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Original Article

Biochemistry Section

Evaluation of Serum Zinc and Antioxidant Vitamins in Adolescent Homozygous Sickle Cell Patients in Wardha, District of Central India

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ABSTRACT

Introduction: Sickle cell anaemia is a condition characterized by haemolytic and vaso-occlusive crisis. Previous studies in different part of the world have reported deficiency of zinc, vitamin C and E but the role of their supplementation in sickle cell disease remains question. Nutritional factors may contribute to clinical manifestation in rural population of developing countries specially in adolescent age group. Thus, the present study was designed in rural population of Wardha district of Maharashtra in adolescent sickle cell homozygous patients in view to evaluate serum zinc and antioxidant vitamins C and E.

Aim: To evaluate the serum zinc and antioxidant vitamins C and E in cases of adolescent homozygous sickle cell disease.

Materials and Methods: The study includes adolescent (between 10-20 years) individuals in two groups of 33 each. Group A included confirmed cases of Sickle Cell Disease (SCD) and Group B included age and sex matched normal healthy controls. Serum zinc, vitamins C and E were analysed in all the subjects of both the groups. Data were expressed as Mean±SD; unpaired t-test was used to compare the two groups. Statistical significance was decided by calculating the p-value.

Results: Serum levels of zinc and antioxidant vitamins E and C were significantly low in sickle cell anaemia patients when compared to normal health controls (p-value<0.001).

Conclusion: Our study shows that the adolescent patients with SCD have significant low levels of zinc and significantly low antioxidant vitamins C and E, which may contribute to some of the manifestations of sickle cell disease.

Keywords: Haemoglobin, Hyperzincuria, Trace element

INTRODUCTION

Sickle cell anaemia is a hereditary condition resulting from the substitution by a single amino acid valine for glutamic acid at sixth position of β chain of haemoglobin. This change leads to HbS polymerization, which on deoxygenation results in sickling. Patients with this condition often present with anaemia due to haemolysis and other complications like growth retardation, recurrent and painful vaso-occlusive episodes, acute chest syndrome and impaired immune function [1,2]. Prevalence of SCD in rural population of Wardha, district of Maharashtra (India) is found to be 4.6% [3].

Various studies have documented deficiency of zinc in patients with sickle cell disease [4,5]. Zinc is a micronutrient and is the most abundant trace element next to iron in nature. It is an important component of metalloproteins, and has a role in maintaining the cellular immunity, immunological integrity and antioxidant activity [6-8]. Patients presenting the symptoms with SCD show growth retardation, hypogonadism, mental lethargy, delayed wound healing, cell mediated immune dysfunctions, and abnormal neurosensory changes, which may be due to deficiency of zinc.

Various studies have reported imbalance between oxidant and antioxidant in patients with SCD, increase in MDA and decrease in antioxidants vitamins C and E [9-11]. Increased Reactive Oxygen Species (ROS) leads to haemolysis, ROS production is increased in hypoxia, infection and deficiency of antioxidants [5].

Adolescents are in a growth phase, so increased demand and low dietary intake may lead to manifestations of the disease. Thus, the present study was designed to evaluate serum zinc and antioxidant vitamins, C and E in patients with sickle cell disease.

MATERIALS AND METHODS

The present comparative cross-sectional study was conducted in Department of Biochemistry for the period of one year from 2016 to 2017, in association with Departments of Medicine, Paediatrics and Pathology of Jawaharlal Nehru Medical College and AVBRH Hospital, Sawangi Meghe, Wardha, Maharashtra, India. Total of 66 adolescents in the age group of 10 to 20 years were enrolled in the study based on the inclusion and exclusion criteria. The study comprised of two groups of 33 each (20 males and 13 female), Group A consisted of confirmed cases of sickle cell disease and Group B included age and sex matched normal healthy controls. Subjects with chronic ailments, like ischemic heart disease, diabetes mellitus, sickle cell disease cases with acute crisis and history of blood transfusion in less than three months and the patients on multivitamin supplementation were excluded from both the groups. Informed consent was obtained from all the subjects.

The study was approved by Institutional Ethical Committee.

Blood collection: Nearly 5 ml of blood was drawn by vein puncture under aseptic conditions, 2 ml in plain tube was allowed to stand for 30 min, centrifuged, serum separated for the assay of zinc, and vitamin E and 2 ml of blood was collected in another bulb of potassium oxalate, centrifuged and plasma separated for vitamin C assay and 1 ml of blood was collected in EDTA bulb for solubility test and electrophoresis.

