PATTERN OF SERUM ZINC LEVEL, PERIPHERAL BLOOD LYMPHOCYTE AND NEUTROPHIL COUNTS AMONG PATIENTS WITH SICKLE CELL DISEASE

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ABSTRACT

Background: Zinc is an important mineral element serving as a cofactor in a number of cellular pathways including those involved in cell growth and proliferation. Sickle cell disease (SCD) is associated with excessive haemolysis and defective kidney function with consequential decrease in body's pool of vital micronutrients. The abnormal loss of zinc in SCD may affect leucopoiesis.

Aim:This study was aimed to determine the relationship between serum zinc and leukocyte subsets (Lymphocyte, neutrophil) in adult patients with SCD in steady state together with their counterpart apparently healthy controls.

Materials and Methods: Blood samples were collected from 33 adult participants with SCD and 33 apparently healthy controls. Lymphocytes and Neutrophils counts were performed using automated haematology analyser (Sysmex KX21N) and serum Zinc level was determined spectrophometrically using the Br-PADAP method.

Results: The results shows statistically significant difference in absolute lymphocyte and neutrophil counts for the two groups were $P < 0.0001$ and $P < 0.0001$, respectively. The serum zinc level was also statistically significant between the groups: $P<0.0002$. However, serum zinc level of subjects with SCD showed no correlation with lymphocyte and neutrophil counts $p<0.0610$ and <0.6775, respectively.

Conclusions: Significant statistical difference was observed, indicating SCD patients have higher WBC count and neutrophil counts and reduced serum zinc and lymphocyte counts. There was no significant correlation between the leucocyte subset counts and serum zinc levels in both the SCD patients and the normal healthy controls.

Keywords: Sickle Cell Disease, Lymphocytes, Neutrophils and Zinc.

INTRODUCTION

Sickle cell disease (SCD) consists of a group of disorders characterized by the presence of sickled haemoglobin. Excessive haemolysis, blockage of microvasculature events and ischemic tissue death are the pathological hallmarks of sickle cell disease (Odi`evre *et al.,* 2011 and Sauntharajah and Vichinsky, 2018). Zinc is an essential micronutrient for growth and development, which plays a vital role in immune functions and resistance to infections in children (Prasad, 2003 and Moon *et al.,* 2018). Zinc deficiency is relatively common in adults with SCD, affecting about 60%–70% of adult subjects (Prasad, 2003). In SCD patients, diagnosis of zinc deficiency is based on zinc levels in lymphocytes and granulocytes (Beck *et al.,* 1997). Increased hemolysis in SCD patients releases a considerable amount of zinc, which circulates in the plasma pool.

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This results in an increase in glomerular filtration of zinc, but its reabsorption is hampered by the renal tubular damage caused by repeated vasoocclusive episodes (Temiye *et al.,* 2010)

Zinc deficiency has multiple effects on proliferative ability of haematopoietic cells as well as their tendency to differentiate toward a cell line (Moon *et al.,* 2018). Its deficiency results not only in decreased lymphocyte concentrations, but also leads to depressed T and B lymphocyte function (MacDonald, 2000). Severely zinc-deficient children have been described to have substantial reductions in the size of the thymus, the central organ for T lymphocyte development (Haase and Rink, 2009), B lymphocyte development in bone marrow is adversely affected by zinc deficiency (Fraker*et al.,* 1997). B lymphocyte antibody responses are inhibited by zinc deficiency (De Pasquale-Jardieu and Fraker, 1997).

Most of the previous studies on the dynamics of serum zinc in sickle cell disease were conducted among patients having one form crisis or the other. There is a dearth of information on the pattern of zinc levels in SCD in steady state. Furthermore, the relationship between serum zinc and leucocyte subsets among patients with sickle cell disease in steady state is not adequately explored.

