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Biochemical evaluation of a steady state sickle cell disease patient

Biochemistry

Apoorva V Shivankar — Jr. Resident, Department of Biochemistry, Government Medical College, Nagpur, Maharashtra, INDIA.

Archana Dhotre — Associate Professor, Department of Biochemistry, Government Medical College, Nagpur, Maharashtra, INDIA.

Manohar Muddeshwar — Professor and HOD, Department of Biochemistry, Government Medical College, Nagpur, Maharashtra, INDIA.

ABSTRACT

Sickle Cell Disease is an inherited hematological condition mostly seen in Central India, Africa, Mediterranean and Middle East region. This study is a comparative study between steady state sickle cell disease patients (100) and healthy controls (100) showing alterations in the nutrient levels of the diseased. Here the serum levels of Zinc, Copper, Calcium and Magnesium were estimated and compared within the groups. Results showed lower levels of serum zinc, calcium and magnesium whereas increased levels of serum copper in the diseased as compared to controls. Conclusively, assessment of these nutrient levels is vital as the supplementation with deficient elements and monitoring of the excess elements may reduce the severity of symptoms and complications associated with the disease, thereby improving the condition and survival of the diseased.

KEYWORDS

Sickle cell disease, Serum zinc, calcium, magnesium, copper, supplementation, monitoring.

INTRODUCTION:

Sickle cell disease is a chronic inherited autosomal recessive hemoglobinopathy without any known complete cure. It is characterized by the presence of sickle shaped red blood cells (RBCs) and presents with hemolytic anemia, increased susceptibility to infections and vaso-occlusive episodes leading to a reduced quality and expectancy of life.[1] Approximately 5% of the world's population carries trait genes for hemoglobin disorders, mainly, sickle-cell disease and thalassemia as stated by World Health Organization (WHO, 2006). According to WHO, over 3,00,000 babies are born per year worldwide with these hemoglobinopathies. The governing bodies of WHO have adopted special resolutions on hemoglobin disorders. The resolution on sickle-cell disease, from the 59th World Health Assembly in May, 2006 meeting of the WHO Executive Board, called upon affected countries and the Secretariat of WHO to strengthen their response to this condition. In addition, a resolution on the prevention and management of birth defects, including sickle-cell disease and thalassemia, was adopted by the 63rd World Health Assembly in May, 2010.[2] In sickle cell disease, hemoglobin, the red blood pigment contained in the erythrocytes, is the one to be affected. It is a protein whose major function is to transport oxygen throughout the body. A molecule of this hemoglobin is made up of two identical α chains and two identical β chains ($\alpha_2\beta_2$ tetramer). In this disease, a single base pair substitution occurs due to which Glutamic acid is replaced by Valine at β6 position of the genes coding for hemoglobin. Erythrocytes containing such mutated hemoglobin assume an irregular crescent like shape under stressful conditions like low oxygen concentration usually present in blood capillaries. It is these sickled red blood cells (RBCs) which are responsible for entire pathophysiology of the disease. Due to their sickled shape, smaller blood vessels are blocked and thus, the corresponding organs are devoid of oxygen supply leading to their impaired functioning. Also these deformed cells being fragile in nature undergo continuous hemolysis which along with the vaso-occlusive crisis is responsible for the generation of oxidative stress.[1,3,4] This sickling is precipitated by RBC dehydration which occurs due to altered RBC membrane permeability and dysregulation of the ionic homeostasis across the membrane. This ionic exchange across the membrane mainly occurs through– K^+- Cl^- co-transporters, Gardos (Ca++ dependent K+) channels and Psickle channels. Both K^+ - Cl^- co-transporters and Gardos channels cause potassium efflux from the cell. This K+ efflux is associated with loss of water causing dehydration. Also sickled cells possess certain non-selective Psickle channels responsible for univalent (Na+, K+, Rb+) and divalent cation (Ca^{2+}, Mg^{2+}) exchange under sickling environment (deoxygenation, polymerization, cell deformation).[5,6] As a result, the concentration of elements in the ECF and ICF may get altered. In addition to this, the hypermetabolic state of sickle cell disease increases the demand and consumption of minerals thus altering their levels.[7] Minerals like Zinc, Copper, Calcium and Magnesium are renquired in living organisms for normal functioning of cellular metabolic processes and maintaining good health. Any alteration in their levels due to altered ionic exchange or state of hypermetabolism as seen in sickle cell disease may aggrevate the disease manifestations. There is paucity of information about the role of these ions in the pathogenesis as well as management of sickle cell disease. Keeping this in mind, the present study was conducted to find out whether there is any difference in the serum levels of zinc, copper, calcium and magnesium between the adult steady state homozygous sickle cell disease patients and their age and gender matched normal healthy controls in an endemic area like Central India. Though some studies were already carried out in this direction, only a few of them were conducted in an endemic area like Central India and most of these studies were confined to the pediatric age group or the patients already in a crisis phase. In an endemic area like Vidarbha, a larger steady state sickle cell disease population belonging to an adult age group is also found. Present study aims at targeting this population for proper management and achieving a better result in the form of decreased sufferings, reduced complications and longer lifespan.

