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Malondialdehyde (MDA), a marker of oxidative stress in Beta Thalassemia major patients

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Abstract

Background: The severe iron overload in beta thalassemia major patients leads to generation of free radicals and peroxidative tissue injury. This process is characterized by metabolic hyperproduction of reactive oxygen species (ROS) and induced lipid peroxidation (LPO). Malondialdehyde (MDA), a terminal compound of lipid peroxidation, is used widely as an index of oxidative status.
Method: serum MDA, iron, ferritin levels were measured in 49 beta thalassemia patients and were compared with age and gender matched 49 healthy individuals.
Result: The MDA level increased significantly in β thalassemia major patients (3.01±0.38) as compared to controls (1.68±0.2). Serum free iron, serum ferritin level was high in study group.
Conclusion: We conclude that in patients with β thalassemia, MDA is the marker of oxidative stress.
Keywords: Beta-thalassemia major; Oxidative stress; Malondialdehyde; Ferritin.

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1. Introduction

Beta-thalassemia major is an autosomal recessive disease causing severe hemolytic anemia, Iron overload, which arises from recurrent transfusion and ineffective erythropoiesis, enhances oxidative stress in thalassemic patients. Peroxidation of membrane lipids represents a primary consequence of cellular oxidative stress. Oxidative stress is defined as "an imbalance between oxidants and antioxidants in favour of the oxidants, leading to a disruption of redox signaling and control and/or molecular damage". [1]

In case of Thalassemia patients, repeated blood transfusions lead to iron overload resulting in immense Reactive Oxygen Species (ROS) generation. At physiological pH, most of the iron is bound and gets chelated in its oxidized form, Fe^{3+} In order to take part in the generation of OH• (Haber–Weiss reaction), the iron must undergo reduction into its reduced form, Fe^{2+}, by superoxide radicals (Fenton reaction). Fe^{2+} can interact with H_2O_2, produced from the spontaneous or enzymatic dismutation of superoxide radicals, to yield OH•. (Figure 1)

The final result of these two reactions is the production of hydroxyl radicals:

$Fe^{3+} + \bullet O^{-2} \rightarrow Fe^{2+} + O2$

$Fe^{2+} + H2O2 \rightarrow Fe^{3+} + OH^- + \bullet OH$ (Fenton reaction)

Net reaction: $\bullet O^{-2} + H2O2 \rightarrow \bullet OH + HO^- + O2$

Ferritin is a marker of iron overload as it is the storage protein for iron. Most ferritin is present in liver, spleen, and bone marrow, and a trace amount is found in the blood as serum ferritin. The greatest concentrations are typically present in the hepatocytes and immune system known as reticuloendothelial cells. Malondialdehyde (MDA), a terminal compound of lipid peroxidation, is used widely as an index of oxidative status [2]. Figure 2 represent stages of MDA formation.

