Abstract

Oxidative stress can be induced in different ways, some of which are connected with known pathologies. Deficiencies in antioxidant or related enzymes are the factors which can result in oxidative stress. Genetic polymorphism is the most common cause of these deficiencies. There were statistically a highly significant elevated (p<0.001) in total antioxidant status(TAS), Bilirubin(Bil). and uric acid(UA) in β-thalassemia major with diabetic patients(No.51), when compared with β-thalassemia major(No.55) and control groups (No.50). The results showed a highly significant increase (p<0.001) of Mn-SOD (SOD2) and EC-SOD(SOD3) activities in β-thalassemia major with diabetic patients when compared with β-thalassemia major patients and control groups. The present study was performed assess whether there is a genetic association between a functional polymorphism (Ala9Val) in the human Mn-SOD (SOD2) and EC-SOD(SOD3) genes in major β-thalassemia, major β-thalassemia with diabetic and control using PCR/RFLP method.

The results shows frequencies of the Ala allele (64.1% vs. 51.1%, p < 0.001) and the Ala/Ala genotype (29.39% vs. 23.51%, p < 0.001) of the SOD2 gene were significantly lower in major β-thalassemia diabetic patients than in major β-thalassemia. In contrast, the Val allele (35.9% vs. 48.9%, p < 0.001) of the SOD2 gene were significantly increased in major β-thalassemia with diabetic patients than in major β-thalassemia and the Val/Val genotype (13.76% vs. 13.76%, p < 0.01) were no significantly between groups. Significance difference in the frequencies appears in Arg213GlySOD3, Arg/Arg, Gly/Gly genotype between major β-thalassemia with diabetic and major β-thalassemia. The present study provides some evidence that genetic polymorphism in the SOD2 and SOD3 genes associated with increased risk of major β-thalassemia with diabetic among major β-thalassemia with high levels of oxidative stress. This suggests that the Ala(-9)Val dimorphism in the SOD2 gene is associated with major β-thalassemia with diabetic.

Keywords: Total antioxidant, Major β-thalassemia, diabetic, genetic polymorphism SOD2 and SOD3 genes
Thalassemia will remain to be one of the major health problems for at least the next few decades, particularly in the developing countries (Kuldeep et al., 2011). β-Thalassemia major is an autosomal disorder caused by mutations in the HBB gene in chromosome 11 (β) which leads to underlie deficiency in β-globin subunits of hemoglobin (Eliezer and Patricia, 2011). The reactive oxygen species (ROS), generated in normal cellular respiration and during metabolism of xenobiotics, cause damage to membranes, mitochondria, and macromolecules including DNA, and thus are potential causes for carcinogenesis (Valko et al., 2004; Shi et al., 2004; De Bont, 2004 and Klaunig 2004).

A large number of antioxidant systems are involved in the scavenging of ROS, including the superoxide dismutase (SOD) family of proteins (Beatti, 2002). These enzymes catalyze the dismutation of O$_2^-$, the superoxide anion, to form hydrogen peroxide (H$_2$O$_2$), which is further converted to water by the action of two other antioxidant enzymes, catalase and glutathione peroxidase (Beatti, 2002).

All mammalian tissues contain three forms of superoxide dismutase (Beyer et al., 1991): Cu/ZnSOD (SOD1), Mn-SOD (SOD2) and extracellular superoxide dismutase (ECSOD or SOD3). Cu/ZnSOD is localized in cytosol, Mn-SOD in mitochondria and ecSOD in extracellular space. Importantly, ECSOD activity is 10-fold higher in the vessel wall than in other tissues, where Cu/ZnSOD and Mn-SOD constitute the majority of SOD activity (Beyer et al., 1991 and Stra et al., 1995). The predominant site of production of EC-SOD is the smooth muscle cell in healthy vessels (Stra et al., 1995). Studies from other laboratories, and our own, indicate that endothelial cells do not produce EC-SOD (Stra et al., 1995). Despite the fact that the EC-SOD is predominantly made by vascular smooth muscle rather than endothelial cells, it binds to the heparin sulfates on the endothelial cell surface and can be internalized by endothelial cells (Oth et al., 1994).

