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Research Article

Micronutrients and Antioxidants Status in Sickle Cell Anaemia Patients with Chronic Leg ulcer

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ABSTRACT

The aetiology of chronic leg ulcer in patients with sickle cell anaemia is multifactorial and complex, prevention and management is a daunting task. Micronutrients and antioxidants contribute to the pathogenesis of sickle cell disease and chronic leg ulcers. Therefore, this study was carried out to determine the serum levels of zinc, copper and vitamin E in sickle cell anaemia patients with chronic leg ulcer. Twelve sickle cell anaemia patients with leg ulcer (SLU), 12 sickle cell anaemia patients without leg ulcer (NLU) and 15 healthy volunteers who were age and sex-matched were recruited for the study. Questionnaires were administered to obtain biodata and history of ulcers with chronic leg ulcers. Venous blood was collected from all respondents and analyzed for complete blood count, zinc, copper and vitamin E levels. The data generated was analyzed using the Statistical Package for the Social Sciences (SPSS) version 23. Descriptive statistics such as frequency, counts, percentages, mean and standard deviation were used to summarize the result. Independent T test and ANOVA were used as appropriate determine significant association between variables. The level of significance was set at P value <0.05. The mean age of the sickle cell anaemia patients with chronic leg ulcer was 31± 10.3 years. There was no statistically significant association between the serum zinc, copper levels and the presence of chronic leg ulcer in sickle cell anemia patients. There was a statistically significant association between the serum vitamin E levels, and the presence of chronic leg ulcer in sickle cell anemia patients' Low serum vitamin E level may play a significant role in the pathogenesis, sustenance and healing of sickle cell chronic leg ulcer. We therefore recommend the supplementation of vitamin E in the management of chronic leg ulcer in patients with sickle cell anaemia.

Keywords: *sickle cell anaemia, chronic leg ulcer, micronutrients, Vitamin E*

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INTRODUCTION

Sickle cell anemia (SCA) is a hereditary blood disorder characterized by abnormality in oxygen carrying capacity of hemoglobin molecule. On deoxygenation, the hemoglobin becomes insoluble and rigid, altering the shape and function of the red cell resulting in the sickle shaped erythrocytes. Sickle cell disease (SCD) is one of the most common autosomal recessive disorders in the world (Lonergan *et al*., 2001). The burden of SCD is highest in sub-Saharan Africa, especially in Nigeria where approximately six million suffer from SCD, while 25 million others are carriers (Aliyu *et al*., 2008). SCA affects about 3% of children in Nigeria and is the commonest genetic disorder in the country (Ogunride *et al*., 2005).

Sickle cell anemia is associated with a number of acute and chronic complications such as chronic leg ulcer. Chronic leg ulcers are common in SCD (Ladizinski *et al*., 2012) with varying geographical distribution, occurring in up to 75% of adults affected with the disease in some population(Minniti *et al*., 2010) .The incidence of CLU among SCD patients studied in Northern Nigeria was 0.45% (Hassan *et al*., 2014). The incidence is considerably higher in males (Hassan *et al*., 2014). The incidence of leg ulceration in tropical countries is further complicated by the high frequency of tropical and chronic non-specific

The cause is multifactorial and complex, prevention is impractical, and management once present is soften difficult (Nolan *et al*., 2006). The ulcers occur spontaneously or as a result of local trauma with subsequent infection and skin necrosis. Recurrence rates are high and range from 40% in Jamaica (Clare *et al*., 2002), to 71.4% in Lagos, Nigeria (Olayemi and Bazuaye 2009), and to 96% in Accra, Ghana (Ankra-Badu, 1992). It is an important contributor to the morbidity and psychological burden of the disease, the negative psychological and social impact is well documented (Marti-carvajal, 2014) . It is a chronic complication that is

difficult to treat, heal slowly over a period of months or years, disrupting quality of life and placing high burden on the health care system.

The exact mechanism by which sickle cell disease causes leg ulcer has not being well elucidated (Madu *et al*., 2013). Various micronutrients such as zinc, selenium and magnesium, vitamins A, C and E play significant role in the prevention and attenuation of peroxidative damage and can potentially enhance wound healing (Thomas and Bishop 2007). Significantly, low circulating levels of antioxidant vitamins A, C and E have been measured in HbSS patients (Natta *et al*., 1992). However direct evidence of any clinical benefit for supplementing these micronutrients is still needed. In the context of sickle cell anemia, far more attention has been focused on zinc than any other mineral. Many health consequences of zinc deficiency have been reported, including immune dysfunction, abnormal or slowed sexual maturation, abnormal growth pattern, poor wound healing and decreased level and activity of zinc metallo-proteins (Prasad, 2008). Interestingly, virtually all of these complications have been associated with the pathophysiology of sickle cell disease. Defective wound healing, indicated by chronic leg ulcers among sickle cell anemia patients, has shown improvement after zinc supplementation (Trent and Robert, 2004).

