

Free Radical Activity in Thalassaemia Major Patients in a Tertiary Care Teaching Hospital, Bhubaneswar

JatindraNath Mohanty¹, Priyanka Samal^{2*}, SaurjyaRanjan Das³, Sweta Padma Routray¹

¹Medical Research Laboratory, IMS and Sum Hospital, SOA deemed to be University, Bhubaneswar, Odisha, India

²Department of Clinical Haematology, IMS and Sum Hospital, SOA deemed to be University, Bhubaneswar, Odisha, India

³Department of Anatomy, IMS and Sum Hospital, SOA deemed to be University, Bhubaneswar, Odisha, India

Corresponding Author

Dr. PriyankaSamal, Department of Clinical Haematology, IMS and Sum Hospital, SOA deemed to be University, Bhubaneswar, Odisha, India

Email Id- priyankasamal@soa.ac.in

Abstract

Thalassemia is an entity involving a collection of inherited diseases caused by defective or absent hemoglobin chain synthesis leading to anemia due to ineffective erythropoiesis. Two hundred and fifty one diagnosed thalassaemic subjects from 6months-40 years of age attending thalassaemia care centres in Bhubaneswar were investigated for Plasma MDA /SOD and RBC SOD. The control group shows SOD levels 5.98 ± 1.72 U/ml (RBC) 3.79 ± 1.42 U/ml (Plasma) & Plasma MDA 6.86 ± 1.72 nmol/ml. In beta Thal patients RBC-SOD was 13.76 ± 2.22 U/ l and Plasma-SOD 08.100 ± 1.88 U/ l, Plasma MDA 15.12 ± 2.28 nmol/ml was observed. Plasma SOD & MDA in thalassaemia major patients is raised due to generation of production of Reactive Oxygen Species (ROS) as a consequence to formation of hemichromes hemolysis of Red Blood Cells and Iron overload.

Keywords: Thalassaemia, MDA, SOD, Reactive Oxygen Species (ROS)

Introduction

According to the WHO, the carrier rate of α -thalassemia is around 1.5% of the world population. It was also suggested that the incidence of individuals born with the severe form of the disease is 60,000 per year. Most of these patients are from regions around the tropical belt, including the Mediterranean Middle East, central Asia, India, and southern China [1]. However, with the era of globalization and easier travel methods, migration is now facilitating the spread of the disease towards the Western countries. Those patients are not entirely dependent on transfusions for survival. α -Hb-stabilizing protein (AHSP) is an erythroid protein that specifically binds α -Hb and prevents its precipitation in vitro, suggesting that it may function by putting a limit to free α -Hb toxicities in vivo. Loss of AHSP worsens the β thalassaemia phenotype as α -Hb-stabilizing protein (AHSP) AHSP specifically protects free α -Hb from precipitation in solution and in live cells (1). Moreover thalassaemia is associated with iron overload due to increased dietary iron absorption and frequent therapeutic blood transfusions. The resulting Iron overload is involved in several

chemical reactions that generate ROS thereby is known to change the cellular redox state causing peroxidative RBC membrane and tissue damage [2]. Giaredini et al (3). Demonstrated that in β -thalassaemia patients MDA (Malondialdehyde) was significantly higher in comparison to healthy controls. Plasma & RBC SOD levels was significantly increased.

Materials and methods

Two hundred and fifty one diagnosed thalassaemic subjects from 6 months-40 years of age who were attending different day care centres at Burdwan i.e. Thalassaemic welfare society, Burdwan Thalassaemia Child Health Care Society Burdwan (Sib Shanker Seba Samity and Burdwan Medical College & Hospital) were pooled and investigated. All these thalassaemic subjects received blood transfusion. Transfusion dependent beta and E-beta thalassaemic subjects, who did not receive any antioxidants in last three months were included in the study. Subjects who received antioxidants within last three months were excluded from the study. One hundred healthy age & sex matched controls who did not receive any antioxidants or vitamins were pooled as controls and investigated. The study protocol was approved by the Ethical Committee of the Institution. All participants gave informed consent. 4ml in K₃ EDTA vials and 4ml in clotted vials were collected. K₃ EDTA blood for Erythrocyte Superoxide dismutase activity assay. Plasma was separated from EDTA blood and sera was separated from clotted sample and were kept at -40°C until further analysis.

Table I : Age & Sex distribution of the study

Age Group	Male	Female
0-10	84	52
11-20	44	25
21-30	12	10
31-40	04	09
>40	07	04
Total	151	100

Table -II Value of SOD cases (U/ml)

Group	RBC-SOD	Plasma-SOD
A (β thalassaemia) N=191	13.76 $\pm$ 2.22	08.100 $\pm$ 1.88
B (E β thalassaemia) n=57	13.92 $\pm$ 2.38	8.27 $\pm$ 1.93
C (Controls) n=100	5.98 $\pm$ 1.72	3.79 $\pm$ 1.42

(β /E β thalpts and control group P-value was highly significant <0.05)

Table -III Value of MDA in β / E β cases:

GROUP	Plasma MDA(nmol/ml)
A (β thalassaemia) n=191	15.12 $\pm$ 2.28
B (E β thalassaemia) n=57	14.97 $\pm$ 2.12
C (Controls) n=100	6.86 $\pm$ 1.72

(β /E β thalpts and control group P-value was highly significant<0.001)

Observations

Table I shows Age and Sex distribution of thalassaemia major Subjects. Maximum number of Patients one hundrethirt six were in the age group 0-10 years and only 11 patients were in the age group above 40years.