MATERIAL AND METHODS:

The present study was conducted in the Department of Biochemistry at a tertiary health care centre (GMCH, Nagpur) with the help of Medicine Department over a period of one and a half year.

Study design:

Hospital based cross sectional study with comparison groups.

Study population:

100 diagnosed and registered cases of homozygous sickle cell disease in a steady state belonging to an age group of 14 to 26 years were taken as cases and another 100 normal age and gender matched healthy volunteers as controls. All subjects of case population were attending the sickle cell outpatient department of the tertiary health care centre.

The study subjects were diagnosed as sickle cell disease (HbSS) cases on the basis of solubility test and hemoglobin electrophoresis.

Inclusion Criteria:

Registered cases of sickle cell disease (HbSS) in steady state, 14 to 26 years of age as study cases and normal age and gender matched healthy individuals as controls.

Exclusion Criteria:
Age < 14 years and > 26 years, heterozygous sickle cell anemia (HbAS),mixed types of sickle cell disease, other hemoglobinopathies, sickle cell patients in crisis phase, documented chronic infection, recent hospitalization, blood transfusion in a period of four months prior to blood sampling, other hemolytic disorders, hepatic or renal disorders, antibiotics or corticosteroids intake prior to blood sampling.

Specimen collection and preservation:
Under all aseptic precautions, about 5 ml of blood was collected in a clean plain bulb by venepuncture after taking due consent. The serum was separated by centrifugation. All the tests were performed on this serum. Serum Zinc, Calcium, Magnesium and Copper levels were estimated in the study by kit method on semiautoanalyser (ICON WP Semi-Automatic Analyzer). All the parameters were estimated on the same day of collection of the sample.

2.6. Parameters were estimated with methods as follows

Sr. No.	Parameters	Methods
1	Serum Zinc	Nitro PAPS colorimetric method
2	Serum Copper	Di-Br-PAESA colorimetric method
3	Serum Calcium	OCPC method Calmagite method
4	Serum Magnesium	Calmagite method

Statistical Analysis:
- Demographic parameters (age, height, weight), clinical parameters and biochemical parameters were presented as mean ± SD.
- Statistical data was recorded on Microsoft Excel programme 2007.
- Unpaired t-test was performed to compare demographic, clinical and biochemical parameters between homozygous sickle cell disease patients and control groups.
- Pearson's correlation coefficient (r) was calculated to assess the correlation between biochemical parameters.
- Tests were two sided. P value <0.05 was considered as statistically significant. The p value < 0.001 was considered as highly significant and the p value > 0.05 was taken as non-significant (NS).
- Graph Pad Prism version 6.0 was used for statistical analysis.
- Sample size calculated using Power and Sample Size Programme version 3.1.2.

