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Micronutrients status along with hematological and biochemical parameters in sickle dubtypes: preliminary report from India

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RESUMEN

Antecedentes:
Objetivo: evaluar las concentraciones de varios micronutrientes en pacientes con anemia drepanocítica y estudiar los parámetros bioquímicos y hematológicos asociados con la gravedad de la enfermedad.
Material y método: estudioÖ.. al que se incluyeron 25 pacientes con drepanocitosis y 30 sujetos controles. El diagnóstico de drepanocitosis se hizo por cromatografía líquida de alta resolución. En todos los pacientes y controles se analizaron las características clínicas y los parámetros hematológicos. Se midieron la cifras de glucosa, bilirrubina total, proteínas totales, transaminasas, fosfatasa alcalina, proteína C reactiva y los micronutrientes: cinc, selenio, cobre, vitamina A y vitamina E. Los pacientes con drepanocitosis se sublasificaron en tres grupos: heterocigotos (n = 6), homocigotos (n = 8) y dobles heterocigotos para hemoglobinopatía S y talasemia beta (n =11). Las transaminasas hepáticas se encontraron elevadas, principalmente la AST. En los pacientes homocigotos la proteína C-reactiva, fosfatasa alcalina y glucosa se encontraron elevadas, y las concentraciones de cinc, selenio y vitamina A estuvieron significativamente disminuidas. Las concentraciones de vitamina E disminuyeron en los pacientes dobles heterocigotos.
Conclusiones: las concentraciones de varios micronutrientes están disminuidas en los pacientes drepanocíticos de la India y asociadas significativamente con parámetros hematológicos y bioquímicos relacionados con la gravedad del padecimiento
Palabras clave: micronutriente, SCD, talasemia beta, dobles heterocigotos

ABSTRACT

The objective of the study was to estimate the level of various micronutrients in sickle cell patients and to study the various biochemical and haematological parameters associated with the disease severity**.** Twenty five sickle cell patients and thirty controls were selected as the subjects of this study. The patients were diagnosed by HPLC. The clinical features and haematological parameters were recorded for all the patients and controls. Glucose (random), Total Bilirubin,Total protein, AST, ALT, Alkaline phosphate and CRP had been evaluated along with micronutrients; zinc, selenium ,Cu, vitamin A and Vitamin E in all the subjects.Sickle cell patients were sub typed into three groups sickle trait (N=6), sickle homozygous (N=8) and Sickle beta thal (N=11). Key enzymes of liver functioning (AST and ALT) were elevated while AST was statistically significant but ALT was non- significant (Pvalue- 0.4598). CRP, Alkaline phosphatase and random glucose levels were increased in sickle homozygous patients. Zn, Sel and Vita-A were significantly lower in sickle homozygous patients. Vitamin E was significantly reduced in sickle beta thal patient [0.418(0.327-0.756)]. Thus it was concluded that the levels of various micronutrients (Zn, Cu, Sel, Vita-A & E) were reduced in Indian SCD patients and associated significantly with the biochemical & hematological parameters and enhance the disease severity.
Key words: Micronutrient, SCD, Sickle beta thalassemia, Sickle homozygous

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Micronutrients are chemical entities, which include minerals and vitamins, required in trace amount in living organisms for maintaining good health. The deficiency of these micronutrients results in varying the severity of various disorders. Sickle cell disease (SCD) is such an inherited hemoglobin disorder characterized by sickled red blood cells in which the severity markedly increases due to micronutrient deficiency. Blood levels of several vitamins and minerals are often low in individuals with SCD, including vitamin A and carotenoids, vitamin B6, vitamin C, vitamin E, magnesium and zinc.[1-8] These deficiencies cause a significant depreciation in blood-antioxidant status in these patients and the resulting oxidative stress may precipitate vaso-occlusion related acute chest syndrome.[9-10] Studies indicate that vitamin-mineral supplements of certain nutrients (vitamins C and E, zinc, magnesium) or treatment with a combination of high-dose antioxidants can reduce the percentage of irreversibly sickled cells.[5,11-14] Zinc is known as an important nutrient for growth and development. A deficiency of zinc in patients with sickle cell disease results in growth retardation (dwarfism), hypogonadism in males, rough skin, poor appetite, mental lethargy and recurrent infections.[7] Prasad et al. first reported zinc deficiency in adult patients with sickle cell disease.[15]

