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Nitric oxide and zinc levels in sickle cell hemoglobinopathies: a relationship with the markers of disease severity

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ABSTRACT

Introduction: Sickle cell disease is a genetically inherited disease affecting millions of people worldwide. Patients with a severe form of the disease present with more complications and end-organ dysfunction.

This study is aimed at determining a relationship between 2 biochemical parameters associated with SCD (i.e. NO and Zn) and disease severity markers such as hematocrit, PF and VOC, with the objective of using NO and/or Zn to better understand the gravity of altered processes of SCD.

Materials and methods: Ninety-eight adult participants of both sexes were recruited for the study and grouped accordingly as HbAA (control), HbSC and HbSS. Questionnaires provided information on PF, frequency of hospitalization, and clinic attendance. Hematocrit, serum NO and Zn were determined using standard techniques. Data were summarized and analyzed using one-way analysis of variance and regression analysis; $p \leq 0.05$ was considered significant.

Results: The rates of hospitalization, PF and clinic attendance were higher among HbSS than HbSC. HbAA had significantly higher levels of hematocrit, Zn and NO than HbSS and HbSC. Zinc and hematocrit for HbSS were significantly lower than HbSC but NO was not significantly different. Hematocrit, NO and Zn of patients in steady, VOC and post-VOC states of HbSC and HbSS were significantly different. A significant association was observed between biochemical parameters (NO, Zn) and hematocrit, VOC but not PF.

Conclusion: The study suggests that Zn and NO are lower in SCD compared with control and can be affected by the severity of the disease. Therefore, periodic testing of NO and Zn may be beneficial in identifying patients with a higher risk of developing end organ damage.

Keywords: nitric oxide; zinc; hematocrit; vaso-occlusive crisis; pain frequency; hospitalization rate; sickle cell disease.

INTRODUCTION

The underlying abnormality of homozygous sickle cell disease (HbSS) is a point mutation of GAG to GTG in the 6 codon of the beta globin gene (Hbβ). This anomaly results in the replacement of the amino acid glutamic acid for a valine, with the eventual formation of hemoglobin S (HbS) instead of hemoglobin A (HbA) [1]. Hemoglobin S forms long polymers when the oxygen tension is low, which occurs due to the hydrophobic interaction of valine and phenylalanine [2]. The affected valine and phenylalanine are in positions 85 and 88 in the globin chain. This interaction/polymerization causes the erythrocytes of HbSS patients to be less flexible than the normal red blood cells (RBCs). Furthermore, polymerization has been linked with biochemical alterations that impair blood flow as well as vaso-occlusion (VO) [3].

The heterozygous sickle cell disease (HbSC) occurs when HbS is inherited along with hemoglobin C (HbC). While the molecular bases of both types of sickle cell disease (SCD) – HbSS, HbSC – are similar but the point mutation of HbC is GAG to AAG, this transition point mutation leads to the substitution of glutamic acid with lysine in the globin chain [1]. According to Nagel et al., this variant of hemoglobin (Hb), i.e. HbC tends to form an amorphous aggregate within the RBC. Furthermore, they opined that K–Cl cotransporter transformation in HbSC disease propels toward RBC dehydration, which in turn elevates the intracellular Hb concentration, making it denser than HbAA-containing RBC [4].

Clinical manifestations of SCD include acute episodes of pain (i.e. frequent pain), pulmonary hypertension, stroke, leg ulcer, and acute chest syndrome (ACS) as well as priapism in males [1, 3]. Stroke as well as leg ulcers is associated with the chronic hemolytic feature of SCD, whereas acute pain crises and ACS have been linked to VO. This dichotomization is not restricted, as they sometimes overlap and therefore may not be useful criteria for the management of SCA or HbSC [5, 6]. The HbSC patients usually present clinical events less frequently when compared with patients with SCA, which is considered a severe form of SCD [7]. Aside from clinical manifestations, clinical chemistry laboratory parameters (such as zinc – Zn, nitric oxide – NO), as well as hematological marker (hematocrit), have been considered as important biomarkers [8, 9]. Recently, there have been calls for their use for the patients' follow-up due to their involvement in many clinical events of SCD such as anemia, hemolysis, leukocytosis and endothelial dysfunction [10, 11]. Essentially, it is hoped that they can be employed to envisage severe clinical manifestations of the disease [12].

