



*Original Research Article*

## **Special Effects of Oral Therapeutic Supplementation of Antioxidants on Attenuation of Iron Overload in Homozygous Beta Thalassemia**

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### **ABSTRACT**

A crucial component in the oxidant susceptibility of the thalassemic red blood cells is the heavy release of heme and iron leads to excessive formation of unpaired  $\alpha$  - globin chains. This release can initiate self amplifying redox reactions which deplete the antioxidant potential. The goal of study was to inspect impact of serum iron, total iron binding capacity (TIBC), ferritin and erythrocyte superoxide dismutase (ESOD) in patients with homozygous beta thalassemia. 81 homozygous beta thalassemia patients were studied before and after supplementation of antioxidants for one month, and status was compared with 90 age and sex matched healthy controls. A significant increase were found in the levels of serum ferritin, TIBC and iron and erythrocyte superoxide dismutase ( $p < 0.001$ ) in patients when compared with controls. After one month supplementation of antioxidants, the levels of superoxide dismutase, iron, TIBC and ferritin ( $p < 0.001$ ) were decreased significantly when compared with controls. Beta thalassemia major children receive multiple blood transfusions are at risk in secondary iron burden and heavy oxidative stress. These effects may be minimized with intake of antioxidants.

**Key Words:** Homozygous Beta thalassemia, Serum ferritin, Oxidative stress, Iron burden.

### **INTRODUCTION**

Thalassemia will remain to be one of the major health problem for at least the next

few decades, particularly in the developing countries. <sup>[1]</sup>  $\beta$  - Thalassemia major is an autosomal disorder caused by mutations in the HBB gene in chromosome 11 ( $\beta$ ) which

leads to underlie deficiency in  $\beta$ - globin subunits of hemoglobin. [2] It is interrelated with profound anemia characterized by extreme pallor, jaundice, irritability, decreased activity or increased somnolence. Hepatosplenomegaly, expanded bone marrow, siderosis, cardiomegaly, impaired erythropoiesis, hemolysis in peripheral circulation and deposition of excess iron in the tissue are usually present. [2,3]

Each year around 100,000 children with thalassemia major are born world over, of which 10,000 are born in India. It is estimated that, there are more than 65,000-67,000 beta thalassemia patients in our country with around 10,000 cases being added every year. [4]

The pathology is associated with decreased hemoglobin production and red blood cells (RBCs) survival rate, which results in excess formation of unaffected  $\alpha$ -globin chains which form unstable homotetramers that precipitate as inclusion bodies. The free  $\alpha$ - globin chains are highly unstable and readily precipitate and release iron in reactive form. [1] The symptoms appear after about 2-4 months of age. [5]

Today, different studies indicate the high levels of oxygen together with haemoglobin containing iron attacking the non saturated fatty acids and RBCs. This result in producing free radicals and reactive oxygen species (ROS) in the blood cell of thalassemic patients which alter the redox status of these patients and intensification of oxidative stress. [6, 7] This leads to congestive heart failure and is one of the most prevalent and important cause of death in beta thalassemia major patients. [8, 9, 10, 11] Beta thalassemia major manifests itself with severe anemia (range 3-7 gm/dl of Hb) and lifelong depends on blood transfusion to sustain life. The goal of transfusion includes correction of anemia, suppression of erythropoiesis and inhibition of gastrointestinal iron absorption. [1, 8]

In patients with iron overload exceeds total iron binding capacity of transferrin and non- transferrin bound iron which causes tissue toxicity leading to increased lipid peroxidation with subsequent consumption of antioxidants. [8,10] In this research we intend to study the antioxidants intake and its affiliation with iron burden on the body by means of serum ferritin, iron and TIBC in children with beta thalassemia major.

## **MATERIAL AND METHODS**

The present research was carried out in the Department of Biochemistry, NSCB Govt. Medical College, Jabalpur, (M.P.) and Department of Biochemistry PDVVPF's Medical College, Ahmednagar, Maharashtra. The Institutional Ethical Committee clearance was obtained and utmost care was taken during experimental procedure according to the Declaration of Helsinki 1975.

