MDA, Oxidative Stress Marker-Role in Diabetic Nephropathy With Special Reference to Type II Diabetes Mellitus

**ABSTRACT**

OBJECTIVE: To investigate the role of oxidative stress marker, MDA levels among type II diabetic patients with and without nephropathy, and to determine its correlation with duration and other biochemical parameters (FBG, HbA1c, MDA, Micro-Albuminuria).

METHOD: Blood Glucose level was estimated by GOD-POD method, Glycosylated haemoglobin by Ion exchange resin method, Microalbuminuria by Pyrogallol red method (end point) and MDA by colorimetric method as described by Ohkawa et al.

RESULTS: The present study showed significant increase in the fasting blood glucose (FBG) and glycated haemoglobin (HbA1c) levels in complicated and non-complicated diabetic patients with nephropathy as compared to healthy controls. The MDA levels were significantly correlated with duration of diabetes in complicated cases which was also highly significant.

CONCLUSION: The study concludes that diabetic patients suffer more from oxidative stress compared to healthy control. Oxidative stress is still higher in diabetic patients with nephropathy than diabetics without nephropathy.

**TEXT:**

Diabetic patients have increased risk of developing various complications that are largely irreversible and are due to micro-vascular (nephropathy, retinopathy, neuropathy) or macro-vascular diseases (ischaemic heart disease, stroke, peripheral vascular disease). Hyperglycaemia seems to be an important causative factor in the development of micro and macro-vascular complications in patients with diabetes. Diabetes mellitus is considered to be one of a kind of free radical diseases which propagates complications with increased free radical formation.

Potential mechanisms by which hyperglycaemia could increase the formation of free radicals and lipid peroxidation are: 1) direct auto-oxidation of glucose, 2) activation of glycation pathways and receptor for advanced glycation end products (RAGE), 3) promotion of the interaction of nicotinamide adenine dinucleotide phosphate (NADPH) oxidase 6) induction and activation of various lipoxygenase enzymes and 7) stimulation of Protein Kinase C (PKC) pathway. The vascular complications are subdivided into two categories viz. micro-vascular and macro-vascular. The non-vascular complications include cataract, glaucoma etc.

Enhanced oxidative stress in diabetes type 2, further, has a variety of important effects in atherogenesis, including lipoprotein oxidation, particularly LDL oxidation. Lipid peroxidation of polyunsaturated fatty acids (PUFA), one of the free radical reactions in vivo, can adequately reflect increased oxidative stress in diabetes. Increased lipid peroxidation damages membrane function by decreasing membrane fluidity and changing the activity of membrane-bound enzymes and receptors. Malondialdehyde (MDA) is a stable end product of lipid peroxidation. It is a three carbon aldehyde that can exist in various forms in an aqueous solution. Serum MDA has been used as a biomarker of lipid peroxidation and has served as an indicator of free radical damage.

Diabetic nephropathy is the most common cause of end-stage renal disease. If untreated, 80% of people who have type-1 diabetes and microalbuminuria will progress to overt nephropathy (i.e., proteinuria characterized by > 300 mg albumin excreted daily), whereas only 20-40% of those with type 2 diabetes will progress over a period of 15 years. Diabetic nephropathy has several distinct phases of development. Kovas GL (2009), concluded that functional changes occur in the neprhon at the level of glomerulus, including podocyte foot process effacement, decrease in podocyte number, thickening of the glomerular basement membrane and mesangial expansion, all occur with the early changes.

**Material and method**

Subjects included for the study were categorised into 2 groups. Hundred age-matched healthy controls were taken (with respect to Type II Diabetics) in group 1. Total 100 type-II diabetic patients were included in group 2 and further divided into two sub groups, fifty non-complicated Type-II diabetic patients in subgroup 2A and 50 complicated Type-II diabetic patients, with nephropathy as a complication, in subgroup 2B as shown in figure:1.

Blood Glucose was estimated by GOD-POD method, Glycosylated haemoglobin by Ion exchange resin method, Microalbuminuria by Pyrogallol red method (end...
point)\(^1\)\(^1\) and MDA by method of Okhawa et al\(^2\). Since MDA is not stable, MDA standard was prepared from 1,1,3,3-Tetramethoxyp propane (TMOP). It was hydrolyzed during the acid incubation step at 45°C, which generated MDA. To each test tube, 0.5 ml of plasma, 0.5 ml of normal saline, 1 ml of 20% Trichloroacetic acid (TCA) and 0.25 ml of TBA reagent (200 mg of Thio bar bituric acid in 30 ml distilled water and 30 ml of Acetic acid) were added. The test tubes were boiled at 95°C for 1 hour. To each of the test tubes 3 ml of n-Butanol was added and mixed well. The tubes were centrifuged at 3000 rpm for 10 minutes. The separated Butanol layer was collected and read in a colorimeter against reagent blank at 540 nm. The MDA concentration was expressed in terms of µmol/L.

**Statistical analysis**

Statistical analysis was performed using the SPSS computer program version 16.0. Pearson correlation coefficient (r) between the parameters of complicated and non-complicated type II diabetes was used to assess the correlation between MDA and other risk factors as shown in Table 1 and Table 2. Student’s t test was performed, to compare the mean between different groups. All analyses were undertaken using \( \alpha < 0.05 \) (two-tailed) as the significant statistical standard.