Hemoglobin S polymerization also causes alteration in RBC membrane [13], an important step that has been associated with the irreversibility of the sickling shape of HbS-containing

erythrocytes. Irreversibly sickled RBC are more adherent and can therefore bind with platelets, vascular endothelial cells and leukocytes. This aggregation leads to physical obstruction of the capillaries, thereby compounding the problem of VO, which is a hallmark of SCD [14]. Vaso-occlusion is further intensified due to unrelenting intravascular hemolysis releasing free heme, Hb and arginase which decrease NO bioavailability, and is directly accountable for endothelial dysfunction [15] and ultimately results in an increased rate of hospitalization [16].

Sickle cell disease presents with a wide range of clinical features and is mostly found in developing countries where medical resources are limited and the people are mostly poor [17]. As a result of a number of factors, ranging from infrastructural deficits to a dearth of adequately trained manpower, the mortality from SCD patients remains high in many other parts of sub-Saharan Africa [18, 19, 20]. Therefore every effort to identify markers that can be used to better understand the severe presentation of the disease seems imperative for better management. This is especially significant in Africa which has the highest burden of SCD worldwide and is still encumbered with the poor utilization of standard-of-care practices for SCD patients [21]. Therefore, this study was designed to assess the levels of serum NO and serum Zn in SCD patients in a painful state (vaso-occlusive crisis – VOC) and post-VOC, compared with those in a steady state and a control group (non-SCD participants), to determe whether the biochemical parameters (NO and Zn) are related to disease severity in both HbSC and HbSS patients. Sickle cell disease has been linked consistently with increased pain frequency (PF), yet there is a dearth of data about any associations between PF and blood biochemistry. Therefore, this study investigates a possible relationship between PF and biochemical parameters – Zn and NO.

MATERIALS AND METHODS

Study design

This study was a comparative cross-sectional study.

Ethical approval/consideration

Ethical approval for the study was received from the Osun state Ministry of Health, Osogbo, Osun state (OSHREC/PRS/569T/164). Confidentiality of information provided by participants was maintained, written informed consent was willingly given by all participants.

Study site and population

Consented participants were recruited from the Haematology and Out-Patient Clinics of the Obafemi Awolowo University Teaching Hospital Complex (OAUTHC) Ile-Ife, Wesley Guild Hospital Ilesha, Osun State University Teaching Hospital Osogbo (UTH) and some other hospitals under the Osun State Hospitals Management Board (OSHMB) in Osun state, Nigeria.

Sampling technique and sample size

Multi-stage random sampling technique was used and the sample size was calculated using the Cochrane formula:

$n = z^2pq / d^2$

Where: n – the minimum required sample size in a population was >10,000; z – the standard normal deviation (1.96); p – the proportion in the target population or prevalence; q – the proportion of failure (1-p); d – the degree of accuracy desired (precision), tolerable margin of error, expected difference

z = 95% (1.96), p = 2% (0.02)

q = 1 – 0.02 = 0.98, d = 5% (0.05)

hence, $n = (1.96)^2 \times 0.02 \times 0.98 / (0.05)^2$

min. sample size = 30 [22].

Participant selection

A total of 98 participants were recruited for the study consisting of 34 HbSS, 32 HbSC and 32 with normal HbAA who served as a control group. All participants were adults, i.e. aged 18 or above. Adequate history, detailed medical examination and laboratory investigations were carried out on these participants.

