Effect of Persistent Hyperglycemia and Lipid Profile and its role in Glycation and Oxidative stress in Chronic Type 2 Diabetes Mellitus subjects

Deepak Kafle
Department of Biochemistry, Chitwan Medical College, Bharatpur, Chitwan, Nepal

ABSTRACT

Introduction: Diabetes mellitus is a disease of carbohydrate metabolism disorder which results in increase in blood glucose level. Hyperglycemia may increase oxidative stress through a variety of ways impairs an antioxidant defenses mechanism. Reactive oxygen species are increased as a result of the oxidative stress caused by hyperglycemia, which activates a number of redox-sensitive cell signaling molecules producing cytotoxic materials.

Methods: 250 randomly visited the OPD at the Chitwan Medical College Department of Medicine were chosen for this cross-sectional study. 10ml of blood sample was drawn from anticubital vein following overnight fasting and was centrifuged at room temperature for 10 minutes at 3000 rpm. The serum was stored at 4oC for biochemical investigations. Fasting blood sugar, lipid profile and HbA1c etc. were analyzed via chemiluminescence Analyzer (CLIA) in the Hospital Laboratory of Chitwan Medical College. With SPSS version 22, statistical analysis was carried out.

Results: It was found from the study that type 2 diabetic subjects have significant increase of fasting blood sugar and malondialdehyde (MDA) but glutathione peroxidase was found to be decreased in type 2 diabetic subjects as compared to healthy controls. Glutathione reductase was found non-significant at (P>0.05) in both the subjects. It was found from the study that association of HbA1c with Total cholesterol, Triglyceride, LDL-C was found to be positively correlated and was significant at P<0.05.

Conclusions: When predicting glycemic control in T2DM patients, lipid profiles (LDL-C) and glycation status can be helpful tools.

INTRODUCTION

Diabetes mellitus is a disorder of carbohydrate metabolism that raises blood sugar levels.1 It is brought on by a lack of insulin secretion, which can be due to defect in the receptors of peripheral tissue insulin uptake or marked failure of the pancreatic -Langerhans islet cells to secrete insulin.2 Because the metabolism of carbohydrates directly influences the metabolism of lipids, carbohydrate metabolism disorder affects lipid levels in diabetes.3 Furthermore, insulin deficiency also leads to higher metabolism of free fatty acid due to rise in the
level of counter regulatory hormone glucagon. Lipid abnormalities have also been seen to play an important role in the macrovascular complication associated with type-2 DM as well.  

Reactive oxygen species (ROS) and the body’s antioxidant defense system are thought to be out of balance under oxidative stress. Reactive oxygen species are increased as a result of the oxidative stress caused by hyperglycemia, which activates a number of redox-sensitive cell signaling molecules and produces harmful materials. Cellular malfunction and damage occur, and as a result, diabetic micro and macrovascular problems. Prolonged exposures to excess glucose and production of reactive oxygen species in type 2 diabetes involve numerous pathways such as protein glycation and formation of advanced glycation end products can lead to metabolic complications throughout the life span in chronic diabetic individuals. So the study was aimed to find out the effect of persistent hyperglycemia and lipid profile and its role in glycation and oxidative stress in chronic type 2 Diabetes Mellitus subjects.

METHODS

A cross-sectional study involving 250 participants was conducted at the Department of Biochemistry in collaboration with the Department of Medicine from May 25 to June 25, 2022. This study project has received approval from Chitwan Medical College’s ethical committee (CMC-IRC/078/079-572). By taking the consent, the patients with diabetic nephropathy who visit the Department of Medicine at CMC were included in this study.

Details of study are as follows:

Experimental designs of study are as follows: (Age matched)

Total number of subjects (experimental): 350

250 –T2DM subjects (>5years)

100- Healthy Control

After an overnight fast, 10 ml of blood will be taken from the antecubital vein and collected in fluoride, and EDTA vacutainers. The blood sample was centrifuged at 3000 rpm for 10 min. at room temperature. The chemiluminescence analyzer (CLIA) was used to determine the lipid profile and biochemical investigations.