**Result:**

Biochemical characteristics and duration of type 2 diabetic patients and healthy control groups are shown in Table 1. FBG and MDA levels were significantly higher (p<0.001) in complicated cases as compared to non-complicated cases figure: 2. The serum MDA levels were significantly correlated (p< 0.05) with duration in diabetes in complicated cases (r =0.959) \(^**\) which was also highly significant (p<0.001).

**Discussion:**

The present study showed significant increase in the fasting blood glucose (FBG), glycated haemoglobin (HbA\(_{1c}\)) and Malondialdehyde (MDA) levels in noncomplicated and complicated diabetics with nephropathy as compared to healthy controls. The microalbuminuria levels were also significantly higher in complicated diabetics with nephropathy as compared to noncomplicated cases. The present study is in agreement with the various authors in their studies like Bhatia S et al\(^13\), Apakkan AS et al\(^14\) and Kornelia Z et al\(^15\) who have all shown the significant higher levels of MDA in complicated diabetes with nephropathy and non-complicated diabetes as compared to healthy controls. MDA levels have also been reported to be elevated in other complications of diabetes like neuropathy, retinopathy, coronary heart disease, hypertension etc., by various authors like Sawant MJ et al\(^6\), Vivian ST et al\(^7\), Mahreen et al\(^8\), Suvarna P et al\(^9\) and Mandal B et al\(^19\).

Further detailed analysis of the data revealed that the Pearson’s correlation coefficient increased from non-complicated to complicated diabetes as far as HbA\(_{1c}\), MDA and Microalbuminuria were concerned. The same held true for the disease duration. Therefore the present study has shown that the MDA level is also increased with duration of diabetes which is also observed in the studies of various authors like Nakjhavani M et al\(^10\), Vivian ST et al\(^11\) and Bhutia et al\(^12\).

**Conclusion:**

The study concludes that diabetic patients suffer more from oxidative stress as compared to healthy controls. Oxidative stress is still higher in diabetic patients with nephropathy than diabetics without nephropathy.

### Table: 1

<table>
<thead>
<tr>
<th>GROUP</th>
<th>CONTROL (n=100)</th>
<th>COMPLICATED (n=50)</th>
<th>NON-COMPPLICATED (n=50)</th>
<th>P - VALUE (n=50)</th>
</tr>
</thead>
<tbody>
<tr>
<td>AGE</td>
<td>43.32±13.20</td>
<td>58.02± 10.80</td>
<td>52.00±11.25</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>FBG</td>
<td>79.40±6.97</td>
<td>198.78± 68.41</td>
<td>178.03±71.77</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>HbA(_{1c})</td>
<td>4.73±0.44</td>
<td>8.36±1.27</td>
<td>7.05±0.40</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>MDA</td>
<td>1.23±0.25</td>
<td>4.49±1.27</td>
<td>2.35±0.23</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>MICRO albuminuria</td>
<td>-</td>
<td>766.87±694.24</td>
<td>19.78±5.09</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>DURATION</td>
<td>-</td>
<td>8.96±5.38</td>
<td>1.85±1.63</td>
<td>&lt;0.001</td>
</tr>
</tbody>
</table>

### Table: 2

Pearson correlation (r) between the parameters of non-complicated diabetics.

<table>
<thead>
<tr>
<th></th>
<th>Age</th>
<th>FBG</th>
<th>HbA(_{1c})</th>
<th>MDA</th>
<th>Micro-albuminuria</th>
<th>Duration</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Sig. (2-tailed)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>FBG</td>
<td>0.97</td>
<td>0.23</td>
<td>0.26</td>
<td>0.31</td>
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<td></td>
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<tr>
<td>HbA(_{1c})</td>
<td>0.47</td>
<td>0.10</td>
<td>0.00</td>
<td>0.00</td>
<td>0.00</td>
<td></td>
</tr>
<tr>
<td>MDA</td>
<td>0.11</td>
<td>0.07</td>
<td>0.00</td>
<td>0.00</td>
<td>0.00</td>
<td></td>
</tr>
<tr>
<td>Micro-albuminuria</td>
<td>0.32</td>
<td>0.01</td>
<td>0.00</td>
<td>0.00</td>
<td>1</td>
<td></td>
</tr>
<tr>
<td>DURATION</td>
<td>0.70</td>
<td>0.80</td>
<td>0.00</td>
<td>0.00</td>
<td>0.00</td>
<td>1</td>
</tr>
</tbody>
</table>

### Table: 3

Pearson correlation (r) between the parameters of type II diabetic nephropathy.

<table>
<thead>
<tr>
<th></th>
<th>Age</th>
<th>FBG</th>
<th>HbA(_{1c})</th>
<th>MDA</th>
<th>Micro-albuminuria</th>
<th>Duration</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Sig. (2-tailed)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>FBG</td>
<td>0.13</td>
<td>0.13</td>
<td>0.05</td>
<td>0.04</td>
<td>0.02</td>
<td></td>
</tr>
<tr>
<td>HbA(_{1c})</td>
<td>0.14</td>
<td>0.89</td>
<td>0.74</td>
<td>0.77</td>
<td>0.88</td>
<td></td>
</tr>
<tr>
<td>MDA</td>
<td>0.14</td>
<td>0.05</td>
<td>0.93</td>
<td>0.96</td>
<td>0.96</td>
<td></td>
</tr>
</tbody>
</table>

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**. Correlation is significant at the 0.01 level (2-tailed).

**Figure 1: Number of cases and controls.**

**Figure 2: MDA levels in complicated and non-complicated cases with respect to controls.**

**References:**