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Zinc Status in Patients with Sickle Cell Disease in Gezira State (Central Sudan)

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Abstract

Introduction

Sickle cell disease is inherited as an autosomal recessive manner. HbS differs from HbA in the substitution of valine for glutamic acid in the sixth position of the β-globin chain. Heterozygous state for the HbS gene. Comprises 25-45% of the total hemoglobin, the rest being Hb-A, Hb-A_2 and Hb-F. .

Zinc status in children with sickle cell disease was determined in 1975, the finding, showed that there was a significant decrease in zinc level in plasma, erythrocyte and hair. However, urinary zinc excretion was increased in sickle cell disease patients and is associated with decreased linear growth, skeletal growth, and muscle mass and sexual and skeletal maturation. . Information regarding zinc status in sickle cell disease in Sudanese subjects is needed.

The objective of this study was to determine the zinc status in patients with sickle cell disease in addition to other related parameters including Haemoglobin level, total white blood cells count, sickling test, and identification of malaria parasite in the blood in the patients and control group. Anthropometric measurements including body weight, body height, and body mass index were also conducted.

Study Design:

This is a case control study designed to study zinc status in patients with Sickle Cell Disease attending Wad Medani Paediatric Teaching Hospital in Gezira State.

Forty four patients diagnosed as sickle cell disease by clinical features and laboratory investigations (Hb Electrophoresis) were enrolled in this study. Fifty normal subjects were taken as control group. Patients with malnutrition and other diseases were excluded.

Results

In the present study the mean of serum zinc concentration in the patients group(40.8 ±20 µg/dl) was significantly lower than control group (55.3 ±32.4 µg/dl) ($P < 0.05$). Haemoglobin concentration in patients (6.6 ± 0.9 g/dl) was significantly lower ($P< 0.001$) compared to control group (11.1±2.1 g/dl). The result of this study also showed that the values of TWBCs count for the patients group (16150 ± 8196 cell / µL) was significantly higher ($P < 0.001$) compared to control group (5750 ± 3537 cell / µL). The mean body weight (16.3 ± 8.2 kg), body height (103 ± 26 cm), and BMIs (14.5 ± 1.9) in the patients were significantly lower compared to control group (23.7 ± 8.0 kg), (120 ± 17 cm), (16.1 ± 3.2) respectively ($P < 0.001$). All patients showed negative blood film for malaria parasite with the exception of only one subject, while three subjects of control group showed positive blood film for malaria parasite

Conclusion

Zinc status in patients with sickle cell disease in central Sudan is significantly lower than that of control group. Patients had lower hemoglobin level, lower body weight and height, and higher total white blood cell count than control group.

All patients showed negative blood film for malaria parasite with exception of only one subject, while three subjects of control group showed positive blood film for malaria parasite.

Because of low zinc level in patients with sickle cell disease, zinc supplementation is recommended. Further studies are needed to see its effect on the different parameters

Introduction.

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Sickle cell disease including the homozygous and heterozygous states of sickle cell gene, were reviewed by Greer and his colleagues, who reported that long before they were known in western hemisphere, sickling disorders were known in Africa by local names denoting the recurrent, unrelenting, and painful nature of the crisis[1].

Internationally, the mutation that results in hemoglobin S is felt to have originated in several locations of Africa and India. Its prevalence is variable, but very high in these countries, due to the survival advantage to heterozygote in regions of endemic malaria. As a result of migration, both forced and voluntary, it is now found worldwide. Recent data indicate that 15% of children born with sickle cell anemia die by the age of 20 years. Median survival age is 42 years for men and 48 years for women, which is 25-30 years lower than expected for the African American population. These data were generated prior to the institution of penicillin prophylaxis. Earlier diagnosis and improved supportive care are expected to substantially decrease mortality for children [2].

Haemoglobin is a conjugated protein composed of four subunits two α and two β-chains; each unit contains iron in the divalent ferrous, (Fe^{+2}) state[3]. The genes for the globin chains occur in two clusters [4]. The α gene complex is situated on the short arm of the chromosome 16 and includes the duplicated α genes (α_1 and α_2), anon-functional pseudo α gene ($4\alpha_1$) and two ζ genes, one of which is probably non-functional. The non-α gene complex is situated on the short arm of chromosome 11 and includesβ, and δ genes the duplicated γ genes (γ^G and γ^A), the ε gene and non-functional pseudo β gene ($4\beta_1$)[5].

