

Effect of iron overload on renal functions and oxidative stress in beta thalassemia patients

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ABSTRACT

الأهداف: التحقق في كمية التلف الخلوي الناجم عن النقل المتسلسل في الدم لدى مرضى الثلاسيميا.

الطريقة: أجريت دراسة مستعرضة في جامعة لاهور، مدينة لاهور، باكستان بين أغسطس 2012 وديسمبر 2012 وخضع ما مجموعه 43 مريضاً بالثلاسيميا لعشر عمليات نقل الدم على الأقل. أجري تحليل كيميائي حيوي شامل من الدم لتسجيل مستويات الكرياتينين واليوريا وحمض اليوريك، الزلال، اختبارات وظائف الكبد، malondialdehyde MDA، والفيريتين.

النتائج: كانت مصل الكرياتينين ($0.732 \pm 0.23 \text{mg/dl}$) وحمض اليوريك ($6.7 \pm 0.94 \text{mg/dl}$, $p < 0.05$) أعلى بكثير في مجموعات المرضى بالمقارنة مع مجموعات التحكم. وكانت مستويات الفيريتين أعلى بكثير (3103.9 ± 1747.4 , $p < 0.05$). وقد لوحظت مستويات الهيموجلوبين في مجموعات الضوابط $1414 \pm 1.3 \text{g/dl}$ وفي المرضى $7.1 \pm 1.03 \text{g/dl}$. لا توجد علاقة واضحة بين العمر والدموية لدى مرضى الثلاسيميا. يرتبط مستوى مصل الفيريتين بإيجابية مع ناقلة في الدم ألانين، الألانين اسبارتاتي، والفوسفاتيز القلوية و MDA $p < 0.05$.

الخاتمة: يعكس كلاً من مصل MDA ومصل الفيريتين ($r = 0.593$, $p < 0.05$) معاملات حاسمة حسب تقدير تلف الخلايا في المرضى الذين يعانون من مرض الثلاسيميا.

Objectives: To check the amount of cellular damage caused by serial transfusions of blood in thalassemia patients.

Methods: A cross-sectional study was conducted in the University of Lahore, Lahore, Pakistan between August 2012 and December 2012. A total of 43 thalassemia patients underwent at least 10 blood transfusions. Comprehensive biochemical analysis of blood was performed to record the levels of creatinine, urea, uric acid, albumin, liver function tests, malondialdehyde (MDA), and ferritin.

Results: Serum creatinine ($0.732 \pm 0.23 \text{mg/dl}$) and uric acid ($6.7 \pm 0.94 \text{mg/dl}$, $p < 0.05$) were significantly higher in patient groups as compared with the control. Ferritin levels were significantly higher in patients as compared with the control (3103.9 ± 1747.4 , $p < 0.05$). Hemoglobin levels were observed in controls $14 \pm 1.3 \text{g/dl}$ and in patients $7.1 \pm 1.03 \text{g/dl}$. No clear relationship exists between age and hematological parameters of thalassemic patients. Serum ferritin level is positively related with serum alanine transaminase, aspartate aminotransferase, and alkaline phosphatase and MDA ($p < 0.05$).

Conclusion: Serum MDA and serum ferritin of patients ($r = 0.593$, $p < 0.05$) reflects that both are crucial parameters estimating the cellular damage in patients suffering from thalassemia.

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Thalassemia is among the most common genetic disorders worldwide. Thalassemia patients require blood transfusion on regular basis for their treatment. Patients develop iron overload as a result of excessive number of blood transfusion. 1mg of iron overload has to be borne by the body for each 1ml of red blood cells.^{1,2} Excess iron gets accumulated in different organs of the body namely; liver, kidney, pancreas, and heart.³ For the conversion of molecular oxygen to highly reactive radical species by Fenton Chemistry iron ions are required, which are not available normally. Iron deposits can exceed the storage and detoxification capacity of ferritin. Consequently, this free iron begins to accumulate in the blood and tissues. Very injurious compounds such as hydroxyl radical are formed as a result of free iron reactions. Patients suffering from beta thalassemia encounter increased levels of lipid peroxidation (LPO) products like malondialdehyde (MDA).⁴ Moreover, when there is an increase in LPO due to oxidative stress, it results in decreased antioxidative substances.^{5,6} Oxidative stress is a natural mechanism that happens in biological system by production of free radicals that interact chemically with other biological molecules, and damage to genetic material, membranes, and proteins leading to oxidative injury. On contrary, as natural defense mechanism biological system produces antioxidants to cope with free radicals/oxidants. Two important essential components of antioxidant defense mechanism of red blood cell are glutathione and its redox enzyme system.⁷

Methods. Study design and sample collection.