Finding a widely available cure for sickle cell anemia still remains a challenge one hundred years after its discovery as a genetically inherited disease. However, growing interest in the nutritional problems of the disease has created a body of literature from researchers seeking nutritional alternatives as a means of decreasing morbidity associated with the disease and also improving quality of life of these patients. This study aims to determine if there is any form of discrepancy in the level of micronutrient and antioxidant in SCA patients with ulcer and those without ulcer, and if it does exist, supplementation of this antioxidant and micronutrient can be incorporated to improve the quality of life of the patients.

MATERIALS AND METHODS

Study Design, participants and location: It was a cross sectional study which included twelve SCA respondents who have had leg ulcer occurring for a duration of at least six month (SLU), twelve SCA respondent without leg ulcer (NLU), and fifteen control respondents with hemoglobin AA phenotype. The respondents were age and sex matched. The study was carried out at the Departments of Hematology and Plastics, Aesthetics and Reconstructive surgery, both of the University College Hospital, Ibadan. The inclusion criteria for SCA respondents recruited for this study were being in steady state and not on hydroxyurea therapy. The exclusion criteria for all respondents were the use of multivitamin supplement in the last one month before the study and history of liver or renal disease.

Data collection: The sample size for the study was determined using standard formula for the calculation of minimum sample size (Kasiulevicius *et al*., 2006). A structured questionnaire was used to obtain information such as sociodemographic characteristics, duration, etiology and recurrence of chronic leg ulcer and age at diagnosis with sickle cell anemia from the respondents. Physical examination was used to determine the location, anatomical site and size of the chronic leg ulcer. Six milliliters of venous blood was collected from the median cubital vein in the anterior cubital fossa after adequate skin preparation, 2mls each was put in a Potassium ethylene diaminetetraacetate (KEDTA) bottle and 4mls a plain bottle. The KEDTA bottle sample was used to determine the Haematocrit, red cell indices, white blood cell count and differentials and platelet count using Sysmexautoanalyzer Kobe, Japan. The blood sample in the plain bottle was allowed to clot for an hour and then spun at 3000rpm for 30 minutes to obtain serum which was stored and subsequently analyzed for zinc, copper and vitamin E.

Analysis of the micronutrient (zinc and copper) was done using Atomic Absorption Spectrophotometer while the analysis of the vitamin E was done using high performance liquid chromatography.

Data analysis

The data generated was analyzed using the Statistical Package for the Social Sciences (SPSS) version 23. Descriptive statistics such as frequency, counts, percentages, mean and standard deviation were used to summarize the result. Independent T test and ANOVA were used as appropriate determine significant association between variables. The level of significance was set at P value <0.05

RESULTS

Demographic Characteristics of Patients: The mean age of the respondents with sickle cell anemia who had leg ulcer, was 31± 10.3years while the mean ages for the respondents with SCA without leg ulcer and healthy controls were 32.7±11.6and 30 .7 ± 8.6year respectively. The differences were not statistically significant. Majority of SLU respondents were females, nine (75.0%) while three (25.0%) were males; the NLU group had 7 females and 5 males while the control group had 9 females and 6 males. The differences in gender were also not statistically significant.

Sites and Characteristics of Ulcers: Eleven (91.7%) of the participants had the ulceration on the left lower limb with one (9%) of these eleven respondents having it on both the left and right lower limb lower, while one (8.3%) respondent had it only on the right lower limb. The most common anatomical site was the left lateral malleoli as this was reported in about 9 (75%) of the patients. The size of the ulcer varied widely ranging from as narrow as 2cm to as wide as 10 cm, with half of the examined ulcer above 4 x4cm in its widest diameter. A recurrence rate of 83.3% was observed in the sickle cell anemia respondents with chronic leg ulcer. The ulcers resulted from trauma (83.3%) spontaneous ulceration (8.3%) and following surgery (8.3%).

Haematological Indices, Micronutrients and Antioxidant Levels in Patients: The hemoglobin, packed cell volume, and red cell count were significantly lower in SLU than NLU and controls ($p<0.001$) while the white cell count was significantly higher in SLU than the NLU and controls (Table 1). There was no statistically significant difference in the serum levels of zinc, copper and vitamin E between the three groups of patients (Table 2).

