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Evaluation of Some Micronutrients in Sickle Cell Disease

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Abstract:
Sickle cell disease (SCD) is an inherited disorder of haemoglobin caused by a single nucleotide substitution of thymidine for adenine (GAG-GTG) of the β-chain that results in the amino acid valine instead of glutamic acid. This congenital haemoglobin mutation alters this balance and creates a pro-oxidant and micronutrient reactive milieu.Our aim is to assess some micronutrients status of sickle cell patients. A total of one hundred (100) subjects were recruited for this study which consists of fifty (50) sickle cell subjects and fifty (50) apparently healthy subjects which served as control. Blood samples were collected by vene-puncture and some micronutrients (Iron, Zinc, Manganese, cobalt, Calcium and Magnesium) were analyzed using Atomic Absorption Spectrophotometer. All parameters were significantly decreased in sickle cell patients when compared with apparently controls. Considering the results obtained in this study, it could be inferred that sickle cell subjects are predisposed to nutritional deficiencies. We therefore advocate routine assessment of micronutrients in sickle cell patients.

Keywords: *Micronutrients, Sickle Cell Disease, Haemoglobin, Iron, Zinc.*

1. Introduction

Sickle cell disease (SCD) is an inherited disorder of haemoglobin caused by a single nucleotide substitution of thymidine for adenine (GAG-GTG) of the β-chain that results in the amino acid valine instead of glutamic acid (Steinberg *et al.,* 2001, Ballas 2002). This leads to alteration in the properties of the haemoglobin tetramer, with the tendencies to polymerize in the deoxygenated state. In homozygotes or when there is co-inheritance of a double heterozygous state (with haemoglobin C, β-thalassemia, D or O), the normal β-chains are replaced by the modified form of the β-chain. SCD is one of the commonest hereditary haemoglobinopathies.

Sickle cell anaemia is primarily a disorder of the red blood cells which are a significant source of free radicals in biological system (Platt *et al., 2004*). Red blood cells have a rich oxygen supply and are densely packed with redox active haemoglobin residues (Wellems *et al*., 2009). The bonding interaction between heme, iron and oxygen in oxygenated haemoglobin is association with an electron transfer. In response, there is an integrated network of the antioxidant system consisting of both enzymes and low molecular weight compounds which help to mitigate oxidative stress and injury to the red cell and tissues in general (Malowany and Butany, 2012). Congenital haemoglobin mutations may alter this balance and create a pro-oxidant and micronutrient reactive milieu (Kumar *et al*., 2009). This may be contributory to the pathophysiology of the abnormalities that underlie the clinical course of sickle cell anaemia,

Micro nutrients consist of vitamins and minerals required by the body in small quantities for the normal function of cellular metabolic processes (Benzie, 2013). Trace elements are usually defined as minerals that are required in amounts between 1 to 100 mg/day by adults (O' dell and Sunde, 1997). They usually function as essential cofactors in the numerous enzyme catalyzed reactions and their absence can result in impairments of metabolic functions which can lead to serious disease conditions (German, 1999). Micro minerals or trace elements include iron cobalt, chromium, copper, iodine, manganese, selenium, zinc molybdenum, calcium and other electrolytes such as sodium and potassium (Debelstein *et al.,* 2007).

Nutritionally essential trace metals are not antioxidants on their own, but are integral parts and are necessary for the proper functions of antioxidant enzymes like catalase, glutathione peroxidase and reductase, and superoxide dismutase (Chan et al., 1999). Thus, it is

necessary that the level of these nutritionally essential trace metals, which are important to these antioxidant enzymes, be assessed in sickle cell disease patients. There is paucity of information in literature concerning the micronutrients status of sickle cell disease patients in this locality. Therefore, we sought to evaluate some micronutrients status in sickle cell patients.