Questio nnaire

Pre-tested questionnaires were administered to all participants for the collection of information such as the use of any medication, signs and symptoms of diseases, etc. A scale was used to measure the frequency of hospitalization. The patients were stratified into the following categories: never hospitalized, hospitalized 4–6 years ago, hospitalized 1–3 years ago and hospitalized less than 1 year ago. The severity of disease of the homozygous and heterozygous SCD groups was assessed by the presence of a painful episode (VOC), PF, rate of hospitalization and hematocrit values. To measure PF, a time scale was used to stratify the patients. The scale was used to divide the patients into 6 pain categories, in which each participant was asked whether he experienced pain at least once every day, every week, every month, twice a year, every year or never (absence of pain). In this study, SCD patient in painful crisis was defined as a pain episode in an individual at the time of recruitment or within 48 h before recruitment in any of the limbs, while steady state was defined as subject who was apparently well, i.e. the absence of any form of crises or any symptom of ill health, recent infection, bone pain, or other problems and no history of blood transfusion in the preceding 3 months prior to recruitment [23, 24]. Information used for severity scoring in homozygous HbSS and heterozygous HbSC study participants (and for study exclusion) was obtained by means of questionnaires.

Exclusion and inclusion criteria

Sickle cell disease patients and controls placed on Zn containing drugs and patients transfused with whole blood or blood products within the preceding 3 months before the study, pregnant women and the presence of renal disease were excluded from the study. Also excluded was any individual using Zn supplementation.

The inclusion criterion was the absence of fever (body temperature >37°C). Fever is associated with acute respiratory infection, malaria, diarrhea, or other clinical conditions known to affect plasma Zn. Controls showed no evidence of

any chronic disease associated with altered Zn metabolism. The Hb genotype of all the subjects was confirmed by cellulose acetate electrophoresis of Hb.

Specimen collection, processing and storage

As suggested by King, control as well as SCD patients were instructed to come for blood collection at least 4 h after their last meal, to forestall the use of a fasting blood sample [25]. During fasting or starvation, muscle tissues are catabolized which results in Zn release which may cumulate to transient and seemingly paradoxical elevations in serum Zn.

Six mL of blood sample was collected from each participant aseptically using the venepuncture technique; 3 mL of the blood was dispensed into a bottle containing dipotassium ethylenediamine tetra-acetic acid anticoagulant, which was subsequently used for hematocrit estimation and Hb electrophoresis. The remaining 3 mL of the blood sample was dispensed into a sterile plain bottle, allowed to clot, and then centrifuged (Bucket centrifuge: Uniscope laboratory centrifuge, Model SM112, Surgifriend Medicals, East Street Okehampton, England) at 2000 × g for 5 min in order to obtain clear non-hemolysis serum. The separated serum samples were transferred into sterile labeled plain bottles and stored frozen at –20°C prior to the time of analyses. To avert possible Zn contamination, materials had been washed clean using 10% nitric acid and thoroughly rinsed with deionized water.

Laboratory analyses of nitric oxide, serum zinc and hematocrit

Serum NO level was estimated by Griess Reaction (ENZO Blood Sciences, UK), using the principle of Griess [26] as described by Sun et al. [27]. The Mapada UV-160 spectrophotometer (Shanghai, China) was used for nitric acid analysis. Serum Zn level was estimated using atomic absorption spectrophotometry – Analyst 400, Perkin Elmer, Singapore [28]. Hematocrit is the proportion of volume occupied by the red cells to the volume of the whole blood. This was determined by the use of microhaematocrit method (Haematospin 1400, Hawksley & Sons, Sussex, England).

Statistical analyses

Statistical analysis was performed on data generated using the Statistical Package for Social Sciences version 20.0 (IBM Corporation, Armonk, NY, USA). Frequency distributions were generated for all categorical variables. The measures of location were determined for quantitative variables such as hematocrit, Zn and NO level. The data were summarized as mean ± standard deviation. Statistical significance between 2 means was assessed using the Student t-test (independent t-test), while one-way analysis of variance was employed to compare multiple (more than 2) means. After ANOVA comparison, where there was a significant difference between means, *post hoc* test (the Dunn's Multiple Comparison Test) was applied to determine where the level of significance occurred. Linear regression was used to determine the association between variables. Differences between values were considered statistically significant where the probability was less than 0.05 ($p < 0.05$).

