Short Communication

Evaluation of Antioxidant Status in Beta Thalassemia Major Patients in Sabah, Malaysian Borneo

Shazia Qaiser1, Mohammad Zahirul Hoque1, Mohammad Iqbal2 and D Kamarudin D Mudin1

1Faculty Of Medicine & Health sciences, University Malaysia Sabah (UMS), Jalan UMS, 88400 Kota Kinabalu, Sabah, Malaysia. 2Biotechnology Research Institute, University Malaysia Sabah (UMS), Jalan UMS, 88400 Kota Kinabalu, Sabah, Malaysia.

ABSTRACT: In beta thalassemia major severe haemolysis and repeated blood transfusions lead to excess iron deposition in various body tissues. This secondary iron overload is thought to be responsible for peroxidative tissue injury and subsequent oxidative stress. The aim of this study was to determine oxidative stress and serum antioxidant levels in patients with beta thalassemia major. Serum levels of reduced glutathione (GSH), catalase and glutathione S-transferase (GST) as well as lipid peroxides were determined. The serum GSH, catalase and GST levels in beta thalassemia major patients were found to be 0.30±0.10 (µmol/ml), 5.84±2.17 (nmol/mg protien) and 86.25±15.10 (nmol/mg protein) respectively. Serum levels of TBARS in beta thalassemia major patients and in controls were found to be 1.34±0.31 (nmol MDA/ml) and 0.81±0.19 (nmol MDA/ml) respectively. We found marked lower serum GSH levels (p <0.05) while significantly higher levels of serum catalase, GST and TBARS (p<0.05) in patients with beta thalassemia major as compared to healthy controls. Our results suggest that the peroxidative status generated by reactive oxygen species in beta thalassemia major patients may lead to a significantly increased production of TBARS which is concomitant with increase in catalase and GST activity.

KEYWORDS: Beta Thalassemia major, reduced Glutathione, Catalase, Glutathione S-transferase, lipid peroxidation, antioxidant enzyme.


CORRESPONDENCE: hoquezahira@hotmail.com

INTRODUCTION

Beta thalassemia major is a common genetic blood disorder which is the most severe form of thalassemia with significantly large number of children dying each year throughout the world.1 It is an inherited disease resulting from homozygosity or compound heterozygosity for beta thalassemia with severe reduction or total absence of beta globin chains. Malaysia has approximately 4.5% carriers of beta thalassemia with affected annual births of 2.1 per 1000.2 The East Malaysian state of Sabah has the highest number of beta thalassemia major patients in Malaysia.3

Blood transfusions are mandatory for survival of beta thalassemia major patients which leads to iron overload, subsequent tissue damage and oxidative stress. Several antioxidant mechanisms in the body arise to protect against oxidative stress.4 Among several markers of oxidative stress, we have focused on some of the important markers namely the reduced glutathione (GSH), catalase (CAT), glutathione S-transferase (GST) and lipid peroxidation (LPO) product. There have been several reports on antioxidant enzymes in beta thalassemia major.5 We were interested to know the status of serum antioxidant enzyme levels in beta thalassemia patients in Sabah, Malaysia.

MATERIALS AND METHODS

One hundred beta thalassemia major patients as well as one hundred healthy controls were included in this present study after informed consent. The patients were from Thalassemia Centre, Hospital Queen Elizabeth, Kota kinabalu, Sabah, Malaysia. This study was carried out in the faculty of Medicine, University Malaysia Sabah. The study protocol was in accordance with the declaration of Helsinki, and was approved by Medical Research Ethics Committee (MREC), Ministry Of Health, Malaysia and Ethical Committee of University Malaysia Sabah.
Fasting blood samples were collected from beta thalassemia major patients and healthy individuals. Sera was then obtained by centrifugation blood samples at 3000 rpm for 30 min and kept at -80°C for the biochemical estimation. All other solvents and chemicals used were either of analytical grade or the highest purity commercially available. Reduced glutathione estimation in serum was performed by the method of Jollow et al. The CAT activity was measured as described by the method of Claiborne. The GST enzyme activity was determined according to the method of Habig et al and the absorbance changes were recorded at 340nm. Estimation of LPO in serum was performed by the method of Buege and Aust. The absorbance read at 535 nm and results were expressed as amount of malondialdehyde (nmol MDA) formed per ml. Protein concentration was estimated by the method of Aitken et al using BSA (1mg/ml) as a standard.

Statistical Analysis
Data was entered and analyzed by excel data base and SPSS statistical software, Windows TM version 17.0 for statistical analysis. Results presented as means and standard deviation (SD). Medians and reference ranges were estimated using the \( t\)-test for data sets. Differences were considered significant when the \( p\) value is \(< 0.05\).

RESULTS AND DISCUSSION
In the present study, we investigated the oxidative stress in beta thalassemia major patients by evaluating the levels of serum GSH, CAT, GST and LPO products. Mean serum concentrations of GSH, CAT, GST and LPO along with minimum and maximum values and standard deviations in beta thalassemia major patients and controls are shown in Table 1. Mean serum concentrations of antioxidant enzyme in thalassemia major patients and controls are with their \( p\)-values are shown in Table 2.