Genetic polymorphism is frequently related to large number of pathologies. Enzymes involved in defense against ROS are not an exception. There has been much interest and research on single nucleotide substitutions (SNPs) in order to understand the maintenance of such polymorphisms in human populations (Cassia et al., 2011). Several SNPs have been reported to result in changes in the levels or the activities of antioxidant enzymes, which can lead to reduction in protection against oxidative stress (Forsberg et al., 2001). Less mutations were found in the gene coding human manganese containing superoxide dismutase (SOD2). Substitution of alanine-16 to valine (so called “Ala variant”) is the most known mutation (Lightfoot et al., 2006). This mutation associated with cancers of breast, prostate, ovaries and bladder, as well as non-Hodgkin lymphoma, mesothelioma and hepatic carcinoma (Lightfoot et al., 2006). The Mn-SOD gene, which is composed of five exons and four introns, is localized to chromosome 6q25 (Church, 1992 et al and Zelko et al., 2002). A T → C substitution, resulting in a Val → Ala change at the -9 position (Val-9Ala), which alters the secondary structure of the protein (Shimoda et al., 1996), has been noted to
affect transport of Mn-SOD into the mitochondria (Rosenblum et al., 1996 and Sutton et al., 2003).

Genetic studies have described that a single AA polymorphism exists in manganese-dependent superoxide dismutase (Mn-SOD). A change of alanine (Ala) to valine (Val) at the 16th amino acid (Ala16Val) of the signal sequence of the Mn-SOD (ninth amino acid from the first amino acid of the mature protein) has been suggested to change the secondary structure of the protein and therefore the mitochondrial targeting of the enzyme (Shimoda et al., 1996). The genes encoding these enzymes are located in different chromosomes and in all of them polymorphisms have been described. Copper-zinc SOD (Cu ZnSOD, SOD1) is encoded on 21q22.1 manganese SOD (Mn-SOD, SOD2) on 6q25.3 and extracellular SOD (ECSOD, SOD3) on 4p16.3–q21 (McKusik, 1998). All these enzymes are expressed in human bronchial epithelium and alveolar macrophages (Lakari et al., 1998). Another polymorphism (Ile58Thr) in exon 3 affects the stability of Mn-SOD and reduces protein amount and enzyme activity (Ho Y-S, 1988 and Borgstahl et al., 1996). Cells that over-expressed the Ile58 allele had higher Mn-SOD activity than did cells that overexpressed the Thr58 allele (Zhang et al., 1999). It is biologically plausible that the Val-9 Ala and Ile58Thr polymorphisms play an important role in ROS detoxification, thus affecting the risk for developing cancer, particularly among individuals with a higher level of oxidative stress or who are deprived of other antioxidative protection, such as through a low level of antioxidant intake.

The aim of study focus on the study the oxidative stress in patients with major β- Thalassemia, and major β- Thalassemia with diabetic and study the genetic polymorphism in Ala(-9) Val and Ile58Thr polymorphisms of the SOD2 gene and Arg 213Gly dimorphism of the SOD3

Materials and methods

Samples

This study included 106 patients suffering from β- thalassemia major (58 male, and 48 female) aged between 3-18 years with mean± SD were 11.36 ± 1.53 years included in this study, who were consecutively referred to the center of inherited blood diseases in General Al-Habuby Hospital in Nasseryia city in Thiqar province at period between 1st of December 2011 up to 30th of June 2012, were investigated. The diagnosis of β- thalassemia major based on peripheral blood film and hemoglobin electrophoresis, and transfusion dependent, all patients were checked for none suffered from other disease. 51 patients suffering from β-thalassemia major with diabetic patients (28 male, and 23 female) were diagnosed on the basis of WHO criteria and not given any medication only treatment with insulin, any subject that have not these criteria are excluded from this study. Eight milliliters blood (divided two parts) was obtained after 8-12 hours, fasting from each subject in thalassemia patients sample was done just before the transfusion. Five milliliters used in the analysis of total antioxidant, albumin, bilirubin, uric acid and superoxide dismutase, the blood samples were centrifuged at 3000×g for minutes and sera were kept in -50°C until analysis. Three milliliters from blood used in the isolation of genomic DNA.

