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A Study of Free radical activity in thalassaemia major patients in South Bengal

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Abstract
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. Loss of AHSP, precipitation free α-Hb iron that generate ROS thereby causing peroxidative RBC membrane and tissue damage leding to Increased MDA(Malondialdehyde) & RBC SOD levels .
Two hundred and fifty one diagnosed thalassaemic subjects from 6months-40 years of age attending thalassaemia care centres in Burdwan were investigated for Plasma MDA /SOD and RBC SOD. The control group shows SOD levels 5.98±1.72U/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.
Conclusion: *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 heamolysis of Red Blood Cells and Iron overload.*
Keywords: *Thalassaemia, MDA,SOD, Reactive Oxygen Species (ROS)*

Introduction

The Thalassaemias are heterogenous group of genetic disorders in which the production of normal heamoglobin is partly or completely suppressed because of defective synthesis of one or more globin chains.

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 there by is

known to change the cellular redox state causing peroxidative RBC membrane and tissue damage[2]. Giaredini et al[3] demonstrated that in β- thalassemia patients MDA(Malondialdehyde) was significantly higher in comparison to healthy controls. Plasma & RBC SOD levels was significantly increased.[4]

MATERIALS AND METHODS

Patients

Two hundred and fifty one diagnosed thalassaemic subjects from 6months-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.

Inclusion criteria: Transfusion dependent beta and E-beta thalassaemic subjects, who did not received any antioxidants in last three months were included in the study

Exclusion criteria: Subjects who received antioxidants within last three months were excluded from the study.

Control:

One hundred healthy age & sex matched controls who did not received 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.

Blood Sampling Procedure

4ml in K_3 EDTA vials and 4ml in clotted vials were collected. K_3 EDTA blood for Erythrocyte Superoxide dismutase activity assay. Plasma was separated from EDTA blood and sera was seperated from clotted sample and were kept at -40°C until further analysis. The Plasma was subjected to MDA, SOD, ESTIMATION OF RBC-SOD[5,6] DETERMINATION OF PLASMA SOD ESTIMATION OF SERUM MDA (THIOBARBITURIC ACID TEST) [7]

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±2.22	08.100±1.88
B (E β thalassaemia) n=57	13.92±2.38	8.27±1.93
C (Controls) n=100	5.98±1.72	3.79±1.42

(β/E β thal pts 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±2.28
B (E β thalassaemia) n=57	14.97±2.12
C (Controls) n=100	6.86±1.72

(β/E β thal pts 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 hundre thirt six were in the age group 0-10 years and only 11 patients were in the age group above 40 years

Table-II shows Plasma/RBC SOD levels in thalassaemia and Control subjects. The conrol

group shows SOD levels 5.98±1.72U/ml (RBC) 3.79±1.42 U/ml(Plasma) In beta Thal patients RBC-SOD was 13.76±2.22 U/ l and Plasma-SOD 08.100±1.88 U/ l was observed. In E β thalassaemia subjects13.92±2.38 RBC-SOD was 13.92±2.38 U/ml and Plasma SOD level was observed to be 8.27±1.93 U/ l. a significant Correlation was found between, Plasma/RBC of SOD of β thal pts and control group P-value was highly significant i.e. <0.05.

Table Ill shows Plasma MDA level in β-thal & E β thal subjects ie 15.12+/-2.28 nmol/ml & 14.97+/-2.21 nmol/ml respectively where as in control Subjects it was observed as 6.86+/-1.72 nmol/ml. The MDA level in patients was significantly high than control group.

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 thalassaemias has 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%) were Sβ-thalassaemia. β-thalassaemia is the commonest 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[4,9,10]. In the present study we have tried to asses 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 in1985 [3] were able to demonstrate elevated MDA levels in β-thalassaemia major patients in comparision to healthy controls. Many workers have shown elevation of SOD & MDA levels in thalassaemia[4,9,10]. In the present study we have tried to asses 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 heamolysis of Red Blood Cells.

Subsequently the Iron overload due to ineffective erythropoesis and repeated blood transfusion cause excessive levels of lable 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 comparision 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.

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