MATERIALS AND METHODS

In this study, we recruited sixty six (66) participants, out of which, 33 were SCD patients and 33 were apparently healthy individualsthat are age and sex matched as control group. Blood sample was collected for Lymphocyte and neutrophils counts and serum Zinc level determination. Electrophoresis was conducted on all the samples and serum Zinc level was determined using a kit from Elabscience®, zinc ion reacts with Br-PADAP (2-(5-bromo-2-pyridylazo-5-(diethylamino) pheno) to produce a colored complex whose spectrophotometric absorbance is directly proportional to zinc concentration when measured at a wavelength of 560nm. The anticoagulant sample was used for full blood count using sysmex KX21N series

RESULTS

The mean Zinc concentration for the test and control group was found to be 2.337 ± 2.111 (µmol/L) and 9.676 ± 5.243 (µmol/L), respectively. While the mean absolute neutrophil count was found to be 10.094 ± 1.519×10^9 cells/L and 5.215 ± 1.870×10^9 cells/L, respectively, P < 0.0001. The mean absolute lymphocyte count was found to be 1.221 ± 0.467 (x10^9/L) and 3.133 ± 0.718 (x10^9/L) for both test and control group respectively, P < 0.0001(Figure 1).

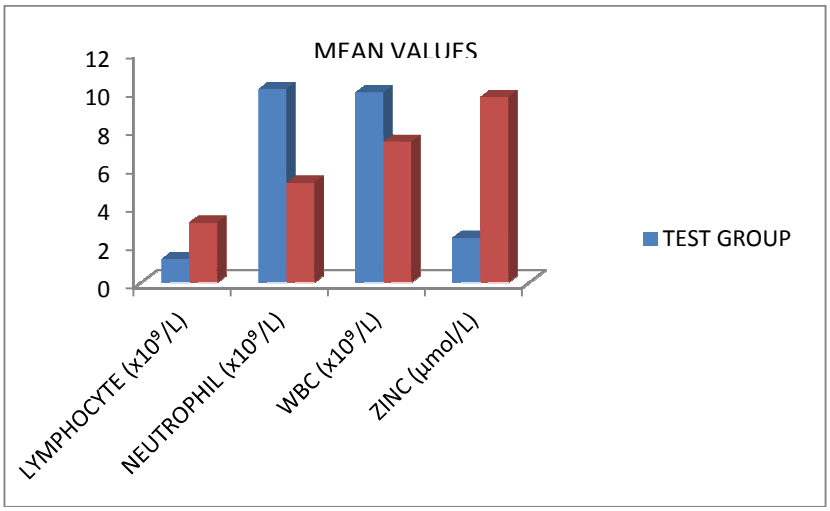

Figure1: Comparison of serum Zinc, Lymphocyte and Neutrophil the test and control group. P < 0.0001

Table 1: Correlation analysis among Test group

Pairing Variables	Correlation coefficient (*r*)	CI	p-value
WBC vs Zn^{2+}	-0.0010	-0.06 to 0.85	0.9955
LYM vs Zn^{2+}	0.0610	-0.07 to 0.09	0.7358
NEU vs Zn^{2+}	0.0752	0.21 to 0.32	0.6775

KEY: WBC = White Blood Cell, LYM = Lymphocyte, NEU = Neutrophil, Zn = Zinc, CI = Confidence Interval, r = Correlation Coefficient.

Table 2: Correlation analysis among Control group

Pairing Variables	Correlation coefficient (*r*)	CI	p-value
WBC vs Zn^{2+}	-0.3117	-0.34 - 0.02	0.0774
LYM vs Zn^{2+}	0.2684	-0.01 - 0.09	0.1310
NEU vs Zn^{2+}	-0.0981	-0.17 - 0.09	0.5861

KEY: WBC = White Blood Cell, LYM = Lymphocyte, NEU = Neutrophil, Zn = Zinc,

CI = Confidence Interval, r = Correlation Coefficient.