Estimation of total antioxidant status:

The total antioxidant status was determined according to the following formula (Bonnefont et al., 2000): (0.63×albumin mmol/l) + (1.02×UA mmol/l)+(1.5×bilirubin mmol/l).

Estimation of Albumin (Alb.): Albumin was measured spectrophotometrically according to Cooper and Morgan (Cooper and Morgan, 1984).
Estimation of bilirubin (Bil.): Bilirubin was measured by sulfanilic acid method by BIOLABO kit (France) (Henry, 1965).

Estimation of uric acid (UA): Uric acid was measured by urease method with BIOLABO kit (France) (Fossait et al., 1980).

Estimation of plasma Mn-SOD (SOD2) activity: The Mn-SOD plasma level determination in the case and control samples were realized using the sandwich enzyme linked immunosorbent assay (ELISA) methodology as described by Taniguchi et al. (Taniguchi et al., 1992).

Estimation of blood EC-SOD (SOD3) activity: Serum EC-SOD enzyme activity was assayed using a two steps enzyme-linked immunosorbent assay with a monoclonal antibody as described by Adachi et al. (Adachi et al., 2012).

Isolation of genomic DNA from whole blood: Genomic DNA was isolated from whole human blood samples Genomic DNA Mini kit (Vogelstein and Gillespie, 1979).

Step 1: RBC Lysis: Three hundred microliter of whole blood (Collect fresh blood in EDTA) was taken in 1.5 ml micro centrifuge tube. Add 900 μl of cell lysis buffer and mix with inversion, and incubate the tube for 10 minutes at room temperature (ice). Centrifuge for 5 minutes at 3000 × g and remove the supernatant completely. Add 100 μl of RBC lysis buffer to re-suspend the cell pellet.

Step 2: Cell Lysis: A volume of 200 μl from GB buffer add to the 1.5 ml microcentrifuge tube and mix by shaking vigorously. Incubate at 60°C for at least 10 minutes to ensure the sample lysate is clear. During incubation, invert the tube every 3 minutes. At this time, pre-heat the required elution buffer (200 μl per sample) to 60°C (for Step 5 DNA Elution).

Step 3: DNA Binding: A volume of 200 μl of absolute ethanol added to the clear lysate and immediately mixed by shaking vigorously for 10 seconds. Place a GD column in a 2 ml collection tube. Transfer all of the mixture (including any precipitate) to the GD column centrifuge at 15,000 × g for 5 minutes. Discard the 2 ml collection Tube containing the flow-through and place the GD. Column in a new 2 ml collection tube.

Step 4: Washing: A volume of 400 μl from W1 buffer to the GD column. Centrifuge at 15,000 x g for 30 seconds. Discard the flow-through and place the GD column back in the 2 ml collection tube. Add 600 μl of wash buffer (ethanol added) to the GD Column. Centrifuge at 15000 × g for 30 seconds. Discard the flow-through and place the GD column back in the 2 ml collection tube. Centrifuge again for 3 minutes at 15,000 x g to dry the column matrix.

Step 5 : DNA Elution: Transfer the dried GD column to a clean 1.5 ml microcentrifuge tube, Add 100 μl of pre-heated elution buffer or TE to the center of the column matrix. Let stand for at least 3 minutes to ensure the elution buffer or TE is absorbed by the matrix. Centrifuge at 15000 × g for 30 seconds to elute the purified DNA.

Genotyping of Mn-SOD: After DNA extraction from blood samples. The Mn-SOD genotyping were determined with polymerase chain reaction (PCR) (Bonnefont et al., 2000).