Table 1:
Hematological parameters of the sickle cell anemia respondents with leg ulcer (SLU), sickle cell anemia respondents without leg ulcer (NLU) and control respondents

Hematological parameters	SLU	NLU	CONTROL	P value
Packed cell volume	19.38±4.00	23.83±5.38	39.28±4.469	< 0.001
Hemoglobin concentration(g/dl)	5.96±1.148	7.43±1.15	12.78±1.73.	<0.001
Red cell count(cells/ litre)	$2.24x10^{12}$ $\pm 0.44x10^{12}$	$2.75x10^{12}$ $\pm 0.58x10^{12}$	$4.86x10^{12}$ $\pm 0.65x10^{12}$	<0.001
white cell count cells(cell/mm^3)	15,175± 6,115	9500± 2400	5,800±1,513.	<0.001
Platelet count(cells/ litre)	$320x10^9 \pm 241x10^9$	$359x10^9 \pm 120x10^9$	$274,x10^9 \pm 80x10^9$	< 0.368
Mean corpuscular volume (fL)	86.6 ± 10.2	86.9 ± 10.2	81.6 ± 4.8	0.197
Mean corpuscular haemoglobin (pg)	26.3 ± 3.38	27.6 ± 2.6	26.3 ± 1.90	0.372
Mean corpuscular haemoglobin concentration (g/L)	31.0 ± 1.19	31.3 ± 1.226	32.2 ±1.05	0.023

Table 2:
Micronutrient and antioxidant levels of the sickle cell anemia respondents with leg ulcer (SLU), sickle cell anemia respondents without leg ulcer (NLU) and control respondents

Parameters	SLU	NLU	CONTROL	P value
Serum zinc µg/l	97.15 ±16.56	97.22 ±23.41	83.81 ± 17.99	0.127
Serum copper µg/l	109.68 ± 17.08	106.04 ± 18.72	104.17 ± 13.86	0.690
Serum vitamin E ng/l	0.554 ± 0.176	0.664 ± 0.159	0.633 ± 0.136	0.131

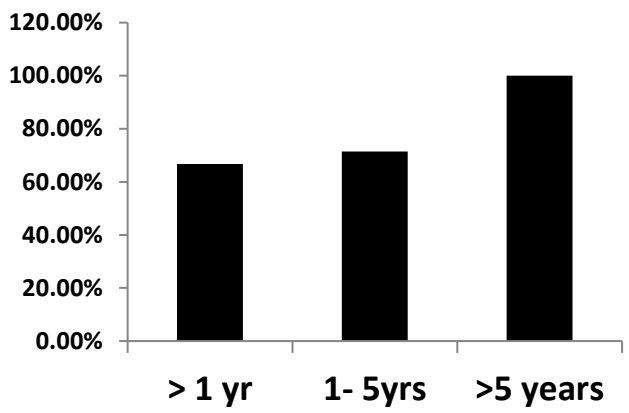

Figure 1:
Percentage proportion of patients with low vitamin e and duration of leg ulcer

Duration of Ulcer in Relation to Micronutrient and Antioxidant Levels: The duration of ulcers was 1-5 years in 58.3%, less than 1 year in 25% and more than 5 years in 16.7% of the patients. The mean duration of the ulcer was 3.07 ± 2.79 years. All the patients who have had the ulcer for less than 1 year had a normal level of zinc, same with those who had had the ulcer for about 1 to 5 years while only one (50%) of the two respondents who have had the ulcer for greater than 5 years had a low level of zinc which was not significant All respondents with SLU had normal level of serum copper irrespective of the duration of ulcer. All patients who had ulcer for more than 5 years had low vitamin E (Fig.1)

Relationship between Leg Ulcer and Serum Zinc Level: Comparison of the low, normal and high levels of serum zinc in patients with leg ulcer, patients without leg ulcer and controls showed that variation in serum level of zinc was not statistically significant associated with the presence of chronic leg ulcer (Table 3).

Table 3:
Relationship between Serum Zinc Level and Sickle Cell Chronic Leg Ulcer.

Status	Serum Zinc (< 70) Low	Serum zinc (70-125) normal	Serum Zinc (> 125) High	Total
SLU	1 (8.3%)	11 (91.7%)	0(0%)	12
NLU	2 (16.7%0	9 (75%)	1 (8.3%)	12
CONTROL	4 (26.7%)	11 (73.3%)	0 (0%)	15
TOTAL	**7**	**31**	**1**	**39**

Pearson chi square value obtained 0.482

Relationship between Leg Ulcer And Serum Vitamin E Level: There were only 2 levels of vitamin E which is low and normal. Seventy –five percent of the patients with chronic leg ulcer had vitamin E level lower than normal compared the 33.3% and 26.7% of patients without chronic leg ulcer and controls respectively (P < 0.05) (Table 4).

