

## **Correlation of oxidative stress with serum antioxidant enzymes level in thalassemia in a tertiary level hospital of western Rajasthan**

**Dr. Mamta Choudhary**

*Senior demonstrator and PhD. Scholar, Department of Biochemistry, S.P. Medical College & Hospital, Bikaner (Rajasthan), India.*

**Dr. Raj Kumar Vyas**

*Senior Professor & Head, Department of Biochemistry, S.P. Medical College & Hospital, Bikaner (Rajasthan), India.*

**Dr. Anil Lahoti\***

*Assistant Professor, Department of Paediatrics, S.P. Medical College & Hospital, Bikaner (Rajasthan), India.*

**Dr. Yogita soni**

*Professor, Department of Biochemistry, S.P. Medical College & Hospital, Bikaner (Rajasthan), India.*

### **Abstract**

**Background:** Thalassemia is an inherited disorder of autosomal recessive gene disorder caused by impaired synthesis of one or more globin chains. Patients with this disease need repeated blood transfusion for survival. This may cause oxidative stress and tissue injury due to iron overload. The association of oxidative stress with serum antioxidant enzymes are very common in thalassemic patients. The levels of these enzymes in thalassemia reveals a significant change.

**Aim:** The aim of this study is to scrutinize the relationship between oxidative stress and serum antioxidant enzymes level in thalassemia.

---

\* Corresponding Author email id: [lahotianil04@gmail.com](mailto:lahotianil04@gmail.com)

**Methods:** In this study there were 50 cases and 50 control subjects were included with the age groups from 3 to 14 years. By using fresh samples, require tests such as malondialdehyde (MDA), glutathione (GSH), superoxide dismutase and catalase were performed by using standard protocol on UV-spectrophotometer. Data were statistically evaluated by Student's t-test and Pearson's correlation coefficient.

**Results:** The level of MDA was found to be increased in thalassemic children as compare to healthy volunteers while antioxidant levels were found to be decreased in children suffering from thalassemia as compared to controls. The MDA level was inversely correlated with these enzymes level.

**Conclusion:** This study shows as the oxidative stress increased, levels of these enzymes were decreased. Studies published on the antioxidant enzymes level in thalassemia patients also showed variable results. The reports are controversial for the relation of oxidative stress with serum antioxidant enzymes status. Hence the present study is undertaken to determine the correlation of oxidative stress with these enzymes in thalassemia patients.

**Key words:** MDA, Glutathione(GSH),Superoxide dismutase, Catalase

## **BACKGROUND**

Thalassemia is a hereditary and severe disorder, resulting from the homozygous state of one of the thalassemia or haemoglobin genes in infancy or childhood.[1] It is accompanied with metabolic irregularation, iron overload, chronic hypoxia and cell damage.[2] All the physiological changes result in ineffective erythropoiesis, haemolysis and anaemia.[3] Typical treatment of  $\beta$ -thalassemia major consists of multiple blood transfusions, a complication of which is iron overload.[4] In thalassemia there is excess production of reactive oxygen intermediates, such as superoxide anion ( $O_2^-$ ), hydroxyl radical ( $OH\cdot$ ), singlet oxygen and hydrogen peroxide ( $H_2O_2$ ) within the erythrocytes, all these events lead to oxidative stress.

This oxidative stress and a possible consequential accelerated apoptosis may contribute to shortened life span of erythrocytes. Malondialdehyde (MDA), a product of lipid peroxidation is generated in excess amounts in supporting the fact that large amount of membrane bound iron is present in thalassemic erythrocytes.[5]

In India, every year more than 10,000 children are born with thalassemia major. It produces severe anemia in its homozygous state.[6] About 190 million people throughout the world have genetic mutations associated with different hemoglobinopathies and more than 90 million of them carry defective genes leading

to thalassemia. Early introduction of chelatory agents control and combat iron overload, inhibit reactive oxygen species generation and regulate lipid per oxidative processes, leading to improved life expectancy.[7,8]

The aim of the present investigation was to study the relationship between oxidative stress by measuring the MDA level, which is the marker of oxidative stress in thalassemia patients and study also measures the enzymatic antioxidant level in thalassemic blood sample.

