

## Antioxidant Status in Children With Homozygous $\beta$ -Thalassemia

\*Veena Dhawan, Kh. Ratan Kumar, R. K. Marwaha, and \*Nirmal K. Ganguly

*From the Departments of \*Experimental Medicine and Advanced Pediatric Center, Postgraduate Institute of Medical Education and Research, Chandigarh 160 012, India.*

*Correspondence to: Dr. R. K. Marwaha, Professor of Pediatrics, Advanced Pediatric Center, Postgraduate Institute of Medical Education and Research, Chandigarh 160 012, India.  
E-mail: rammarwaha1@rediffmail.com*

*Manuscript received: March 18, 2004, Initial review completed: July 12, 2004;*

*Revision accepted: June 29, 2005.*

*The status of enzymatic and non-enzymatic anti-oxidants was evaluated in 41 patients with transfusion dependent  $\beta$ -thalassemia. An additional 20 age-matched children, with non-hemolytic anemia, served as controls. Fresh blood samples, obtained in the morning, were processed immediately. Plasma was stored at  $-80^{\circ}\text{C}$ . Levels of vitamins A and E were assayed simultaneously by HPLC. RBC vitamin A was not measurable in 29 (70.7%) thalassemics and in all the controls. Plasma vitamin A levels were lower in thalassemics than in controls ( $p < 0.05$ ). Vitamin E in RBCs was not measurable in 13 (31.7%) cases. The mean level of RBC vitamin E was 3 times lower in thalassemics. Similarly, SOD enzyme activity in thalassemics, was at least 1.5 lower in comparison to the activity documented in controls ( $p < 0.05$ ). The observations indicate that thalassemics have enhanced oxidative stress. Administration of selective antioxidants and a balanced diet may preclude oxidative damage.*

**Key words:**  *$\beta$ -thalassemia, Red blood cells, Superoxide dismutase, Vitamin A, Vitamin E.*

**I**NCREASED susceptibility to oxidative stress is a major factor in the etiopathogenesis of hemolysis of red blood cells (RBCs) in thalassemics(1,2). Auto-oxidation of globin chains, iron overload and low levels of adult hemoglobin (HbA) enhance the oxidative damage(3,4). Deficiency of vitamin E in thalassemics and enhanced resistance to oxidative insult, after supplements of vitamin E, have been documented(5-8). In contrast, other investigators have reported that hemolysis, secondary to oxidative stress, is not prevented in spite of administering large doses of vitamin E. The divergent observations suggest that other antioxidants, *e.g.*, Superoxide dismutase (SOD), neutralize oxidative stress. In this communication, we report the salient observations in a study designed to determine the status of enzymatic and non-enzymatic antioxidants in transfusion dependent  $\beta$ -thalassemics.

### Subjects and Methods

Forty one thalassemic children were enrolled as study subjects. An additional 20 age-matched children, with non-hemolytic anemia, served as controls. Administration of vitamin A or E supplements in the preceding 4 weeks or a recent febrile illness precluded recruitment. A written informed consent was obtained.

The enzymatic (SOD activity) and non-enzymatic status (assay of vitamin A and E), were determined in cases and the controls. Fresh blood samples were collected in EDTA vacutainers and transported, protected from light. RBCs and plasma were separated immediately. Plasma was stored at  $-80^{\circ}\text{C}$ .

Levels of vitamins A and E were simultaneously assayed by high performance liquid chromatography (HPLC), using the method of Bieri, *et al.*(9). Vitamin A was

measured at absorbance 325 nm whereas vitamin E was measured at absorbance 292 nm. The results in red blood cells and in plasma were expressed as  $\mu\text{g/mL}$  of RBC or  $\mu\text{g/dL}$  of plasma, respectively. Superoxide dismutase (SOD) activity was assayed in red blood cells (RBCs), by the method described by Flohe and Otting(10). SOD activity in the RBCs was expressed as enzyme units/mg of protein.

Descriptive data was analyzed by percentage, mean and standard deviation. Group comparisons involved analysis of variance (ANOVA) followed by appropriate tests for significance. For discrete variables, Chi-square test followed by pair wise Chi-square test was employed. Relationship between variables has been assessed by means of Pearson's product moment correlation coefficient.

### Results

Some salient features observed in children with thalassemia were: (i) gender bias with a M:F ratio of 2. 4:1 (ii) mean age at initial diagnosis was  $4 \pm 12$  months and (iii) the mean number of transfusions in the preceding year was  $17.7 \pm 3.9$  units.

Levels of vitamin A and E in plasma and red blood cell of thalassemics and control are

depicted in *Table I*. SOD enzyme activity was measurable in all the cases and in controls. In thalassemics, SOD activity ranged between 0.42 to 2.59 with a mean  $\pm$  S.D. of  $1.71 \pm 0.47$  units/mg protein. In comparison, the range and mean  $\pm$  SD in controls, were 1.54 to 3.57 and  $2.64 \pm 0.57$  units/mg protein, respectively. The mean SOD enzyme activity was 1.5 times lower than in controls. The difference was statistically significant ( $P < 0.001$ ).