Cross-sectional study design was selected and implicated on the samples. Samples were collected from Sundas Foundation of Thalassemia, Lahore, Pakistan. Detailed physical examination was performed and all the available blood tests reports were taken. A total of 43 thalassemia patients that satisfied the inclusion criteria were included in the study and compared with 8 healthy control individuals with same gender, age, and body mass index (BMI). Informed written consent was taken from all the patients and controls participating in the study according to the Helsinki declaration. The study was also approved by the ethical committee of the University of Lahore, Lahore, Pakistan. The study was performed between August 2012 and December 2012.

Inclusion criteria. Blood was taken from patients who had undergone with at least 10 blood transfusions.

Exclusion criteria. The patients who received less than 10 blood transfusions, or with other health problems were excluded from the study.

Biochemical analysis. All the biochemical tests were performed at the Biochemistry and Physiology of Stress Tolerance Lab, Institute of Molecular biology and Biotechnology (IMBB), The University of Lahore, Pakistan. Serum levels of creatinine (Cr), urea (U), uric acid (UA), and albumin were measured by spectrophotometer by using commercial Redox kits. Liver function tests (LFT's) including serum alanine transaminase (ALT), aspartate aminotransferase (AST), and alkaline phosphatase (ALP) were also measured by using commercial Redox kits. Serum level of MDA was measured by standard method.⁶ Enzyme linked colorimetric immunoassay was used for serum ferritin evaluation.

Statistical analysis. The data is represented as Mean \pm SD and determined by the Independent T-test and Mann-Whitney Test and correlations (Spearman) between patients of thalassemia and control individuals using the Statistical Package for Social Sciences (SPSS Inc., Chicago, IL, USA) version 17. The significance difference was taken as $p < 0.05$.

Results. The results of the study shows that serum urea and serum albumin were not differ statistically between patients and controls ($p > 0.05$) (Table 1). Serum creatinine (0.732 ± 0.23 mg/dl) and uric acid (6.7 ± 0.94 mg/dl, $p < 0.05$) are significantly higher in patient groups and as compared with control. Ferritin levels are significantly higher in patients as compared with control (3103.9 ± 1747.4 , $p < 0.05$). Hemoglobin levels (Hb) in controls are 14 ± 1.3 g/dl and patients are 7.1 ± 1.03 g/dl (Table 1).

Table 1 - Circulating biochemical and stress markers between control and thalassemics.

| Parameters | Control (n=8) | Thalassemic patients (n=43) | P-value |
|---------------------|--------------------|-----------------------------|---------|
| Ferritin (ng/ml) | 129.25 \pm 21.38 | 3103.9 \pm 1747.40 | 0.000* |
| Hb (g/dl) | 14 \pm 1.3 | 7.1 \pm 1.03 | 0.000* |
| Urea (mg/dl) | 23.3 \pm 5.99 | 26 \pm 5.39 | 0.233 |
| Creatinine (mg/dl) | 0.50 \pm 0.22 | 0.73 \pm 0.23 | 0.017* |
| Uric acid (mg/dl) | 4.56 \pm 0.96 | 6.72 \pm 0.94 | 0.000* |
| ALT (IU/L) | 30.37 \pm 7.20 | 98.20 \pm 31.93 | 0.000* |
| AST (IU/L) | 26.0 \pm 5.70 | 110.74 \pm 31.32 | 0.000* |
| ALP (IU/L) | 320.0 \pm 37.43 | 455.51 \pm 123.75 | 0.000* |
| Albumin (g/dl) | 3.83 \pm 0.26 | 4.03 \pm 0.26 | 0.071 |
| MDA (μ mol/ml) | 1.23 \pm 0.17 | 4.86 \pm 0.98 | 0.000* |

Data were compared as (Mean \pm Standard deviation), Hb - Hemoglobin levels, ALT - alanine transaminase, AST - aspartate aminotransferase, ALP - alkaline phosphatase, MDA - malondialdehyde, *significant ($p < 0.05$)

Discussion. Patients suffering from β -thalassemia major (β -TM) have to face another problem referred as iron overload/hemochromatosis due to excessive blood transfusion, and it is generally called second disease.⁸ Ferritin is an intracellular protein that stores as well as release iron in a controlled fashion as per requirement of biological system. However, ferritin is evaluated in serum as indirect measurement of iron deficiency and/or iron overload. Single measure, in case of β -thalassemia, is insufficient and unreliable for iron levels. As a novel approach, magnetic resonance imaging (MRI) could be employed for measuring iron contents in the body because MRI is considered as more sensitive as compared with routine ferritin evaluation from serum.⁹ Low levels of ferritin in serum reflect lesser risk for various clinical problems as well as longer survival for patients with thalassemia.¹⁰ Long lasting anemia, iron overload, toxicity of deferoxamine (an iron-chelating agent) and chronic hypoxia lead to renal dysfunctions in patients with β -TM.¹

