Assessment of Oxidative Stress Among Type 2 Diabetes Mellitus Patients Attending in A Rural Teaching Hospital, Sangareddy.

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Abstract:
Background: Oxidative stress is associated with the poor glycemic control in diabetes mellitus. Severe oxidative stress may cause cellular inflammation and damaging blood vessels in type 2 diabetic patients.
Objectives: The present study was aimed to assess oxidative stress among type 2 diabetic patients by estimating MDA along with the lipid profile and blood glucose.
Materials and methods: The present study was carried out at MNR Medical college & hospital situated in Sangareddy, part of Medak District, Telangana state. The 80 study subjects were selected from the medicine ward of MNR hospital during the period from February 2016 to July 2016.
Results: A significantly higher level of MDA, total cholesterol, triglycerides, low-density lipoprotein and very low-density lipoprotein was estimated in type 2 diabetic patients (cases) when compared with non diabetic patients (controls) (p<0.0001). The mean HDL of cases was significantly lower than that of controls (p<0.0001).
Conclusion: Our study concludes that good glycemic control will prevent in alteration in lipid peroxidation and the lipid metabolism. This helps in prognosis and preventing complications in type 2 diabetes mellitus.
Keywords: Oxidative stress, Malondialdehyde (MDA), Fasting blood sugar (FBS), Lipid profile, Type 2 diabetes mellitus.

I. Introduction
Globally diabetes mellitus became one of the major health problems and endemic with rapidly increasing prevalence in both developed and developing countries [1]. According to World Health Organisation (WHO), India has a high prevalence of diabetes and declared India as the diabetes capital of the world. In India, type 2 diabetes mellitus rapidly increasing day by day due to reduced physical activity and increased obesity. According to International Diabetes Federation (IDF) in India 40 million people are living with Diabetes mellitus and are projected to increase to 70 million by 2020 [2].

Diabetes mellitus is a metabolic disorder characterized by persistently elevated levels of blood glucose with disturbances of lipid, carbohydrate and protein metabolism as a result of a defect in insulin production or insulin action or both. The long-term complication of diabetes mellitus includes dysfunction, damage to various organs, especially the heart, kidneys, eyes, nerves and blood vessels and are responsible for the majority of morbidity and mortality associated with the disease [3]. During diabetes; constant hyperglycemia causes increased production of free radicals especially reactive oxygen species (ROS) [4]. ROS are generated during oxidative phosphorylation in mitochondria and other metabolic processes [5]. ROS are highly reactive and can damage membrane lipids, nucleic acid and enzymes [6,7]. Oxidative stress (OS) is a medical term used for the damage of cells caused by ROS. OS is defined as a loss of imbalance between free radicals generating and free radicals scavenging systems i.e. increased free radicals production or reduced activity of antioxidant defence or both. OS is having negative effects on carbohydrates, lipids, proteins. It is also involved in the progression of different chronic diseases and apoptosis. OS induces cell injury has been reported to have a role in the pathogenesis of a number of diseases including cardiovascular, inflammatory diseases, diabetes and neurodegenerative pathologies. Oxidative tissue and organs damage play roles in diabetes mellitus and its complications. Hyperglycemia can cause OS particularly by evidence that several biochemical pathways activated during hyperglycemia can increase the production of free radicals [8,9,10].So the present study was aimed to assess oxidative stress among type 2 diabetic patients by estimating malondialdehyde and lipid profile.

II. Materials and methods
The present study was carried out at MNR Medical college & hospital situated in Sangareddy, part of Medak District, Telangana state (600 beds teaching hospital catering to rural population). A total of 80 study subjects of both gender groups were selected from the medicine ward of MNR hospital during the period from
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February 2016 to July 2016. This study was approved by institutional ethical committee and investigations were carried out in the biochemistry laboratory, MNR Medical College & Hospital, Sangareddy, Telangana State.

2.1 Collection of Blood Sample:

Blood samples (about 8ml) were collected after a 12 hour overnight fast under aseptic conditions, dispensed into clean dry tubes and allowed to clot and care is taken to procure serum. An informed consent was taken from study subjects for tests performed and the study was approved by institutional ethical committee. Investigations are carried out on the serum samples by standard kit methods and analyses were performed on ERBA RA-150 semi-autoanalyzer, in the biochemistry laboratory, MNR Medical College & Hospital.

2.2 Parameters measured:

In the present study following parameters were measured:

1. Fasting Blood Sugar (FBS)
2. Malondialdehyde (MDA)
3. Total Cholesterol (TC)
4. Triglycerides (TG)
5. High Density Lipoprotein-cholesterol (HDL-C)

Estimation of fasting blood sugar was done by GOD/POD method [11]. Serum Malondialdehyde was measured by Kei Satoh method et al. [12]. Serum total cholesterol was measured by CHOD – PAP method [13]. Triglycerides were measured by GPO-Trinder method [14], HDL-Cholesterol measured by Phosphotungstic acid method [15] and the values of low-density lipoprotein cholesterol (LDL-C) and Very-low-density lipoprotein cholesterol (VLDL-C) can be calculated by using Friedewald’s equation [16] as follows;

- LDL - Cholesterol = total cholesterol – (HDL-cholesterol + triglycerides/ 5)
- VLDL-C = Triglycerides/5.