RESULTS:

Demographic and anthropometric data
Cases and controls were matched for age and sex. They showed statistically significant difference in their height, weight and BMI

Table 1:

Parameters	Cases (100) [Mean ±SD]	Control (100) [Mean ± SD]	P value	Significance
Mean Age (years)	19.59 ± 3.18	19.35 ± 3.57	0.6164	not significant
Height (m)	1.59 ± 0.09	1.63 ± 0.08	0.0012**	significant
Weight (kg)	42.51 ± 7.83	47.6 ± 9.08	<0.0001***	significant
BMI(kg/m^2)	16.67 ± 1.90	17.71 ± 2.41	0.0009***	significant

Vital parameter Hemoglobin
Hemoglobin levels were significantly lower in sickle cell disease cases

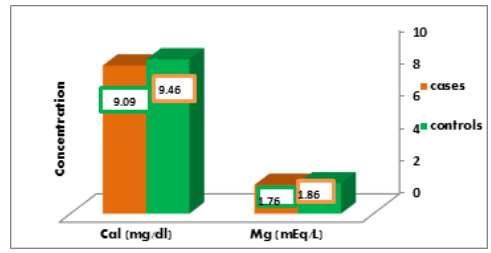
Table 2:

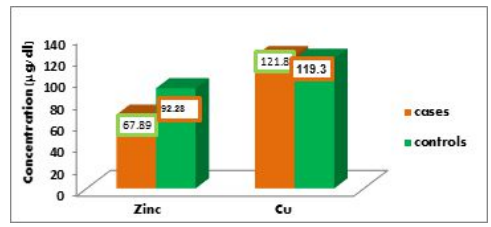
Vital parameter	Cases [mean ± SD] gm/dl	Controls [mean ± SD] gm/dl	p-value	Significance
Hemoglobin	8.12 ± 0.87	12.23 ± 2.04	<0.0001***	significant

Biochemical Parameters:
Serum zinc, calcium and magnesium levels were reduced whereas serum copper levels were increased in patients as compared to controls.

Table 3:

Analytes	Cases [mean ± SD]	Controls [mean ± SD]	p-value	Significance
Se. Zinc [µg/dl]	67.89 ± 11.25	92.28 ± 9.44	<0.0001***	significant
Se. Copper [µg/dl]	121.8 ± 6.49	119.3 ± 9.62	0.027*	significant
Se. Calcium[mg/dl]	9.09 ± 0.96	9.46 ± 1.02	0.0093**	significant
Se. Magnesium [mEq/l]	1.76 ± 0.29	1.86 ± 0.37	0.04*	significant



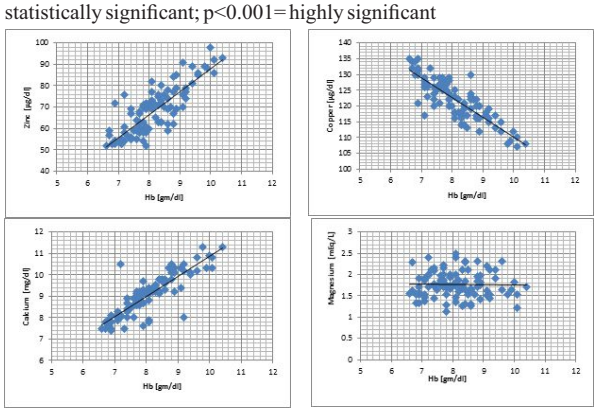


Correlation of severity:
When serum zinc, copper, magnesium and calcium levels in cases were correlated independently with hemoglobin levels, zinc and calcium levels showed a significant positive correlation whereas copper levels showed a significant negative correlation. No significant correlation was found between hemoglobin and serum magnesium levels.

Table 4:

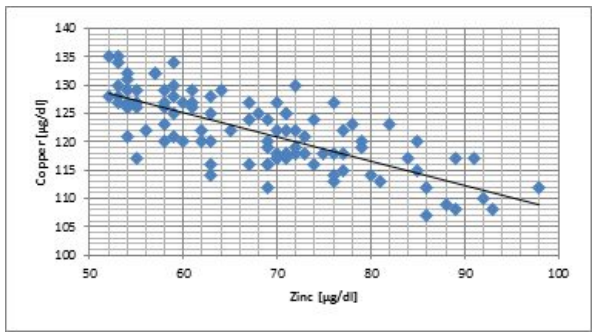
Parameter	Haemoglobin	Signicance
Se. Zinc	r = 0.83, p = <0.0001***	significant
Se. Copper	r = -0.83, p = <0.0001***	significant
Se. Calcium	r = 0.85, p = <0.0001***	significant
Se. Magnesium	r = -0.02, p = 0.79	not significant

Where **r** - Karl Pearson correlation Co-efficient (-1 to +1), p<0.05= statistically significant; p<0.001= highly significant



A significant negative correlation was also observed between the levels of serum zinc and copper of the patients.