1-To amplify the polymorphic Ala(-9) Val region, the primer sequences were SOD2-16 F 5'-CCAGCAGGCAGCTGGCACCG-3' and SOD2-16 5'-TCCAGGCGGC CGTAGTCGTAGG-3'.

2-To amplify the polymorphic Ile 58 Thr region of Mn-SOD gene, the primer sequences were SOD2-58 F 5'-AAGCTCTCCATTATCTAATAGC-3 and SOD2-58R 5'-TCAGTGACGCTGAAGAGAT-3'.
3-Master mix consisting of 67mM Tris-HCl (pH 8.8), 16.7 Mm ammonium chloride, 1 mM magnesium chloride, 0.1% Tween-20, 10% dimethyl sulfoxide, 0.2 mM of each dNTP, 5 pmol of each primer, 100ng of genomic DNA, and 2.5 units of Taq polymerase.

Detection of Ala(-9)Val and Ile 58 Thr dimorphism (Bonnefont et al., 2000)
1-The PCR product was digested with Bsh TI for the Ala(-9)Val.
2-The PCR product was digested with Eco32I for the Ile 58 Thr.

17 µL of PCR product, 2 µL of buffer O'/Tango™ (BshTI) and 1 µL restriction endonuclease (1 unit/µL) were mixed and incubated for 12 h at 37°C.

Analysis of amplified product: Successful PCR amplification (digested DNA product) was performed and confirmed by electrophoresis in a 2% agarose gel and visualized by ethidium bromide staining (Budowle et al., 1991)

Genotyping of EC-SOD (Bonnefont et al., 2000)
1-To amplify the polymorphic Arg 213 Gly EC-SOD region, the primer sequences of SOD3-213 were F 5'-GGCTGGCCTGCTGCGTGGTGG-3' and SOD3-213R 5'-CCTTGCACTCGCTCTCGCGCG-3'.
2-Master mix consisting of 10mM Tris-HCL (pH 8.8), 50 mM KCl, 1.5 mM magnesium chloride, 0.1% Tween-20, 10% dimethyl sulfoxide, 0.2 mM of each dNTP, 5 pmol of each primer, 100ng of genomic DNA, and 2.5 units of Taq polymerase.

Statistical analysis: The data of the study was subjected to statistical analysis is expressed as mean ± SD. Statistical analyses were done on a computer by using the Student ‘t’ test. Conditional logistic regression models applied to estimate odds ratios and 95% confidence interval (95% CI) were measure the strength of the relationship between the Mn-SOD or EC-SOD polymorphisms and β-thalassemia major and β-thalassemia major with diabetic patients.

Results
Part one: Table 1 show the total antioxidant status (TAS), Albumin (Alb.), bilirubin (Bil.) and uric acid (UA) were determined in control (C), β-thalassemia major (BTM) and β-thalassemia major with diabetic patients (BTM+DM).

The results showed a highly significant increase (p<0.001) in TAS, Bil. and UA in β-thalassemia major and β-thalassemia major with diabetic patients, when compared with control group. Also a highly significant increase (p<0.001) in TAS, Bilirubin. and UA in -thalassemia major with diabetic patients, when compared with β-thalassemia major. The result show a highly significant increase (p<0.001) of SOD2 and SOD3 activities in β-thalassemia major with diabetic patients when compared with β-thalassemia major patients and control groups. Also cTAS show a highly significant increase when compared with the other groups in patients. No significant difference is observed in Alb. concentration in patients groups when compared with control group. Also there is a highly significant increase (p<0.001) in TAS and Bil. levels in male patients when compared with females.
Table (1): Biochemical parameters of control (C), β- thalassemia major (BTM) and β- thalassemia major with diabetic (BTM+DM) patients in male (M) and female (F).