RESULTS

Some of the results of the study are presented below in Figures 1, 2, 3, 4. Some other results can be found in Tables 1 and 2. Figures 1 and 2 revealed that the rate of hospitalization of participants (3 years prior to the commencement of the study) was higher among HbSS (82%) than for HbSC (44%). Only 3% of HbSS and 31% of HbSC patients had never been hospitalized. Figure 3 featured the PF of SCD patients; 97% of HbSS patients experienced an SCD-related pain episode at least once every month while the figure for the same period among HbSC patients was 53%. Meanwhile, Figure 4 showed the rate of clinic attendance of SCD patients; HbSS patients (95%) had a higher rate of clinic attendance than HbSC (81%). In Table 1, it was revealed that compared with control HbAA, HbSS and HbSC had significantly lower levels of hematocrit ($p < 0.001$), serum Zn ($p < 0.001$) and NO ($p < 0.001$) using ANOVA. The multiple comparison (*post hoc*) test showed no significant difference in the NO levels of HbSS and HbSC ($p = 1.000$); although for Zn and hematocrit the levels of both parameters were significantly lower for HbSS than HbSC at $p = 0.012$ and $p < 0.001$, respectively.

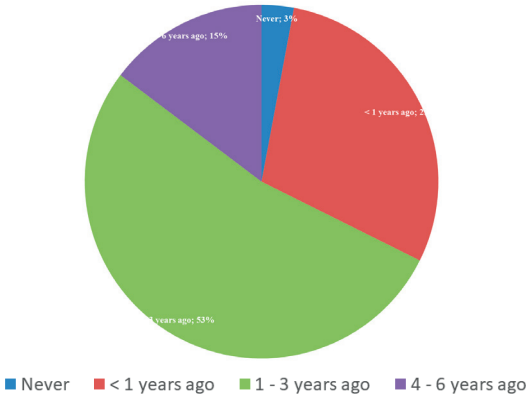


FIGURE 1. Frequency distribution of sickle cell disease-related hospitalization among homozygous sickle cell disease participants

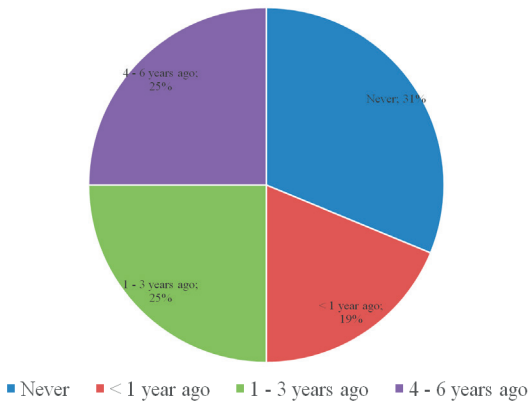


FIGURE 2. Sickle cell disease-related hospitalization among homozygous sickle cell disease participants