2. Materials and Methods

This study was carried out among patients visiting Sickle Cell Centre, Benin-City, Edo State. A total of one hundred (100) subjects were recruited for this study which consists of fifty (50) sickle cell subjects and fifty (50) apparently healthy subjects which served as control. Out of the total fifty subjects30 were males and 20 were females. The control subjects comprised of 20 males and 30 females. Ethical clearance was obtained from the Ministry of Health ethical committee, Benin City and informed consent was obtained from participants after explanation of the purpose and procedures of the study. Inclusion criteria include sickle cell disease while exclusion criteria are sickle cell disease patients with underlying diseases.

2.1. Sample Collection

Blood samples (5mls) were collected by vene-puncture into an accurately labeled plain container for both subjects and control. The blood samples were centrifuged at 4000rpln for l0minutes at room temperature within two hours of collection and the serum separated into clean plain containers, kept frozen until required for analysis. Analysis was carried out for iron, manganese, zinc, cobalt and magnesium

2.2. Biochemical Analysis

The concentrations of serum trace elements (iron, manganese, zinc, cobalt and magnesium) were determined using atomic absorption spectrophotometer (AAS) using direct method as described by Kaneko, (1999).

2.3. Statistical Analysis

The Student's t - test at 0.05 level of significance was used to compare results in both the control and the test groups (all results were reported asmean ± standard deviation); using a computer program named SPSS for windows release 21.0. Confident interval of Values P< 0.05 was considered significant.

3. Results

Table 1 show the mean ± SD for Iron in sickle cell subjects as 86.66 ± 10.84 µg/ dl while that of the control is 172.22 ± 2.54.16 µg/ dl; and when compared statistically, there was significant difference(P<O.O5) between the values obtained. Also there was a significant difference (P< O.O5) in the mean ±SD of serum manganese of sickle cell subjects (7.10 ± 0.72 µg/L) and the control (06.96± 16.88µg/L) when compared. The mean ± SD obtained for zinc in sickle cell subjects are 75.20 ± 10.84 I1g/L. while that of the control is 119.27 ± l5.68 µg/L; and when compared statistically, there was significant difference (P<O.O5) between the values obtained. Also there was a significant difference (P< O.O5) when the mean ± SD of serum cobalt in sickle cell subjects (0.24 ± 0.03 µg/L) were compared with that of the control (0.36±0.04µg/L). Furthermore, the mean ± SD obtained for serum calcium among sickle cell subjects and control are 7.22 ± 0.90 mg/dl and 10.89 ± 1.31 mg/dl respectively. When compared statistically there was significant difference (P< 0.05) between the values.

Parameters	Control	Test	T-Value	P-Value
	(N-50)	(N-50)		
Iron (µg/dl)	172.22+2.54	86.66+10.84	43.24	0.000*
Mn(µg/dl)	16.96+16.88	7.10+0.72	74.72	0.000*
Zn (µg/dl)	119.27+15.68	75.20+10.84	31.240	0.000*
Co(µg/dl)	0.36+0.04	0.24+0.03	22.04	0.000*
Ca2+(mg/dl)	10.89+1.31	7.22+0.90	22.25	0.000*
Mg (mmol/l)	3.61 ± 0.45	4.69 ± 0.64	5.68	0.000*

Table 1: Comparison of Micronutrient Status among Sickle Cell Subjects with the Control
** Significant*

Similarly, there was a significant difference (P< 0.05) when the mean ±SD of serum magnesium in sickle cell subjects (3.61 ± 0.45 mmol/l) were compared with that of the control (4.69 ± 0.64 mmol/L).