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Control (C)</th>
<th>BTM</th>
<th>BTM+DM</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>TAS (nmol/l)</td>
<td>1.47±0.34</td>
<td>1.60±0.43</td>
<td>2.07±0.36</td>
<td>p&lt;0.05</td>
</tr>
<tr>
<td>M=1.58±0.27</td>
<td>M=1.76±0.32</td>
<td>M=1.58±0.27</td>
<td>p&lt;0.001</td>
<td></td>
</tr>
<tr>
<td>F=1.36±0.32</td>
<td>F=1.44±0.21</td>
<td>F=1.36±0.32</td>
<td>p&lt;0.001</td>
<td></td>
</tr>
<tr>
<td>Alb. (g/dl)</td>
<td>5.07±0.53</td>
<td>4.89±0.45</td>
<td>4.68±0.32</td>
<td>p&lt;0.05</td>
</tr>
<tr>
<td>M=5.16±0.34</td>
<td>M=4.80±0.51</td>
<td>M=5.16±0.34</td>
<td>p&lt;0.001</td>
<td></td>
</tr>
<tr>
<td>F=4.99±0.42</td>
<td>F=4.79±0.34</td>
<td>F=4.99±0.42</td>
<td>p&lt;0.001</td>
<td></td>
</tr>
<tr>
<td>Bli. (mg/dl)</td>
<td>0.57±0.12</td>
<td>1.23±0.11</td>
<td>1.58±0.25</td>
<td>p&lt;0.001</td>
</tr>
<tr>
<td>M=0.62±0.21</td>
<td>M=1.30±0.21</td>
<td>M=0.62±0.27</td>
<td>p&lt;0.001</td>
<td></td>
</tr>
<tr>
<td>F=1.26±0.26</td>
<td>F=1.10±0.26</td>
<td>F=0.53±0.36</td>
<td>p&lt;0.001</td>
<td></td>
</tr>
<tr>
<td>UA (mg/dl)</td>
<td>4.04±0.54</td>
<td>5.02±0.36</td>
<td>5.89±0.34</td>
<td>p&lt;0.001</td>
</tr>
<tr>
<td>M=4.26±0.43</td>
<td>M=5.47±0.41</td>
<td>M=4.26±0.43</td>
<td>p&lt;0.001</td>
<td></td>
</tr>
<tr>
<td>F=3.82±0.39</td>
<td>F=4.58±0.24</td>
<td>F=3.82±0.39</td>
<td>p&lt;0.001</td>
<td></td>
</tr>
<tr>
<td>SOD2 (ng/ml)</td>
<td>63.65±0.37</td>
<td>96.34±0.34</td>
<td>109.24±0.41</td>
<td>p&lt;0.001</td>
</tr>
<tr>
<td>M=62.32±0.26</td>
<td>M=97.42±0.32</td>
<td>M=62.32±0.26</td>
<td>p&lt;0.001</td>
<td></td>
</tr>
<tr>
<td>F=67.83±0.48</td>
<td>F=97.44±0.23</td>
<td>F=67.83±0.48</td>
<td>p&lt;0.001</td>
<td></td>
</tr>
<tr>
<td>SOD3 (ng/ml)</td>
<td>67.08±11.13</td>
<td>97.94±12.21</td>
<td>102.78±13.1</td>
<td>p&lt;0.001</td>
</tr>
<tr>
<td>M=98.45±10.99</td>
<td>M=76.34±10.21</td>
<td>M=98.45±10.99</td>
<td>p&lt;0.001</td>
<td></td>
</tr>
</tbody>
</table>

Part Two: Ala(-9)Val SOD2(MnSOD) allele and genotypes in patients with control and β- thalassemia major. Genotype data for Ala(-9)Val SOD2 were available from 55 patients with β- thalassemia major and 50 as a control. In the control and β-thalassemia major Ala allele and Ala/Val genotype were the most common in control and patients groups. The Ala allele (odd ratio 0.8913; 95%CI) and Ala/Ala genotype (odd ratio 1.587; 95%CI) as shown in table 2.
Table(2) : Ala(-9)Val SOD2 allele and genotype frequencies in control, and beta-thalassemia major.