Table 4:
Relationship Between Serum Vitamin E Levels and sickle cell chronic leg ulcer

STATUS	Serum vitamin E (<5.4)	Serum vitamin E (5.5-17) normal	Total
SLU	9 (75%)	3(25%)	12
NLU	4 (33.3%)	8(66.7%)	12
CONTROL	4 (26.7%)	11(73.3%)	15
TOTAL	17	22(%)	39

Pearson chi square value obtained 0.029

DISCUSSION

The mean age of 31 years of respondents with sickle cell leg ulcer from this study is similar to the studies by Hassan *et al*.,(2014), in Zaria and Ideawor *et al*.(2002), in Benin who both reported a mean age of 28.3 and 28 respectively. This is most likely due to the fact that most individual at this age are highly active and more susceptible to trauma. The female preponderance observed is not in keeping with the male preponderance reported by Hassan *et al*(2014) and Waziri *et al*.,(2017) both carried out in Zaria, as well as the male preponderance found by Durosinmi *et al*.,(1991) in Ibadan. However, Ideawor *et al*.,(2002) reported a slight female preponderance in his study in Benin, he also reported that the left leg was the commonest site of occurrence of ulcer. This is similar to the observation of the lateral malleoli on the left leg as the most common anatomical site in our study which was similar to what was observed by Bazuaye *et al*,(2010) however, Hassan *et al*,(2014) reported the right medial malleoli as the commonest site.. The absence of subcutaneous tissue, a very thin skin in the region and poor vascularization makes the lateral malleoli a very susceptible area for chronic sickle cell leg ulcer. The mean duration of leg ulcer reported by this study is not consistent with the study by Ogunkeyede *et al*.,(2017) which reported a median age of seven years, although, this study shows a reduction in the level of vitamin E and zinc with increasing duration of SLU, however there was no statistically significant association between the duration of the chronic sickle cell leg ulcer and the serum levels of zinc and vitamin E.

The etiology and high recurrence rate of the SLU have not changed over time as this study is in keeping , with that of Olatunya *et al*., (2018) and Bazuaye *et al*.,(2010) , although Hassan *et al*.,(2014) reported that half of the respondent's ulcer happened spontaneously in their own study in Zaria. High recurrence rate of the leg ulcer of 96% was reported in Accra, Ghana (Ankra-Badu, 1992) while 40% was reported in Jamaica (Clare *et al*., 2002) .

The hemoglobin concentration, red blood cell count and packed cell volume were both significantly reduced in respondent with sickle cell anemia who had CLU when compared with the control group and those with sickle cell anemia without leg ulcer. This could be due to blood loss from ulcer as bleeding may occur during dressing. Mean corpuscular volume and mean corpuscular haemoglobin was slightly higher in respondents with chronic leg ulcer in our study, although not statistically significant but the observation is similar to the finding by Cones *et al*.,(2013) and that of Olatunya *et al*.,(2018). The slightly higher level of mean corpuscular heamoglobin concentration in the control respondents was statistically significant which is in agreement with Waziri *et al*., (2017). The significantly high white cell counts in observed in the SLU respondents which is in agreement with several available studies and could be explained by the presence of an infective process (Ademiluyi *et al*., 1988).The platelet count was not significantly different among the three groups of respondents which is consistent with findings by Hassan *et al*.,(2010) Madu *et al*.,(2013) and Olatunya *et al*. (2018) suggesting that platelet count may not play a role in the pathogenesis of sickle cell chronic leg ulcer Our finding of comparable levels of serum zinc and copper in respondents with sickle cell with chronic leg ulcer, sickle cell without chronic leg ulcer and the control group is in contrast to available literature by Waziri *et al*.,(2017) and Rojas *et al* (1999) which have all reported zinc deficiency in patients with sickle cell anemia with chronic leg ulcer. This suggests that zinc and copper are unlikely to be important in the pathogenesis and sustenance of sickle cell chronic leg ulcer in our patients. There was no statistically significant association between the ulcer and serum level of zinc and copper making low level of zinc a poor predictor of predisposition of sickle cell anemia patient to chronic leg ulcer as reported by Waziri *et al*., (2017).

Serum vitamin E level which was slightly lower in the SLU respondents than the control group is in agreement with the study by Rojas *et al*.,(1999) that reported a deficiency of antioxidant vitamin E in patients with sickle cell anemia chronic leg ulcer. There was a statistically significant association between proportion of patients with sickle cell leg ulcer and low serum vitamin E Level, this finding suggest that low serum vitamin E may play a significant role in the pathogenesis, sustenance and healing of sickle cell chronic leg ulcer. This is supported by studies by Paolino *et al*.,(2015) who observed a good response with the use of vitamin E as a co adjuvant in the treatment of sickle cell leg ulcer, and Ette *et al*.,who reported that there was significantly lower level of antioxidant vitamins including vitamin E in chronic leg ulcer although this was not specific to SLU and thus recommended the addition of vitamin E in the treatment of chronic leg ulcer.

In conclusion, low serum vitamin E level was associated with the presence ulcer in patients with SCD and may play a significant role in the pathogenesis of sickle cell chronic leg ulcer. We therefore recommend the supplementation of vitamin E in the management of chronic leg ulcer in patients with sickle cell anaemia.

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