ANTIOXIDANT STATUS IN THALASSEMIC PATIENTS
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Abstract
Background: increased membrane lipid peroxidation in patients with thalassemia has been reported suggesting that superoxide radicals generated in excess following auto oxidation of isolated hemoglobin chains is an important contributor to the hemolytic process.

Objective: This study was undertaken to evaluate the extent of lipid peroxidation and antioxidant status of patients with beta-thalassemia in comparison to healthy people.

Methods: Red cell superoxide dismutase (SOD) activity and red cell catalase activity were measured in the biochemistry department for the period from January 2003 to October 2003, 76 patients with beta-thalassemia, 14 patients with beta-thalassemia minor and 19 healthy controls were studied.

Results: Erythrocytes of patients with beta-thalassemia major had significantly higher SOD than control (p<0.0004). Red cell catalase activity of thalassemia minor patients was significantly higher than that of the control (p<0.05). In thalassemic patients, the more anemic patients have significantly higher SOD activity, but this correlation was not present between anaemic patients & catalase activity.

Conclusion: Red cell superoxide dismutase activity was greatly increased in homozygous beta-thalassemia, and inversely correlated with severity of anaemia.

Keyword: SOD, Catalase, Thalassemia.


Introduction
Auto-oxidation of biomembranes is considered to be the primary factor involved in cellular senescence and breakdown[1,2]. An increased production of highly activated forms of oxygen released during the oxidation of hemoglobin to methemoglobin in thalassemic red blood cells (RBC)[3-8] has stimulated much interest in superoxide dismutase (SOD) and cellular antioxidant for the control of such deleterious radical reactions. The aim of this study was to evaluate the extent of lipid peroxidation and antioxidant status of patients with beta-thalassemia in comparison to healthy people. Also to find any correlations between the level of these antioxidants with the appropriate time of transfusion.

Patients & Methods
During the period of ten months from January 2003 to October 2003, 109 subjects were included in this study; 76 patients with beta-thalassemia major, 14 patients with beta-thalassemia minor were taken from hematology center of Ibn-Balady Hospital, and 19 healthy controls were taken from laboratory healthy staff in Al-Kadhimiya Teaching Hospital.

Venous blood was collected from patients before blood transfusion and then hematological studies were done including; red cell count , white cell count , mean corpuscular volume and haematocrit were determined in a coulter counter ,MS9. haemoglobin concentrations were measured on a haemoglobinometer. Haemoglobin types and quantitation of different types...
were identified by variant haemoglobin testing system. Enzyme assay: the method for SOD measurements is based on the ability of the enzyme to inhibit the reduction of nitroblue tetrazolium by superoxide radical, which is generated by the reaction of photoreduced riboflavin and oxygen\cite{9}. The method for measuring catalase activity is based on the ability of catalase to decompose hydrogen peroxide\cite{10}. Units of the enzyme assays were reported as mg/g Hb, mg/ml red blood cells.

**Statistical Analysis**
All values are given as mean ± SD (standard deviation). The differences were assessed by unpaired student t-test. P< 0.05 was considered to be statistically significant. The correlation probability and correlation coefficient (R) of any two variables was computed by an AMSTRAD PCW 8256 computer using the AMSTAT computer program.

**Results**
The mean SOD of erythrocytes of thalassemia major patients was 1478.4 IU/ml, SD (standard deviations) = 346.79, SE (standard error) = 48.09, range: (1012.07-2556.82). The mean SOD of erythrocytes of thalassemia minor patients was 1189.0 IU/ml, SD (standard deviations) = 212.84, SE (standard error) = 56.88, range: (871.51-1568.72). The mean SOD of erythrocytes of healthy controls was 1261.7 IU/ml, SD (standard deviations) = 165.81, SE (standard error) = 40.22, range: (984.58-1547.19). The mean Catalase of erythrocytes of thalassemia major patients was 5.0 mg/ml, SD (standard deviations) = 1.63, SE (standard error) = 0.23, range: (1.35-8.56). The mean Catalase of erythrocytes of thalassemia minor patients was 6.0 mg/ml, SD (standard deviations) = 1.78, SE (standard error) = 0.48, range: (3.35-10.08). The mean Catalase of erythrocytes of healthy controls was 5.3 mg/ml, SD (standard deviations) = 1.63, SE (standard error) = 0.39, range: (3.58-8.71). Figures 1 and 2 show the mean values of SOD and Catalase activities.

![Figure 1: Bar Chart showing mean values of SOD for thalassemia major, thalassemia minor and control subjects](image)

<table>
<thead>
<tr>
<th>SOD</th>
<th>Mean</th>
</tr>
</thead>
<tbody>
<tr>
<td>MAJ</td>
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</tr>
<tr>
<td>MIN</td>
<td>1189.0</td>
</tr>
<tr>
<td>CONT</td>
<td>1261.7</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Enzyme</th>
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<th>SD (standard deviations)</th>
<th>SE (standard error)</th>
<th>Range</th>
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<td>Catalase (MAJ)</td>
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</tr>
</tbody>
</table>
Figure 2: Bar chart showing mean values of catalase for thalassemia major, thalassemia minor and control subjects

Erythrocytes from thalassemia major have significantly higher SOD than control (p<0.0004), also erythrocytes of thalassemia minor had a higher SOD than that of the control but statistically was not significant (p= 0.3138), while erythrocytes of thalassemia major patients had a higher SOD than that of thalassemia minor patients but statistically was not significant (p= 0.36817).