The study has been performed on total 171 subjects which included 81 age and sex matched (48 males and 33 females) healthy controls and 90 (53 males and 37 females)  $\beta$ - thalassemia major children which were previously diagnosed by High Performance Liquid Chromatography (HPLC) and electrophoretic patterns. All patients were under the strict supervision of medical professionals during this period. The patients were blood transfusion dependent aged between 3-12 years. The average hemoglobin concentration ranged between 3 -7.7 gm/dl. All the patients having history of cardiovascular diseases, hypertension, thyroid dysfunction, diabetes mellitus which induce oxidative stress were excluded from the study.

After obtaining a written consent from all the participants, total 5ml blood was withdrawn aseptically from the antecubital vein from each subject, out of this

approximately 2 ml blood in EDTA (0.47mol/l K3-EDTA) container and 3 ml blood in plain blue. The samples were centrifuged at 3000 rpm for 10 min to separate serum and RBC's respectively. The separated serum was collected in polythene tube with cork and stored at -20°C. The serum with no sign of hemolysis was used for analysis of all the parameters.

Serum ferritin concentration was performed by ELISA in batches of ten each along with healthy controls. Iron was estimated by Ramsay's Dipyrindyl Method. [12] Estimation of Total Iron Binding Capacity (TIBC) done by Ramsay's Dipyrindyl Method. [13] Erythrocyte SOD activity was measured by Kajari Das. [14]

The analyses of all parameters except ferritin were done manually using the chemicals of Qualigens Fine Chemicals Co., Mumbai. The parameters were run on UV visible Spectrophotometer (Systronix).

The assessment of the above parameters except controls were conducted before and on 30<sup>th</sup> day of the antioxidant

supplementation in the form of an antioxidant tablet A-Z b.i.d. which was composed of predominantly antioxidant vitamins and trace elements. The statistical analysis was carried out by using the SPSS (Statistical Package for Social Sciences) software. The Student 'z' test was applied for the statistical analysis and the results were expressed in mean ± SD, p values (p <0.001) were considered as highly significant.

## RESULTS

Table-1 shows significantly elevated (p<0.001) levels of serum ferritin, Iron, TIBC and erythrocyte superoxide dismutase than healthy controls. On the 30<sup>th</sup> of antioxidant supplementation, it was observed in patients that, a significant decrease in the levels of ferritin, iron, TIBC and erythrocyte superoxide dismutase (p<0.001) as compared with before supplementation results.

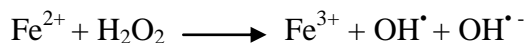
**Table 1. Depicts Indices of oxidative stress and antioxidant status in blood samples of control and homozygous beta thalassemia patients before and after supplementation.**

Parameters	Controls (n=90)	Group I Beta thalassemia major patients (n=81). Before supplementation of antioxidants.		Group II Beta thalassemia major patients (n=81). After 30 <sup>th</sup> day supplementation of antioxidants.	
	(Mean ± SD)	(Mean ± SD)	'p' value	(Mean ± SD)	'p' value
Serum Ferritin (ng/ml)	137.54± 64.87	3597±1076.08	p<0.001	2543±568.19	p<0.001
Serum Iron (µg%)	123.49 ± 17.12	183.63±38.93	p<0.001	149.47±15.39	p<0.001
Serum TIBC (µg%)	324.28 ± 66.25	469.19±57.13	p<0.001	377.51± 39.61	p<0.001
ESOD (unit/gm Hb)	1038±196	2872±554	p<0.001	2397±196	p<0.001

Statistical comparison was done between controls, group I and II  
 Group I - Beta thalassemia major patients before supplementation of antioxidants.  
 Group II - Beta thalassemia major patients after supplementation of antioxidants.  
 Values are expressed in mean with standard deviation (mean ± SD).  
 p<0.001- considered as highly significant.  
 n= number of subjects.