SOD enzyme activity was correlated with hemoglobin concentration and RBC vitamin E levels. A negative correlation was observed between enzyme activity and the levels of erythrocytic vitamin E ( $\gamma = -0.22$ ) and hemoglobin ( $\gamma = -0.02$ ), respectively.

### Discussion

Red blood cells in thalassemics have morphologic abnormalities which result in increased susceptibility of thalassemic red cells to exogenous peroxidant threat(1,11-13). Formation of oxygen radicals in  $\beta$ -thalassemia and consequent formation of hemichromes lead to hemolysis of RBCs(1). The two mechanisms for inadequate peroxidant defence, in thalassemics, are insufficient vitamin E levels in RBCs and in plasma and decreased activity of several enzymes including superoxide

**TABLE I—Vitamin A and E Levels in Plasma and Red Blood Cells.**

Parameter	Patients (n = 41) (Mean $\pm$ SD)	Controls (n = 19) (Mean $\pm$ SD)	P value
RBC vitamin A* ( $\mu\text{g/mL}$ ) RBC	$147.92 \pm 99.95$	Not detected	<0.01
Range	3.66 to 329.98		
Plasma vitamin A ( $\mu\text{g/dL}$ ) RBC	$57.37 \pm 53.37^\dagger$	$115.03 \pm 69.83^\ddagger$	<0.01
Range	3.07 to 203.40	13.80 to 219.4	
RBC vitamin E# ( $\mu\text{g/mL}$ ) RBC	$68.81 \pm 48.31$	$213.46 \pm 181.84$	<0.01
Range	1.13 to 201.80	19.10 to 642.60	
Plasma vitamin E ( $\mu\text{g/mL}$ ) RBC	Not detected	$52.56 \pm 3.0$	<0.01
Range			

\*n=12; RBC vitamin A was not detected in 29 patients,  $^\dagger$ n=39;  $^\ddagger$ n=18, #n=28

dismutase(5-8).

In a study population of 23 and 56 thalassemics, respectively, low levels of plasma vitamin E were observed in 4 (17.4%) and 26 (46.4%) patients. On the basis of their observations, Stocks, *et al.* concluded that vitamin E was, by no means, the only factor for protection against anti-oxidant stresses(13).

In our study, RBC vitamin E was not detected in nearly 1/3rd of the thalassemics whilst all the controls had measurable vitamin E. The mean level of RBC vitamin E was atleast 3 times lower in thalassemics than in the controls. Decreased levels of RBC vitamin E in thalassemics have been reported by several investigators(9,13-15). Bieri, *et al.*(9) observed that the normal ratio between RBC and plasma vitamin E was 0.2:1.0. The low levels of RBC vitamin E, in thalassemics, are a surrogate indicator of low plasma levels of vitamin A as well.

SOD enzymes are the first line of defence against oxidant stress. Low levels of SOD activity with high free oxygen radicals consume vitamin E in the process of lipid peroxidation(11). The enhanced consumption with consequent reduced levels of vitamin E suggests that supplements of vitamin E may improve pre-transfusion hemoglobin. Rachmilevitz, *et al.*(14) documented a 3-6 fold increase in RBC vitamin E levels after supplements without a concomitant beneficial impact on interval between transfusions. In contrast, other investigations(8,16) have reported an increase in hemoglobin levels and in the interval between transfusions. These somewhat divergent findings suggest that vitamin E is not the only antioxidant factor which protects RBCs against hemolysis(13).

In normal circumstances, vitamin A is not measurable inside RBCs(9). In our study, RBC vitamin A was not detected in any of the

controls and in 29 thalassemics. In addition, the mean plasma vitamin A levels were significantly lower in thalassemics than in the controls ( $P < 0.05$ ). The mean plasma level of vitamin A, in our patients was 57.37  $\mu\text{g/dL}$ . An almost identical mean value of 53.8  $\mu\text{g/dL}$  was observed by Bieri, *et al.*(9) whilst Jagdeesan and Reddy(17) reported a mean value of 21.0  $\mu\text{g/dL}$ . The factors implicated in the etiopathogenesis of low levels of plasma vitamin A were (i) increased consumption whilst mounting a defence against oxidative stress (ii) poor dietary intake of vitamins A and E and (iii) low levels of serum lipids with secondary decrease in plasma levels of vitamin A (18).

In comparison to controls, the mean SOD enzyme activity was at least 1.5 times lower in the thalassemics evaluated in our study. The findings, pertaining to erythrocytic SOD enzyme activity, reported by other investigators are varied. They range from high SOD activity (19,20) to no difference in patients and controls(21,22). Vanella, *et al.*(23) studied the effect of polyamines on autohemolysis in normal and thalassemic children and observed significant differences in SOD activity.

In conclusion, our observations indicate that thalassemics are in a state of enhanced oxidative stress. The administration of selective antioxidants, along with an appropriate, nutritionally balanced diet would represent a promising approach towards counteracting oxidative damage and its deleterious effects on the disease status.