Known cases of sickle cell diseases were re-confirmed by cellulose acetate Hb electrophoresis using Tris -EDTA borate buffer of pH 8.9 by Marengo –Rowe technique [12] and S band confirmation was done by Nalbandian solubility test [13]. Healthy controls were also screened for sickle cell disease.

Serum zinc was analysed by using commercially available diagnostic kit compatible with semi-automated analyser by Nitro PAPS methodology [14]. Zinc in an alkaline medium reacts with Nitro-PAPS to form a coloured complex whose intensity is directly proportional to the amount of zinc present in sample.

Vitamin C was measured by Aye kyaw method [15]. The Phosphotungstic Acid (PTA) used serves the dual purpose of a protein precipitant and ascorbic acid extractant colour developing agent where, PTA is found to be specific and sensitive for ascorbic acid determination in the plasma with good reproducibility. Vitamin E was analysed by Jargar JG et al., modified method of serum vitamin E estimation [16]. This method is based on previous Baker and Frank method [17] and the method of Martinek [18] by using 2,2'-bipyridyl, ferric chloride, and xylene. The complex of ferrous ions generated in this reaction with 2,2'-bipyridyl was determined by using a plain non-enzyme-linked immunosorbent assay microplate at 492 nm using (Robonik) ELISA reader.

STATISTICAL ANALYSIS

Data were expressed as mean±SD, unpaired t-test was used to compare the two groups and p-value less than 0.05 is considered to be statistically significant.

RESULTS

In our study, the subjects in both the groups were evenly distributed across the mean age matching well in both males and females. The average age group of male SCD patients was (in years) (14.9±3.35) and whereas (14.6±4.05) in female patients when compared to healthy controls male was (15±3.32) and (14.69±3.99) in female [Table/Fig-1]. Significant difference in serum zinc levels in SCD patients was observed (83.09±9.26) µg/dl when compared against healthy controls (104.06±6.27) µg/dl. Serum zinc concentration (in µg/dl) was also found to be significantly low in both male (84.3±9.56) and female (81.23±8.81) SCD patients when compared to male (104.2±7.13) and female (103.85±4.95) healthy controls [Table/Fig-2-4].

Antioxidant vitamins C and E were found to be significantly low in adolescent sickle cell anaemia patients, when compared with healthy controls. Vitamin C levels were found to be significantly low in male (0.873±0.179) and female (0.7± 0.143) SCD patients when compared with male (1.055±0.193) and female (0.99± 0.19) healthy controls. Vitamin E levels were significantly low in male (8.813±1.211) and female (8.43 ±1.014) SCD patients when compared with healthy male (11.902±1.662) and female (11.546±2.156) controls [Table/Fig-3,4].

DISCUSSION

In the present study, it was observed that the levels of serum zinc were significantly low in patients with sickle cell disease, compared with normal healthy control group. The results obtained are in agreement with the study by Hasanato RM [2].

Durosinmi MA et al., study supports our finding where they showed that the plasma concentrations of zinc are significantly low in sickle cell anaemia when compared to controls [19].

In developing countries like India, nutritional deficiency of zinc is caused by malabsorption syndrome compounded by intestinal infections. Some studies have documented hyperzincuria in patients with SCD [20], which was not a part of present study and zinc deficiency in sickle cell anaemia could partly be contributed by hyperzincuria. Since, zinc has a role in maintaining the cell mediated immunity, deficiency affects the growth and function of T and B cell.

In the patients with sickle cell disease there is an oxidant and anti-oxidant imbalance, zinc reduces the oxidative stress by induction of production of metallothionein which acts as a scavenger of $OH^{\cdot}$(hydroxyl ion) and suppress the production of inflammatory

Parameters	Adolescent homozygous sickle cell disease (n=33)		Healthy controls (n=33)	
Age (yrs.)	14.88 ± 3.54		14.82 ± 3.58	
Sex Distribution	Male (20)	Female (13)	Male (20)	Female (13)
Age (yrs.)	14.95 ± 3.35	14.62 ± 4.05	15.00 ± 3.32	14.69 ± 3.99

[Table/Fig-1]: Age and sex distribution of adolescent homozygous sickle cell disease and healthy controls.