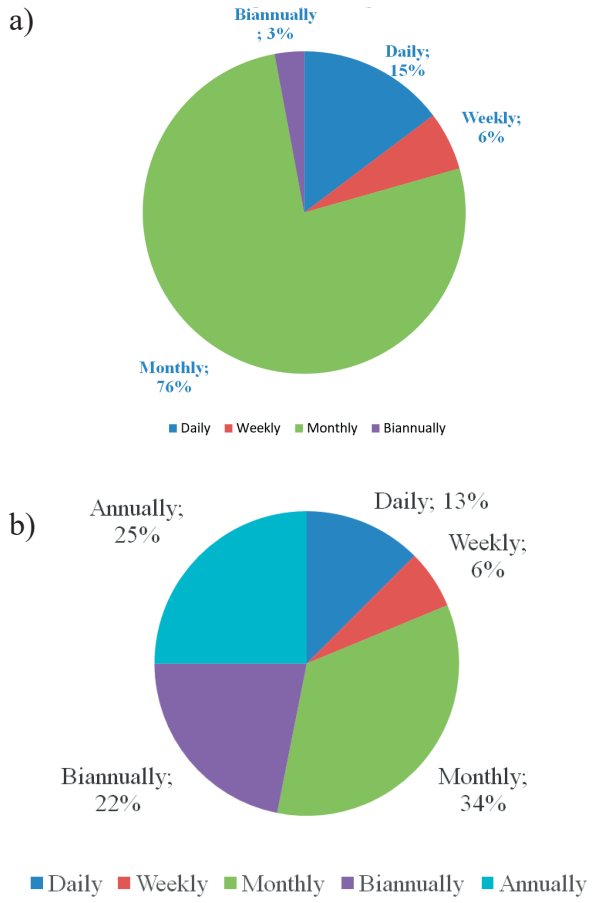
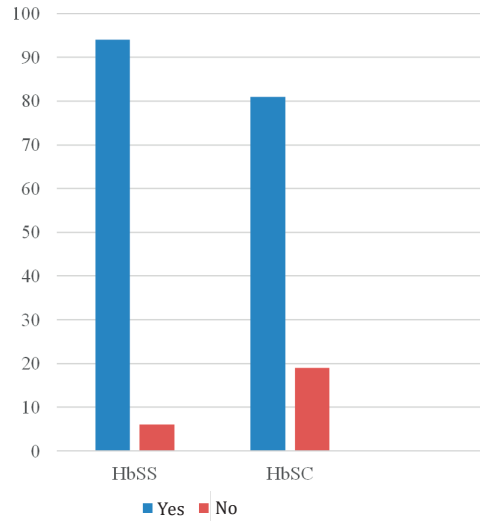


FIGURE 3. Frequency of pain among: a) HbSS participants; b) HbSC participants



HbSS – homozygous sickle cell disease; HbSC – heterozygous sickle cell disease

FIGURE 4. Clinic attendance among participants with sickle cell disease

TABLE 2. Hematocrit, nitric oxide (NO) and serum zinc (Zn) concentration of control group and sickle cell patients

Laboratory parameters		Mean ±SD	f-value	p
Hematocrit (%)	HbAA HbSS HbSC	39.66 ±4.92 24.06 ±2.91 29.10 ±3.79	133.27	<0.001*
NO (µmol/L)	HbAA HbSS HbSC	52.65 ±9.83 43.93 ±10.27 43.75 ±9.82	8.34	<0.001*
Zn (µg/L)	HbAA HbSS HbSC	85.37 ±12.91 55.62 ±11.45 64.93 ±13.42	47.80	<0.001*

HbAA – control (n = 32); HbSS – homozygous sickle cell disease (n = 34); HbSC – heterozygous sickle cell disease (n = 32)
* p < 0.05 is significant

Hematocrit, Zn, and NO values of steady, VOC and post-VOC stages of HbSC were significantly different ($p < 0.05$), as shown in Table 2 below using ANOVA. The use of the multiple comparison test revealed that the hematocrit of patients in steady state HbSC were significantly higher than those in VOC ($p = 0.001$) and post-VOC groups ($p = 0.001$), although VOC and post-VOC values were not significantly different ($p = 0.686$). On the other hand, NO values of patients in steady state of HbSC were higher than post-VOC ($p < 0.001$) but not different from VOC values ($p = 0.358$), even though NO levels of VOC and post-VOC stages of HbSC were not significantly different ($p = 0.125$). Meanwhile, the Zn status of patients in steady state HbSC was significantly higher than for VOC ($p < 0.001$) and post-VOC ($p < 0.001$), although VOC and post-VOC Zn levels were not significantly different ($p = 0.325$).