*Contributors:* VD and RKM conceptualized the study. Implementation was done by KRK under the supervision of VD, RKM and NKG. RKM will act as guarantor of the paper. All contributed in the review of the manuscript.

*Funding:* PGIMER, Chandigarh.

*Competing interest:* None.

### Key Messages

- Thalasemics are in a state of enhanced oxidative stress.
- Oxidative stress is enhanced in transfusion dependent thalasemics.
- Plasma vitamin A levels were significantly lower in thalasemics.
- Erythrocytic vitamin E was not measurable in one-third of the thalasemics. The mean level of the RBC vitamin E was 3 times lower in thalasemics.
- Similarly, super-oxide dismutase activity was at least 1.5 times lower than the mean documented in the controls.
- There is a need for a multicentric study to determine the benefit of administering the selective anti-oxidants.

### REFERENCES

1. Brunori M, Falcino G, Fioretti E. Formation of superoxide in autooxidation of isolated alpha and beta chains of human haemoglobin and its involvement in hemichrome precipitation. *Eur J Biochem* 1975; 53: 99-104.
2. Rachmilewitz EA, Lubin BH, Shohet SB. Lipid peroxidation in beta-thalassemia major. *Blood* 1976; 47: 495-505.
3. Shinar E, Rachmilewitz EA. Oxidative denaturation of red blood cells in thalassemia. *Semin Haematol* 1990; 27: 70-82.
4. Vives Corrons JL, Miquel-Garcia A, Pujades MA. Increased susceptibility of microcytic red blood cells to in vitro oxidative stress. *Eur J Hematol* 1995; 55: 327-331.
5. Miniero R, Canducci E, Ghigo D. Vitamin E in beta-thalassemia. *Acta Vitaminol Enzymol* 1982; 4: 21-25.
6. Kahane I, Rachmilewitz EA. Alterations in the red blood cell membrane and the effect of vitamin E on osmotic fragility in beta-thalassemia major. *Isr J Med Sci* 1976; 12: 11-15.
7. Suthutvoravut U, Hathirat P, Sirichakwal P. Vitamin E status, glutathione peroxidase activity and the effect of vitamin E supplementation in children with thalassemia. *J Med Assoc Thai* 1993; 76: 146-152.
8. Giardini O, Cantani A, Donfrancesco A. Biochemical and clinical effects of vitamin E administration in homozygous beta thalassemia. *Acta Vitaminol Enzymol* 1985; 7: 55-60.
9. Bieri G, Tolliver JT, Catignani GL. Simultaneous determination of alpha tocopherol and retinol in plasma or red cells by high pressure liquid chromatography. *Am J Clin Nutr* 1979; 32: 2143-2149.
10. Flohe L, Otting F. Superoxide dismutase assays. *Methods in Enzymology* 1984; 105: 93-97.
11. Rachmilewitz EA, Shohet SB, Lubin BH. Lipid membrane peroxidation in beta-thalassemia major. *Blood* 1976; 47: 495-505.
12. Rachmilewitz EA. The role of intracellular hemoglobin precipitation, low MCHC, and iron overload on red blood cell membrane peroxidation in thalassemia. *Birth Defects Orig Artic Ser* 1976; 12: 122-133.
13. Stocks J, Offerhan EL, Modell CB, Dormandy TC. Susceptibility to autooxidation of human red cell lipids in health and disease. *Br J Hematol* 1972; 3: 713-724.
14. Rachmilewitz EA, Shifter A, Kahane I. Vitamin E deficiency in beta thalassemia major: Changes in hematological and bio-chemical parameters after therapeutic trial with alpha-tocopherol. *Am J Clin Nutr* 1979; 32: 1850-1858.
15. Zannos Mariolea LF, Tzortzatos K, Denvaki K-Svolaki, Katerellos CH, Kavallari M, Matsanitois N. Serum vitamin E levels with beta-thalassemia major, a preliminary report. *Br J Hematol* 1977; 26: 193-199.

BRIEF REPORTS

16. Giardini O, Cantani A, Donfrancesco A. Vitamin E therapy in homozygous beta thalassemia. *N Engl J Med* 1981; 305: 644 (letter).
  17. Jagadeesan V, Reddy V. Inter-relationship between vitamin E and A:A clinical study. *Clin Chimica Acta* 1978; 90: 71-74.
  18. Chakraborty D, Bhattacharyya M. Antioxidant defence status of red blood cells of patients with beta thalassemia and E-beta thalassemia. *Clin Chim Acta* 2001; 305: 123-129.
  19. Gerli GC, Baretta L, Bianchi M. Erythrocyte superoxide dismutase, catalase and glutathione peroxidase activities in beta thalassemia (major and minor). *Scand J Hematol* 1980; 25: 87-92.
  20. Vanella A, Rizza V, Livotti S. Effect of polyamines on autohemolysis studies on normal and thalassemic children. *Acta Hematol* 1980; 63: 226-229.
-