



## Alterations in antioxidant enzyme status with lipid peroxidation in $\beta$ thalassemia major patients

A. B. Patne<sup>1\*</sup>, P.J. Hisalkar<sup>1</sup>, S.B. Gaikwad<sup>2</sup> and S.V. Patil<sup>3</sup>

1, Dept. of Biochemistry, ACPM Medical College & Hospital, Dhule, (MH) - India

2, Dept. of Biochemistry, Government Medical College Aurangabad, (MH) - India

3, Dept. of Community Medicine, ACPM Medical College & Hospital, Dhule, (MH) - India

### Abstract

$\beta$  thalassemia major is an inherited disease resulting from reduction or total lack of beta globin chains. Patients with this disease need repeated blood transfusion for survival, this may cause oxidative stress. Although some reports suggest endogenous free radical damage in thalassemia, there remains discrepancy in the status of antioxidant enzymes. The prevalence of  $\beta$  thalassemia major is high in tribal and non tribal population in area around Dhule, Nandurbar and Jalgaon districts in Maharashtra. So the present study was initiated to evaluate the status of antioxidant enzymes with lipid peroxidation in  $\beta$  thalassemia major patients. Blood samples were collected from 100 subjects (50  $\beta$ -thalassemia major patients and 50 healthy controls). Serum levels of iron, total iron binding capacity (TIBC), ferritin, MDA and TAS activity were determined using conventional methods. Serum Iron, MDA were significantly increased while TIBC, SOD and GPX activities were significantly decreased in  $\beta$ -thalassemia major patients as compared to healthy individuals. Our results suggest that iron overload causes peroxidative damage in beta-thalassemia and antioxidant systems try to compensate for reducing lipid peroxidation to lower tissue damage.

Key-Words: Thalassemia major, Oxidative stress, Glutathione peroxidase (GPx), Superoxide dismutase (SOD)

### Introduction

$\beta$  thalassemia is one of the most common genetic disorder in India.<sup>1</sup> Increasing evidence in both experimental and clinical studies suggests that oxidative stress and free radical release plays a major role in thalassemia. Abnormally high levels of these free radicals and the simultaneous decline of antioxidants defence mechanism can lead to membrane permeability alterations, influx of calcium and activation of protein kinases and proteases which leads to its complications.<sup>2,3</sup> These processes are characterized by metabolic hyper production of reactive oxygen species (ROS) and induced lipid peroxidation (LPO). 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 thalassaemic erythrocytes. Trace metals, especially Iron are implicated as causative agents in excessive generation of free radical which are capable of causing oxidative damage to erythrocytes.<sup>4</sup>

Oxidative stress exceeds the capacity of the antioxidant defences (content of antioxidants and activity of antioxidant enzymes). It activates diverse damaging processes in cells, including oxidation of intracellular and surface components of the red blood cells in  $\beta$  thalassemia major patients.<sup>5-7</sup> Antioxidants are complex and diverse group of molecules that protect key biological sites from oxidative damage. They scavenge free radicals and other reactive oxygen species (ROS).<sup>8</sup> Early introduction of chelatory agents control and combat iron overload, inhibit ROS generation and regulate LPO processes, leading to improved life expectancy.<sup>9</sup> Enzymatic antioxidants include catalase, superoxide dismutase and glutathione peroxidase. Superoxide dismutase, a copper-zinc and manganese-containing enzyme, reacts with superoxide radical to form hydrogen peroxide, which is then converted to water by glutathione peroxidase (a glutathione dependent selenoprotein), or catalase, a heme enzyme. The prevalence of  $\beta$  thalassemia major is high in tribal and non tribal population in area around Dhule, Nandurbar and Jalgaon districts in Maharashtra. Very few studies have been carried on the role of oxidative stress in  $\beta$ -thalassemia major in this region. Therefore, the study was planned to determine the antioxidant

### \* Corresponding Author

E.mail: anupatne@yahoo.com

Mob.: +91-9422993974

status of the following enzymes in beta thalassemia major.

- Superoxide dismutase (SOD)
- Glutathione per oxidase (GPx)

### Patients and Methods

Blood samples were collected from 48  $\beta$ -thalassemia major patients. They were clinically diagnosed by clinical history, requirement for regular blood transfusions and laboratory tests including complete blood count (CBC) and hemoglobin electrophoresis from Shri Bahusaheb Hire Government Medical College & Hospital, ACPM Medical College & Hospital and Navjeevan Blood Bank, Thalassaemia Center, Dhule (Maharashtra), during the period June 2010- April 2012.

These patients were compared with healthy normal participants on the basis of age, sex, dietary conditions and life styles. None of the patients or control subjects enrolled in this study received antioxidant supplementations that could affect the results. Mean age of patients and participants was in range between 10 to 31 years. Exclusion criteria were having diabetes mellitus, hypothyroidism, hyperthyroidism, renal failure and hereditary hyperlipidemia. This study was approved by the Ethics Committee.

**Biochemical Investigations:** Serum lipid peroxide was measured by precipitating lipoproteins with trichloroacetic acid and boiling with thiobarbituric acid which reacts with malondialdehyde to get pink colour as per the method of Kei Satho.<sup>10</sup> Serum Iron and TIBC were measured by Ferrozine Method, using CREST BIOSYSTEMS kit. Superoxide dismutase (SOD) was measured by using RANSOD kit and Glutathione peroxidase (GPx) was measured by using RANSEL kit (Randox Laboratories Ltd. Crumlin, United Kingdom) This method is based on Paglia DE and Valentine W N.<sup>11</sup>

**Statistical Analysis:** The data obtained in our study was analyzed for its statistical significance using 'z' test. P value less than 0.05 was considered the level of significance.