## **METHODS**

### **Study place and Design**

This study was carried out in department of biochemistry in collaboration with the department of Paediatric out patients department of S.P. Medical college and attached P.B.M. Hospital, Bikaner.

It was cross-sectional study, includes 100 subjects. Out of 100 subjects, 50 subjects will be patients of thalassemia, needs repeated blood transfusion for survival and another 50 subject will be age and sex matched healthy volunteers as control group. The control group will be taken from patient's attendants, staff, students and may be from private labs which conduct routine serum check up of healthy persons.

### **Inclusion criteria**

- Clinically diagnosed thalassemia patients those receiving regular blood transfusion.
- Age 3 to14 years.

### **Exclusion criteria**

- Very sick patients.
- Children having history of anemia, abnormal blood counts and abnormal electrophoresis results.
- Patients receiving antioxidant, vitamin supplementations and any herbal medicine.
- Patients taking drugs which affecting levels of trace elements.
- Patients associated with any acute and chronic illness, liver, kidney, cardiovascular and endocrine disorders.
- Haemolysed blood samples.

**Procedural steps**

Patients with confirmed diagnosis of thalassemia on regular blood transfusion were enrolled and consent was taken. Selection of subjects based on inclusion and exclusion criteria noted in the study through following assessment methods:

- Detailed clinical history
- General physical examination
- Sample collection and storage
- Blood from thalassemic patients was collected, for comparison, control blood was drawn from healthy individual.
- Blood collection was performed using venipuncture needles with all aseptic precautions, in plain vial with informed consent of attendants.
- The samples were left standing for one hour, serum was separated at 2500 rpm centrifugation, precautions were taken to avoid haemolysis and preserved at -20°C.

**Investigations**

- Serum Malondialdehyde, Glutathione(GSH), Superoxide dismutase, Catalase levels.

**Method of assay**

Estimation of serum Serum Malondialdehyde, Glutathione(GSH), Superoxide dismutase, Catalase levels were done by using commercially available reagents and kits on UV-spectrophotometer.

**Statistical Analysis**

Data were entered on MS office 2007 excel worksheet in the form of master chart. These data were classified and analyzed as per aims and objectives. Quantitative data were expressed in the form of Mean $\pm$  SD. 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.

**RESULTS**

Serum mean  $\pm$ SD value of MDA was  $8.99\pm 1.33$  nmol/ml with range of 6.45-11.54 nmol/ml in healthy subjects and  $17.16\pm 1.79$  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 no.1.

The mean serum Glutathione level was found to be  $13.97\pm 2.52$  U/L with a range of 9.15-18.66 U/L in normal healthy children (control group). The mean serum glutathione was decreased to  $4.79\pm 0.87$  U/L with a range of 3.05 - 6.94 U/L in thalassemic children (study group) as shown in table no.1. The highly significant decrease ( $P < 0.0001$ ) glutathione level was observed in thalassemic subjects or case group when compared to healthy controls.(table no.-1)

The mean  $\pm$  SD value of Superoxide dismutase level in healthy control subjects was  $207.85\pm 20.14$ U/mL in the range of 166.6 to 233.4 U/ml and in thalassemic children it was found to be  $128.52\pm 13.92$  U/mL with the range of 99.8 to 154.5 U/mL as described by table no.-1. Superoxide dismutase level showed highly significant decrease ( $P < 0.0001$ ) in thalassemic subjects when compared to healthy control subjects. (table no.-1)

The mean serum Catalase level was found to be  $118.17\pm 17.04$  U/L with a range of 94.31 to 145.38 U/L in normal healthy children. The mean serum magnesium was decreased to  $42.32\pm 12.25$  U/L with a range of 24.36 to 63.58 U/L in thalassemic children (study group) as shown in table no.-1. The highly significant decrease ( $P < 0.0001$ ) in Catalase level was observed in thalassemic subjects or case group when compared to healthy control subjects showed in table no.-1.