<table>
<thead>
<tr>
<th></th>
<th>Alpha-thalassemia major(55)</th>
<th>Control(50)</th>
</tr>
</thead>
<tbody>
<tr>
<td>OR (95% CI)</td>
<td>0.8913</td>
<td>0.724</td>
</tr>
<tr>
<td></td>
<td>64.1 % (64)</td>
<td>65.3 % (73)</td>
</tr>
<tr>
<td></td>
<td>35.9% (39)</td>
<td>34.7% (34)</td>
</tr>
</tbody>
</table>

Ala(-9)Val SOD2 genotype distribution

<table>
<thead>
<tr>
<th></th>
<th>Alpha-thalassemia major(55)</th>
<th>Control(50)</th>
</tr>
</thead>
<tbody>
<tr>
<td>OR (95% CI)</td>
<td>1.587</td>
<td>1.013</td>
</tr>
<tr>
<td></td>
<td>29.39% (15)</td>
<td>56.85% (31)</td>
</tr>
<tr>
<td></td>
<td>33.42% (17)</td>
<td>52.93% (27)</td>
</tr>
<tr>
<td></td>
<td>13.76% (8)</td>
<td>13.65% (6)</td>
</tr>
<tr>
<td></td>
<td>Ala/Ala</td>
<td>Ala/Val</td>
</tr>
<tr>
<td></td>
<td>Ala/Val</td>
<td>Val/Val</td>
</tr>
</tbody>
</table>

The results of restriction analysis of Ala(-9)Val polymorphism in the SOD2 gene after digestion with BshTl as shown in figure 1.

![Figure 1: Genotyping of SOD2 gene, for analysis of Ala(-9)Val polymorphism,; lanes 1, 4, 5, 8, 9, 11, and 14, Ala/Ala genotype; lanes 2, 7, 10, and 13, Ala/Val genotype; lanes 3, 6, and 12, Val/Val genotype. Lanes M: standard size reference marker (50 bp). (sample after digestion with BshTl) electrophoresis in a 2% agarose gel.

Table 3 shows the frequencies of the Arg/Arg and Gly/Gly genotype were significantly lower in patients with beta-thalassemia major when compared with control groups (p<0.05), while the frequencies of the Gly allele and heterozygous Arg/Gly genotype was highly significant higher in patients with beta-thalassemia major patients (p<0.001) when compared with control.
Table (3) : Arg 213 Gly SOD3 allele and genotype frequencies in control, and beta-thalassemia major.

<table>
<thead>
<tr>
<th>Arg 213 Gly – SOD3 Allele distribution</th>
</tr>
</thead>
<tbody>
<tr>
<td>OR (95%CI)</td>
</tr>
<tr>
<td>0.765</td>
</tr>
<tr>
<td>0.624</td>
</tr>
</tbody>
</table>

Table 4 show the results of Ala(-9)Val SOD2 allele and genotypes in patients with control and beta-thalassemia major +diabetic. The result show a highly significantly difference in Val allele (48.9%) and Ala/Val genotype (62.73%) in patients with β- thalassemia major + diabetic when compared with β- thalassemia major (35.9% ,56.85% respectively) and control groups (34.7%,52.93% respectively).While a lower significant difference in Ala allele and Ala/Ala genotype frequencies in patients with β- thalassemia major + diabetic (51.1%,23.51% respectively) when compared with β- thalassemia major (64.1%,29.39% respectively) and control groups (65.3%,33.42% respectively).No significance difference is observed in Val/Val genotypes between different groups.