The biological importance of iron is well known in the synthesis of hemoglobin. Copper is known to be essential in the proper functioning of different metalloenzymes, which include ceruloplasmin involved in iron metabolism whereas its deficiency is known to cause anemia.[16] Certain minerals, copper, iron, magnesium selenium as well as some antioxidants and vitamins (C, E, folate, B6, B12) have been found to effectively relieve the oxidative stress that prevails in SCD.[17-19]

The pathology of sickle cell hemoglobin is considerably variable. The clinical expression of HbS is reported to be variable even within the same population. The effect on hematological and biochemical parameter values is also variable and is influenced by the severity of the disease and the occurrence of the crisis. In the heterozygous state, the presence of HbS in low concentration in red cells does not result in the sickling phenomenon under normal conditions and so the carriers of HbS are generally asymptomatic with normal hematological parameters values. The homozygous state has been defined as an incapacitating disease in which the hematological and several biochemical parameter values are abnormal.[20-22]

Acute phase reactants such as C-reactive protein (CRP) is one of the important biochemical parameter which is moderately increased in stable symptom free sickle patients and significantly increased during painful vasoocclusive crisis and C-reactive protein (CRP) indicated inflammation.[23-25] Serum bilirubin levels is markedly increased due to increased rate of breakdown of hemoglobin and serves as an important biochemical indicator of SCD. In addition, hepatic dysfunctions may occur due to sequestration of the liver by the sickled cells. Bone infarction due to vasocclusion of the capillaries is a common finding and is associated with bone pains in SCD which results in increased alkaline phosphatase level due to constant damage repair by osteoblasts.[26,27] The rate of glucose consumption and pyruvate and lactate production in red blood cells of normal, sickle cell trait and SS disease subjects were measured and Glucose consumption was found to be increased significantly in red cells of sickle cell patients than the sickle cell trait and normal persons.[28] Aspartate aminotransferase (AST) and Alanine aminotransferase (ALT) levels are reported to be elevated in SCD patients with liver enlargement. In Indian scenario there are very few reported literature on the subject of micronutrient status in sickle cell patients and its relationship with hematological and biochemical parameters so in this study it was aimed at finding the levels and status of these micronutrients in Indian SCD patients.

MATERIAL AND METHOD

Sickle cell Patients attending haematology OPD (Out Patient Department), All India Institute of Medical Sciences, New Delhi for routine follow-up were selected as subject of the study after getting the signed consent form. Twenty five sickle cell patients which were diagnosed by High Performance Liquid Chromatography (HPLC) along with 30 healthy individuals were selected as the control for the study. Control population were chosen from the healthy relatives of patients had never diagnosed any disease. All the patients and control were adults with similar age group. Duration of the study was one year.

Venus Blood samples were collected in 8 ml anticoagulant free glass tube and serum was separated 2 ml blood was collected in EDTA vials for Complete blood count

and red cell indices were measured by automated Analyzer (SYSMEX K-4500, Kobe Japan) using Transasia diagnostic kit. Quantitative assessment of hemoglobin variants; HbF, HbA, HbA2, and HbS was performed by HPLC (Bio-Rad-Variant™ from Bio Rad, CA, USA) using Bio-Rad diagnostic kit. All biochemical investigation were done by modular-P-800 auto analyzer from Roche (USA) using Roche diagnostic kit. Serum samples were diluted 50 folds using 1% nitric acid for the analysis of zinc, copper and selenium by Thermo Scientific ICPMS X-Series -2 (Bremen, Germany). Gallium was used as the internal standard and seronorm trace element serum (Level 1 and 2) was used as the reference material for ensuring accuracy of analysis. Analysis of Vitamin A and E was carried out on LC-6AD Binary Gradient HPLC System (Shimadzu, Kyoto, Japan) using the Bieri et. al method.[29] CRP was analyzed using Immulite-1000 (Siemens, USA).

Statistical analysis were done by Pearson chi square and Fisher's exact test. P- values below 0.05 were considered as statistically significant.

All haematological and biochemical parameters were evaluated in the department of haematology while micro nutrient parameters evaluated in the ICMR reference laboratory campus -2.