Besides these, the correlation between serum MDA and serum antioxidant enzymes were also determined in healthy control subjects as well as thalassemic subjects. All these were found to be positive non significantly correlate with MDA concentration in healthy subjects while these were negative and statistically significantly correlate with MDA in thalassemic children as shown in table no.-2,3 and figure no.-1,2,3.

**Table no.-1**  
**Biochemical data of Antioxidant enzymes in Thalassemic patients and Healthy control subjects**

<b>Enzymes</b>	<b>Thalassemic Patients(Mean±SD) (n=50)</b>	<b>Healthy control subjects (Mean±SD) (n=50)</b>	<b>P-Value</b>	<b>Significance</b>
Glutathione (GSH) U/L	4.79±0.87	13.97±2.52	<0.0001	HS
Superoxide dismutase (SOD)U/mL	128.52±13.92	207.85±20.14	<0.0001	HS
Catalase (CAT)U/L	42.32±12.25	118.17±17.04	<0.0001	HS
Malondialdehyde (MDA)nmol/mL	17.16±1.79	8.99±1.33	<0.0001	HS

**Table No.-2**  
**Correlation of MDA with Antioxidant enzymes levels of Healthy control subjects**

<b>S. No.</b>	<b>Correlation</b>	<b>r-value</b>	<b>P value</b>	<b>Inference</b>
1	MDA v/s GSH	+0.10	>0.01	NS*
2	MDA v/s SOD	+0.01	>0.01	NS*
3	MDA v/s CAT	+0.19	>0.01	NS*

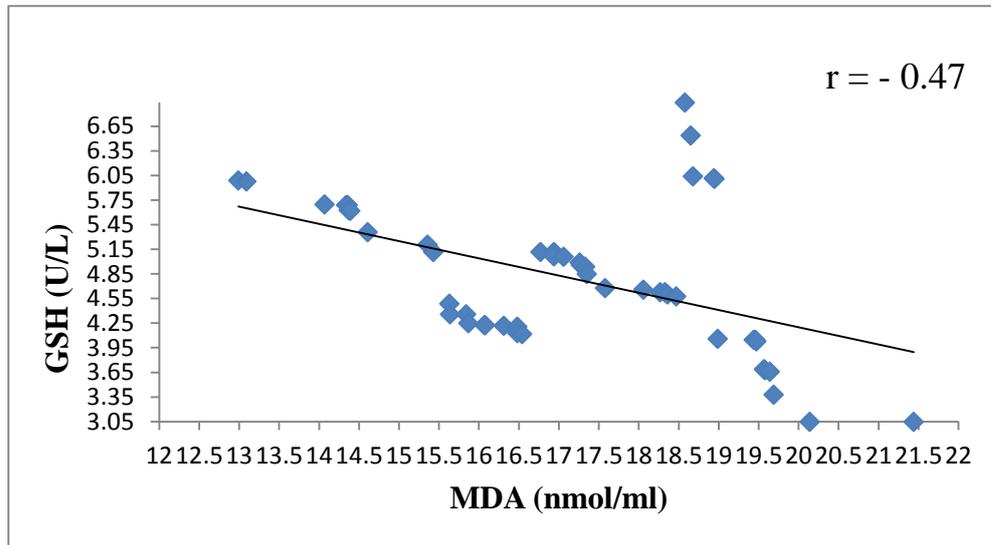
**Table No.-3**  
**Correlation of MDA with Antioxidant enzymes levels of Thalassemic patients**

<b>S. No.</b>	<b>Correlation</b>	<b>r-value</b>	<b>P value</b>	<b>Inference</b>
1	MDA v/s GSH	-0.47	< 0.0001	HS*
2	MDA v/s SOD	-0.64	< 0.0001	HS*
3	MDA v/s CAT	-0.62	< 0.0001	HS*