Hb transports O_2 from respiratory organ to peripheral tissues. Acts as one of the buffers in blood, accounting for about 70% of its buffering power [3]. HbS differs from HbA in the substitution of valine for glutamic acid in the sixth position of the β-globin chain[5].

Hbs has poor solubility in deoxygenated state and can polymerize within the red cell leading to its characteristic shape, rigid membrane, generation of oxidant substances and abnormal adherence of the red cells to vascular endothelium[6] Sickle cell is inherited as an autosomal recessive[7].

Heterozygous state for the HbS gene, comprises 25-45% of the total haemoglobin, the rest being Hb-A, Hb-A_2 and Hb-F.

Sickle cell trait does not cause anaemia and in general is asymptomatic[5]. In vivo sickling occurs only at very high altitude and low oxygen pressures. Hematuria is the most common symptom owing to sickling in the renal papillae and is found in about 1% of people with sickle cell trait [6].

Homozygous Sickle cell anaemia (HbSS) is the most common type of the sickle cell disease[4].

The in vivo Sickling is responsible for the clinical manifestation of the disease, these are: chronic anaemia[1, 10, 11]. Organ damage and episodes of pain[1, 5,7,8,9].

Other variants of sickle cell, Hb-C disease, HbSE, and HSD Clinically they resemble homozygous sickle cell disease but are less severe and the patient are mildly anaemic. Most have a relatively normal life with only very occasional painful crisis. The electrophoretic pattern may be confused with that of homozygous sickle cell disease

Zinc is an essential trace element, it was found to be biologically important more than 100 years for the growth of certain bacteria.

In 1920s it was demonstrated to be required for the growth of experimental rats. But, it was not until early 1960s that zinc was shown to be an essential nutrient of man[12].

It is important in activating certain serum enzymes[13], needed for DNA synthesis, synthesis and metabolism of proteins. Acts as growth factor. Required for cell division[14], for development and functioning of reproductive system[12]. Supports a healthy immune system[15]. Needed for wound healing[16], and for normal taste and smell[17].

Zinc status in children with sickle cell disease was determined in 1975, the finding, showed that there was a significant decrease in zinc level in plasma, erythrocyte and hair, and however, urinary zinc excretion was increased in anemia patients[18].

In 1979 zinc status was studied in 47 children with homozygous sickle cell disease. Decreased hair and plasma zinc was demonstrated in these children and hyperzincuria was found in the older patients. This study indicates that zinc deficiency in patients with SCD is probably due to hyperzincuria[19].

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Results of controlled trial with zinc supplementation for 14-19 years old sickle cell anemia subjects who were retarded in growth showed that zinc supplementation significantly improved longitudinal growth and body weight of these subjects[20].

Zinc and Copper status in 57 patients with sickle cell anemia and in 45 control patients from eastern province of Saudi Arabia were studied. Plasma zinc and copper levels in patients were found to be close to those of the control subjects. Similarly, there was no difference neither in urinary zinc level nor in the ratio of Cu: Zn in patients and control subjects. This is in contrast to the situation which existed in North American black subjects with sickle cell anemia who were known to have zinc deficiency as well as a further decrease in zinc level during sickle cell crisis. The near normal level of zinc and copper found in Saudi sickle cell patients therefore exclude zinc deficiency and confirmed that this population exhibited a milder form of SCD[21].

In 1988 zinc status was studied in 80 children with sickle cell disease (SCD) and 44 disease free siblings control aged 3-18 years. They found, zinc status in SCD was not related to inadequate dietary intake. The origin of low serum zinc levels in children with SCD is most likely related to increased urinary zinc excretion, chronic intravascular hemolysis[22].

Copper, zinc and iron and their interrelations in SCD patients were studied, iron deficiency, hypercopperemia and low plasma zinc level foud are attributed to their short stature[23].

Leonard and his colleagues studied the relation of plasma zinc status to growth and maturation in children with sickle cell disease. The result showed that, decreased plasma zinc is common, and is associated with decreased linear growth, skeletal growth, and muscle mass and sexual and skeletal maturation [24].

Evaluation of zinc and anti-oxidant vitamins in patients with severe sickle cell anemia indicated that there was a significant decrease in plasma level of vitamin A, C, and E and in serum level of zinc on these patients. Serum copper levels were significantly elevated compared to control[25].