Parameter	Serum Copper [µg/dl]	Significance
Serum Zinc [µg/dl]	r = -0.7444, p <0.0001***	Significant



DISCUSSION:

Considering the fact that sickle cell disease is endemic in Central India, present study was conducted to estimate the levels of serum zinc, copper, calcium and magnesium in adult homozygous sickle cell

disease patients (14 years – 26 years of age) in a steady state here along with their correlation with the hemoglobin content in the diseased. Results showed significantly decreased levels of serum zinc, calcium and magnesium whereas increased copper levels in the diseased population with significant correlation between hemoglobin and zinc, calcium and copper levels. Also, a significant negative correlation was found between serum zinc and copper levels.

Zinc:

Zinc is an important constituent of erythrocytes. Chronic hemolysis seen in sickle cell disease causes increased release of zinc in the plasma pool resulting in its excessive filtration at the glomerulus. However, its reabsorption from the renal tubules is hampered owing to the renal tubular damage caused by repetitive vaso-occlusive episodes leading to zincuria and lowered serum zinc levels.[8-10]Zinc is known to protect the body against oxidative stress by inhibiting lipid peroxidation in erythrocytes and liver and thus stabilizes biomembranes.[9,11] Also being an important cofactor for the enzyme Superoxide Dismutase,zinc plays a significant role as an antioxidant. Thus, the level of serum zinc further decreases along with other antioxidants because of its consumption by the pro-oxidant phenomenon of sickle cell disease.[4,9,11-13] Significant lower levels of serum zinc may also be due to an increased demand and consumption of zinc for accelerated hematopoiesis and tissue repair seen in sickle cell disease.[9,11] In addition to the above causes chronic inflammation, increased protein turnover, urinary zinc losses, inadequate zinc intake because of a poor diet, inadequate net intestinal absorption and an increased intracellular shift of zinc into liver[9] are also suggested as some of the causes for zinc deficiency in sickle cell disease patients.[9,14-16]In addition to these, zincuria induced by hydroxyurea (drug used in management of sickle cell disease)is also reported as a possible cause of zinc deficiency by Silliman *et al*. (1993).[4,17-19] This deficiency of zinc in patients might be a cause of growth retardation (dwarfism), hypogonadism in males, rough skin, poor appetite, mental lethargy, male infertility and recurrent infections often seen in diseased patients.[7] Zinc is also known to possess an antisickling phenomenon exerted by its anticalcium action through its effect on calmodulin. Increased intracellular calcium is responsible for sickling. This intracellular entry of calcium is controlled by calcium pump located on RBC membrane which itself is regulated by calmodulin. Zinc is known to inhibit the activity of this calmodulin. As sickling presents with zinc deficiency, this calmodulin gets so overactivated that the membrane is destroyed causing excessive entry of calcium inside the cell. It is suggested, therefore, that supplementation of zinc, by its anticalcium action can thus prevent sickling (through its effect on calmodulin).[7,12,13] Also, Brewer and Oelshlegel discovered that calcium binding to red cell membrane is responsible for the formation of irreversible sickled cells and that the antisickling effect of zinc in sickle cell anemia is due to its ability to antagonize calcium binding to red cell membrane.[9] This antisickling effect along with the zinc deficiency seen in the diseased supports the role of zinc supplementation in the management. Our findings were also in agreement with the work done by Nnodim et al. (2014),[4] Temiye et al. (2011),[9]Arinola et al. (2008),[11] Bot et al.(2013),[18] and Idonije et al. (2011).[19]

