinc, selenium, manganese, vitamins A, C, E, and superoxide dismutase (SOD) and glutathione peroxidase (GPx), but higher plasma concentration of copper compared to HbAA. Mean plasma concentrations of manganese, vitamin A, vitamin C, vitamin E, SOD and GPx were significantly lower in SCA children with severe disease compared to those with mild and moderate disease ($F = 10.629$, $p < 0.001$), ($F = 5.636$, $p = 0.005$), ($F = 10.438$, $p < 0.001$), ($F = 6.424$, $p = 0.003$), ($F = 7.006$, $p = 0.002$) and ($F = 4.934$, $p = 0.010$) respectively. Also, significant negative correlation existed between frequency of VOC and plasma concentration of Manganese ($r = -0.428$, $p < 0.001$), Vitamins A ($r = -0.309$, $p = 0.005$), Vitamin C ($r = -0.456$, $p < 0.001$), Vitamin E ($r = -0.354$, $p = 0.001$), superoxide dismutase ($r = -0.320$, $p = 0.004$) and glutathione peroxidase ($r = -0.333$, $p = 0.003$).
Conclusion: Deficiency of plasma antioxidants exists in SCA and plasma levels of some antioxidants have inverse relationship to frequency of VOC.

1. Introduction

Sickle cell anaemia (SCA) is a homozygous, monogenic, autosomal recessive disorder that results from point mutation in the genetic code, with substitution of glutamic acid by valine at position six of beta globin chain. This leads to the formation of sickled haemoglobin molecules (HbS) that are susceptible to precipitation when deoxygenated with change in the disc shape of erythrocytes to sickled forms [1]. Due to occlusion of the capillary beds by these rigid sickled erythrocytes, the disease is associated with chronic inflammation, ischemia-reperfusion injury and excessive production of Reactive Oxygen Species (ROS) like superoxide and hydrogen peroxide. These processes are aggravated when patients are exposed to various pathologic conditions such as hypoxemia, infection, acidosis and dehydration [2–4].

Previous reports have also shown that patients with SCA also have inefficient anti-oxidant defense system as a result of decreased production and/or increased consumption of the body's antioxidants such as trace elements (zinc, copper, manganese, colbalt, selenium), vitamins (vitamins A, C and E) and antioxidant

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Peer review under responsibility of Pediatric Hematology Oncology Chapter of Indian Academy of Pediatrics.

https://doi.org/10.1016/j.phoj.2019.03.003
2468-1245/

enzymes (superoxide dismutase, catalase and glutathione peroxidase) even when they are in steady state [5]. The low levels of these antioxidants leads to chronic oxidative stress which play a significant role in the development of microvascular dysfunction, damage to cellular macromolecules and multiple organ dysfunction [5].

Vaso-occlusive crisis is a prominent manifestation of SCA with multiple organ affectations and is a major cause of mortality in these patients [6]. These have impacted on both the patients and care givers, which is worse in developing countries such as Nigeria, due to inadequate social welfare and health care service. It is associated with frequent hospital visits, poor school attendance, affectation of patients' social and peer interaction and reduction in overall quality of life of these patients [7,8].

Although several studies have shown reduction in plasma antioxidants and trace elements levels in patients with SCA [1,9,10], research data relating the levels of antioxidants (trace elements, vitamins and enzymes) to clinical severity of the disease, most especially in children, is scanty. The main goal of this study therefore is to determine the relationship between the frequency of vaso-occlusive crises and plasma levels of antioxidant trace elements (Zinc, copper, manganese and selenium), antioxidant vitamins (vitamins A, C and E) and antioxidant enzymes (superoxide dismutase and glutathione peroxidase).

2. Materials and methodology

2.1. Study location

This study was conducted in the Departments of Chemical Pathology and Paediatrics of the Obafemi Awolowo University Teaching Hospitals Complex, Ile-Ife, Nigeria.

2.2. Study design

It was a descriptive cross-sectional comparative study involving subjects with SCA and apparently healthy controls. A total of 160 participants aged 1–15 years were recruited consecutively into the study. They comprised of 80 children with SCA (HbSS) in steady state who met the selection criteria as cases and 80 age- and sex-matched HbAA as controls. The Hb genotype of both participants (cases and controls) was diagnosed by haemoglobin electrophoresis with cellulose acetate at alkaline pH (8.6). Patients with sickle cell anaemia and febrile illness, history of blood transfusion in three months preceding recruitment into the study, those on hydroxyurea or chronic blood transfusion and those on trace element or antioxidant supplement were excluded from the study.

