Association of serum MDA with Magnesium, Calcium and Zinc levels in thalassemic and healthy children

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Abstract

Introduction: The thalassemias are group of haemoglobin disorders, initially found in 1925 in United States and Italy. It is the genetic disorder which passed down through families. In this disease deficient haemoglobin formation occurs which leads to gross destruction of R.B.C., subsequently leads to anaemia. The treatment of thalassemic patients involves mostly blood transfusion, lead to accumulation of excess iron in the body tissues. This secondary iron overload is responsible for peroxidative damage by increased production of reactive oxygen species within the erythrocytes leading to oxidative stress. This increased oxidative stress will cause damage of many organs including lungs, liver and brain.

Materials and Method: This study was conducted in Department of Biochemistry with collaboration of Dept. of paediatrics S.P. Medical College and attached Hospital, Bikaner. Samples taken for study includes total 100 subjects, including 50 thalassemic and 50 healthy children as controls with age groups from 3 to 14 years. Serum malondialdehyde (MDA), Mg, Ca and Zn were estimated by using standard enzymatic kits and instruments.

Results: The mean ± SD of serum MDA, Magnesium, calcium and zinc levels were found 8.99±1.33 nmol/ml, 2.04±0.22 mg%, 9.54 ± 0.53 mg% and 105.11±9.89 µg% respectively in healthy children and 17.16±1.79 nmol/ml,1.19±0.14 mg%, 6.88±0.61 mg% and 61.76±5.44 µg% respectively in thalassemic children.

Conclusions: Children who were suffering from thalassemia disease, had increased level of MDA level and decreased levels of magnesium, calcium and zinc level with respect to healthy children. The inverse relation were found between MDA and magnesium, calcium and zinc levels in thalassemic subjects.

Keywords: Thalassemia, Malondialdehyde, Magnesium, Calcium, Zinc

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Introduction

The Thalassemias group of inherited autosomal recessive hematologic disorders that cause haemolysis leads to anemia because of the decreased or absent synthesis of a globin chain.(1) Imbalance of globin chains causes haemolysis and impair erythropoiesis.(2) It is found in homozygous and heterozygous state. The heterozygous state, is less severe and asymptomatic than homozygous.(3) About 5% globin disorders are found in total of the world's population, in that approximately 2% has alpha and beta thalassemic individuals. Thalassemia equally affects men and women.(4)

Major prevalence of thalassemia are found in South-East Asia and Mediterranean countries. In developing countries like in India, this disease increases in alarming rate due to lack of proper health care and knowledge and illiteracy. According to study by WHO, over all carrier frequency of thalassemias is 3–4% (3.9%) in India, which denote the current national population between 35.1 and 46.8 million carriers of the disorder nationwide.(5)

Patients suffering from this disease need repeated blood transfusion for survival. This may cause oxidative stress and injury due to excessive iron overload, altered antioxidant enzymes, and other serum trace element levels.

The significant alteration are found in the levels of trace elements mainly magnesium, calcium and zinc in thalassemia. The administration of selective antioxidants along with these supplements reduces the extent of oxidative damage and related complications in thalassemia patients. The reports are found very controversial for the association of oxidative stress with serum magnesium, calcium and zinc levels status. Hence the present study is undertaken to determine the association of oxidative stress with these trace element levels in thalassemia patients.

Materials and Method

This study was designed on 100 patients, in the department of biochemistry in collaboration with the Paediatric department of S.P. Medical College and attached P.B.M. Hospital, Bikaner. Out of this 50 were healthy individuals and remaining were the subjects suffering from thalassemia, who were on repeated blood transfusion therapy attending or admitted in paediatric wards and O.P.D. includes age between 3-14 years.

The drugs effecting level of trace elements, clinical past history of anaemia, abnormal blood counts or any

other diseases were excluded from study. After taking patients consent, blood sample were taken by aseptic procedure and subjected for estimation of serum MDA, magnesium, calcium and zinc levels in healthy and thalassemic subjects.

