Zinc Level is a Poor Predictor of Leg Ulcer in Patients with Sickle Cell Anemia

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Abstract

Background: Sickle cell leg ulcers (SCLUs) are a major chronic debilitating complication in patients with sickle cell anemia (SCA). These ulcers lead to stigmatization and depression. Zinc deficiency has been implicated as a cause of SCLU. This study determined the predictability of leg ulcers and zinc levels among SCA patients in Zaria. **Methods:** This was a case–control study in which 100 participants (50 patients with HbSS and 50 controls with HbAA) were enrolled over a 3-month period by convenience sampling technique. Semi-structured questionnaires were used to obtain participants' ages, gender, and presence or absence of leg ulcers. Serum zinc levels were assayed using spectrophotometry. Data obtained were analyzed using IBM SPSS software version 20.0. Means and standard deviations were used to summarize the data, independent sample t-test was used to compare means, and logistic regression was used to assess whether zinc levels can predict the prevalence of leg ulcer among patients. $P \leq 0.05$ was considered statistically significant. **Results:** The mean age of the participants was 24.92 ± 6.2 years while that of the control was 22.92 ± 4.9 ($P = 0.172$) with a range of 18–42 years in both groups. Leg ulcers were present in 10/50 (20.0%) of the patients with SCA. The mean zinc levels of patients with SCA with and without leg ulcers were 12.0 ± 4.0 μmol/l versus 15.5 ± 7.4 μmol/l ($t = 2.060$, $P = 0.050$). A binary logistic regression model using zinc as a predictor could explain only 4.7%–7.5% of the variability in leg ulcers but did not improve classification of cases. There was a negative nonsignificant association between zinc levels and leg ulcers, $B = -0.098$, odds ratio $= 0.906$, 95% confidence interval (0.789, 1.042), $P = 0.167$. **Conclusion:** Serum Zinc level is a poor predictor of leg ulcers in patients with SCA, thus suggesting other yet unstudied factors as likely better predictors.

Keywords: Sickle cell anemia, sickle cell leg ulcer, zinc

INTRODUCTION

Sickle cell disease (SCD) is the most common single gene disorders in humans. It has varying clinical presentations that range from acute recurrent vaso-occlusive crisis which may cause hypoxic injury or infarction that can affect the brain, eyes, lungs, spleen, bone marrow, penis, or other tissues, leading to sequelae such as acute cerebral syndrome, acute chest syndrome, splenic infarction, or priapism.[1] Other complications such as leg ulcer, cholelithiasis, and osteomyelitis can also be found. Sickle cell leg ulcer (SCLU) is a major complication that is relatively common, often debilitating and a cause of stigmatization among affected individuals.[2]

SCLU is seen in those with the hemolytic phenotype associated with increased serum lactate dehydrogenase levels, increased serum bilirubin, reticulocytosis, and reduced nitric oxide level.[3] Chronic hemolysis, which increases demand and utilization, along with the secondary loss of zinc in the urine due to renal tubular damage following recurrent infarction had been implicated as a cause of abnormal zinc levels in plasma, erythrocytes, and hair due to increased urinary excretion, compared with controls.[4]

Zinc has been found to improve wound healing, decrease the incidence of infection, improve the age of attaining secondary sexual characteristics, reverse dark adaptation of the eyes, and accelerate growth.[5,6] However, local data are scarce regarding the role of zinc in leg ulcers. Hence, we aimed determined the predictability of serum Zinc levels on the presence of SCLUs among patients with sickle cell anemia (SCA).

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Table 1: Sex distribution of leg ulcer in the patients studied

Leg ulcer	Frequency (%)
Present	
Male	6 (12.0)
Female	4 (8.0)
Absent	
Male	13 (26.0)
Female	27 (54.0)
Total	50 (100)
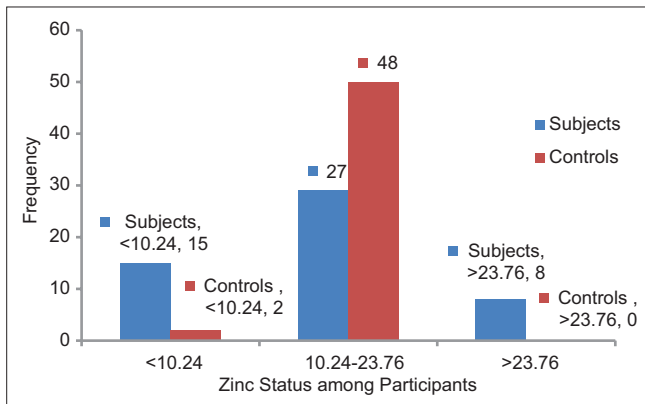
Figure 1: Distribution of zinc levels among all the participants. Key: (1) Low: <10.24 μg/mol, (2) Normal: 10.24–23.76 μg/mol, (3) High: >23.76 μg/mol

METHODS

It was a case–control study using semi-structured questionnaires, physical examination, and laboratory investigations. One hundred participants (50 each of steady state adult SCA patients who were confirmed by alkaline hemoglobin electrophoresis and age- and sex-matched Hb AA controls attending sickle cell clinic of Ahmadu Bello University Teaching Hospital, Zaria) were enrolled into this study after obtaining informed written consent. Consenting patients with SCA aged ≥18 years in steady state were recruited. Those in crisis or on zinc supplementation were excluded from the study. Similarly, consenting Hb AA individuals with age ≥18 years were recruited as control excluding persons on zinc supplementation. Ethical approval for this study was obtained from the Health Research Ethics Committee of Ahmadu Bello University Teaching Hospital Zaria.