Steady state was defined as period without any acute illness, pain, infection of at least 4 weeks before recruitment and no transfusion in the preceding 3 months [11].

2.3. Data collection

The general characteristics including the age, gender, socioeconomic status and frequency of vaso-occlusive crises (number of pain episode in the 12 months preceding recruitment into the study) were obtained and filled in a pretested study proforma. Socioeconomic status was determined using the occupation of the father and the highest academic qualification of the mother and were classified into upper, middle and lower social classes according to Olusanya et al. [12]. Also, participants with SCA were grouped into underweight (<5), healthy weight (5- <85), overweight (85-<95) and obesity (>95) using BMI-percentile.

Children with SCA in steady state were grouped into those with mild, moderate and severe disease using the number of vaso-occlusive crises/pain episodes they had in the 12 months preceding recruitment into the study [13]. Those who had 3 or more pain episodes in a twelve months period are regarded as having severe disease while those with 1–2 pain episode(s) and no pain episode are said to have moderate and mild disease respectively [13,14].

About 5 mls of venous blood was collected from the antecubital vein and was dispensed into lithium heparin bottle. This was subsequently centrifuged within 1 h at 2000 revolutions per minute for 10 min which was validated by use of tachometer and timer calibrator. The supernatant plasma was separated in aliquots into acid washed (10% (v/v) HNO_3) plain screw cap specimen bottles to remove contaminants and subsequently stored frozen at $-20\,^{\circ}C$ freezer (with two daily regular temperature monitoring via the inbuilt thermometer in the freezer) for analysis of antioxidants in batches. Antioxidant trace element (Zn, Cu, Se, Mn) levels were measured by atomic absorption spectrophotometric (AAS) technique using Buck 210/211 AAS by Buck Scientific Inc. 58 Fort Point St, East Norwalk, USA. Antioxidant vitamins (vitamins A, C and E) levels were measured by High Performance Liquid Chromatography using 616/626 HPLC machine by Waters Incorporate, California, USA. Superoxide dismutase and glutathione peroxidase activities were measured by Enzyme Linked Immunosorbent Assay (ELISA), using Kit purchased from Cell Biolabs Inc. (Cat No: STA 340), 7758 Arjons Drive, San Diego, CA 92126, and Biovendor research and diagnostic products (Cat No: RAG012R) respectively.

2.4. Ethical consideration

The ethical clearance for this study was obtained from the Ethics and Research Committee of the Obafemi Awolowo University Teaching Hospitals Complex, Ile-Ife; reference number IRB/IEC/0004553. The participants were fully informed about the study both verbally and with the use of patient's information sheet. Written informed consent and assent were obtained from the parents/caregivers and older patients (≥7 years) respectively.

2.5. Statistical analysis of data

Data collected was analyzed using descriptive and inferential statistics contained in the Statistical Package for Social Sciences (SPSS), version 20.0 (SPSS Chicago Inc., IL, and U.S.A). Means and standard deviations were compared using the independent sample *t*-test or analysis of variance (ANOVA) as applicable. The degree of correlation of continuous data was determined by Pearson's correlation analysis. Statistical significance was established when probability, p value was less or equal to 0.05.

3. Results

3.1. General and demographic characteristics of study population

Eighty children with SCA in steady state and 80 age and gender-matched apparently healthy HbAA were recruited and studied. In all, there were 86 males (43 in each group) and 74 females (37 in each group) with an overall male: female of 1.2: 1. Also, male to female ratio of both participants and those of controls were 1.2: 1 respectively. There was no significant difference in the gender distribution between the participants and controls ($\chi^2 = 0.00$, $p = 1.00$). The age of the study population was between 1 and 15 years with a mean of 6.47 ± 3.17 years. The mean age of participants (6.46 ± 3.16years) and controls (6.48 ± 3.20years) were similar, $t = -0.03$ $p = 0.98$. Majority of the participants were between the ages of 5–9 years (52.5%) while 30% were underfives (<5years) and 17.5% were adolescents. Also, there was no significant difference between the age groups of the study participants and controls

Table 1
Mean plasma micronutrient and antioxidant enzyme levels in the two study groups.