Table 2 shows the micronutrient parameters obtained for sickle cell male subjects and its comparison with the male control. There was significant difference (P< 0.05) when the mean ± SD of serum iron in sickle cell male subjects (87.65 ± 9.28 µg/ dl) were compared with that of the male control (131.10 ± 13.52 µg/ dl). The mean± SD of serum manganese of male subjects (7.17 ± 0.62 µg/L) were significantly lower when compared with the male control (14.07 ± 0.90 µg/L). There was significant difference (P< 0.05) when the mean ± SD of serum zinc in sickle cell male subjects (76.20 ± .28 µg/dl) were compared with that of the male control (119.65 ± 13.52 µg/L).The mean ± SD of serum cobalt of male subjects (0.24 ± 0.03 µg/ dl) were significantly lower when compared with the male control (0.36 ± 0.01 µg/L). The mean ±SD of serum calcium among sickle cell male subjects and control are 7.30 ± 0.77 mg/ dl and

10.92 ± 1.13 mg/ dl respectively. When compared statistically there was significant difference (P<0.05) between values obtained. Similarly, there was a significant difference (P< 0.05) when the mean ± SD of serum magnesium in sickle cell male subjects (3.65 ± 0.39 mmol/L) were compared with that of the male control (4.80 ± 0.40 mmol /L).

Parameters	**Male Control (n-20)**	**Male Test (n-30)**	**t-value**	**P-value**
Iron(µg/dl)	131.10±13.52	87.65±9.28	20.42	0.000*
Manganese (µg/dL)	14.07±0.90	7.17±0.62	48.64	0.000*
Zinc (µg/dl)	119.65±13.53	76.20±9.28	20.42	0.000*
Cobalt (µg/dl)	0.36±0.04	0.24±0.03	19.95	0.000*
Calcium (mg/dL)	10.92±1.13	7.30±0.77	20.39	0.000*
Magnesium (mmol/L)	4.80±0.40	3.65±0.39	12.95	0.000*

Table 2: Comparison of Micronutrient Status among Male Sickle Cell Subjects with Male Control
** Significant*

Table 3 shows the mean ±SD obtained for sickle cell female subjects and its comparison with the female control. There was significant difference (P< 0.05) when the mean ± SD of serum iron of sickle cell female subjects (84.94± 13.44 µg/ dl) were compared with that of the female control (199.64 ± 328.76 µg/L). The mean±SD of serum manganese of female subjects (6.99 ± 0.90 µg/L) were significantly lower when compared with the female control (18.83 ± 21.81 µg/L). There was significant difference (P< 0.05) when the mean ±SDof serum zinc of sickle cell female subjects (73.49 ± 13.44 µg/L) were compared with that of the female control (119.01 ± 17.35 µg/L). The mean ±SDof serum cobalt of female subjects (0.23 ± 0.04 µg/ L) were significantly lower when compared with the female control (0.36 ±0.05 µg/L). The mean±SD obtained for serum calcium among sickle cell female subjects and female control is 7.08 ± 1.12 mg/dl and 10.97 ± 1.45 mg/ dl respectively. When compared statistically there was significant difference (P< 0.05) between values obtained. Similarly, there was a significant difference (P<0.05) when the mean ±SDof serum magnesium of sickle cell female subjects (3.53 ± 0.56 mmol/L) were compared with that of the female control (4.61 ± 0.73 mmol/L).

Parameters	**Female Control (n-30)**	**Female Test (n-20)**	**t-value**	**P-value**
Iron (µg/dl)	131.64±328.76	84.94±13.44	28.30	0.000*
Manganese (µg/dl)	18.83±21.81	6.99±0.90	43.82	0.000*
Zinc (µg/dl)	119.01±17.53	73.49±13.44	11.23	0.000*
Cobalt (µg/dl)	0.36±0.05	0.23±0.04	11.21	0.000*
Calcium (mg/dl)	10.87±1.45	7.08±1.12	11.23	0.000*
Magnesium	4.61±0.73	3.53±0.56	6.23	0.000*
(mmol/L)				

Table 3: Comparison of Micronutrient Status among Female Sickle Cell Subjects with female Control
** Significant*