RESULTS

Twenty five sickle cell patients were subtyped, out of which 6 were sickle trait [HbS=37.81(10-45.2)], 8 sickle homozygous [HbF =8.8(6-14.6) and HbA2= 2.65(1. 9-3)] and 11 were sickle beta thal [HbF= 22.5(20-39.7) and HbA2 = 4.5(3.3-6.8]. Patients were different states from India i.e Karnataka (6), Bihar (5), Orissa (4) Madhya Pradesh (4) Jharkhand (3) and Delhi (3). In study design we distributed them in 4 group, group -1 sickle trait, group 2- sickle homozygous, group -3 sickle beta, group -4 healthy control. We evaluated hematological, biochemical, micronutrient and clinical parametrs in all above mentioned groups.

The demographic variables are given in table 1, which showed weight and height to be statistically significant. (P = 0.0003 and 0.0079 respectively)

The biochemical variables (Table 2) showed that random glucose level (104 g%) was high in sickle homozygous. Total Bilirubin (2.2 mg %), Aspartate aminotransferase (AST = 68 IU) and Alanine aminotransferase (ALT 37 I.U.) was high in sickle beta thal patients but ALT was non-significant (P = 0.4598) whereas AST and total bilirubin were statistically significant. (P = 0.0012 and 0.0001 respectively). Alkaline phosphate (367 IU) was high in sickle homozygous patients among all the groups and the P-value was in the border range (0.05). C-reactive protein (CRP) (10.96 mg/L) was significantly increased in sickle homozygous patients. (P=0.0292)

Hematological parameters were evaluated in all patents and control group (Table-3). All the parameters were statistically significant except MCH, MCHC and WBC. HCT value was similar across all the patient groups. Low RBC levels were seen in sickle homozygous (3.365 millions/µL) and sickle beta thal (3.3 millions/µL). Similarly, Hemoglobin level were also low in sickle homozygous (9.1 g/dL) and sickle beta thal (9.1 g/dL). MCV was lowest in sickle beta thal (70.5 fl) whereas MCHC value was highest in sickle beta thal (33.1 g/dL). Lowest platelets were recorded in sickle beta thal (117 Ths/µL).

Micronutrient variables (VIT-A, VIT-E, Zn, Sel and Cu) were evaluated in all the groups and the results are tabulated in Table 4. All the variables were statistically significant except Cu in the patient group. Vitamin A (18.105 µg/dL), Zn (519.7 µg/L) and Selenium (60.885 µg/L) were low in sickle homozygous patients whereas Vitamin E (0.418 mg/dL) was lowest in sickle beta that patients.

Various clinical features associated with sickle cell disease were recorded for all the patients. (Table 5) Anemia and pain (33.33 %) were the main clinical findings among the sickle trait patients whereas two patients had normal profile. Half of the Sickle homozygous patients were having painful episode and fever as the major clinical finding whereas three patients were reported to have anemia. In the third group, the most important findings were splenomegaly and jaundice affecting 45.45% of these patients. None of the controls showed any clinical symptoms associated with the sickle disease.

Three (27.27%) of the sickle beta and two (25%) Sickle homozygous patients received multiple blood transfusions. None of the sickle trait patient received blood transfusions. Four (50%) Sickle homozygous patients and four (36.36%) sickle beta patient did not receive blood transfusion.

Table 1. Demographic variables

Features	*Median (range)*				*P-value*
	Group-1 (N=6)	*Group-2 (N=8)*	*Group-3 (N=11)*	*Group-4 (N=30)*	
Age(Yrs)	25(15-38)	22(18-32)	21(17-35)	26(15-40)	0.0859
Sex M	3(50)	7(87.50)	7(63.64)	18(60)	
F	3(50)	1(12.50)	4(36.36)	12(40)	
Weight (Kg)	50(45-67)	49(40-61)	45(40-65)	60(40-70)	0.0003
Height (Cm)	162.5(130-170)	162.5(130.5-180)	155(140-170)	170.5(150-177.5)	0.0079

Table 2. Biochemical variables

Features	*Median (range)*				*P-value*
	Group-1 (N=6)	*Group-2 (N=8)*	*Group-3 (N=11)*	*Group-4 (N=30)*	
Glucose (random) (gm %)	92(63-131)	104(80-130)	100(50-151)	68.5(28-106)	0.0003
Total Bilrubin (mg %)	1.2(0.6-1.9)	1.4(0.7-1.6)	2.2(1.1-6.1)	0.6(0.2-2.2)	0.0001
Total protein (gm%)	7.15(6.4-7.9)	7.25(5.9-7.7)	7.1(6.2-7.8)	7.4(6-7.9)	0.9067
AST (IU)	58.5(24-102)	45(33-86)	68(26-111)	34(12-72)	0.0012
ALT (IU)	32(20-55)	28.5(17-59)	37(16-66)	28.5(7-50)	0.4598
Alkaline Phosphate (IU)	301(104-628)	367(178-596)	336.5(169-792)	224(139-346)	0.0500
CRP (mg/l)	0.69(0.379-1.5)	10.96(0.3-17.4)	3.15(0.427-9.78)	0.683(0-1.2)	0.0292