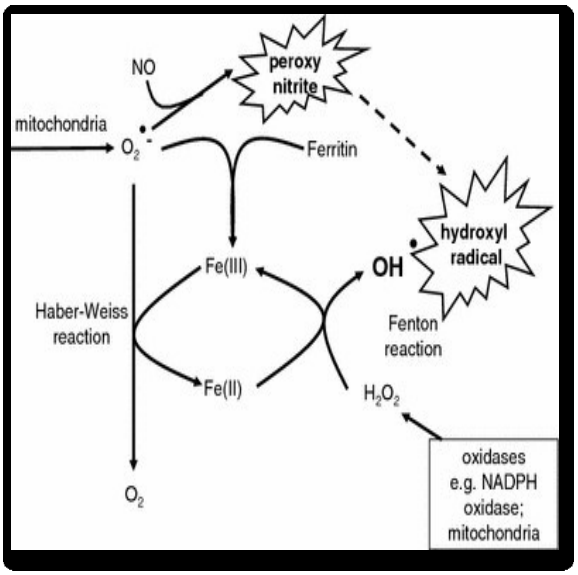


Figure 1: Haber–Weiss reaction

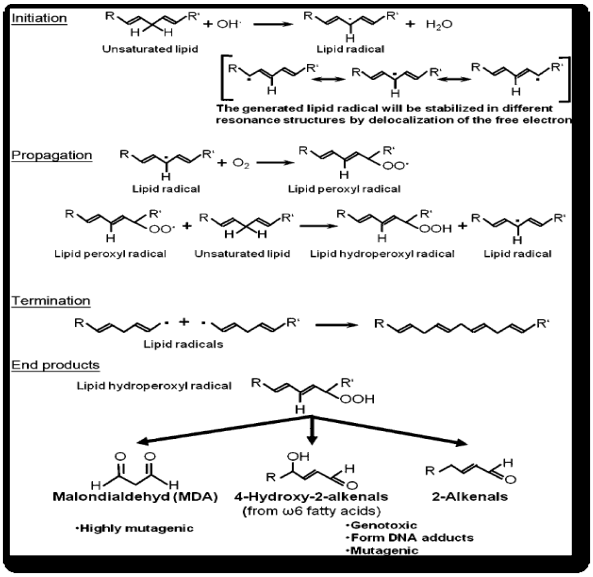


Figure 2: Stages of MDA formation

2. Materials and Method

After obtaining Institutional Ethical Committee approval and written informed consent from the patients/relatives, this cross sectional, analytical study was conducted in total 98 patients in the Department of Biochemistry in a Tertiary Care Centre during the study period of 1 year from September 2017 to August 2018. They were divided in to two equal groups. Study group: 49 patients of either sex, age 5 to 25 years, diagnosed with beta thalassemia major with blood transfusion and on iron chelating agents. Control group: 49 non thalassemic, normal healthy age and sex matched controls. The blood in plain bulbs were kept for 15 minutes to allow clotting and to prevent hemolysis and then subjected to centrifugation at 3000 to 4000 rpm for 15 to 20 minutes and serum was collected. The serum samples were analyzed as per procedure of biochemical parameters. The reports thus obtained after estimating the biochemical parameters from serum such as Iron, ferritin, MDA in patients and compared with normal healthy controls.

Table 1: Methods for estimation of above parameters

S. No.	Parameters	Method used
1	Iron	Ferrozine Method [3]
2	Ferritin	Immunoturbidimetric Method [4]
3	MDA	Modified Sadasivadu et al [5]

3. Observation and Result

Level of lipid peroxidation expressed in serum MDA levels which were increased in β thalassemia major patients as compared to controls. Student‘t’ test was analyzed using Open epi info version 2.3, year 2009. P values less than 0.05 were considered as statistically significant. We found significant correlation of ferritin with Iron and MDA. Iron correlates significantly with MDA. Ferritin has a positive correlation with MDA and Iron. (Table 3)

Table 2: Comparison of serum levels of biochemical parameters between two groups

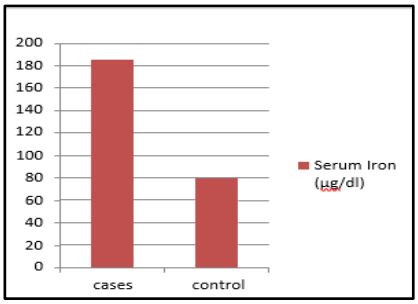
Parameters (Serum)	β thalassemia major (n=49)	Normal controls (n=49)	P value
Iron (µg/dl)	185.65 ± 58.75	79.12 ± 28.73	<0.001
Ferritin (ng/ml)	3431.65 ± 1567.7	128.57 ± 26.8	<0.001
MDA (nmol/L)	3.01 ± 0.38	1.68 ± 0.2	<0.001

Table 3: Pearson Correlation: Serum Ferritin with other variables in cases

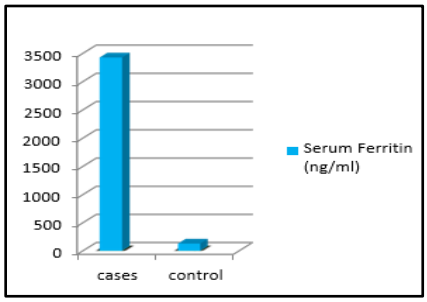
Variables	Pearson Correlation factor	P value (two tailed)
Ferritin with Iron	0.631	< 0.001**
Ferritin with MDA	0.0.488	<0.001**
MDA with Iron	0.345	0.015*

Remark: ** Correlation is significant at the 0.01 level (2-tailed), *. Correlation is significant at the 0.05 level (2-tailed).

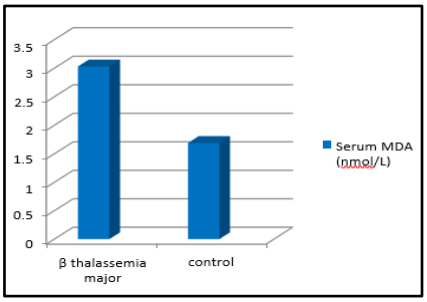
The following graphs represent the level of lipid peroxidation expressed in thalassemia major cases and healthy control in parameters like serum Iron, Ferritin and MDA respectively in graph (A, B, C). Whereas the graphs 1 and 2 depict the correlation between Ferritin and MDA, Iron and MDA.