## DISCUSSION

Iron burden is the life limiting complication frequently found in thalassemia. The ability to estimate the distribution of excess iron to predict its consequences and therefore to tailor treatment accordingly is surprisingly imprecise. [10] There are two main mechanisms by which iron overload develops in beta thalassemia major, increased iron absorption due to ineffective erythropoiesis and repeated blood transfusion. [15] Normally iron stored in the body is maintained within the range of 200-1500mg by adequate adjustment of intestinal iron absorption, since no excretory mechanism exists. [10] Excess iron has deposited as hemosiderin and ferritin in the liver, spleen, and endocardium. [1] The accumulation of toxic quantities of iron cause tissue damage which leads to formation of ROS such as superoxide anions ( $O_2^-$ ), hydroxyl radicals ( $OH^\bullet$ ), singlet oxygen and hydrogen peroxide ( $H_2O_2$ ) which induces oxidative stress in thalassemia major patients via Fenton reaction-



This oxidative stress and possible consequential accelerated apoptosis may contribute to shortened erythrocyte life span, primary or secondary amenorrhoea, osteoporosis, and other endocrine disorders. [16]

Our findings strongly supports with Kuldeep K. Gupta et al, Eliezer A. Rachmilewitz et al, and Rafaella Mariani et al, that there were significantly increased serum iron and TIBC levels in patients with beta thalassemia major as compared with healthy controls. [1, 2, 15]

Ferritin is the main iron storage protein in the body. Its synthesis regulated

by quantities of iron by means of the interaction of cytoplasmic protein bound to the messenger ribonucleic acid (mRNA), currently identified as iron regulatory protein with specific structure of mRNA, called iron responsive element. It has a central role in iron homeostasis because it binds to an sequesters intracellular iron. A very high serum ferritin level was found in our study and it was also supported by Nadeem Ikram et al, Fliliz Simsek et al, I.F. Esterao et al, E George et al, M. A. Livrea et al. [10, 16, 18, 19, 20], that iron indices were markedly increased and the mean concentration of serum ferritin was elevated more than 20 times than healthy controls. After one month oral supplementation of antioxidants the values were reduced but not upto the controls.

The repeated blood transfusion increases the proportion of younger erythrocytes which leads to vivo lipid peroxidation and the compensatory increases in the level of ESOD. [21] Earlier studies and our investigations shows significantly elevated activity of ESOD in thalassemia major victims as compared to the controls before the antioxidant supplementation. [1, 15, 16] This supports the hypothesis made by various studies Nandita Das et al and Gerli GC et al that, enhanced production of hydrogen peroxide by the activity of ESOD which can inhibit various peroxidase enzyme activities; it may contribute to further augmentation of oxidative stress. [21, 22]

Depleted activity of ESOD on 30<sup>th</sup> day after the supplementation of antioxidants was seen in patients group. Supplementation of antioxidants may neutralize the formation of superoxide radical and  $H_2O_2$ .

## CONCLUSION

The core of the current study lies in the fact that, there was enhanced oxidative

stress in the form of serum iron, TIBC, ferritin in patients before treatment. Because of repeated blood transfusion for survival results in increased free iron overload and giving credence to serum ferritin level which causes oxidative stress leading to development of abnormalities in the body. Till date the technique available for this genetic disorder is iron chelation, bone marrow transplantation and stem cell therapy. But these treatments are burdensome, very expensive and hence a person depends on regular blood transfusion throughout life. The regular antioxidant supplementation as adjunct therapy to the homozygous beta thalassemia patients may improve the antioxidant status by neutralizing the free radicals formation followed by protection of RBCs from hemolysis and recovers the hemoglobin concentration.