DISCUSSION

It has been established that patients with sickle cell anaemia have deficient Zinc level and this may contribute to immune impairment and growth retardation (Prasad, 2003). The body of an adult human (70 kg) contains about 2–3 g of zinc, which is absorbed from our dietary sources in the proximal small intestine, either at the distal duodenum or proximal jejunum (Krebs *et al.,* 1998 and Maywald and Rink, 2015) and released into the blood. Studies in patients with SCD have yielded mixed results with a majority indicating lower levels of serum zinc in SCD with few others showing no difference with control subjects.

The present study found low mean zinc concentration in test group as compared with controls. This is similar to the findings of Emokpae *et al.,* 2019 where they assessed the pattern of serum zinc, copper and disease severity among patients with sickle cell anemia. This is inconformity with the study conducted by Ogunrinde, (2013) in Zaria, who analyzed erythrocytes for zinc concentration in children with SCA in a steady state, he observed that the mean erythrocyte zinc concentration in SCA subjects was lower than that of the controls. He also found an age related increase in erythrocyte zinc in both controls and SCA subjects. Our findings also agrees with the study of Phebus, (2007) who found lower serum zinc in patients with SCD in steady state compared to controls Contrary to previous findings, Temiye *et al.* (2005) found no significant difference in mean serum zinc among controls and SCA children in steady state.

This variation may be associated with increased hemolysis in SCD patients which releases a considerable amount of zinc into the plasma pool (Yuzbasiyan-Gurkan *et al.,* 1989). Zinc deficiency in SCD in some studies was thought to be related to factors such as increased urinary zinc excretion, chronic intravascular haemolysis, and/or zinc mal-absorption (Yuzbasiyan-Gurkan *et al.,* 1989).

Total white blood cell count and neutrophil and were found to be higher in test group compared to control subject, this is in conformity with the findings of Omotola and Wiraola. (1992) who reported increase white blood cells count in the test group compared to control. This is also similar to the findings of Omoti. (2005) and Akinbami *et al.* (2012).

Rise in neutrophil count is at the very center of sickle cell disease pathogenesis. Elevated count is found to occur even among patients in steady state; neutrophil in sickle cell disease have strong association with vaso-occlussive crises, ischemic stroke, acute chest syndrome and other acute and chronic manifestations of sickle cell disease (Zhang *et al.,* 2015). In sickle cell disease, neutrophils are not elevated in counts rather; they also express a persistent activation phenotype characterized by increased adhesiveness to vascular endothelium; thus creating a nucleus for attachment by the young and sticky reticulocytes of SCD patients (Kato, 2015). The mechanism for this intriguing finding is not fully elucidated. However, recent studies have started to unveil the basis for the dysregulated innate immunity in SCD (van Beers *et al.,* 2015). Gene expression studies have revealed a 200 fold increase toll like receptor 4 (TLR4) which is membrane bound pattern recognition receptor essential for activation of the TLR4-Inflammasome system (Hounkpe *et al.,* 2015).

The mean absolute lymphocyte count was found to be low in test group compared to control group. This is inconformity with the study of Kaaba and Al-Harbi. (1993) where they found a decreased lymphocyte count in SCD patients compared with controls, but contrary to the study of Koffi *et al.* (2003) in Cote d'Ivoire who reported decrease lymphocytes count in control group and increase in test group. The predominant lymphocyte population in the peripheral blood are the T cell, and they are found to be reduced in among SCD patients in steady state (Saidu *et al.,* 2019). The reduction in the B cell population may not be as pronounced, as the adaptive immune response in sickle cell disease is found to be skewed towards antibody production (Th2) and inflammation (Bao *et al.,* 2013).

CONCLUSION

This research observed significant statistical difference in WBC count, Neutrophils count, lymphocyte count and Serum Zink between the test and control group. However, there was no significant correlation between the leucocyte subset counts and serum zinc levels in both the SCD patients and the normal healthy controls.

RECOMMENDATION

Based on the data obtained from this study, it is recommended that patients suffering from Sickle Cell Disease should be encouraged in oral zinc intake.

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