We determined the serum levels of reduced glutathione (GSH), catalase and glutathione S-transferase (GST) as well as lipid peroxides. The serum GSH, catalase and GST levels in beta thalassemia major patients were found to be 0.30 \(\pm\) 0.10 (µmol/ml), 5.84 \(\pm\) 2.17 (nmol/mg protein) and 86.25 \(\pm\) 15.10 (nmol/mg protein) while in healthy controls they were 1.34 \(\pm\) 0.29 (µmol/ml), 4.76 \(\pm\) 0.52 (nmol/mg protein) and 31.97 \(\pm\) 7.12 (nmol/mg protein) respectively (Figure 1, 2 and 3).

### Table 1. Serum concentrations of GSH, GST, CAT and LPO in beta thalassemia major patients and healthy controls.

<table>
<thead>
<tr>
<th>Variables</th>
<th>Patients Mean ± SD</th>
<th>Min</th>
<th>Max</th>
<th>SD</th>
</tr>
</thead>
<tbody>
<tr>
<td>LPO (nmol MDA/ml)</td>
<td>1.35 ± 0.31</td>
<td>0.48</td>
<td>1.78</td>
<td>0.31</td>
</tr>
<tr>
<td>Catalase (nmol/mg protein)</td>
<td>5.85 ± 2.19</td>
<td>1.89</td>
<td>9.91</td>
<td>2.19</td>
</tr>
<tr>
<td>GSH(µmol/ml)</td>
<td>0.30 ± 0.11</td>
<td>0.088</td>
<td>0.617</td>
<td>0.11</td>
</tr>
<tr>
<td>GST (nmol/mg protein)</td>
<td>86.25 ± 15.18</td>
<td>45.13</td>
<td>109.86</td>
<td>15.18</td>
</tr>
<tr>
<td>LPO (nmol MDA/ml)</td>
<td>0.82 ± 0.48</td>
<td>0.3</td>
<td>1.21</td>
<td>0.19</td>
</tr>
<tr>
<td>Catalase (nmol/mg protein)</td>
<td>4.77 ± 4.03</td>
<td>4.03</td>
<td>6.3</td>
<td>0.53</td>
</tr>
<tr>
<td>GSH (µmol/ml)</td>
<td>1.35 ± 0.176</td>
<td>0.176</td>
<td>1.89</td>
<td>0.29</td>
</tr>
<tr>
<td>GST (nmol/mg protein)</td>
<td>31.97 ± 22.68</td>
<td>22.68</td>
<td>61.58</td>
<td>7.16</td>
</tr>
</tbody>
</table>

Note: Min = minimum, Max = maximum, SD = standard deviation.

### Table 2. Mean Serum Concentrations of antioxidant enzymes with their \( p\)-values in Beta Thalassemia major patients (n=100) and controls (n=100).

<table>
<thead>
<tr>
<th>Variables</th>
<th>Patients Mean ± SD</th>
<th>Controls Mean ± SD</th>
<th>( t)-Test</th>
<th>( p)-Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>GSH (µmol/ml)</td>
<td>0.30 ± 0.11</td>
<td>1.34 ± 0.29</td>
<td>33.22</td>
<td>&lt; 0.05</td>
</tr>
<tr>
<td>GST (nmol/mg protein)</td>
<td>86.25 ± 15.18</td>
<td>31.97 ± 7.12</td>
<td>32.33</td>
<td>&lt; 0.05</td>
</tr>
<tr>
<td>CAT (nmol/mg protein)</td>
<td>5.84 ± 4.76</td>
<td>4.76 ± 0.53</td>
<td>4.81</td>
<td>&lt; 0.05</td>
</tr>
<tr>
<td>LPO (nmol MDA/ml)</td>
<td>1.34 ± 0.31</td>
<td>0.81 ± 0.19</td>
<td>14.50</td>
<td>&lt;0.05</td>
</tr>
</tbody>
</table>

![Figure 1. Mean Serum Concentrations of Reduced Glutathione (GSH) in Beta Thalassemia Major Patients (n=100) and Controls (n=100).](image)
The serum levels of LPO in beta thalassemia major patients and in controls were found to be 1.34 ± 0.31 (nmol MDA/ml) and 0.81 ± 0.19 (nmol MDA/ml) respectively (Figure 4). The result of our study showed markedly lower serum GSH levels (p<0.05) while significantly higher levels of serum catalase, GST and LPO (p<0.05) in patients with beta thalassemia major as compared to healthy controls (Table 2).

**CONCLUSION**

Oxidative stress in patients with beta thalassemia major is mainly caused by peroxidative injury due to secondary iron overload. The data implies marked decrease in reduced glutathione as first line of defence against free radicals in beta thalassemia major patients which eventually leads to tissue damage. Increased CAT activity may be a compensatory mechanism in response to oxidative stress while increased GST and LPO levels may be suggestive of undergoing cellular damage. It will be of considerable interest to examine the serum levels of other antioxidant enzyme for further evaluation.

**ACKNOWLEDGEMENTS**

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**REFERENCES**