Red cell catalase activity of thalassemia minor patients was significantly higher than that of the controls (p<0.05), also was higher than that of thalassemia major patients but statistically was not significant (p= 0.1234).

In thalassemic patients, the more anaemic patients had significantly higher SOD activity (Figure-3), but this correlation was not present between anaemic patients and Catalase activity (Figure-4).

Figure 3: there is negative correlation between hemoglobin (G/dl) and SOD of erythrocytes in thalassemia major patients
Antioxidant Status … Al-Mudalal et al

Figure 4: there is no correlation between hemoglobin (G/dl) and catalase of erythrocytes in thalassemia major patients

There was no significant correlation between SOD activity, Catalase activity and the last time of blood transfusion (Figures 5 and 6).

Figure 5: there is no correlation between SOD of thalassemia major patients with the last time of transfusion

Figure 6: there is no correlation between Catalase of thalassemia major patients with the last time of transfusion
Discussion
In beta-thalassemic red blood cells (RBC), increased precipitation of alpha chain associated with increased oxidation of heme iron has been cited as the principal mechanism for generating activated species of molecular oxygen\textsuperscript{[3-6]}, conceivably, these activated forms of oxygen can be formed during transport of oxygen through the membrane as well as within the cell. In this context, the role of small molecules endowed with antioxidant activity would be more effective in protecting membrane lipids from undergoing free radical chain reactions. In contrast, SOD, being effective in the dismutation of superoxide anion in the cell, may be not accessible to the lipid matrix of the membrane due to its size and charged groups. Consequently, the effective radical scavenging action of SOD within the membrane would be markedly diminished\textsuperscript{[11]}. Measurements of the specific activity of SOD in thalassemic RBC show a higher percent inhibition of cytochrome c per milligram of protein as compared with normal individuals. The increase of SOD specific activity found in RBC of thalassemic patients may be associated with increased production of superoxide anion. An induction of SOD was demonstrated in experiments on oxygen toxicity\textsuperscript{[12-14]}. The results of this study concerning the increased specific activity of SOD in thalassemic RBC donot agree with reports from other laboratories\textsuperscript{[15]} which have observed no differences in the level of SOD in normal and thalassemic RBC. Conceivably, the different assay methods employed in such comparison might explain the discrepancy, these methods describes by Nishikimi\textsuperscript{[22]} and by Misra\textsuperscript{[23]}, both methods are based on inhibition by the enzyme of color development by chromogenic substance reacting with $O_2^-$.

In the method of Nishikimi, Nitro Blue Titrazolume aqueous solution giving blue formazan which can be monitored at 560nm. In the method of Misra; epinephrine at pH 10.2 acts both as the source of $O_2^-$ and as the detecting system giving adrenochrome which can be monitored at 480nm. The interesting observation that emerges from this work is that high SOD activities found in thalassemic RBC apparently do not protect them from increased rate of autohemolysis, and polyamines might be more effective in protecting biomembranes against the deleterious effect of free radicals. The findings of this study indicate that patients with more sever disease, beta thalassemia major, having a greater excess of alpha globin chain, also have higher activities of SOD but lower catalase activity than the milder genotype, beta thalassemia minor. Increased red cell SOD values in thalassemic patients have previously been explained as a reaction to, or compensation for the increased production of superoxide radicals, the amount of which is related to excess globin chain\textsuperscript{[16,19]}. This study showed significantly lower red cell catalase activity (although higher than normal subjects), in patients with the more sever form of the disease expressed both as per g Hb and per ml red blood cells and that was similar to other study\textsuperscript{[21]} . A possible explanation for lower red cell catalase activity found in the more severe genotype of beta thalassemia is that the greater amount of hydrogen peroxide might produce direct toxic damage to catalase\textsuperscript{[17,18]}, the concentration of this is considerably reduced in conditions of high oxidative stress\textsuperscript{[20]}. The increase of erythrocyte superoxide dismutase activities is most likely due to abnormalities specific to thalassemic red cells rather than an increased number of younger red cells for
reticulocytes and nucleated red blood cells did not affect the enzyme activity. Patients with beta-thalassemia major disease with lower haemoglobin concentration had significantly higher superoxide dismutase activities 16. In all 76 subject with beta-thalassemia major, haemoglobin concentrations and superoxide dismutase activities were inversely correlated (r=−0.60) (p<0.001). This indicates that the amounts of superoxide generated in red cells may, at least partly, determine severity of disease; the increased superoxide dismutase activity in thalassemia is a response to superoxide generated in greater amounta because of accumulation of excessive globin chains and iron in the red cells.

**Conclusion**

1. Red cell superoxide dismutase activity was greatly increased in homozygous beta-thalassemia.
2. Lower red cell catalase activity was found in the more severe genotype of beta-thalassemia.
3. The more anaemic patients had significantly higher SOD activity.

**References**

19. Gerli, G.C., Mongiat, R., and Sandri, M.T.: Antioxidant system and serum trace elements in alpha-thalassemia and Hb...