Antioxidants	Cases Mean ± SD	Control Mean ± SD	% difference (%)	p- value
Zinc (μmol/L)	15.47 ± 2.06	16.17 ± 1.76	4.4	**0.023**
Copper (μmol/L)	22.66 ± 2.69	18.25 ± 4.36	21.6	< **0.001**
Selenium (μmol/L)	0.87 ± 0.18	1.21 ± 0.19	32.7	< **0.001**
Manganese (nmol/L)	14.07 ± 3.84	16.62 ± 5.13	16.6	**0.001**
Vitamin A (μmol/L)	1.03 ± 0.27	1.18 ± 0.32	13.6	**0.026**
Vitamin C (μmol/L)	38.36 ± 9.81	63.20 ± 16.96	48.9	< **0.001**
Vitamin E (μmol/L)	12.58 ± 3.20	16.16 ± 3.67	24.9	< **0.001**
SOD (U/μL)	0.28 ± 0.10	0.41 ± 0.10	37.7	< **0.001**
GPx (ng/mL)	3.42 ± 1.77	4.67 ± 2.39	30.9	< **0.001**

SD- Standard deviation; SOD- Superoxide dismutase, GPx- Glutathione peroxidase. Percentage (%) difference was calculated using omni calculator.

($\chi^2 = 0.13$, $p = 0.94$).

Majority, 84 (52.5%) of the cases and controls were from the middle socio-economic class. The overall socio-economic status distribution was statistically similar ($\chi^2 = 4.796$, $p = 0.093$).

3.2. Heamatological data in study participants

The mean haematocrit level in study participants with SCA was significantly lower (23.88 ± 3.45%) compared to those with HbAA (35.56 ± 3.03%), ($p < 0.001$); On the contrary, the mean total white blood cell and platelet counts were significantly higher in children with SCA ($14.89 \pm 5.74 \times 10^9$/L and $348.29 \pm 149.16 \times 10^9$/L respectively) compared to HbAA controls ($6.10 \pm 2.09 \times 10^9$/L and $256.89 \pm 93.49 \times 10^9$/L respectively); both with $p < 0.001$.

3.3. Anthropometric data and plasma antioxidant concentration

The weight of children with SCA ranged from 9.0 to 44.0 Kg with a mean weight of 20.10 ± 7.57 Kg while the weight of HbAA control ranged from 8.0 to 46.5 Kg with mean weight of 22.43 ± 8.81 Kg. Also, the minimum and maximum height in children with SCA were 0.70 M and 1.58 M respectively while the minimum and maximum height in HbAA controls were 0.71, and 1.59 M respectively. There was no significant difference between the weight and height of the two study participants ($p = 0.075$ and $p = 0.262$ respectively).

Furthermore, using BMI- percentile which assesses nutritional status in children, 21.1% of children with SCA were under weight, 60% were of healthy weight and 3.8% were overweight. None of them was obese. The distribution of the measured mean plasma antioxidants micronutrients and enzymes across the different BMI-percentiles was statistically similar; (Table 2).

3.4. Mean plasma antioxidant concentration in the two study groups

Mean plasma concentrations of zinc (15.47 ± 2.06 μmol/L), selenium (0.87 ± 0.18 μmol/L) and manganese (14.07 ± 3.84 nmol/L) in subjects with SCA were significantly lower than in controls (16.17 ± 1.76 μmol/L), (1.21 ± 0.19 μmol/L) and (16.62 ± 5.13 nmol/L) respectively; ($p \leq 0.05$). On the contrary, subjects with SCA had significantly higher mean plasma copper concentration (22.66 ± 2.69 μmol/L) than in controls (18.25 ± 4.36 μmol/L), ($p < 0.001$); (Table 1).

Also, mean plasma concentrations of vitamins A (1.03 ± 0.27 μmol/L), vitamin C (38.36 ± 9.81 μmol/L) and vitamin E (12.58 ± 3.20 μmol/L) were significantly lower in subjects with SCA than in controls (1.18 ± 0.32 μmol/L), (63.20 ± 16.96 μmol/L) and (16.16 ± 3.67 μmol/L) respectively; with $p = 0.026$, <0.001 and < 0.001 respectively; (Table 1).