Although it has been known for more than 6 decades that zinc is essential for growth of micro-organisms, plants, and animals, it was believed that zinc deficiency in humans could never occur. It is now clear that nutritional deficiency of zinc is widely prevalent and its morbidities are severe.

Zinc deficiency leads to growth retardation; hypogonadism in males, cell-mediated immune disorder, impaired wound healing and reduced insulin function.

Information regarding zinc status in sickle cell disease in Sudanese subjects is needed.

Objective.

The main objective is to determine Serum zinc concentration in patients with SCD, and to study other related parameters likeWBCs count, Haemoglobin level, Weight and Height, BMIs in patients and control groups

SUBJECTS, MATERIALS, AND METHODS

Study Design and subjects:

This is a case control study designed to study zinc status in patients with Sickle Cell Disease attending Wad Medani Paediatric Teaching Hospital in Gezira State.

Forty four patients diagnosed as sicklers by clinical features and laboratory investigations (Hb Electrophoresis) were enrolled in this study. Fifty normal subjects were taken as control group.

Exclusion criteria: Patients with malnutrition and other diseases were excluded from this study.

Verbal consent was taken from the care takers of the study groups.

Materials:

Blood Sample:

Five ml of venous blood were drawn from each patient using disposable sterile syringe and divided into two parts; the first part 2 ml, was transferred to polyethylene container coated with anticoagulant (EDTA) and were used for haematological test and identification of malaria parasite. The remaining 3 ml was centrifuged. Serum was kept in -20c, analyzed for zinc at the National Research Centre using atomic absorption spectrophotometer. [27]

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Microscope was used for sickling test[26]. And identifying of malaria parasite[28].
Hb electrophoresis was used to distinguish between different variants of haemoglobin[26]. Haemocytmeter was used for TWBCs count[28]. Colorimeter was used for the estimation of Hb[26]

For each patient questionnaire was designed to get informations about age, sex and tribe.

Anthropometric measurements of study subjects:

All subjects were weighed on equilibrated portable balance scale[30].
Standing height was measured without shoes using a tape measure fixed to the wall[30]. Body Mass Index (BMI) was calculated).

Statistical Analysis:

Different variables were presented as mean ± standard deviation. Analysis of variance (ONE WAY ANOVA) was applied for comparison between different groups. The confidence limit was 95%, the P value was considered significant at a value less than 0.05.
Statistical analysis was done using SPSS program (ver. 9) under windows (XP) computer system.

Results

Forty four patients diagnosed as sickle cell disease (SCD) recruited from Wad Medani Paediatric Teaching Hospital, were selected in this study. Patients with negative sickling test and normal electrophoretic pattern of haemoglobin were excluded from this study.

The minimum age of study subjects was one year old, the maximum 17 years old and the mean was 6.0 ± 4.6. years. The highest incidence of disease was shown between the ages from 1 – 5 years (Table 1,). 28 (63.6%) were male and 16 (36.4%) were female. Of all patients only one patient had positive film for malaria parasite.

Table 1 Age and Sex in SCD patients

Sex	Male		Female		Total	
Age group	No	%	No	%	No	%
1 – 5 years	20	71.4	4	25	24	54.5
6 – 10 years	6	21.4	6	37.5	12	27.3
11 – 17years	2	7.1	6	37.5	8	18.2
Total	28	100	16	100	44	100

The studied subjects extended along 14 different tribes, about 58.5% of them belonged to three tribes, (26.8%) Hawsa, (17.1%) Bargo, and (14.6%) Selehab (Figure 1)