The GOD-POD method was used to assess fasting blood sugar levels. Using the method developed by Hateman DG et al.,(1974) glutathione peroxides (Gpx) were calculated. Glutathione reductase (GR) was estimated by the method of Horn HD (1963). Plasma malondialdehyde (MDA) was estimated by Jean CD et al. (1983). Triglyceride, Total Cholesterol, HDL cholesterol, LDL cholesterol was measured by automated Biochemistry analyzer on Dimension R Clinical Chemistry. The National Glycohemoglobin Standardization Program established a specific standardized measurement set that was used to measure glycated hemoglobin (HbA1c) utilizing ion-exchange high-performance liquid chromatography in a D-10 system (BioRad Laboratories Inc., Hercules, CA, USA). Statistical analysis was done using student t-test to estimate differences between the groups. All parameters were written in mean± standard deviation. The significance criterion was P < 0.05.

RESULTS

Table 1: Descriptive analysis of oxidative stress markers and Glycation between type 2 diabetic and Healthy control subjects.

<table>
<thead>
<tr>
<th>S.N</th>
<th>Parameters</th>
<th>Healthy Controls (n=100)</th>
<th>T2DM (n= 250)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Age</td>
<td>40-60 yrs</td>
<td>40-60 yrs</td>
</tr>
<tr>
<td>2</td>
<td>Duration</td>
<td>----</td>
<td>&gt;6 yrs</td>
</tr>
<tr>
<td>3</td>
<td>BMI</td>
<td>26.98 ± 1.70</td>
<td>29.98 ± 4.00*</td>
</tr>
<tr>
<td>4</td>
<td>FBS</td>
<td>96 ±13.10</td>
<td>143.13 ±12.62*</td>
</tr>
<tr>
<td>5</td>
<td>MDA</td>
<td>4.31±1.10</td>
<td>6.92±1.33**</td>
</tr>
<tr>
<td>6</td>
<td>Gpx</td>
<td>6.46 ±0.84</td>
<td>4.897±0.895*</td>
</tr>
<tr>
<td>7</td>
<td>GR</td>
<td>6.55±1.02</td>
<td>6.90±0.895NS</td>
</tr>
<tr>
<td>8</td>
<td>HbA1c</td>
<td>4.2± 1.1</td>
<td>6.5±1.5*</td>
</tr>
</tbody>
</table>

* Significant at P<0.05

**Significant at P<0.001
Table 2: Descriptive analysis of lipid profile parameters between type 2 diabetic and Healthy control subjects

<table>
<thead>
<tr>
<th>S.N.</th>
<th>Parameters</th>
<th>Healthy Controls (n=100)</th>
<th>T2DM (n= 250)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Total cholesterol (mg/dL)</td>
<td>180±25</td>
<td>195±33</td>
</tr>
<tr>
<td>2</td>
<td>Triglyceride (mg/dL)</td>
<td>135±20</td>
<td>195±28*</td>
</tr>
<tr>
<td>3</td>
<td>HDL-C (mg/dL)</td>
<td>42±3.4</td>
<td>33±2.5*</td>
</tr>
<tr>
<td>4</td>
<td>LDL-C (mg/dL)</td>
<td>110± 12.14</td>
<td>128±13.18*</td>
</tr>
</tbody>
</table>

*Significant at P<0.05

Table 3: Correlation between lipid profile with HbA1c level

<table>
<thead>
<tr>
<th>S.N</th>
<th>Variable</th>
<th>HbA1c</th>
<th>r (Correlation Coefficient)</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Total cholesterol (mg/dL)</td>
<td>N=250</td>
<td>0.461</td>
<td>0.041*</td>
</tr>
<tr>
<td>2</td>
<td>Triglyceride (mg/dL)</td>
<td></td>
<td>0.236</td>
<td>0.001*</td>
</tr>
<tr>
<td>3</td>
<td>HDL-C (mg/dL)</td>
<td></td>
<td>-0.541</td>
<td>&lt;0.001*</td>
</tr>
<tr>
<td>4</td>
<td>LDL-C (mg/dL)</td>
<td></td>
<td>0.613</td>
<td>0.031*</td>
</tr>
</tbody>
</table>

*Significant (p<0.05)

It was found from the study that type 2 diabetic subjects have significant increase of fasting blood sugar and malondialdehyde (MDA) as compared to healthy controls without diabetes. Both the parameters are showing highly significant at (P<0.05) whereas antioxidant enzyme glutathione peroxidase was found to be decreased in type 2 diabetic subjects as compared to healthy controls and it was highly significant at (P<0.05). Glutathione reductase was found non-significant at (P>0.05) among type 2 diabetic and healthy controls and was within normal range (3-13 U/g of Hb) in both subjects. In our study the body mass index (BMI) was found to be increased in type 2 diabetic subjects as compared to healthy controls and it was highly significant at (P<0.05).