In Table 2 it can be observed that statistical analysis of data using ANOVA revealed that hematocrit, Zn, and NO values of steady, VOC and post-VOC stages of HbSS were significantly

TABLE 1. Hematocrit, nitric oxide (NO) and serum zinc (Zn) concentration of adult SCD (HbSS and HbSC) patients at steady, VOC and post-VOC stage

Laboratory parameters	HbSS (n = 34)			HbSC (n = 32)		
	mean ±SD	f-value	p	mean ±SD	f-value	p
Hematocrit (%) steady **VOC** **Post-VOC**	25.32 ±2.15 20.00 ±2.19 23.50 ±2.17	24.998	<0.001	30.32 ±3.05 23.75 ±2.06 24.50 ±0.71	25.323	<0.001*
NO (µmol/L) steady **VOC** **Post-VOC**	49.12 ±6.64 31.82 ±7.24 36.98 ±10.27	22.257	<0.001	45.64 ±8.94 38.95 ±11.46 29.70 ±1.98	8.697	0.001*
Zn (µg/L) steady **VOC** **Post-VOC**	61.66 ±8.53 44.25 ±4.06 44.87 ±9.43	29.831	<0.001	68.34 ±12.24 48.93 ±9.44 54.35 ±0.50	11.857	<0.001*

SCD – sickle cell disease; HbSS – homozygous sickle cell disease; HbSC – heterozygous sickle cell disease; VOC – vaso-occlusive crises; post-VOC – post-vaso-occlusive crises
* p < 0.05 is significant

different ($p < 0.05$). Subsequent *post hoc* analysis of hematocrit revealed that steady-state levels were higher than VOC ($p < 0.001$) and post-VOC ($p = 0.028$) levels. Additionally, post-VOC values were higher than VOC values ($p < 0.001$). On the other hand, NO values in steady state were higher than in VOC ($p < 0.001$) and post-VOC ($p < 0.001$) states, even though NO levels for VOC and post-VOC states were not different ($p = 0.267$). Meanwhile, serum Zn level at steady state was significantly higher than VOC ($p < 0.001$) and post-VOC ($p < 0.001$) states, although post-VOC and VOC levels of Zn were not significantly different ($p = 0.992$).

Regression analysis of data revealed that between hematocrit and NO ($p = 0.001$) or Zn ($p = 0.003$) the relationship was statistically significant for HbSS. Similarly for HbSC, a significant association was observed between hematocrit and NO ($p = 0.022$) or Zn ($p = 0.001$). The significant relationship (i.e. p-values) between VOC and NO or Zn for both SCDs was established as $p = 0.001$ and $p = 0.001$ (HbSC) and $p = 0.001$ and $p = 0.001$ (HbSS), respectively. Meanwhile, PF was not associated with Zn or NO in HbSS ($p = 0.071$; 0.423) and in HbSC ($p = 0.220$; 0.400), respectively, but an association was observed between PF and hematocrit among the HbSS patients ($p = 0.033$) but not HbSC ($p = 0.901$) patients.

DISCUSSION

The results of the study revealed that SCD lowered the values of hematocrit and Zn in SCD patients compared with control. The result of the study is in agreement with those of Garba et al. [29] and Okocha et al. [30] who observed low Zn levels among SCD patients.

Zinc is significant for SCD at different levels. It plays an important role in wound healing, immune system, reproduction, growth, etc.; meanwhile many of the clinical events of SCD such as leg ulcer, frequent infection, and delayed growth have been linked to abnormal Zn levelsas well [31]. Furthermore, Zn inhibits lipid peroxidation of cells as well as Ca binding to the membranes of RBC (an important step before the formation of irreversibly sickled RBCs). Both processes (lipid peroxidation, irreversible sickle shape), if allowed to progress due to low serum Zn, can result in low RBC counts. Also important is the protective property of Zn toward RBC by stabilizing its biomembranes.