Table 3. Hematological variables

Features	*Median (range)*				*P-value*
	Group-1 (N=6)	*Group-2 (N=8)*	*Group-3 (N=11)*	*Group-4 (N=30)*	
WBC (Ths/µl)	7.4(5.3-11.5)	9.2(4.321.1)	7.4(3.9-20.13)	8.39(3.94-12.75)	0.4431
RBC (millions/µl)	4.18(4.26-6.02)	3.365(2.58-3.82)	3.3(2.11-5.59)	4.99(3.96-7.78)	0.0001
HGB (g/dl)	12.8(10.1-15.1)	9.1(4.7-13.1)	9.1(5.6-12.3)	14.45(13.3-17.2)	0.0001
HCT (%)	28.55(24.3-42.70)	28.6(20.6-38.6)	28.3(17.8-35.2)	47.15(29.7-56.1)	0.0001
MCV (fl)	82.15(64.6-90.8)	73.4(13.6-97.6)	70.5(25.6-85.3)	95.45(80.5-100)	0.0001
MCH (pg)	26.75(25.3-30)	30.75(20.4-42)	26.1(20.6-86.2)	29.55(26.9-32.8)	0.1748
MCHC(g/dl)	31.55(25.3-33)	31.45(26.1-39.9)	33.1(30.3-39.9)	29.9(33-35.3)	0.0212
PLT(Ths/µl)	235(133-330)	187.5(84-330)	117(46.280)	245(74-402)	0.0072

DISCUSSION

This study was a preliminary investigation of the valuable micronutrient status in association with hematological and biochemical parameter's that affects the clinical manifestation in sickle cell patients.

The demographic variables showed that the height and weight of the sickle cell patients were significantly lower when compared to control, which is well known.[7] Next we looked at the biochemical parameters in which sickle beta that patients had high levels of the key enzymes AST and ALT, which helps in finding out the normal functioning of

Table 4. Micronutrient variables

Features	*Median (range)*				*P-value*
	Group-1 (N=6)	*Group-2 (N=8)*	*Group-3 (N=11)*	*Group-4 (N=30)*	
VIT-A (µg/dl)	25.825(12.68-45.58)	18.105(9.37-36.24)	21.69(12.34-45.58)	30.445(17.09-74.25)	0.0388
VIT-E (mg/dl)	0.621(0.393-0.816)	0.703(0.409-0.756)	0.418(0.327-0.756)	0.799(0.504-1.201)	0.0001
Zn(µg/l)	546.3(442.8-582.4)	519.7(402.4-1077)	638.8(574.7926.3)	637.35(486.6-1033)	0.0077
Sel(µg/l)	61.125(48.69-82.28)	60.885(35.45-92.76)	74.24(52.37-105.2)	88.03(48.7-130.1)	0.0045
Cu(µg/l)	1005.5(770.4-1608)	887.45(686.7-1239)	895.2(817.2-1610)	946.8(359.6-1251)	0.6739

Table 5. Clinical findings

Clinical features	*Frequency (%)*				*Total (%)*
	Group-1 (N=6)	*Group-2 (N=8)*	*Group-3 (N=11)*	*Group-4 (N=30)*	
Anemia	2(33.33)	3(37.50)	2(18.18)	0	7(12.73)
Fever	1(16.67)	4(50)	2(18.18)	0	7(12.73)
Pain	2(33.33)	4(50)	4(36.36)	0	10(18.18)
Splenomegaly	0	1(12.50)	5(45.45)	0	6(10.91)
Jaundice	0	2(25)	5(45.45)	0	7(12.73)
Chest pain	0	1 (12.50)	2(18.18)	0	3(5.45)
Osteonecrosis	0	1 (12.50)	0	0	1(1.82)
G6PD	0	1 (12.50)	0	0	1(1.82)
Joint pain	0	1 (12.50)	0	0	5(9.09)
Weakness	0	1 (12.50)	1(9.09)	0	2(3.64)
Hyper – pigmentation	0	0	1(9.09)	0	1(1.82)
Hepato-megaly	0	0	2(18.18)	0	2(3.64)
Pallor	0	0	2(18.18)	0	2(3.64)
Stones	0	0	1(9.09)	0	1(1.82)
normal	2(33.33)	0	1(9.09)	30(100)	33(60)