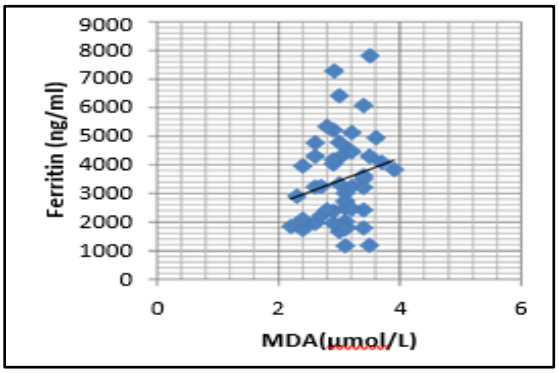
Graph A



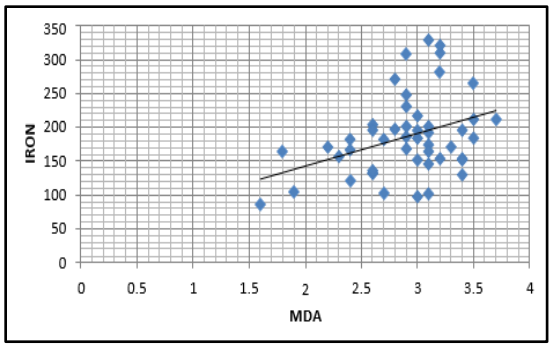
Graph B



Graph C



Graph 1: Correlation between Ferritin and MDA



Graph 2: correlation between Iron and MDA

4. Discussion

Iron induced oxidative stress is known to be one of the most important factors determining cell injury in thalassemic patients. Studies have confirmed the progression of oxidative stress in patients with β-thalassemia major, activation of free radical processes and lipid peroxidation.[6] Malondialdehyde (MDA), the end product of lipid peroxidation is a marker of oxidative stress. There are increasing reports indicating imbalance between free radical generation and antioxidant defence mechanism in β thalassemia major patients.

In the present study, MDA is increased from 1.68 ± 0.2 to 3.01 ± 0.38 in β thalassemia major when we compared with control group (graph no.4). We got positive correlation of MDA with serum ferritin and serum iron which is statistically significant (Table No 3).

In 1976, Rachmilewitz et al observed 2 fold increases in MDA level and considerable loss in serum α tocopherol levels. Autooxidation may be initiated by free radicals, which are constantly formed in normal red cell, prevalent when stable haemoglobins are present. They suggested that supplementation of Vitamin E is recommended to prevent the membrane damage.[7] Also M.M.A. Attia et al reported malondialdehyde concentration 3.4 times higher in β-thalassemia patients than in healthy controls. After a period of twelve months of vitamins treatment, improvements in all non-enzymatic antioxidants levels was noticed when compared with untreated β-thalassemia patients and MDA was decreased significantly. [8]

Another study conducted by Cighetti et al confirmed free and total MDA and Non Transferrin Bound Iron (NTBI) levels were higher in the Thalassemia Major (TM) patients than in the Thalassemia Intermedia (TI). In the TM patients the free MDA levels correlated positively with serum iron, whereas the total MDA correlated positively with NTBI. [9]

An agreement comes from P. B. Walter et al stating MDA level higher in β thalassemia major than controls. Regression analysis comparing plasma malondialdehyde (MDA) (nmol/l) with liver iron concentration shows higher liver iron concentrations are associated with more plasma MDA. Livrea *et al* explained Ferritin levels were positively correlated with the amount of MDA as the deleterious effects of high tissue iron levels.[6] Its correlation with MDA was positive (Graph 5). Pavlova [10], Adaletmerall [11], Cighete [12], Attia et al [8] exhibited positive correlation of MDA with ferritin.

It has been already known that oxidative stress is increased in patients with iron overload. We know that during the course of metabolism, superoxide anion is converted to H_2O_2 by ubiquitous enzyme superoxide dismutase. Normally H_2O_2 is converted to innocuous compounds by the action of catalase and peroxidase. But if free iron is available, it reacts with H_2O_2 to form hydroxyl radicals. This is a result of mounted concentration of highly reactive Fe^{2+} ions which catalyze the Fenton and Haber weiss reaction and leads to generation of ROS. Moreover our body does not excrete iron in any form hence it remains in circulation; it is stored in the body with the help of ferritin in liver. Only in case of females iron loss is noted during menstruation. Our study results harmonises with the above fact as free iron was found to be increased in males and females inspite of daily iron chelation therapy.

Our result is supported by various studies undertook since 1996 by Livrea et al [6], Asma Kassab-Chekir[13], Pavlova[10], Trivedi[14] and Mahdi [15], Sengsuk [16], Hageman [17].

Ferritin is the intracellular protein responsible for the sequestration, storage and release of iron. Ferritin protects the cell against insoluble ferric oxide and oxyhydroxide formation, as well as against the production of oxygen radicals. In our study, serum ferritin levels were 3431.65 ± 1567.7 in β thalassemia major, 128.57 ± 26.8 in controls and were highly significant (Table 2). The increase of ferritin creates ideal conditions for radical oxygen species formation to damage erythrocytes. The study by Ford et al suggests that in beta- thalassemia, the first target organ dysfunction of diminished antioxidant reserves is the liver, enhanced by an iron overload leading to an acute free radicals action. [18]

5. Conclusion

Our study was conducted to assess oxidative stress in thalassemia major patients. Measurement of MDA level reflected free radical generation. By comparing and correlating Malondialdehyde (MDA) levels in case and control, we can conclude that MDA is definitely a marker of oxidative stress in beta thalassemia patients.

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