Estimation of malondiadehyde was done by thiobarbituric acid (TBA) assay method described by Buege and Aust (1978) on UV-Spectrophotometer. Basic principle of the method is the reaction of one molecule of malondialdehyde and two molecule of thiobarbituric acid to form a red MDA-TBA complex, which can be measure at 535 nm.[6]

Magnesium and Zinc level were estimated by Atomic Absorption Spectrophotometer (AA-7000) as described by Fernandez, based on the principle in which a liquid sample is aspirated and mixed as an aerosol with combustible gases (Air-C_2H_2 or C_2H_2-N_2O). The mixture is ignited in a flame of temperature ranging from 2100 to 2800^0C. During combustion, atoms of the element of interest in the sample are reduced to the atomic state.[7,8,9,10]

The serum Calcium was estimated on semi auto-analyser (SA-20 Clinding) by using Calcium AS III, single ready to use reagent kit, in which Arsenazo III, reacts with calcium ions at neutral pH to form a blue coloured complex.[11,12]

Results were arranged in MS office 2007 excel worksheet in the form of master chart. Mean± SD were calculated by appropriate formulas of stats and Inference were drawn with the use of appropriate test of significance. The strength and direction of linear relationships between variables were evaluated using Pearson's correlation coefficient.

Result

Serum mean ±SD value of MDA was 8.99±1.33 nmol/ml with range of 6.45-11.54 nmol/ml in healthy subjects and 17.16±1.79 nmol/ml with a range of 12.99-21.44 nmol/ml in the children of thalassemia disease. The MDA level was increased highly statistically significant, as evident by P-value (P <0.0001), indicated in Table 1.

Table 1: Biochemical data of Magnesium, Calcium and Zinc in Thalassemic patients and Healthy control subjects

Elements	Thalassemic Patients(Mean±SD) (n=50)	Healthy control subjects (Mean±SD) (n=50)	P-Value	Significance
Malondialdehyde (MDA)nmol/mL	17.16±1.79	8.99±1.33	<0.0001	HS*
Magnesium(mg%)	1.19±0.14	2.04±0.22	<0.0001	HS*
Calcium(mg%)	6.88±0.61	9.54±0.53	<0.0001	HS*
Zinc(µg%)	61.76±5.44	105.11±9.89	<0.0001	HS*

Table 2: Correlation of MDA with Mg, Ca and Zn levels of Healthy control subjects

S. No.	Correlation	r-value	P value	Inference
1	MDA v/s Mg	+0.04	>0.01	NS*
2	MDA v/s Ca	+0.09	>0.01	NS*
3	MDA v/s Zn	+0.18	>0.01	NS*

Table 3: Correlation of MDA with Mg, Ca and Zn levels of Thalassemic patients

S. No.	Correlation	r-value	P value	Inference
1	MDA v/s Mg	-0.21	< 0.001	S*
2	MDA v/s Ca	-0.23	< 0.001	S*
3	MDA v/s Zn	-0.57	< 0.0001	HS*