liver. Since these patients have liver dysfunction, the levels of these enzymes are elevated. Total bilirubin was elevated in sickle beta thal as expected since the heavy burden of pigment resulting from increased red cell destruction lead to extreme hyper-bilirubinemia with SCD, when combined with acute hepatic damage or common bile duct obstruction. Under similar circumstances, increased haemolysis from any cause may lead to markedly elevated levels of serum bilirubin.[30] The Alkaline phosphatase was high in the sickle homozygous as bone complications are more common in this subtype of SCD. Alkaline phosphatase levels is considered as a sensitive marker of bone turnover and could be especially useful as valuable non-invasive biochemical marker for identifying sickle cell patients with bone complications.[31]

CRP was elevated in the sickle homozygous patients because these patients suffer from vaso-occlusion, which results in damage to the endothelial walls of the blocked capillaries, which initiates an inflammatory response leading to the increased levels of this protein.[32] This study mainly focused on the micronutrient levels in the various subtypes of SCD. Vitamin E levels were reduced in sickle beta thal patients due to higher consumption of this vitamin.[33, 34] Splenomegaly, jaundice, stones and chest pain followed by pallor, hyper-pigmentation and hepatomegaly were more commonly present in sickle beta thal patients

and may be associated with vitamin E. Supplementation with this vitamin has been shown to restore plasma vitamin E levels, improve clinical outcome, and reduce the number of irreversible sickled red cells.[18, 35-37]

Vitamin A was significantly reduced in all subtypes whereas sickle homozygous patients had the lowest level of this vitamin. Vitamin A Level has been reported to be low and associated with increased hospitalizations, poor growth and hematological status in patients with sickle cell disease. It is important to note that zinc is required for the synthesis of retinol binding protein (RBP), and the decreased level of zinc found in patients with Sickle cell anemia may affect the level of RBP and vitamin A in these patients despite adequate intake.[38-40]

In this study, zinc was significantly present in low levels in the sickle homozygous subtype. This may be attributed to the fact that zinc deficiency is associated with sickle cell anemia and appears to play a role in various aspects of the illness. The preliminary research has correlated low zinc levels with poor growth in children with sickle cell anemia.[41] Zinc supplementation is reported to decrease the number of infections in adults with sickle cell anemia.[42] Copper was reduced in the sickle homozygous subtype but when compared to controls its levels were not statistically significant. Alayash et. al have reported that zinc and copper levels in patients were found to be close to those of the control subjects which is similar to our finding, but a contrasting situation exists in North American Black subjects with sickle cell anemia.[43]

Selenium is another important micronutrient associated with SCD and it has been seen from earlier studies that the levels of selenium was significantly lower in SCD than those of controls.[44] This findings also show consistent decrease in selenium levels in all the subtypes of SCD.

Another important association of SCD is with the various hematological parameters, which help in assessing the severity of the disease. Red blood cell indices (MCV, MCHC and MCH) help classify types of anemia, a decrease in the oxygen carrying capacity of the blood. In this study, the results show that MCV is significantly decreased in the sickle beta thal patient, which is the characteristic feature of microcytic anemia. Patients with Sickle cell Anemia have reduced hemoglobin or hematocrit (HCT) levels which were observed in the results. The sickle homozygous subtype has increased WBC count. It is known that WBC contribute to SCD by adhering to blood vessel walls and obstructing the lumen, aggregating with other blood cells with more effective blockage of the lumen, stimulating the vascular endothelium to increase its expression of ligands for adhesion molecules on blood cells, and causing tissue damage and inflammatory reaction which predispose to vaso-occlusion.[45]

CONCLUSION

From this study it was concluded that the levels of various micronutrients (Zn ,Cu,Sel, Vita-A and E) were reduced in indian SCD patients. It was also found the biochemical and hematological parameters were associated significantly with the micro nutrients level and play significant role to modulate disease severity in SCD patients. A larger population study is required to confirm the preliminary finding in this study.

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