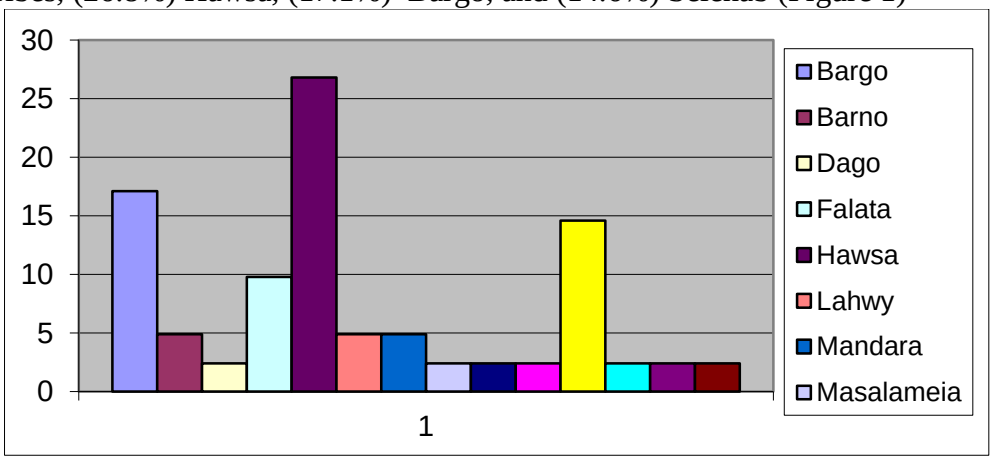

Figure (1): Distribution of the respondents according to their tribes

Zinc and Hb were found to be lower in patients compared with control group, while TWBCs counts were higher in patients than control (table2)

Table -2: Biochemical parameters in patients and control groups (Mean ± SD)

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Parameter	Patients		Control	
	No	Mean ± SD	No	Mean ± SD
Zinc µg/dl	44	40.8 ± 20.	50	55.3 ± 32.4*
Hb g/dl	42	6.6 ± 0.9	50	11.1 ± 2.1***
TWBCs(Cell/µl)	42	16150 ± 8196	50	5750 ± 3537.9***

No: number of subjects
*: significant at P < 0.05
***: significant at P < 0.001

Anthropometric measurements showed highly significant differences in the mean between the two groups.

Table -3: Anthropometric measurements in patients and control groups (Mean ± SD)

Parameter	Patients		Control		Significance
	No	Mean ± SD	No	Mean ± SD	
Weight(Kg)	40	16 ± 8	47	24 ± 8	S***
Height(cm)	40	103 ± 26	50	120 ± 17	S***
BMIs	40	15 ± 2	47	16 ± 3	S**

No: number of subjects
S**: significant at P < 0.01
S***: significant at P < 0.001

Discussion

In this study all patients were homozygous sickle cell anaemia patients. They belongs to Afro –Arab ,Afro-Asiatic ,and African tribes [34,35]Due to excessive haemolysis of sickled red blood cells ,all patients in study group are having significantly low heamoglobin as compared with the control .This results agrees with other studies in sudan[35,36,37]

Zinc deficiency has been known of 40 years but ignored by global health organization [22] .In patient with sickle cell disease zinc deficiency is well known from different studies[18,19,20].In Sudan there is no study looked at the level of zinc in patient with sickle cell disease. In the present study the mean serum zinc concentration in the patients group (40.8 ±20 µg/dl) were significantly lower (P < 0.05) compared to control group (55.3 ±32.4 µg/dl) and reported normal values. This is also in agreement with Phebus et al[22] finding in Pittsburgh who reported that serum zinc concentration of 77.8µg/dl of sickle cell disease patients versus (82.2 ± 9.8µg/dl). Our results also agree with those of Prasad [18] and Leonard et al[24] who attributed this low levels to increased urine excretion of zinc in sickler patient. Our result disagree with Alayash et al [21] from the eastern province of Saudi Arabia who found that plasma zinc level in patients were found to be close to those of control subjects, and attributed this to mild form of sickle cell anaemia exhibited by the patients.

The result of this study showed that the values of TWBCs for the patients group was significantly higher as compared to control group (P < 0.001).which agrees with studies done in Sudan[36, 38] which showed increased incidence of bacterial infections as compared with normal children. The same study showed partial protection against malaria in children with sickle cell disease, it agrees with our finding as we observed that only one patient has a positive blood film for malaria..

The mean body weight and height in the patients were significantly lower as compared to control group (P < 0.001), and standard reference value for BMI[32.] This is in agreement with study in Sudan and others[33, 38] which can be explained in the context of zinc deficiency.

Conclusions

It can be concluded from this study that:

- Zinc status in patients with sickle cell disease in central Sudan is significantly lower than that of control group.
- Patients had lower haemoglobin level, lower body weight and height, and higher total white blood cell count than control group.

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- All patients showed negative blood film for malaria parasite with exception of only one subject, while three subjects of control group showed positive blood film for malaria parasite.

Recomendations

Because of poor zinc status in patients with sickle cell disease, zinc supplementation is recommended. Further studies are needed to see its effect on the different parameters.

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