Earlier reports suggested that Zn supplementations lower the incidence of VOC, reduce the frequency of infection, promote wound healing, as well as enhance growth, indicating a definite role for Zn in pathophysiology of many SCD complications [32]. This may account for the correlation between Zn and disease severity when VOC was used as a marker. Additionally, it may explain why VOC group had a significantly low level of Zn compared with the steady state. And therefore may be an indication that altered Zn level plays a somewhat direct role in the pathogenesis of VOC of SCD. Ugwu et al. also reported that SCD resulted in low Zn and hematocrit levels. That the effect of SCD on Zn is not necessarily limited to the time of crises was evident since patients in a steady state had low Zn compared with HbAA, which is in agreement with the report by Ugwu et al. [11].

According to Ugwu et al., there seemed to be an association between low hematocrit and low Zn level in SCD, an observation in line with the results of the present study [11]. This is not surprising, lipid peroxidation and irreversibly sickled erythrocytes that have been linked with low Zn levels, frequently affect RBCs survival rates and may be the basis of low hematocrit among SCD patients and the association between serum Zn and hematocrit levels. Low hematocrit levels observed in both HbSS and HbSC agree with past findings such as the report by Oluwagbenga et al. [33]. Low hematocrit can be linked with the short life span of RBCs and intravascular hemolysis. A positive association between Zn and hematocrit may not be due to chronic hemolysis only but may be ascribed to its consequent loss with urine. Additionally, it is widely recognized in literature that the importance of Zn in the hemolytic process is derived from its ability to hinder the action of calmodulin which stimulates the Ca-ATPase that regulates the Ca pump system of erythrocytes. An influx of Ca into RBCs during the sickling process as well as the overactivation of calmodulin leads to membrane derangement, increased hemolysis and low hematocrit values.

While our results suggest that SCD adversely affected NO, shown in significantly low levels of NO in SCD patients compared to control, there was no significant difference in NO value between HbSS and HbSC, i.e. between severe and milder forms of the disease. This means that NO may be a more sensitive marker of hemoglobinopathies. Although HbSS is a more severe form of the disease, the NO levels in both forms (HbSS, HbSC) were not significantly different. This indicates that irrespective of the type of hemoglobinopathy, NO is profoundly affected. Aside from being a probably more sensitive marker of hemoglobinopathy, the role of NO in the pathophysiology of SCD has resulted in calls from various quarters that therapies focused on reducing the destruction of NO, increasing its production, or amplifying NO response may have a more profound impact on SCD management [34]. It is worth remembering that Hb generated from intravascular hemolysis rapidly consumes NO, leading to a whole cascade of events that inhibit blood flow. Low NO levels in SCA have been reported also by Sant'Ana et al. [35]. The significantly lower levels of hematocrit and Zn in HbSS compared with HbSC seem to provide biochemical evidence for why HbSS rather than HbSC is the severe form of SCD.

The VOC state is a condition where an SCD patient has intermittent painful episodes due to acute vascular obstruction. As many as 5 in 10 patients with SCD have endothelial dysfunction due to impaired bioavailability of endogenous NO, partially due to scavenging of NO by cell-free plasma Hb [34]. The half-life of NO that is in seconds does not explain why the NO of patients in steady state HbSC was not different from VOC values, even though NO is continuously generated in endothelial cells from L-arginine by the enzyme NO synthase. Nitric oxide produced from endothelium activates soluble guanylyl cyclase in smooth

muscle after binding to its heme group, leading to enhanced intracellular cyclic GMP. Thus generated cyclic GMP activates cGMP-dependent kinases that reduce intracellular calcium levels in smooth muscle, leading to relaxation, vasodilation, and enhanced regional blood flow [36].