NS*= Non significant, S*= Significant, HS*= Highly Significant

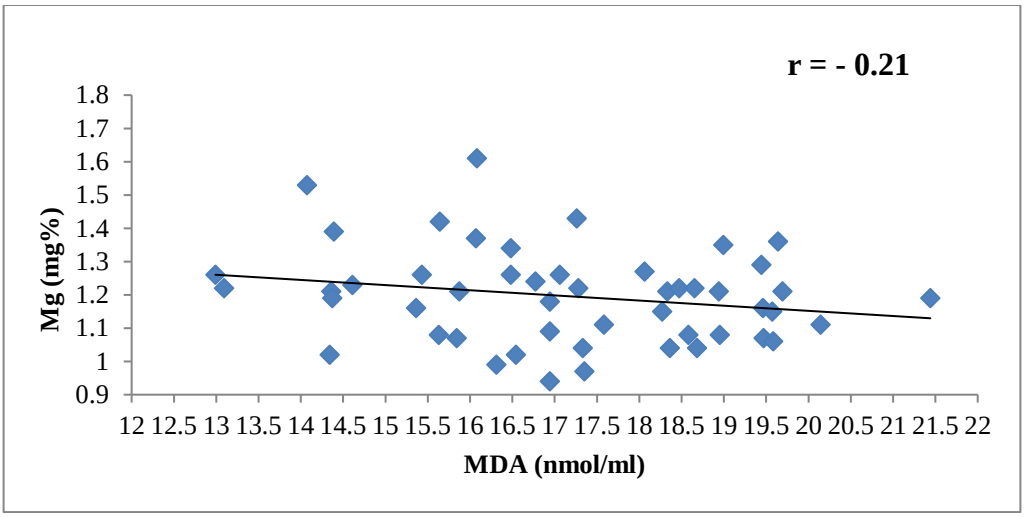

Fig. 1: Correlation between serum MDA and Mg in Thalassamic patients

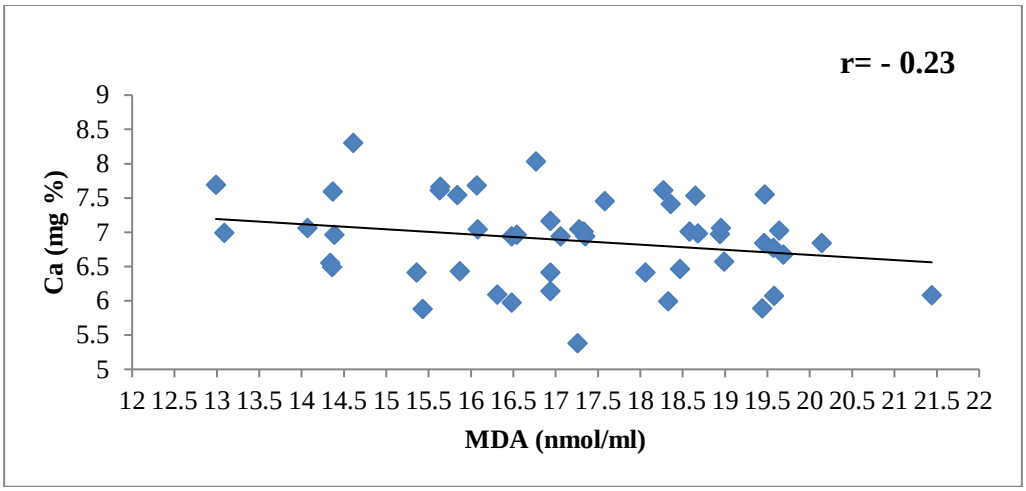

Fig. 2: Correlation between serum MDA and Ca in Thalassamic patients

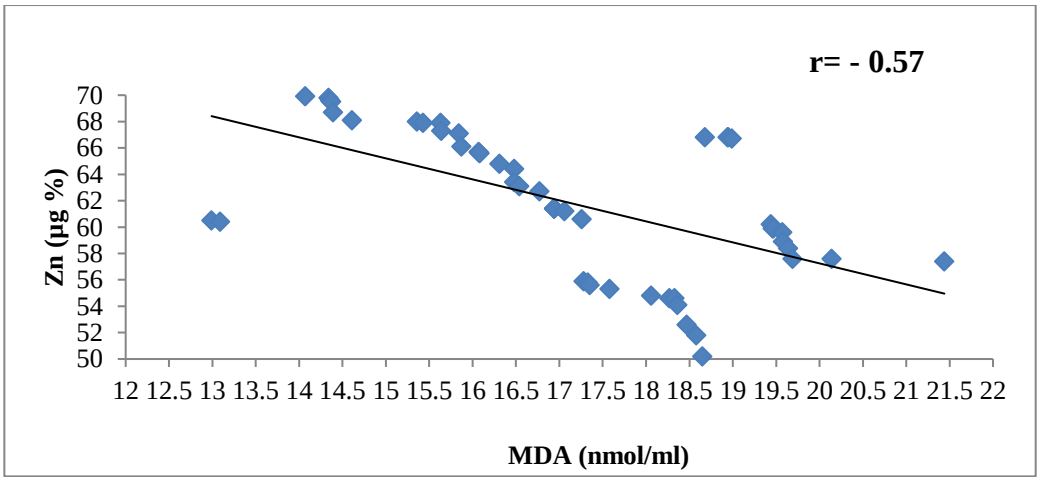

Fig. 3: Correlation between serum MDA and Zn in Thalassamic patients

The mean serum magnesium level was found to be 2.04±0.22 mg% with a range of 1.71 to 2.64 mg% in normal healthy children and it was decreased to 1.19±0.14 mg% with a range of 0.94to 1.61 mg% in thalassemic children. The highly significant decrease ($P<0.0001$) Mg level was observed in thalassemic subjects when compared to healthy controls as shown in Table 1.