Three milliliters of venous blood was collected into a plain sample bottle from each participant while observing standard aseptic procedures. The sample was allowed to stand for 1 h at room temperature to clot and subsequently centrifuged at 3000 g for 5 min using the universal 320 Hettich benchtop centrifuge (Hettich, Germany). The serum was transferred to another plain bottle and stored at −20°C in a chest freezer for colorimetric zinc estimation within 2 days with a spectrophotometer (Spectro UV-11, MRC Israel). Data obtained were analyzed using IBM SPSS version 20.0 (Armonk, NY: IBM Corp.). Means and standard deviations were used to summarize the data, Independent sample *t*-test to compare means, and logistic regression to determine if zinc levels can predict leg ulcers. $P \le 0.05$ was considered statistically significant.

RESULTS

The mean age of the participants was 24.9 ± 6.2 years. The mean zinc levels for SCA and controls were 14.86 ± 3.38 μg/mol and 17.00 ± 6.76 μg/mol, respectively ($P = 0.04$). The zinc status among participants was classified into low, normal, and high using the reference interval calculated from the control group (reference level = 10.24–23.76). Of the 50 patients, 8 (16.0%) had high zinc levels, 15 (30.0%) had low, while 27 (54.0%) had normal zinc levels [Figure 1].

Leg ulcers were present in 10/50 (20.0%) of patients with SCA [Table 1]. The mean zinc levels of SCA patients with and without leg ulcers were 12.0 ± 4.0 μmol/l versus 15.5 ± 7.4 μmol/l ($t = 2.060$, $P = 0.050$). Binary logistic regression model using zinc as a predictor could explain only 4.7% to 7.5% of the variability in leg ulcers but did not improve classification of cases ($\chi^2 = 4.903$, df = 8, $P = 0.768$ with Cox and Snell $R^2 = 0.047$, Nagelkerke $R^2 = 0.075$). However, the model did not improve classification of cases (80.8% before and after inclusion of the predictor). There was a negative nonsignificant association between zinc levels and leg ulcers, $B = -0.098$, odds ratio = 0.906, 95% confidence interval (0.789, 1.042), $P = 0.167$.

DISCUSSION

Ten (20.0%) participants had leg ulcers of which 6 (60%) were males. Chronic leg ulcer was found to be common in the age group of 18–25 years with a decreasing prevalence with age. This could be explained by the fact that, this age group is very active and prone to injuries most especially at the lower extremities. This is significantly higher than the 0.45% report by Hassan *et al*. in Zaria and 9.6% and 7.6% reported by Bazuaye *et al*. and Idaewor *et al*., respectively.[7-9] This may be due to the different seasons of patient recruitment and geographical location of the studies. The difference in sex and ages may be due to release of injurious cytokines due to interaction between sickle cells and granulocytes, anemia, thrombocytosis, lower fetal hemoglobin, trauma, and genetic factors (HLA B35, CW47, Male sex).[8] Zinc levels could not predict those who were likely to develop leg ulcers because many factors play a role in causation of ulcers in patients with sickle anemia.

It was observed in this study that the mean zinc levels for participants were lower than that of controls (4.86 ± 7.63 and 17.00 ± 6.7 (μmol/l), respectively, and $P = 0.04$). The mean zinc levels found in the participants are higher than the observation by Edamisan *et al*., with a mean value of 4.94 ± 2.1 μmol/l.[10] Twenty-seven participants (54.0%) had normal levels and 15 (30.0%) had low zinc levels. The relatively normal values

seen in our participants could be as a result of increased awareness about health status and diets. Moreover, the staple foods in the community such as whole grain cereals, legumes, and pulses have high zinc concentration.[11] While others such as rice, fish, root and tubers, and green leafy vegetable have moderate amounts of zinc.[12] Fifteen (30.0%) of our study participants have low zinc levels, and this is also in keeping with the findings by Prasad *et al*., Leonard *et al*. and Ogunrinde *et al*., who all reported zinc deficiency among SCA patients.[4,6,13] Low zinc level is caused by chronic hemolysis which increases demand and utilization and zincuria due to renal tubular damage following recurrent infarcts.[14]

Conclusion

Zinc level is a poor predictor of leg ulcers in patients with SCA, thus suggesting other yet unstudied factors as likely better predictors.

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Conflicts of interest
There are no conflicts of interest.

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