Furthermore, mean plasma concentrations of SOD (0.28 ± 0.10U/μL) and GPx (3.42 ± 1.77 ng/mL) in subjects with SCA were significantly lower when compared to the controls (0.41 ± 0.10U/μL) and (4.67 ± 2.39 ng/mL) respectively, with $p < 0.001$; (Table 1).

3.5. Relationship between frequency of vaso-occlusive crises and antioxidants

The number (frequency) of painful episodes that children with SCA had in the twelve months preceding recruitment into the study ranged from 0 to 10. Twenty six (32.5%) had no episodes of pain and 54 (67.5%) had one or more episode(s) of pain. Out of these 54 participants, 32 had 1 to 2 pain episodes while 22 had ≥3 pain episodes.

In subjects with SCA, mean plasma concentrations of manganese in children with 3 or more pains episodes in the twelve months preceding recruitment into the study (11.44 ± 3.03 nmol/L) was significantly lower than in those with 1–2 pain episodes (14.28 ± 3.71 nmol/L) and no pain episodes (16.02 ± 3.43 nmol/L); ($p < 0.001$). Also, mean plasma concentration of vitamin A, C and E in children with 3 or more pains episodes in the twelve months preceding recruitment into the study (0.90 ± 0.29 μmol/L), (31.17 ± 4.71 μmol/L) and (10.68 ± 1.98 μmol/L) respectively were significantly lower when compared to the levels in those with 1–2 pain episodes (1.14 ± 0.25 μmol/L), (40.25 ± 6.96 μmol/L) and

Table 2
Mean plasma micronutrient and antioxidant enzyme levels in BMI- percentile groups.

Antioxidants	Underweight Mean ± SD	Healthy weight Mean ± SD	Overweight Mean ± SD	ANOVA (F– test)	p- value
Zinc (μmol/L)	15.34 ± 2.15	15.59 ± 1.98	13.95 ± 3.18	0.948	0.392
Copper (μmol/L)	23.19 ± 2.82	22.62 ± 2.67	20.41 ± 1.23	1.404	0.252
Selenium (μmol/L)	0.89 ± 0.17	0.87 ± 0.18	0.80 ± 0.14	0.367	0.694
Manganese (nmol/L)	14.03 ± 3.89	14.11 ± 3.91	13.37 ± 3.15	0.053	0.948
Vitamin A (μmol/L)	1.01 ± 0.26	1.04 ± 0.28	1.03 ± 0.27	0.104	0.901
Vitamin C (μmol/L)	34.85 ± 5.58	39.07 ± 10.57	44.14 ± 9.23	1.805	0.171
Vitamin E (μmol/L)	12.39 ± 2.97	12.69 ± 3.34	11.90 ± 1.43	0.130	0.878
SOD (U/μL)	0.26 ± 0.07	0.29 ± 0.10	0.28 ± 0.95	0.643	0.529
GPx (ng/mL)	3.01 ± 1.65	3.54 ± 1.76	4.86 ± 3.22	1.517	0.226

SD- Standard deviation, SOD- Superoxide dismutase, GPx- Glutathione peroxidase.

Table 3
The mean of plasma antioxidants in the participants groups of pain episodes.

Antioxidants	No pain episodes Mean ± SD	1 to 2 pain episodes Mean ± SD	3 or more pain episodes Mean ± SD	ANOVA (F– test)	p value
Zinc (μmol/L)	15.32 ± 2.01	15.72 ± 1.84	15.30 ± 2.45	0.369	0.693
Copper (μmol/L)	22.40 ± 2.35	22.74 ± 2.83	22.84 ± 2.94	0.174	0.840
Selenium (μmol/L)	0.90 ± 0.16	0.88 ± 0.20	0.84 ± 0.17	0.731	0.485
Manganese (μmol/L)	16.02 ± 3.43	14.28 ± 3.71	11.44 ± 3.03	10.629	**< 0.001**
Vitamin A (μmol/L)	1.02 ± 0.22	1.14 ± 0.25	0.90 ± 0.29	5.636	**0.005**
Vitamin C (μmol/L)	42.13 ± 12.67	40.25 ± 6.96	31.17 ± 4.71	10.438	**< 0.001**
Vitamin E (μmol/L)	13.62 ± 2.93	13.08 ± 3.57	10.68 ± 1.98	6.424	**0.003**
SOD (U/μL)	0.31 ± 0.11	0.30 ± 0.09	0.22 ± 0.06	7.006	**0.002**
GPx (ng/mL)	4.08 ± 1.82	3.63 ± 1.95	2.55 ± 1.13	4.934	**0.010**

BOLD values are statistically significant. SD- Standard deviation, SOD- Superoxide dismutase, GPx- Glutathione peroxidase.