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

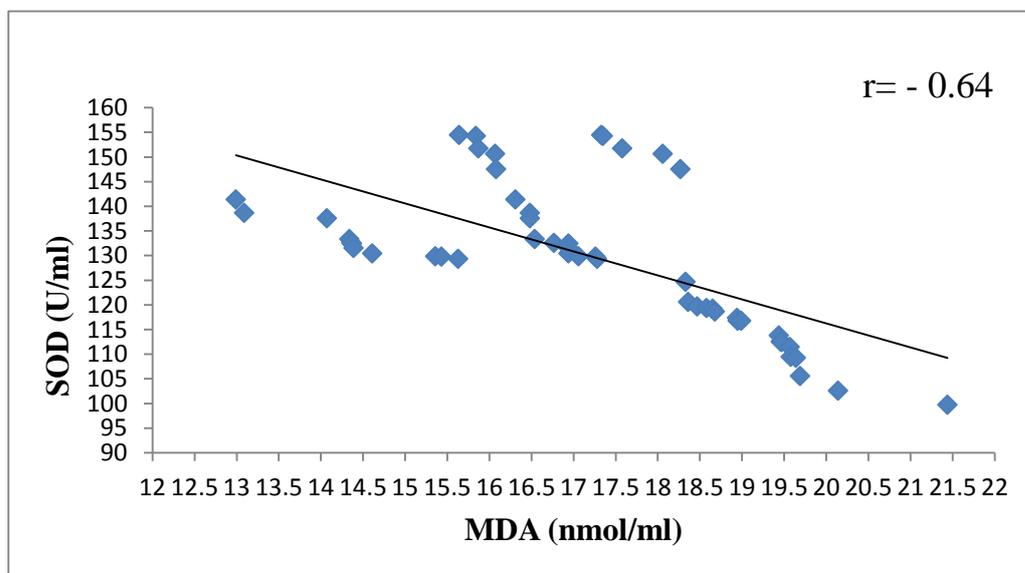
**Figure No.-1**

**Correlation between serum MDA and Glutathione in Thalassemic patients**

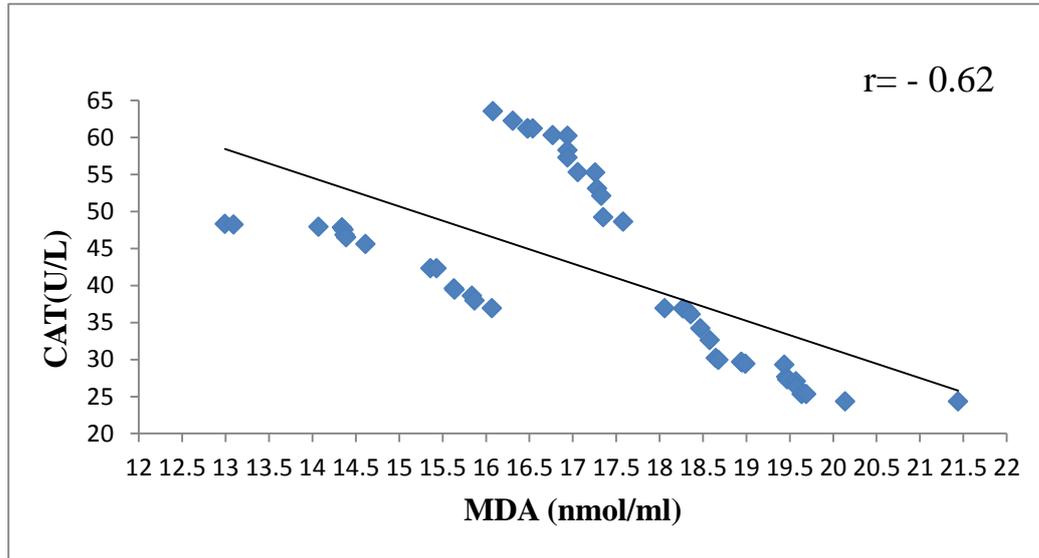


**Figure No.-2**

**Correlation between serum MDA and Superoxide dismutase in Thalassemic patients**



**Figure No.-3**  
**Correlation between serum MDA and Catalase in Thalassemic patients**



## DISCUSSION

Malondialdehyde level was higher in the study group than control groups, these results agree with previous studies. In one of the previous studies, MDA was found to be higher in regularly transfused thalassemia patients than in the thalassemia intermedia patients.[9] As a result of continuous blood transfusions, the patients might be subjected to peroxidative tissue injury by the secondary iron overload. These findings might support the idea that iron overload in thalassemia leads to an enhanced generation of reactive oxygen species and oxidative stress. It also correlate with the study of Elham Abed Mahdi in 2014.[10]