Table 4 shows the mean ±SD obtained for male and female sickle cell subjects. There was no significant difference (P> 0.05) when the mean ± SD of serum iron in sickle cell male subjects (84.94 ± 13.44 µg/dl) were compared with that of the female subjects (87.65 ± 9.28 µg/dl). The mean ± SD of serum manganese of male subjects (7.17 ± 0.62 µg/L) was higher when compared with the female subjects (6.99 ± 0.90 µg/L). There was no significant difference (P> 0.05) when the mean ± SD of serum zinc of sickle cell male subjects (76.20 ± 9.28 µg/L) were compared with that of the female subject (73.49 ± 13.44 µg/L). The mean ± SD of serum cobalt of male subjects (0.24 ± 0.03 µg/L) were higher when compared with the female subjects (0.23 ± 0.04 µg/L). The mean ± SD of serum calcium among sickle cell male subjects and female subjects are 7.30 ± 0.77 mg/ and 7.07 ± 1.12 mg/ dl respectively. When compared statistically there was no significant difference (P> 0.05) between values obtained. Similarly, there was no significant difference (P> 0.05) when the mean ± SD of serum magnesium of sickle cell male subjects (3.65 ± 0.39 mmol/I) were compared with that of the female subjects (3.54 ± 0.56 mmol/l).

Parameters	Male Control (n-30)	Female Test (n-20)	t-value	P-value
Iron (µg/dl)	87.65+928	84.94+13.44	0.67	0.519**
Manganese (µg/dl)	7.17+0.62	6.99+0.90	0.67	0.516**
Zinc (µg/dl)	76.20+9.28	73.49+13.44	0.67	0.519**
Cobalt (µg/dl)	0.24+0.03	0.23+0.04	0.47	0.647**
Calcium (mg/dl)	7.30+0.77	7.08+1.12	0.66	0.526**
Magnesium (mmol/l)	3.65+0.39	3.53+0.56	0.66	0.526**

Table 4: Comparison of Micronutrient Status among Male and Female Sickle Cell Subjects
*** Non- Significant*

4. Discussion

Sickle cell disorder is a heamoglobinopathy caused by point mutation in the β-chain of the globin gene. It is an autosomal recessive inheritance, and clinical severity varies widely from the sickle cell trait (heterozygous) to sickle cell anemia (homozygous) (Wang, 2004).Our results show low level of serum Iron in sickle cell patients when compared to the apparently healthy controls.This is in tandem with thestudy of Olaniyi and Arinola, (2010). Also there were significantly decreased levels of iron when the male sickle patients and female sickle cell patients are compared with their respective controls (as shown in table 2 and 3) but there were no significant differences observed when the male and female sickle cell patients were compared. A plausible explanation for this low level of iron may be due to constant use of iron in the formation of normoblast after occlusive crisis experience by the patients. Iron is important inthe synthesis of Haemoglobin while copper and zinc play very important roles in iron metabolism (Chan *et al*., 1999, Okochi and Okpuzor, 2005).Therefore, continuous use of iron in haemoglobin synthesis in SCD patients may explain low plasma iron in these patients.

Manganese is an antioxidant as it is incorporated into antioxidant enzymes, such as superoxide dismutase and glutathione peroxidase (Halliwell and Gutteridge, 2007). Our report observed a significantly decreased level of manganese in sickle cell patients when compared with their apparently healthy counterparts. Also when the male and female sickle cell patients were compared with their respective sex, there was a significantly decreased levels of serum manganese in the sickle cell patients but there was no significant difference observed when male and female sickle cell patients were compared. These reduced levels of manganese could be due to consumption as antioxidants to ameliorate oxidative stress, which follows free radical load in sickle cell disease patients.