Table 4
Correlation between the number(s) of vaso-occlusive crises in the twelve months preceding the study and plasma levels of antioxidants.

Variables	r- value	p- value
Zinc (μmol/L)	0.090	0.429
Copper (μmol/L)	0.122	0.282
Selenium (μmol/L)	−0.172	0.128
Manganese (μmol/L)	−0.428	**< 0.001**
Vitamin A (μmol/L)	−0.309	**0.005**
Vitamin C (μmol/L)	−0.456	**< 0.001**
Vitamin E (μmol/L)	−0.354	**0.001**
SOD (U/μL)	−0.320	**0.004**
GPx (ng/mL)	−0.333	**0.003**

Pearson's correlation coefficient (r); SOD- Superoxide dismutase, GPx- Glutathione peroxidase.

(13.08 ± 3.57 μmol/L) respectively and in those with no pain episode (1.02 ± 0.22 μmol/L), (42.13 ± 12.67 μmol/L) and (13.62 ± 2.93 μmol/L) respectively; ($p = 0.005$, $p < 0.001$ and $p = 0.003$ respectively). In addition, mean plasma concentration of SOD and GPx in children with 3 or more pains episodes in the twelve months preceding recruitment into the study (0.22 ± 0.06 U/μL) and (2.55 ± 1.13 ng/mL) respectively were significantly lower when compared to the levels in those with 1–2 pain episode(s) (0.30 ± 0.09 U/μL) and (3.63 ± 1.95 ng/mL) respectively and those with no pain episode (0.31 ± 0.11 U/μL) and (4.08 ± 1.82 ng/mL) respectively; ($p = 0.002$ and $p = 0.010$ respectively). On the contrary, there was no significant difference in the plasma concentration of Zn, Cu and Se in the children with 3 or more pains episodes in the twelve months preceding recruitment into the study (15.30 ± 2.45 μmol/L), (22.84 ± 2.94 μmol/L) and (0.84 ± 0.17 μmol/L) respectively compared to those with 1–2 pain episode(s) (15.72 ± 1.84 μmol/L), (22.74 ± 2.83 μmol/L) and (0.88 ± 0.20 μmol/L) respectively and no pain episode (15.32 ± 2.01 μmol/L), (22.40 ± 2.35 μmol/L) and (0.90 ± 0.16 μmol/L) respectively; ($p = 0.693$, $p = 0.840$ and $p = 0.485$ respectively); (Table 3).

Using Pearson's correlation coefficient, there was moderate significant negative correlation between the number of vaso-occlusive crises 12 months prior to the study and plasma concentration of manganese ($r = -0.428$, $p < 0.001$), vitamin C ($r = -0.456$, $p < 0.001$) and vitamin E ($r = -0.354$, $p = 0.001$); and mild significant negative correlation with plasma concentration of vitamins A ($r = -0.309$, $p = 0.005$), superoxide dismutase ($r = -0.320$, $p = 0.004$) and glutathione peroxidase ($r = -0.333$, $p = 0.003$). In contrast, there was no significant correlation between the number (frequency) of vaso-occlusive crises and plasma concentration of zinc ($r = 0.090$, $p = 0.429$), copper ($r = 0.122$, $p = 0.282$) and plasma selenium ($r = -0.172$, $p = 0.128$); (Table 4), (See Figs. 1 and 2).

