Oxidative Stress in Patients with Type 2 Diabetes Mellitus

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Abstract

Background: To assess the reduced glutathione (GSH) levels as marker of oxidative stress in patients of diabetes mellitus.

Methods: In this cross sectional study 40 subjects were divided into two groups. One group was designated as control while the other was diabetic. Glycemic status was measured to confirm their normal and diabetic states respectively. Plasma reduced GSH level was measured by using standard ELISA kits.

Results: Level of reduced GSH was decreased in the diabetic group (18.4 ± 1.35) as compared to the control group (27.5 ± 1.38).

Conclusion: Levels of reduced GSH were significantly decreased in patients of diabetes mellitus as compared to normal healthy controls.

Key Words: Diabetes mellitus, oxidative stress, reduced glutathione marker.

Introduction

Oxidative stress is an imbalance between the production of reactive oxygen and ability of the biological system to detoxify the reactive intermediates or to repair the resulting damage. The destructive aspect of oxidative stress is the production of reactive oxygen species (ROS), which include free radicals and peroxides, that can damage nucleic acids, proteins and cell membranes.1 Oxidative stress occurs when ROS production exceeds than their removal by the cellular defense mechanism. In humans, oxidative stress is involved in many diseases, such as diabetes mellitus (DM), atherosclerosis, myocardial infarction (MI), heart failure, Parkinson's disease, chronic fatigue syndrome, Alzheimer's disease and fragile X syndrome.2,3

DM is a continuous source of oxidative stress to the body and there is increased generation of ROS. These ROS have a very short half-life and cannot remain as such and react rapidly with DNA, protein, and lipids, thereby leading to oxidative damage. Oxidative stress occurs in diabetic patients due to increase in the steady-state levels of ROS, which is a result of decreased anti-oxidant defense mechanism and increased free radical generation. These ROS are involved in the development of diabetic complications like blindness, renal failure, neuropathy and myocardial infarction.2,4

Hyperglycemia is the initiating cause of oxidative stress in diabetes. It causes repeated acute changes in cellular glucose metabolism and long-term accumulation of glycosylated biomolecules and advanced glycation end products (AGEs). In the presence of uncontrolled hyperglycemia, the increased formation of AGEs and lipid peroxidation products exacerbate intracellular oxidative stress, disruption in cellular signaling and homeostasis followed by inflammation and tissue injury such as endothelium dysfunction, arterial stiffening and microvascular complications.5

In diabetics, there are also other multiple pathways enhancing ROS production, which includes; protein kinase C-dependent activation of NADPH oxidase, enhanced glucose auto-oxidation, uncoupled endothelial nitric oxide synthase activity, increased mitochondrial superoxide production and stimulation of eicosanoid metabolism.4

Patients and Methods

This cross sectional study was conducted at Department of Biochemistry & Molecular Biology, Army Medical College, Rawalpindi in collaboration with Combined Military Hospital (CMH), Rawalpindi and Centre for Research in Experimental and Applied Medicine (CREAM), Army Medical College, Rawalpindi for blood sampling and biochemical assays respectively. Total duration of study was 01 year and was performed on 40 adult volunteer human subjects of both sexes, with a body mass index between 19 - 40 kg/m². They were divided into two groups containing 20 subjects each. One group was designated as control (healthy) group while the other
was diseased (diabetic) group. Control group (Group I) comprised of 20 normal healthy humans having normal fasting blood glucose levels (between 70 – 100 mg/dl). Pregnant women, person suffering from any chronic illness or disease or taking any sort of medication / supplements were excluded. Diabetic Group (Group II) had 20 patients suffering from type 2 diabetes mellitus having fasting blood glucose in a range of 126 – 400 mg/dl, despite taking their usual anti-diabetic medication. Patients on insulin therapy, pregnant women, persons suffering from any other chronic illness or taking any medication other than that of diabetes were excluded. Patients of both sexes with known type II diabetic history and fulfilling all the inclusion criteria were screened by determining their fasting blood glucose by a glucometer. Similarly, healthy individuals were screened by determining their fasting blood glucose before selection for the study. Reduced glutathione (GSH) was measured by enzyme linked immuno-sorbent assay (ELISA) method. A p value of ≤ 0.05 and ≤0.01 was considered significant and highly significant respectively.

**Results**

The mean reduced Glutathione in control group was 27.5 ng/ml, while in diabetic group it was 18.4 ng/ml (Table 1). There was a highly significant difference (p < 0.001) between the control and diabetic groups (Table 2).

**Table 1: Oxidative stress marker (GSH) of control & diabetic group.**

<table>
<thead>
<tr>
<th>Study Group</th>
<th>Parameter</th>
<th>Sample Size</th>
<th>Mean</th>
<th>Standard error of mean (SEM)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>Reduced Glutathione (GSH) (ng/ml)</td>
<td>20</td>
<td>27.5</td>
<td>1.383</td>
</tr>
<tr>
<td>Diabetic</td>
<td></td>
<td>20</td>
<td>18.4</td>
<td>1.346</td>
</tr>
</tbody>
</table>

**Conclusion**

Levels of reduced GSH are significantly decreased in diabetics as compared to normal healthy persons.

**Discussion**

Glutathione, an antioxidant, helps protect cells from reactive oxygen species (ROS) such as free radicals and peroxides. In healthy cells and tissues, more than 90% of the total glutathione pool is in the reduced form (GSH) and less than 10% exists in the disulfide form (GSSG). An increased GSSG-to-GSH ratio is considered indicative of oxidative stress. In our study, blood level of reduced GSH was in the range of 14 – 38 ng/ml with a mean value of 27.5 ± 1.383 for the control group compared to 9 – 31 ng/ml with a mean value of 18.4 ± 1.346 for the diabetic group. GSH was significantly reduced in diabetics as compared to normal healthy persons (p < 0.001). Studies found an association between non enzymatic glycation and oxidative stress in the pathogenesis of diabetic complications. Enzymatic glycation is found to be linked to both glucose auto oxidation and proteins glycation processes, which in turn proceed to the formation of free radicals. This coupled with long term high glucose levels results in the major complications of diabetes. Studies carried out by Sasaki, S. et al and Brownlee, M. et al also support these facts.

Tagliabue, M. et al in their study presented that levels of GSH are significantly decreased in type 2 diabetics. In conjugation to this, Soliman, GZ. and Ozdemir, G. carried out experiments to evaluate the role of GSH levels in the pathophysiology of type 2 diabetes. They emphasized on the link between exaggerated oxidative stress and hyperglycemia leading to clinical complications of type 2 diabetes. Sekhar, et al concluded from their studies in uncontrolled diabetes that glutathione synthesis is decreased in these patients. Decreased levels of reduced glutathione levels aggravate diabetic complications.

**References**