Our results show, a significantly decreased levels of serum zinc concentration in sickle cell patients when compared with apparently healthy controls. This is in agreement with the report of Prasad *et al*., (1967) and Bot *et al*., (2013) who related zinc deficiency in sickle cell disease. This results to manifestations such as growth retardation, hypogonadism in males, hyperammonemia, abnormal dark adaptation and cell mediated immune disorder. Similarly, the biochemical evidence for zinc deficiency in patients with SCD includes low zinc concentrations in plasma, erythrocytes, lymphocytes and granulocytes (Zemel *et al*., 2002, Singhi *et al*., 2003). In a report by Prasad *et al.,* (1967), low activities of zinc dependent enzymes such as carbonic anhydrase, alkaline phosphate and thymidine kinase have also been observed. Due to the low level of Zinc in sickle cell patients, a higher than normal activity of plasma ribonuclease has been observed in patients with sickle cell disease, because zinc is known inhibitor of this enzyme activity (Parad *et al*., 1967). Zinc deficiency can also be the result of the adverse effect of hydrourea which increase zinc excretion as reported by Silliman *et al*. (1993). In a study on sickle cell disease by Parad *et al*., (1975) on the effect of zinc supplementation on the growth and body composition of children with sickle cell disease, they observed that zinc deficiency, resulted in growth retardation, which is a major clinical problem in sickle cell patients. Prasad *et al*., (1975) therefore affirm that zinc supplementation could help sickle cell patients resume normal growth. In addition, these patients showed a reduction in crippling attacks of severe abnormal pain and vomiting, which had hitherto caused them to be hospitalized more often than not.

Our result shows significantly decreased level of serum cobalt in sickle cell patients when compared with apparently healthy subjects. Cobalt is a part of the vitamin B_{12} molecule as cobalamin and has no other known function in humans (Farell *et al*., 2010). Free cobalt cannot be incorporated in body's' vitamin B_{12} pool, hence diet has to supply body's B_{12} needs. Therefore, the significant decrease observed for cobalt in sickle cell subjects could suggest low dietary intake of vitamin.

We observed a significantly decreased level of serum magnesium in sickle cell diseasesubject when compared with the control.This is in agreement with the work of Defrancheschi *et al*, (1994) and Nnodim *et al*., (2014). This probably contributes to redblood cell dehydration and a concomitant increase in the symptoms of sickle cell disease. Sickle red bloodcells are fragile and dehydrated. It has been shown that magnesium is not only useful in reducing the painful episode in sickle cell subjects, but also affects the hydration of red blood cells.

Also, our study observed a significantly decreased serum calcium levels in sickle cell subjects when compared with the control. This is in accordance with the reports of Nduka *et al.*, (1995) whichreported a similar trend of calcium in sickle cell subjects. These observations could suggest that calcium homeostasis is affected in sicklecell disease (Nduka and Ekeke, 1987; AI-Dabbagh *et al*., 1989; Muhammed *et al.,* 1993). A tendency towards hypocalcaemia in these sickle cell subjects has been found in others studies (Nduka and Ekeke, 1987; AI-Dabbagh *et al*., 1989; Muhammed *et al*., 1993). The fact that patients with sickle cell disease are not universally hypocalcaemic may be explained by the complex nature of calcium homeostasis and the possible role of vitamins and hormones, as suggested by Al-Dabbagh and his colleagues(1989). The similarity of our results to those of earlier studies (Nduka and Ekeke, 1989; Al-Dabbagh *et al*., 1989; Muhammed*et al.,* 1989) and the absence of variation in the calcium levels between crisis and

steady state periods of sickle cell disease strongly suggest that the commonest presenting feature of the disease is the bone manifestations which is an inherent aspect of the disease (Nduka and Ekeke, 1987; Muhammed *et al*., 1993).

Genetic diseases especially heredity blood disorder such as Sickle Cell Disease (SCD) is a significant problem in many countries. Their chronic nature with no prospect for cure, make them important causes of morbidity and mortality. Considering the results obtained in this study, it could be inferred that sickle cell subjects are predisposed to nutritional deficiencies. This is supported by the significant decrease levels of iron, manganese, zinc, cobalt, calcium, magnesium.

5. Recommendation

We therefore advocate that management of sickle cell disease should include consideration for early and prompt diagnosis and improvement on general health conditions such as improved sanitation, healthy nutrition, immunization, prophylaxis against infection and public education programmes aimed at preventing early mortality. Research programmes are also needed to develop comprehensive treatment plan tailored to Africa with particular reference to Nigeria. It is advisable to include trace elements supplements co the therapies used for the management of sickle cell disease patients.

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