Table (4) : Ala(-9)Val SOD2 allele and genotype frequencies in control, and beta-thalassemia major +diabetic

<table>
<thead>
<tr>
<th>Ala(-9)Val – SOD2 Allele distribution</th>
</tr>
</thead>
<tbody>
<tr>
<td>OR (95%CI)</td>
</tr>
<tr>
<td>0.765</td>
</tr>
<tr>
<td>0.624</td>
</tr>
</tbody>
</table>

Table 5 showed the results of Arg 213 Gly-SOD3 allele and genotypes in patients with control and beta-thalassemia major with diabetic. The results showed a highly significantly difference in Gly allele (50.0%) and Arg /Gly genotype (58.8%) in patients with β- thalassemia major with diabetic and in β- thalassemia major (60.15%,65.89% respectively) when compared with control group (36.3%,55.6% respectively).Also the result show a lower significant difference in Arg allele and Arg/Arg genotype frequencies in patients with β- thalassemia major with diabetic (50.0%,19.84% respectively) and in β- thalassemia major (39.85%,23.21% respectively) when compared with control groups (63.7%,31.5% respectively).
Table(5) : Arg 213 Gly-SOD3 allele and genotype frequencies in control, and beta-thalassemia major+diabetic

<table>
<thead>
<tr>
<th>Arg 213 Gly-SOD3 Allele distribution</th>
<th>OR (95%CI)</th>
<th>Beta-thalassemia major with diabetic (51)</th>
<th>Control(50)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Arg</td>
<td>0.765</td>
<td>50.00 % (51)</td>
<td>63.7 % (71)</td>
</tr>
<tr>
<td>Gly</td>
<td>0.624</td>
<td>50.00 % (51)</td>
<td>36.3 % (35)</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Arg 213 Gly-SOD3 genotype distribution</th>
<th>OR (95%CI)</th>
<th>Beta-thalassemia major with diabetic (51)</th>
<th>Control(50)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Arg/Arg</td>
<td>1.587</td>
<td>19.84 % (10)</td>
<td>31.5 % (19)</td>
</tr>
<tr>
<td>Arg/Gly</td>
<td>1.013</td>
<td>58.8 (30)</td>
<td>55.6 % (27)</td>
</tr>
<tr>
<td>Gly/Gly</td>
<td>0.918</td>
<td>21.36% (11)</td>
<td>12.9 % (4)</td>
</tr>
</tbody>
</table>

The results of restriction analysis of Arg 213Gly polymorphism in the SOD3 gene after digestion with Eco521 as shown in figure 2.

![Figure 2: Genotyping of SOD3 gene, for analysis of Arg213Gly polymorphism.](image)

Discussion

The antioxidant enzyme play an important role in scavenging ROS produced under oxidative stress(Ramchandra et al.,2012). Oxidative stress is defined as the interruption of balance between oxidants and reluctant within the body due to the excess production of peroxides and free radicals. This imbalance will cause damage to cellular components and tissues in the body leading to oxidative stress(Q.Shazia et al.,2012). In patients with β-thalassemia major where frequent blood transfusion are required due to severe anemia.

In the present study total antioxidant, superoxide dismutase in patients with β-thalassemia major and showed elevated when compared to control group. This increased may be a compensatory mechanism in response to increase oxidative stress in these patients. Also the results showed a highly significance increased in the total antioxidant and superoxide dismutase in patients with β-thalassemia major +diabetic, this indicated that oxidative stress induced by high level of glucose may
increase superoxide radical production with diabetic this findings was agreement with the other studies.( Ramchandra et al.,2012;Seghouchni et al.,2002;Domingues et al.,1998). Some investigators suggested that assessment of total antioxidant capacity of plasma may be more useful than measuring antioxidants individually since their synergistic interaction could be determined [Kampa et al.,2002;Erel 2004;Fatemeh et al.,2011].Cakmak et al (Cakmak et al.,2010) reported that no significant differences in TAC between thalassemia and control groups, in spite of increase level of Oxidant Status (OS) and Oxidative Stress Index(OSI).