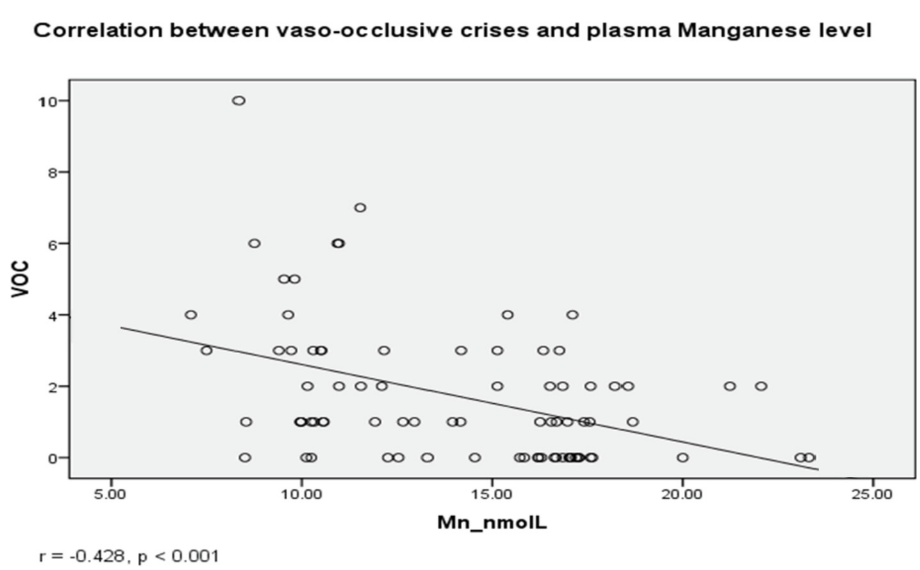

Fig. 1. Scatter plot diagram showing negative correlation between vaso-occlusive crises and plasma Manganese level.

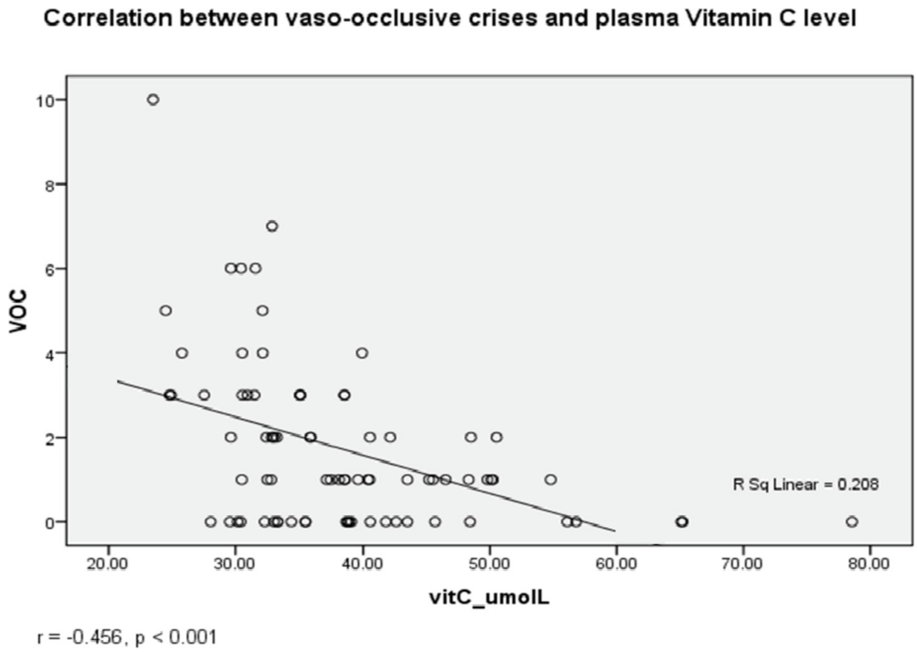

Fig. 2. Scatter plot diagram showing negative correlation between vaso-occlusive crises and plasma Vitamin C level.

4. Discussion

SCA is a global disease of public health importance. The antioxidant status of these patients has been shown to be one of the factors that contribute to the pathophysiologic mechanism of the disease [1].

Previous studies reported a reciprocal relationship between zinc and copper in patients with SCA, which is similar to what was observed in this study [15–18]. On the contrary there was no significant different in a study done in Congo and Saudi Arabia between patients with SCA and HbAA controls [18,19]. It has been suggested that both zinc and copper compete with the same binding site on proteins and increase in plasma concentration of copper is associated with zinc deficiency in plasma and tissues [20]. Zinc deficiency in SCA is due to increased urinary zinc excretion, and/or Zinc malabsorption from the small intestine [21]. Copper is also incorporation in superoxide dismutase, hence its antioxidant effect [22].