It was found from the study that association of HbA1c with Total cholesterol, Triglyceride, LDL-C was found to be positively correlated and was significant at P<0.05. The correlation between HbA1c and Triglyceride was highly positive and was highly significant (r = 0.23, P<0.001) whereas the correlation between HbA1c and HDL-C was negative and was highly significant (r = -0.54, P<0.001).

DISCUSSION

Reactive oxygen species (ROS) and free radicals, the byproducts of oxidative stress become destructive when they are not neutralized by the antioxidant mechanisms.10 The detrimental biological effects of reactive oxygen species include DNA damage, oxidation of proteins, renal vasoconstriction, and peroxidation of cell membrane lipids. Alternate mechanisms for the metabolism of glucose, such as PKC activation and advanced glycation end-products, show that hyperglycemia in diabetes is the contributing factor to the production of reactive oxygen species.11 Our study showed moderate decrease of Glutathione reductase in type 2 Diabetic subjects as compared to healthy controls but glutathione peroxidase level was significantly decreased in both the groups which might be due to hyperglycemia induced oxidative stress and glycation of antioxidant enzymes. The effects of oxidative stress on lipids are mainly expressed by the induction of lipid peroxidation which was in consistent with (Desai N et al. 2005).12

Advanced glycation end-products (AGEs) are created by the non-enzymatic condensation of free amine groups from nucleic acids, proteins, or lipids via the carbonyl groups of reducing sugars, followed by further rearrangements that produce stable, irreversible end-products. AGEs play a role in numerous pathophysiological processes and diseases, including diabetes, cancer, cardiovascular disease, and others. AGEs are recognized by a variety of cellular receptors and activate numerous signaling pathways related to inflammation and oxidative stress.13 The high level of MDA in diabetic nephropathy individuals is a suggestive hallmark of oxidative stress in long-term type-2 diabetes. ROS created in hyperglycemia enhances peroxidation of cellular membrane lipids as well as boosting the oxidation of proteins that yield protein carbonyl derivatives. Our findings are in consistent with the research published by Mima A. in 2013.14

In our study HbA1c was found significant in type 2 Diabetics as compared with controls. Glycation of proteins is dependent on the persistence of hyperglycemia, duration, and metabolic disorders.15 A high glucose level causes proteins to glycate non-enzymatically by Schiff base to
Amadori rearrangement, resulting in glycated hemoglobin (HbA1c). The amount of glycated proteins (HbA1c) reflect the extent of exposure to glucose within 8–12 weeks duration. In type 2 diabetic patients, persistent hyperglycemia activates a number of mechanisms, including the polyol pathway and glucose autoxidation, which causes protein glycation with the production of advanced glycation end products. It was found that association of HbA1c with lipid profile components like Total cholesterol, Triglyceride, LDL-C was found to be positively correlated and was significant in type 2 diabetic subjects. People with diabetes who have poor glycemic control frequently have dyslipidemic condition, which involves an increase in triglycerides (TG), low-density lipoprotein cholesterol (LDL-C), and a drop in high-density lipoprotein cholesterol (HDL-C).

The occurrence of the dyslipidemia in diabetes can be explained by changes in plasma lipoprotein that occurs in diabetic patients during fasting and post-prandial condition, due to defects in insulin action and hyperglycemia. When fatty acids and cholesterol from food are absorbed in the small intestine, postprandial conditions result in the formation of TG and cholesteryl esters, which are later converted to chylomicrons. Chylomicrons are a lipoprotein substrate that cause TG and fatty acid lipolysis in adipocyte and muscle cells. Insulin regulates lipoprotein activity via protein synthesis and gene expression. The activity of lipoprotein lipase is decreased when insulin resistance arises, which is the reason for increases in TG and decreases HDL in a diabetic state.

CONCLUSIONS

Persistent hyperglycemia induces intracellular reactive oxygen species (ROS), protein glycation and metabolic disorders in type 2 diabetic subjects. Antioxidant Gpx enzyme levels are found to be decreased due to hyperglycemia induced oxidative stress however, the level of glutathione reductase was found within the normal range in both the subjects. The Dyslipidemia in type 2 diabetes might be associated with insulin resistance. Lipid profiles (LDL-C) and glycation were positively correlated which can be a useful tool in predicting glycemic control in patients with T2DM.

REFERENCES

14. Mima A. Inflammation and Oxidative Stress