This study showed an increased concentration in bilirubin and uric acid in thalassemia patients, increased due to the hemolysis that occurs in patients ,also may be due to the decrease in the activity of cytochrome C oxidase. Also the study showed markedly greater bilirubin concentration was found in males when compared with females ,may be due to hormonal changes due to metabolism of bilirubin(Fatemeh et al.,2011).Previous studies have shown the effect of uric acid on total antioxidant capacity in patients with renal dysfunction .Therefore, in order to attenuate this effect, in this study used cTAS(Bonnefont et al.,2000),from result suggest that elevation of TAS cannot be solely explained as a result of increased uric acid and bilirubin. Compensatory excess of TAS to oxidative stress could also be the reason for difference between our findings and previous studies.

In the genetic study the results showed the Ala allele of the SOD2 gene was more widespread than the Gly allele in control . This feature is common to all other Asian populations (Chinese and Japanese) than in most European populations (Cakmak et al.,2010;Hori et al.,2000;Hiroi et al.,1999). In contrast, the frequency of the Ala variant is significantly higher in Caucasian populations (Germans, Swedes, Lithuanians, Finns,Russian and Saamis) (Van ,1999;Grasbon et al.,1999;Dimitry et al.,2001 ).Populations of the world vary considerably in their predisposition to diseases and in allele frequencies at pharmacogenetically important loci, probably as a result of genetic drift, and also because of adaptation to local selective factors such as climate and available nutrients(Suarez-Kurtz ,2004).The Ala allele varies among ethnic groups (Zhao et al.,2005) and has been associated with increased risk of different diseases related to oxidative stress and abnormal free radical defense mechanisms (Choi et al.,2008).The Val form (change from alpha-helix to beta-sheet) is less efficiently transported into mitochondria than the Ala form (alpha-helical structure )of the enzyme (Shimoda ,1996).The Val variant of the Mn-SOD may be present in a lower concentration in mitochondria. If this is the case, then homozygous Val/Val should have lower resistance to oxidative stress than patients with other Mn-SOD variants(Suarez-Kurtz ,2004).

The Ala/Val variation in the Mn-SOD leader signal affects the processing efficiency of the enzyme. Studies of processing have suggested basal level of the Mn-SOD activity may be highest for Ala/Ala, followed by Ala/Val, and then Val/Val (Hiroi et al.,1999).This study showed in the β- thalassemia major patients, the Val Mn-SOD allele predisposed patients to the development to diabetes mellitus. MnSOD is the major antioxidant in the mitochondria. Because the mitochondria carry out oxidative phosphorylation, which is essential to the vitality of the cell, they are a major source of endogenous ROS generation. (Wang et al.,2001). MnSOD is transcribed in the nuclear matrix and transported into the mitochondria via a mitochondrial signal sequence (MSS), where it exerts its antioxidant function (McKusik ,1998).Inefficient targeting of Mn-SOD may leave mitochondria inadequately defended against superoxide radicals. This may lead to protein oxidation, mitochondrial DNA mutations and damage, common in the pathogenesis of
diabetic neuropathy [Q.Shazia et al.,2012; Seghrouchni et al.,2002; Dimitry et al.,2001)].

**Conclusion**

1- Increased endogenous antioxidants such as uric acid, bilirubin, SOD2, SOD3 in the patients can also lead to increase level of TAS. Regular monitoring of antioxidant defense and administration of proper antioxidant may protect thalassemia patients from consequences of oxidative damages. These finding suggest that serum SOD2, SOD3 activities may be as marker of severity of β- thalassemia major to develop the β- thalassemia major with diabetic.

2- The present study provides some evidence that genetic polymorphism in SOD2 and SOD3 genes and associated with increased risk of diabetic among β- thalassemia major patients with high level of oxidative stress.

3- Ala(-9)Val substitution in the SOD2 gene was associated with β- thalassemia major with diabetic millets.

**Acknowledgements**

Thank to Dr. Naeem Salih Al-Zaidi (Assistant lecture in clinical biochemistry) for collection blood samples.

**References**


Lakari E., Paakko P., and Kinnula VL. 1998: Manganese superoxide dismutase, but not CuZn superoxide dismutase, is highly expressed in the granulomas of