Parameters	Adolescent homozygous sickle cell disease (n=33)	Healthy controls (n=33)	p-value
Serum Zinc (µg/dl)	83.09 ± 9.26	104.06 ± 6.27	p<0.001
Plasma Vitamin C (mg/dl)	0.829 ± 0.172	1.030 ± 0.194	p<0.001
Serum Vitamin E (µg/ml)	8.662 ± 1.137	11.762 ± 1.848	p<0.001

[Table/Fig-2]: Comparison of serum zinc, vitamins C and E concentration in healthy controls and adolescent homozygous sickle cell patients.

Parameters	Male Adolescent SS Patients (N-=20)	Male Healthy Control (N=20)	p-value
Serum Zinc (µg/dl)	84.3 ± 9.56	104.2 ± 7.13	p<0.001
Plasma Vitamin C (mg/dl)	0.873 ± 0.179	1.055 ± 0.193	p=0.0038
Serum Vitamin E (µg/ml)	8.813 ± 1.211	11.902± 1.662	p<0.001

[Table/Fig-3]: Comparison of serum zinc, vitamins C and E concentration in healthy male controls and male adolescent homozygous sickle cell patients.

Parameters	Female Adolescent SS Patients (N=13)	Female Healthy Control (N=13)	p-value
Serum Zinc (µg/dl)	81.23 ± 8.81	103.85 ± 4.95	p<0.001
Plasma Vitamin C (mg/dl)	0.7 ± 0.143	0.99 ± 0.19	p=0.0024
Serum Vitamin E (µg/ml)	8.43 ± 1.014	11.546 ± 2.156	p<0.001

[Table/Fig-4]: Comparison of serum zinc, vitamins C and E concentration in healthy female controls and female adolescent homozygous sickle cell patients.

cytokines by inhibition of NF-κB (nuclear factor κB) activation [2,10,20]. Thus, in patients with SCD disease, deficiency of zinc may be due to hyperzincuria and due to excessive haemolysis and excess excretion which results from oxidative stress [16] and also increased demand and less intake in adolescents.

Antioxidant vitamins C and E were observed to be significantly low in patients with SCD, when compared with healthy controls. Our finding is in accordance with the study by Gizi Anna et al., who showed significant reduction in antioxidant vitamin C and E in SCD patients on comparison with healthy controls [11].

A study by Ray D et al., found the significant low levels of vitamin C and E in both homozygous and heterozygous SCD patients when compared to healthy controls, which is in accordance with the results obtained in this study [21].

Our results are in agreement with study by Hasanato RM [2] and Rane Hundekar PS et al., which reported that vitamin C and E are significantly low in severe sickle cell disease patients when compared with healthy controls [2,22]. Factors contributing to deficiency may be due to inadequate intake, increased demand, and increased excretion [2].

Muskiet FAJ et al., done in Netherland concluded that supplementation of zinc, alpha -tocopherol, vitamin C, soyabean oil and fish oil had a effect on decreasing number of irreversible sickle cells attributed to zinc and zinc might influence heme synthesis, but still they did not found any change in haemoglobin and RBC turnover [23].

Arruda MM et al., in their study of supplementation with vitamins C and E in sickle cell anaemia patients of Brazil found no improvement in anaemia and despite the deficiency they did not recommended

supplementation [24].

Muskiet FAJ as well as Arruda MM studies done in the developed countries did not come to final conclusion for cause of deficiency of zinc, vitamin C and E in SCD patients and hence they did not recommended the supplementation of same [23,24]. Whereas, our study is done in developing part of India especially in rural area of Wardha district of India where the nutritional factors such as those associated with poor diet, liver and gastrointestinal disorders, and anorexia nervosa can lead to zinc deficiency [25].

The diets of patients from low socio-economic group can be low or partly deficient in vitamins C and E. Low levels of vitamins C and E may not be enough to combat the ROS. Thus, the effects of antioxidants deficiency may be multiplied and manifested in the adolescent SCD patients, particularly because of altered oxygen metabolism.

LIMITATION

1. Limited sample size.
2. Enzymatic antioxidant not analysed.
3. Oxidant parameters not assayed.

CONCLUSION

Thus, the present study shows patients with SCD have low levels of zinc, which may be due to the increased demand during adolescent age and low intake or absorption resulting from disease, which in turn contribute to some of the manifestations of SCD and significantly low antioxidant vitamins C and E the reasons for which need to be affirmed in developing rural areas where situation is altogether different from developed countries and supplementation can be tried.

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