The present study reported a deficiency in levels of reduced glutathione, which is 2.3 times lower than in healthy controls. These results go hand in hand with previous study, which suggested that glutathione (GSH) is a major intracellular reducing agent, which is very sensitive to oxidative pressure and has several important function such as: protection against oxidative stress, regulation of gene expression, induction of apoptosis activation and proliferation in T lymphocytes.[11]

Our results are in agreement with those of Dhawan et al.[12],who found that the mean SOD enzyme activity was at least 1.5 times lower in the thalassaemic than in controls. The findings pertaining to SOD enzyme activity reported by other investigators are varied. They ranged from high SOD activity to no difference in patients and controls.[13,14]

Catalase, widely distributed in all cells, is present in high amounts in erythrocytes. It is responsible for detoxification of hydrogen peroxide in the cells. In present study, catalase was significantly lower in thalassemia patients as compared with healthy controls could be due to increase in the lipid per oxidation product malondialdehyde, which can form cross links, therapy inactivating several membrane bound enzymes.[15,16]

Our study also correlate the study of A.B.Patne et al. in 2012, who studied on 50 beta thalassemia major and 50 healthy controls and found that serum superoxide dismutase and glutathione activities were significantly decreased in thalassemia patients as compared to healthy individuals.[17] These results also goes with the Faiza Waseem et al. in 2011, who also found the levels of these enzymes were significantly lowered ( $p < 0.001$ ) in thalassemia patients compared to control groups.[18]

These results suggested that as the MDA concentration increases, serum antioxidants levels were decreased in children suffering from thalassemia. Low level of glutathione seems to result from the enzyme inhibition or reduced activity due to excessive production of hydrogen peroxide. Decreased levels of superoxide dismutase might be due to inactivation by the increased super oxide anion production leading to an increase in oxidative stress. Decrease in the activity of catalase could be due to increase in the lipid per oxidation product malondialdehyde, which can form cross links, therapy inactivating several membrane bound enzymes.

## **CONCLUSION**

This study indicates that oxidative stress in patients with thalassemia is mainly caused by per oxidative injury due to secondary iron overload. Production of free radicals cause alteration in serum antioxidant enzymes status which play an important role in the pathogenesis of thalassemia. There is limited data available concerning oxidative stress, antioxidant status, degree of peroxidise damage, and role of antioxidant enzymes in thalassemia patients. Studies on antioxidant enzymes like glutathione, superoxide dismutase and catalase reveal significant change in plasma concentration. The administration of selective antioxidant along with essential antioxidant enzymes, in order to reduce the extent of oxidative damage and the related complications in thalassemia still need further evaluation.

## **ACKNOWLEDGEMENT**

We thank all members of staff at the faculty of medical Biochemistry department of Sardar Patel Medical college, Bikaner, Rajasthan for their help and support, and colleagues at Laboratory Unit of P.B.M. Hospital Bikaner for excellent technical assistance in conducting the antioxidant enzymes status analysis.