The mean± SD value of Calcium level in healthy control subjects was 9.54 ± 0.53 in the range of 8.64-10.3 mg%, and in thalassemic children it was found to be 6.88±0.61 mg% with the range of 5.38-8.30 mg%. Calcium level showed highly significant decrease (P<0.0001) in thalassemic subjects when compared to healthy control subjects described by Table 1.

The mean Zinc level was found to be 105.11±9.89 µg% with a range of 84.6-119.8 µg% in control subjects and it was found to be 61.76±5.44 µg% with a range of 50.2-69.9 µg% in the children of thalassemia disease. The mean Zinc level was statistically highly significantly decreased as evident by P-value (P <0.0001), indicated in Table 1.

A correlation between serum MDA and magnesium, calcium, zinc were determined in healthy control subjects as well as in thalassemic children. These correlation coefficient were found to be positive, statistically non significant, in controls and inverse, statistically significant for magnesium, calcium and highly significant for zinc as shown by p value in thalassemic children. (Table 2, 3 and Fig. 1, 2, 3)

Discussion

Malondialdehyde levels were higher in the study group than control groups, these results agree with previous studies, which reported increased plasma malondialdehyde level.[13,14] MDA is a good indicator of oxidative damage and also, a product of lipid peroxidation, is generated in excess amounts in thalassemia.

MDA cross links with membrane components and makes rigid RBCs compared with normal. This erythrocyte deformability is a major determinant of anemia in thalassemia.[15] In one of **study**, free and total MDA was found to be higher in transfused thalassemia major patients than in the thalassemia intermedia patients.[16] As a result of continuous blood transfusions, the patients might be subjected to peroxidative tissue injury by the iron overload. These results support the idea that iron overload in thalassemia leads to an increased production of reactive oxygen species and oxidative stress. It also correlate with the study of Elham Abed Mahdi in 2014.[17]

The decrease in magnesium levels may result from dietary insufficiency of magnesium and the inappropriately high dose of desferrioxamine drug used in treatment. Previous study showed that magnesium levels were within normal levels.[18] In another study, magnesium depletion in thalassemia patients was documented by low serum magnesium levels, abnormal magnesium tolerance tests, and low symptoms responsive to magnesium therapy.[19]

Our study also shows resemblance with results of Bandebuche S et al. (2013) who studied on 9 patients of clinically diagnosed beta thalassemia major and age matched control and results revelled statically significant decrease in serum total calcium in thalassemia patient as compared to normal.[20]

Our data showed that in patients with thalassemia, there was a significant decrease in the levels of serum Zn. These results are in agreement with other studies published elsewhere Zn deficiency in thalassemia may not only be due to high iron levels, but may also be due to multi-factorial causes such as, hyperzincuria, hepatic dysfunction and impaired Zn absorption. It may be related to insufficient amount of daily intake of zinc.[21,22]

These results suggested that as the MDA concentration increases, serum calcium, magnesium and zinc levels will be decreased. These deficiency in thalassemia may be due to iron overload (due to repeated blood transfusion), but also be due to multifactorial causes such as endocrinal abnormalities (hypoparathyrodism), hyperzincuria resulted from the release of zinc from haemolysed red cells, hepatic dysfunction and impaired absorption from gastro intestinal tract. It is possible that these elements are also chelated with iron and removed by urine, so if these are measured in urine of the patients, they may be increased.

Conclusion

In conclusion, there is limited data available concerning oxidative stress, antioxidant status, degree of per-oxidase damage, and role of these elements in thalassemia patients. Studies on elements like magnesium, calcium and zinc reveal significant change in plasma concentration of these trace elements in thalassemia patients. Thus, these antioxidant enzymes and trace elements can be recognized as potential and effective therapeutic targets or strategies for the treatment of this disease.

The administration of antioxidants along with essential trace elements including magnesium, calcium and zinc supplementations, reduce the effects of oxidative damage and the complications in thalassemia requires more research and investigations.

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