In the present study, high serum creatinine was observed only in 12% of the patients, which were in the age group of 15-20 years and those in the chelation therapy for more than 15 years. In the present study, the mean serum uric acid levels were significantly higher in the patient (6.7 ± 0.94 mg/dl) group than in

the controls (4.5 ± 0.96 mg/dl). In addition to rapid erythrocyte turnover, proximal tubular damage may lead to hyperuricemia in these patients, because the filtered uric acid can be reabsorbed from the proximal tubules. Serum alanine transaminase, aspartate aminotransferase, and alkaline phosphatase abnormality in results suggested that patients developed chronic liver malfunctioning. The correlation between ALT activity and serum ferritin ($r=.421$; $p=.002$) level refers to hepatocellular injury (Table 2). Chronic iron overload in hepatocytes leads to cirrhotic outcomes as well as hepatic carcinoma that is referred as hepatoma. Hence, rate of mortality is associated with hepatocellular carcinoma (HCC), diabetes, cardiomyopathy, and liver dysfunctioning.¹¹ Moreover, increasing serum ferritin level seems to be associated with liver cirrhosis.¹² There is a strong association between serum ferritin and MDA, it is proved that both these parameters are important to estimate cellular damage in thalassemia. Moreover, enhanced plasma LPO marker MDA has also been observed previously in patients suffering from thalassemia.^{7,13,14} No clear relationship exists between age and hematological parameters of thalassemic patients. Serum ferritin level is positively related with serum ALT, AST, ALP, and MDA ($p<0.05$). Serum MDA and serum ferritin of patients ($r=.593$, $p<0.05$) reflects that both

Table 2 - Correlation matrix circulating biomarkers in beta thalassemia patients.

| Biomarkers | MDA | Urea | Creatinine | Uric acid | ALT | AST | ALP | Albumin | Hb | Ferritin |
|------------|-----|----------------|------------------|----------------|------------------|------------------|-----------------|-----------------|-------------------|-------------------|
| MDA | 1 | 0.106 0.460 | 0.384** 0.005 | 0.257 0.069 | 0.347* 0.013 | 0.464** 0.001 | 0.279* 0.047 | -0.036 0.800 | -0.285* 0.042 | 0.593** 0.000 |
| Urea | | 1 | 0.112 0.435 | 0.010 0.943 | 0.380** 0.006 | 0.085 0.552 | 0.208 0.143 | 0.186 0.192 | -0.028 0.843 | 0.202 0.154 |
| Creatinine | | | 1 | 0.170 0.233 | 0.197 0.167 | 0.430** 0.002 | -0.061 0.670 | 0.066 0.646 | -0.024 0.868 | 0.248 0.079 |
| Uric acid | | | | 1 | 0.457** 0.001 | 0.386** 0.005 | 0.105 0.465 | 0.101 0.481 | -0.579** 0.000 | 0.285* 0.043 |
| ALT | | | | | 1 | 0.586** 0.000 | 0.180 0.206 | 0.013 0.926 | -0.415** 0.002 | 0.421** 0.002 |
| AST | | | | | | 1 | 0.188 0.187 | -0.014 0.923 | -0.315* 0.024 | 0.392** 0.004 |
| ALP | | | | | | | 1 | 0.173 0.226 | -0.217 0.125 | 0.308* 0.028 |
| Albumin | | | | | | | | 1 | -0.249 0.078 | -0.104 0.470 |
| Hb | | | | | | | | | 1 | -0.402** 0.003 |
| Ferritin | | | | | | | | | | 1 |

MDA - malondialdehyde, ALT - alanine transaminase, AST - aspartate aminotransferase, ALP - alkaline phosphatase, Hb - Hemoglobin levels

are crucial parameters estimating the cellular damage in patients suffering from thalassemia (Table 2). We used limited sample size due to financial constraints and the limitation of patients suffering from thalassemia that have undergone at least 10 blood transfusions and does not show any other health problems. Further research should include more patients with less than 10 blood transfusions.

In conclusion, patients with thalassemia show various clinical signs and symptoms that seem to be unavoidable due to the regular transfusion of blood and its associated physiological as well as pathological outcomes. Having insights into various clinical aspects of thalassemia it can be concluded that antioxidative therapy may be more helpful for long survival of patients with thalassemia with conventional iron chelating therapy.

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