Copper:

Our study showed an increased level of serum copper in the patients. Studies conducted by Nnodim et al. (2014)[4] and Bot et al. (2013)[18] also showed significantly elevated copper levels in sickle cell patients. Metallothionein, a copper-binding protein, traps copper within intestinal cells, blocking its absorption in blood. Zinc is needed for the production of this metallothionein. Zinc is also known to compete with copper for common site of binding on proteins as both have similar valence states.[18,20] As sickle cell disease is a state of zinc deficiency, lesser amount of metallothionein is produced resulting in lesser copper trapping and thus elevated serum copper levels in these patients.[12,18,20,21] This indicates that an elevated copper level may be secondary to the zinc deficiency found in sickle cell disease patients. This elevated level may lead to free radical production, oxidative damage aggravating sickling, forms deposits and generalised symptoms suggesting the need of monitoring the levels regularly.[12,22] As absorption of copper is dependent on zinc levels, a correlation study was performed between serum copper and serum zinc levels in the present study and the results found were showing a significant negative correlation supporting an antagonistic effect of zinc on copper absorption as a possible cause of copper excess.

Calcium:

(Ca^{2+} + Mg^{2+})-ATPase pump of sickled cells show a lower response to its activator, a cytoplasmic protein present in RBCs. This reduced activation of (Ca^{2+} + Mg^{2+})-AT Pase leads to decreased efflux of calcium from the sickled cell causing cellular calcium accumulation and thus hypocalcemia.[23]Again this increased intracellular calcium activates Gardos channel causing potassium efflux and thus precipitates sickling.[24] Under sickling environment, the non-specific P selective channels are activated and they bring about calcium influx from ECF to within the cell. This abnormally high cation permeability in red blood cells (RBCs) from patients with sickle cell disease (SCD) occupies a central role in pathogenesis of hypocalcemia.[5,6] An increased calcium binding affinity of the sickled red cell may also contribute to the diminished calcium levels in serum.[25] Zinc and Magnesium[26] deficiency may also cause hypocalcemia via (Ca^{2+} + Mg^{2+})-ATPase. Studies indicate that supranormal PTH, and impaired intestinal absorption of calcium and vitamin D in sickle cell patients might be responsible for the lowered calcium concentration and the skeletal changes seen in the diseased.[25,27]. The skeletal changes due to hypocalcemia necessitate its supplementation to the diseased. Oladipo et al. (2005),[25] have also documented reduced serum calcium levels in sickle cell disease patients in their studies.

Magnesium:

In this study, magnesium levels were found to be significantly lower in sickle cell disease patients. Results of our study corroborated with the studies conducted by Nnodim et al. (2014),[4] Idonije et al. (2011)[19] and Zehtabchi et al. (2004).[24] Certain studies suggest genetic errors in magnesium metabolism, diminished dietary consumption, increased renal losses,[24]increased clearance, excretion fraction, excretion rates of magnesium in sicklers[28] as the supposed causes for hypomagnesemia. *Activation of* P_{sickle} channels in the diseased may also promote Mg^{2+} depletion.[5]Magnesium inhibits K^+ - Cl^- co-transporters. Thus, magnesium deficiency leads to uninhibited overactivity of these transporters causing potassium efflux (disinhibition of K^+–Cl^- cotransport), thus cell dehydration, sickling and polymerization.[4,12,24] Reducing this deficiency by supplementation can thus decrease sickling.

CONCLUSION:

- In the present study the following findings were concluded
- The altered serum levels of these nutrients signify their role in the pathophysiology of sickle cell disease and indicate towards the additional ways to improvise the existing management of this incurable disease.
- Supplementation of Calcium, Magnesium and Zinc may improve the bone marrow density, hydration status as well as the physical manifestations of patients respectively.
- Serum Copper levels should be routinely monitored to prevent complications due to excess.
- Thus, these nutritional management alternatives can serve as additive measures of decreasing morbidity, improving the quality of life and reducing the economic burden of the patients. Still,further studies on changes in the intracellular concentrations of these analytes in this disease are needed for designing a better management strategy.

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