This finding about manganese, selenium, vitamins A, C and E, SOD and GPx is similar to reports in previous studies [23–27]. The reduced level of manganese and antioxidant vitamins in sickle cell anaemia individuals have been attributed to increased utilization and/or consumption during oxidative stress [23,28]. Selenium plays significant role in the prevention of oxidative modification of lipids and reduction of inflammation [24]. Its deficiency is related to mortality in patients with SCA [10,17]. Reduced SOD levels in patients with SCA may result from its degradation by oxidants during sickling of erythrocytes. Erythrocytes become exposed to high level of hydrogen peroxide which reacts with haemoglobin to form Heinz bodies and insoluble hemichromes. This results in more ROS formation and the viscous cycle continues [29].

Frequency of VOC is an important index of disease severity in patients with SCA [4]. Although previous studies have reported significantly lower levels of antioxidants in patients with SCA in VOC compared to those in steady state [10,15,30], and a study in Ibadan, Nigeria determined the relationship of total antioxidant status (TAS) to frequency of VOC [31], studies relating the levels of individual antioxidants to severity of disease using frequency of VOC are scarce. In this study, it was observed that children with SCA with severe disease (3 or more pain episodes in the 12 months preceding recruitment into the study) had lower plasma levels of Mn, vitamins A, vitamin C, vitamin E, SOD and GPx compared to those with no pain episodes and few pain episodes (mild/moderate disease). Also, the plasma levels of these antioxidants correlated negatively with frequency of VOC.

Workers from Southwestern Nigeria reported that patients with SCA who presented in the hospital with VOC had higher manganese levels than in SCA in steady state and HbAA controls [32]. However, to the best of the author's knowledge, there are no previous reports on the relationship of manganese to frequency of VOC. This is a novel finding.

Manganese is a vital element in nutrition and is found in biological systems in 2^+ and 3^+ valency states. It is essential for growth, development and metabolism; serving as a cofactor for several classes of enzymes in the body such as oxidoreductase, transferase, hydrolases, lyases. Its incorporation in superoxide dismutase enzymes makes it an important component of human antioxidant system [33]. Also, it is vital for the formation of connective and bony tissues. Patients with manganese deficiency have characteristic phenotypic expression such as impaired growth, skeletal abnormalities and impaired wound healing which are also exhibited in patients with sickle cell anaemia [34]. Cellular effects of Mn deficiency also include decreased bone resorption, decreased synthesis of organic matrix and predisposition to osteoporosis [35]. Although the finding in this study suggests that levels of manganese may influence the rate at which patients present with pain crises, the mechanism at which this occur is however a subject for further research.

In addition, antioxidant vitamins inhibit haemoglobin polymerization, scavenge free radicals, improve vascular endothelial vasodilation and reduce RBC dehydration [36,37]. These mechanisms underlie development of VOC. Also, Glutathione, an important intracellular antioxidant, works in synergy with SOD to reduce the concentration of free radicals in the blood [38]. Reduced GPx levels is associated with less intracellular scavenging activities on the continuously generated ROS thus creating wide spread oxidative stress with its attendant generalized molecular and cellular damage [38,39]. These suggest that patients with low levels of vitamins, SOD and GPx may be predisposed to frequent vaso-occlusive crises.

Our study has some limitations. First, none of our patients was on hydroxyurea, which is a globally acclaimed disease-modifying drug recommended to prevent rates of VOC, anaemia, acute and chronic SCD complications including end organ damage. A comparative study involving the use of antioxidants with or without hydroxyurea would have improved the message in this study. Secondly, the cross-sectional design did not allow for inference about causality to be conclusively made.

In conclusion, the severity and frequency of VOC have been linked to both genetic (β-globin gene haplotype, presence or absence of blood group O and levels of HbF) and several non-genetic factors [13,40]. The findings in this study suggest that deficiency of some antioxidant (such as Mn, vitamins A, C, and E, SOD and GPx) is one of the non-genetic factors associated with VOC in children with SCA; it is possible that those with more severe disease have an increased requirement as a result of oxidative stress or they have higher losses of micronutrients which may contribute to disease severity. Regular assessment and monitoring of levels of these antioxidants in children with SCA to predict patients that are likely to have increased occurrence of VOC is also recommended.

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