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#### REFERENCES

1. Kuldeep KG, Amit M, Archana T. Production of reactive oxygen species, its effects, drugs and plant extract used as an antioxidant, chelator on thalassaemic patients: A Review. *International J of Pharma Sciences and Research* 2011; 2(9):2278-2285.
2. Eliezer AR, Patricia G. How I treat thalassemia. *Blood* 2011;118:3479-3488.
3. Tangvarasittichai SU. Thalassemia Syndrome. *Open journal system*. Online 23/3 2012.
4. Naveen T. Prevention of thalassemia in India. *Indian Pediatrics* 2007; 44:647-648.
5. Ghone RA, Kumbar KM, Suryakar AN et al. Oxidative stress and disturbance in antioxidant balance in beta thalassemia major. *Indian J of Clinical Biochemistry* 2008 ; 23 (4): 337-340.
6. Lee j, Koo K, Min DB. Reactive oxygen species, aging, and antioxidant nutraceuticals. *Comprehensive Reviews in Food Science and Food Safety* 2004; 3: 21-32.
7. Lien Ai PH, Hua He, Chuong PH. Free radicals, antioxidants in disease and health. *Int J of Biomedical Science* 2008; 4(2):89-96.
8. Faiza W, Karira AK, Raihan S. Antioxidant status in beta thalassemia major: A single- center study. *Indian J of Pathology and Microbiology* 2011; 54(4):761-763.
9. Veena D, Ratan K, Marwaha RK et al. Antioxidant status in children with homozygous beta- thalassemia. *Indian pediatrics* 2005; 42:1141-1145.
10. Nadeem I, Khalid H, Muhammed Y et al. Ferritin levels in patients of beta thalassemia major. *Int J of Pathology* 2004; 2(2):71-74.
11. Akram K, Tahereh T, Fatemeh RR et al. The relationship between the antioxidants intake and blood indices of the children with thalassemia in sabzevar and mashhad. *Pakistan J of Nutrition*. 2010; 9(7):716-719.
12. Varley H. Ramasays dipyriddy method for iron. In: *Practical clinical biochemistry* 4<sup>th</sup> Ed. 1967: 472.

13. Varley H. Ramasays dipyrityl method for total iron binding capacity. In: Practical clinical biochemistry 4<sup>th</sup> Ed. 1967: 475.
14. Kajari Das. A modified spectrophotometric assay of superoxide dismutase using nitrate formation by superoxide radicals. Indian J of Biochem and Biophys 2000; 57: 201-4.
15. Raffaella M, Paola T, Matteo P et al. Iron metabolism in thalassemia and sickle cell disease. Medit J Hemat Dis 2009; 1: Open journal System.
16. Filiz S, Gulyuz O, Sabri K et al. Oxidant and antioxidant status in beta thalassemia major patients. Ankara Universitesi Tip Fakultesi Mecmuasi 2005 ; 58 : 34-38.
17. Rachmilewitz EA, Stern OW, Adamsky V et al. Role of iron in inducing oxidative stress in thalassemia. New York Acad. Science 2005; 1054:118-123.
18. Estevao IF, Peitl PJ, Bonini Domingos. Serum ferritin and transferrin saturation levels in  $\beta^0$  and  $\beta^+$  thalassemia patients. Genetics and Molecular Research 2011; 10(2): 632-639.
19. George E, Wong HB, George H et al. Serum ferritin concentrations in transfusion dependent beta thalassemia. Singapore Med J 1994; 35:62-64.
20. Livrea MA, Tesoriere L, Pintaudi AM et al. Oxidative stress and Antioxidant status in beta thalassemia major: Iron overload and depletion of lipid soluble antioxidants. Blood 1996; 88(9): 3608-3614.
21. Nandita D, Chowdhury TD, Chattopadhyay AD. Attenuation of oxidation stress – induced changes in thalassemic erythrocytes by Vitamin E. Pol J Pharmacol 2004; 56:85-96.
22. Gerli GC, Beretz L, Bianchi M et al. Erythrocyte superoxide dismutase, catalase and glutathione peroxidase activities in beta thalassemia (major and minor). Scand J of Haematol 2009; 5(1):87-92.

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