Table-II shows Plasma/RBC SOD levels in thalassaemia and Control subjects. The conrol group shows SOD levels 5.98 $\pm$ 1.72U/ml (RBC) 3.79 $\pm$ 1.42 U/ml(Plasma) In beta Thal patients RBC-SOD was 13.76 $\pm$ 2.22 U/ l and Plasma-SOD 08.100 $\pm$ 1.88 U/ l was observed. In E β thalassaemia subjects13.92 $\pm$ 2.38 RBC-SOD was 13.92 $\pm$ 2.38 U/ml and Plasma SOD level was observed to be 8.27 $\pm$ 1.93 U/ l. a significant Correlation was found between, Plasma/RBC of SOD of β thalpts and control group P-value was highly significant i.e. <0.05. Table III shows Plasma MDA level in β -thal& E β thal subjects ie 15.12 $\pm$ 2.28 nmol/ml &14.97 $\pm$ 2.21 nmol/ml respectively where as in control Subjects it was observed as 6.86 $\pm$ 1.72 nmol/ml. The MDA level in patients was significantly highthan controlgroup.

Discussion

Thalassaemias are commonest single gene disorder, the production of normal heamoglobin is partly or completely suppressed due to defective synthesis of one or more globin chains. According to the chain which is defective, several types of thalassaemiashas been described. In the present study we pooled 251 cases of thalassaemia of which 191(76.09%) were β -thalassaemia,57(22.70%) E β -thalassaemia and 3 (1.19%) wereS β -thalassaemia. β -thalassaemia is thecommonest type of thalassaemia in our area, which corroborates well with the Indian and global scenario.(7) β -thalassaemia is a common heamoglobinopathy caused by β -globin gene mutations, which has increased Reactive Oxygen Species (ROS) production by excess of α -globin precipitation. (8) Formation of oxygen radicals in β -thalassaemia and consequent formation of hemichromes lead to haemolysis in RBCs (7) In thalassaemia there is insufficient Vitamin E levels in RBCs and plasma and low levels of reduced Glutathione (GSH) have also been demonstrated (8).Which Corroborates with the inadequate peroxidant defense. Many workers have shown elevation of SOD& MDA levels in thalassaemia(4). In the present study we have tried to assess the free radical activity in thalassaemia major subjects. Excess of globin chain and iron accumulation in RBCs is

responsible for enhanced SOD activity, they recorded significantly high SOD activity in red cells and plasma ($P < 0.001$) Giardini et al in 1985 (3) were able to demonstrate elevated MDA levels in β -thalassaemia major patients in comparison to healthy controls. Many workers have shown elevation of SOD & MDA levels in thalassaemia (8). In the present study we have tried to assess the free radical activity in thalassaemia major subjects. Our observations corroborated with them. Antioxidant therapy may be considered as an adjuvant to the other existing management protocol to augment patient's antioxidant defenses and to reduce oxidative stress in an effort to decrease morbidity and mortality.

Conclusion

The oxidative stress in thalassaemia major patients is due to generation of free oxygen radicals as a consequence to formation of hemichromes lead to hemolysis of Red Blood Cells. Subsequently the Iron overload due to ineffective erythropoiesis and repeated blood transfusion cause excessive levels of labile plasma iron, this iron penetrates into the cells further promoting production of Reactive Oxygen Species (ROS). In the present study maximum number of Patients i.e, one hundred and thirty six were in the age group 0-10 years RBC-SOD, Plasma SOD & MDA in thalassaemia major patients was raised in comparison to control group. Antioxidant therapy may be considered as an adjuvant to the other existing management protocol to augment patient's antioxidant defenses and to reduce oxidative stress in an effort to decrease morbidity and mortality.

References

1. Kihm AJ, Kong Y, Hongw, Russell JE, et al : An abundant erythroid protein that -haemoglobin. *Nature*; 417 : 758 – 763; 2002
2. Amer J., Gold farb. A., Fibach E.: Flow cytometric measurement of reactive oxygen species production by normal and thalassaemia red blood cells. *Eur. J. Haematol* ; 70 : 84 – 90; 2003.
3. Giardini O, Cantani A, Don francesco A, Martino F. et al : Biochemical and clinical effects of vitamin E administration in homozygous beta-thalassaemia. *Acta vitaminol Enzymol* ; 7 : 55, 1985.
4. Lardi S, Ferchichi S, Khelil AH et al Oxidant, antioxidant status and metabolic data in patients with beta thalassaemia. *Clinica Chimica Acta* : Vol 338, Issue 1–2. 79–86; 2003.
5. An gastiriotis M, Modell B Global Epidemiology of Haemoglobin disorders *Annals of New York Academy of Sciences* ; 850 : 25–69.
6. Cunningham MJ, Macklin EA, New feld EJ, Cohen A. R., Complications of beta thalassaemia major in North America *Blood*; 104 : 34 – 39 : 2004.
7. Liverca MA, Tessoriere L, Pintaui AM Chae Oxidative stress and antioxidant. -thalassaemia major : iron overload and depletion of lipid – soluble antioxidants : *Blood* No 1988 : 3608 – 14 ; 1996.
8. Yen chitsomanus Pand Wasi P : Increased erythrocyte superoxide dismutase activities in beta O-thalassaemia/haemoglobin E and in HbH diseases ; *J. Clinical pathol* ; 36 (3) : 329-33, 1983.