### Results and Discussion

The observations and inference obtained from this study are summarized in the following table:

Parameter	$\beta$ -thalassemia major patients (n = 48)	Controls (n = 30)
Age (year)	21.1 $\pm$ 4.6	21.7 $\pm$ 3.1
Sex (M/F)	34/14	21/09
Hb (g/dL)	8.3 $\pm$ 1.2 *	11.2 $\pm$ 0.6
Serum Iron ( $\mu$ g/dl)	156.4 $\pm$ 19.8 *	104.7 $\pm$ 14.3

Serum TIBC ( $\mu$ g/dl)	239.1 $\pm$ 43.6 *	293 $\pm$ 24.2
Serum MDA (nmol/ml)	3.4 $\pm$ 1.1 *	1.9 $\pm$ 0.4
SOD (U/ml)	111 $\pm$ 2.7 *	206.5 $\pm$ 5.5
GPx (U/ml)	4053.95 $\pm$ 161.9 *	7388 $\pm$ 135.3

Data are presented as means  $\pm$  SD

\* Significant difference compared with controls ( p < 0.05 )

It is clear that a significant decrease of hemoglobin concentration in thalassaemic patients as compared to controls. On the other hand serum iron & MDA were significantly higher as well as serum TIBC, SOD & GPx were significantly lower in thalassaemic patients as compared to controls.

In thalassaemic patients regular blood transfusions along with chelation therapy drastically improve the quality and duration of life. But Iron overload is a serious complication of long term blood transfusion which increases free radical production and peroxidative damage of tissues. In such condition, depletion of endogenous antioxidants may be expected. The intracellular antioxidative mechanisms normally prevent damage due to a dangerous combination of oxygen and iron (hemoglobin or hemoglobin derived). The released heme has been shown to directly inhibit a number of cytoplasmic enzymes, further disrupting normal cellular homeostasis and predisposing the cell to additional injury. In addition to the released heme, the  $\alpha$ -hemoglobin chain-mediated production of reactive oxygen species ( $O_2$ ,  $H_2O_2$ ) catalyzes the production of the hydroxyl radical ( $OH^*$ ) and reactive lipid radicals. These species are believed to mediate much of the damage seen in thalassaemic erythrocytes.<sup>12</sup> Removal of these oxygen metabolites is the function of antioxidant enzymes such as SOD and GPx.

SOD: Superoxide dismutases are the proteins co factor with copper, zinc manganese, iron or nickel. In humans it exists in three different forms including SOD1 found in cytoplasm, SOD2 present in cytoplasm, and SOD3 is extracellular. Superoxide is the main reactive oxygen species which react with nitric oxide radical and forms peroxynitrite thereby causing oxidative stress and cellular damage. SOD is the essential antioxidant that decreases the formation of ROS and oxidative stress thus protecting the cells from damage. Erythrocyte SOD protects the erythrocyte from being damage during oxidative stress. SOD activity in patients with  $\beta$  thalassemia major is decreased, resulting in pronounced inhibition of the blood antioxidant capacity. Our results are in agreement with those of



Dhawan *et al.*, who found that the mean SOD enzyme activity was at least 1.5 times lower in the thalassaemic than in controls.<sup>13</sup> The findings pertaining to erythrocytic SOD enzyme activity reported by other investigators are varied. They ranged from high SOD activity to no difference in patients and controls.<sup>14,15</sup> GPx: This antioxidant enzyme belongs to a group of antioxidant selenoenzymes that protects the cells from damage by catalyzing the reduction of lipid hydroperoxides. This action requires the presence of glutathione. GPx levels in the body are in close relation with the glutathione which is the most important antioxidant present in the cytoplasm of the cells. The stability of the cellular and sub cellular membranes depend mainly on GPx and the protective antioxidant effect of GPx depends on the presence of selenium. GPx also protects the heart from damage by stress due to oxygen free radicals through its antioxidant effect. In our study, significantly reduced levels of GPx were observed in patients with  $\beta$  thalassemia major as compared with controls. Our findings are in confirmation with the study of Garelnabi *et al.*<sup>16</sup> Low level of GPx seems to result from the enzyme inhibition or reduced activity due to excessive production of hydrogen peroxide. This study showed significantly lower levels of all the antioxidants-vitamin E, GPx and SOD in thalassaemic children compared with the matched healthy controls. In contrast to these findings, we found a significant elevation of signs of iron overload and cell damage (serum Iron and MDA) in patients with  $\beta$  thalassemia major when compared with controls. Our study confirm that, 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 leads 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.<sup>17,18</sup> Iron are also implicated as causative agents in excessive generation of free radical which are capable of causing oxidative damage to erythrocytes.<sup>19</sup> Our results agree with those of Das N et al (2004) and Livrea MA et al (1996). This oxidative stress will cause growth failure as well as liver, cardiovascular, endocrine and neurological complications in  $\beta$  thalassemia major.<sup>20</sup> Peroxidative damage of lipids is indicated by the increase in serum MDA and decreased antioxidant defence mechanism play an important role in the pathogenesis of  $\beta$  thalassemia major. It requires adequate treatment in thalassaemic so that the early deaths especially from iron induced cardiomyopathies will be prevented.<sup>21</sup>

### **Conclusion**

This study indicates that in patients with  $\beta$  thalassemia impairment of the antioxidant enzymes associated with elevated plasma levels of lipid peroxidation. The administration of selective antioxidants along with essential trace elements and few minerals in order to reduce the extent of oxidative damage and the related complications in  $\beta$  thalassemia major.

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