This is aside from the fact that NO plays a role in some other cellular events that promote blood flow, mainly through the suppression of platelet aggregation, expression of cell adhesion molecules on endothelial cells, and secretion of procoagulant proteins [34], a role that may be impaired because of reduced NO bioavailability, which provides a possible explanation for an association between NO and disease severity observed in the present study. More importantly, it has been reported that SCD patients are affected by reduced NO reserves. Blood plasma concentrations of NO precursor, the L-arginine, are decreased also in SCD, especially during VOC in which their levels are inversely related to pain symptoms. It was reported that dysfunctional vascular endothelium may contribute to clinical events suffered by SCD patients. Low serum concentrations of NO of SCD have been attributed to decreased plasma L-arginine, consumption of NO by cell-free Hb, and reactive oxygen species.

The higher rate of hospitalization among HbSS patients compared with HbSC is supported by earlier papers. For example Abd Elmoneim et al. showed that SCD complications are the determinant factors of hospitalization rates. According to them, causes of hospitalization vary significantly and that infection is an important factor especially in SCD children in Saudi Arabia and the developing world. A breakdown of the results of the study carried out among hospitalized SCD patients in Western Province of Saudi Arabia revealed 49% of the children presented with acute pain crisis; acute chest crisis was evident in 20.9%; 17.5% were admitted because of infection, while anemia accounted for 8.1%. Many of these clinical manifestations that were recognized as causes of hospitalization in Saudi Arabia were equally identified among HbSS patients recruited for the present study. The rates of PF and hospitalization were in agreement especially among HbSS patients, which buttresses the submission of Abd Elmoneim et al. that at least 49% of the children presented with acute pain crisis [16].

Regression analysis just like Pearson's correlation coefficient can be used to determine relationship. Results of the study revealed significant association between biochemical parameters (NO, Zn) and markers of disease severity namely hematocrit, VOC and PF. The outcome of the study indicates that Zn and NO are associated with the markers of disease severity among both HbSS and HbSC patients. Meanwhile, among HbSC patients these inexpensive and commonly available biochemical tests can be used to envisage disease severity only if the rate of clinic attendance is increased. In Osun state, the rate of clinic attendance among HbSC patients was lower compared to individuals with HbSS. These are laboratory-based parameters (NO, Zn, hematocrit), which are mostly determined in hospital settings in the communities where the study was carried out.

CONCLUSION

The low serum levels of Zn and NO that were observed in SCD patients as compared with healthy individuals indicated that SCD can significantly alter these analytes. The results of regression analysis further support the association between SCD and Zn and NO. Providing a biochemical basis for the more severe nature of HbSS compared with HbSC, are the results of Zn levels which were significantly lower than in HbSS than HbSC. The non-significant difference between NO of HbSS and HbSC warrants further investigation.

Limitations of the study

The study was limited by the small number of the recruited SCD patients. In addition, the use of few markers of disease severity rather than all of them, which was due to limited resources available to carry out the study means that data obtained cannot be used in sufficient measure to make inference about the effects of NO and Zn on all markers of the severe SCD state. Unfortunately, lack of a consensus scoring system for assessing disease severity in patients with SCD informed the decision to use VO crisis, rate of hospitalization and PF as the markers of SCD severity in this study.

CONTRIBUTION TO KNOWLEDGE

This study further confirms the involvement of NO and Zn in the pathophysiology of SCD. The association between the depletion in the concentrations of NO and Zn and the severe stage of SCD suggests that serum NO and Zn could serve as reliable disease severity markers in the management of SCD patients in many parts of the world due to high accessibility of tests, their low costs and high reliability. Early diagnosis of patients at risk of disease severity may improve prognosis, management and quality of lives of patients.

RECOMMENDATION

Sickle cell disease patients should be encouraged to undergo periodic testing of these bioanalytes – NO and Zn. They can be useful in identifying patients with higher risk of developing end organ dysfunction. This could help in the improving the management of the disease, prognosis, life-quality, and life expectancy among SCD patients. Additionally, further research efforts on the effects of medical or pharmaceutical interventions that can boost the concentrations of these analytes in SCD patients should be encouraged.

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