**DECLARATIONS**

Funding: Self funding

Conflict of interest: None declared

Ethical approval: The study was approved by the departmental and institutional Ethics Committee

**REFERENCES**

- [1] Phumala N., Porasuphatana S., Unchern S., Pootrakul P., Fucharoen S., Chantharaksri U. (2003): Hemin: a possible cause of oxidative stress in blood circulation of beta thalassemia/hemoglobin E disease, *Free Radic. Res*; 37(2): 129–35.
- [2] Weatherall D.J., Clegg J.B. (1982): Thalassemia revised, *Cell*; 29: 7–9.
- [3] Petkov GH., Efremov GD., Efremov DG., Dimovski A., Tchaicarova P., Tchaicarov R., Rogina B., Agarwal S., Kutlar A., Kutlar F. et al. (1990): Beta-thalassemia in Bulgaria, *Hemoglobin*; 14(1): 25–33.
- [4] Livrea MA., Tesoriere L., Pintaudi AM., Calabrese A., Maggio A., Freisleben HJ., D'Arpa D., D'Anna R., Bongiorno A. (1996): Oxidative stress and antioxidant status in beta thalassemia major: iron overload and depletion of lipid-soluble antioxidants, *Blood*; 88(9): 3608–14.
- [5] Livrea MA, Tesoriere L, Pintaudi AM, Calabrese A, Maggio A, Freisleben HJ, et al. Oxidative stress and Antioxidant status in beta thalassemia major: Iron overload and depletion of lipid - soluble antioxidants. *Blood* 1996, 88: 3608-14.
- [6] Widad NM, Al-Naama L, Meaad. Trace element in patients with beta thalassemia major. *Haem* 2003 ; 6(3) : 376-83.
- [7] Das N, Chowdhury TD, Chattopadhyay A, Datta. Attenuation of oxidation stress - induced changes in thalassemic erythrocytes by Vitamin E. *Pol J Pharmacol* 2004 ; 56 : 85-96.
- [8] Ambekar SS, Phadke MA, Balpande DN, Mokashi GD, Khedkar VA, et al. The prevalence and Heterogeneity of Beta Thalassemia Mutation in The Western Maharashtra Population : A hospital based study. *IJHG* 2001; 1(3) : 219-23.
- [9] G. Cighetti, L. Duca, S. Bortone and M. Handyani, Oxidative Status and Malondialdehyde in  $\beta$ - Thalassemia Patients, *Eur. J. Clin. Invest.*, 32, 55-60 (2002).
- [10] Elham abed mahdi, Relationship between oxidative stress and antioxidant Status in beta thalassemia major patients, *acta chim. Pharm. Indica*: 4(3), 2014, 137-145.

- [11] W. Droge and M. Breitk, Glutathione and Immune Function, *Proc. Nutr. Soc.*, 59, 40-56 (2000).
- [12] V. Dhawan, Kh. R. Kumar, R. K. Marwaha, S. Naravan and M. Kamgar, Antioxidant Status in Children with Homozygous Thalassemia, *Ind. Pedi.*, 42, 1141-1145 (2005).
- [13] S. Simsek, G. Ozturk, S. Kemahli and H. Lardi, Oxidant and Antioxidant Status in  $\alpha$  Thalassemia Major Patients, *J Ankara Univ. Fac. Med.*, 58, 34-38 (2005).
- [14] G. Gerli, L. Beretta, M. Bianchi and K. Reoselate, Erythrocyte Superoxide Dismutase, Catalase and Glutathione Peroxidase Activities in  $\alpha$ -Thalassemia (Major and Minor), *Scand. J. Haematol.*, 25, 87-92 (1980).
- [15] K. Kikugawa, H. Kousugi and T. Asakura, Effects of MDA, a Product of Lipid Peroxidation on the Function and Stability of Hemoglobin, *Arch Bio. Biophys.*, 229, 227-232 (1984).
- [16] C. Pfafferott, H. Meiselman and P. Hochstin, The Effect of MDA on Erythrocyte Deformability, *Blood*, 59, 62-69 (1982).
- [17] B. Patne , P.J. Hisalkar , S.B. Gaikwad and S.V. Patil; *International journal of pharmacy and life sciences*, Alterations in antioxidant enzyme status with lipid peroxidation in  $\beta$ - thalassemia major patients, 2012, Vol. 3, Issue 10: October.
- [18] Faiza Waseem, Karira A. Khemomal, Raihan Sajid; Antioxidant status in beta thalassemia major: A single case study, *Indian journal of pathology and microbiology*-2011, 54(4).