2.3 Statistical Analysis:

The collected data were analyzed by SPSS software version 16.0. All results were presented as mean ± standard deviation (SD). A p-value of less than 0.0001 was considered as a statistically significant.

III. Results

In the present study, total 80 subjects were divided into two groups, 30 controls (non-diabetic) and 50 cases (diabetic) with the age range of 30 – 70 years. Out of 30 non-diabetic controls, 14 were males and 16 females and in 50 diabetic cases, 32 were males and 18 females as shown in the [Table 1].

Table 1: Age and Gender wise distribution of controls and cases.

| Table 1: Age and Gender wise distribution of controls and cases. |
|-----------------|-----------------|-----------------|-----------------|
| Age             | Controls (n=30)  | Cases (n=50)    |
|                 | Males (n=14)    | Females (n=16) | Males (n=32)    |
| 30-40           | 05              | 04              | 05              |
| 41-50           | 04              | 06              | 08              |
| 51-60           | 05              | 02              | 12              |
| 61-70           | 03              | 01              | 03              |
| Total           | 30              | 30              | 50              |

The mean ± SD of FBS among diabetic cases was 178.1 ± 41.1 mg/dl. Whereas that of control was 81.30 ± 8.70 mg/dl. The mean ± SD of diabetic cases was significantly higher than non-diabetic controls (p< 0.0001). The mean ± SD of MDA among diabetic cases was 15.22±6.04 mg/dl. Whereas that of non-diabetic control was 6.89±2.26 mg/dl. The mean ± SD of MDA among diabetic cases was significantly higher than non-diabetic controls (p< 0.001). The mean ± SD of total cholesterol among diabetic cases was 203.26±35.42 mg/dl and that of non-diabetic control was 152.63±31.31 mg/dl. The mean ± SD of total cholesterol among diabetic cases was significantly higher than non-diabetic controls (p< 0.0001). The mean ± SD of Triglycerides among diabetic cases was 167.76 ±38.04 mg/dl and that of control was 106.83±20.03 mg/dl. The mean ± SD of Triglycerides in diabetic cases was significantly higher than controls (p< 0.001). The mean ± SD of HDL among diabetic cases was 42.50 ±8.06 mg/dl and that of control was 46.43±7.93 mg/dl. The mean HDL of cases was significantly lower than that of controls (p< 0.001) and is statistically significant. The mean ± SD of LDL among diabetic cases was 126.60±31.16 mg/dl and that of controls was 86.00±26.46 mg/dl. The mean ± SD of LDL of diabetic cases was significantly higher than that of controls (p< 0.001). The mean ± SD of VLDL among diabetic subjects was 33.56±7.66 mg/dl and mean ± SD of VLDL of controls was 21.77±4.83 mg/dl. The mean ± SD of VLDL of diabetic cases was significantly higher than controls (p< 0.0001) as shown in table 2.
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Table 2: Comparison of serum FBS, MDA and Lipid profile levels between controls and Cases.

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Controls (Non Diabetic) (n=30)</th>
<th>Cases (Diabetic) (n=50)</th>
<th>t- value</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Fasting Blood Sugar (mg/dl)</td>
<td>81.30 ±28.70</td>
<td>178.1 ±41.1</td>
<td>8.9152</td>
<td>&lt;0.0001 *S</td>
</tr>
<tr>
<td>MDA (nmol/L)</td>
<td>6.89 ±2.26</td>
<td>15.22 ±6.04</td>
<td>7.2329</td>
<td>&lt;0.0001 *S</td>
</tr>
<tr>
<td>Total cholesterol (mg/dl)</td>
<td>152.63 ±31.31</td>
<td>203.26 ±35.42</td>
<td>6.4569</td>
<td>&lt;0.0001 *S</td>
</tr>
<tr>
<td>Triglycerides (mg/dl)</td>
<td>106.83 ±20.03</td>
<td>167.76 ±38.04</td>
<td>8.1081</td>
<td>&lt;0.0001 *S</td>
</tr>
<tr>
<td>HDL (mg/dl)</td>
<td>46.43 ±7.93</td>
<td>42.50 ±8.0</td>
<td>2.1256</td>
<td>&lt;0.0001 *S</td>
</tr>
<tr>
<td>LDL (mg/dl)</td>
<td>86.00 ±26.46</td>
<td>126.60 ±31.16</td>
<td>5.9592</td>
<td>&lt;0.0001 *S</td>
</tr>
<tr>
<td>VLDL (mg/dl)</td>
<td>21.77 ±4.83</td>
<td>33.56 ±7.66</td>
<td>7.5678</td>
<td>&lt;0.0001 *S</td>
</tr>
</tbody>
</table>

*S= Significant.

IV. Discussion

In the present study, patients with diabetes mellitus show statistically significant increase in MDA (serum lipid peroxide) levels. Similar findings were observed by Mahendra D. Bikkad et al., [17]. Few of the most probable causes for the increased MDA level in diabetes mellitus are as follows: in diabetes mellitus abnormally increased levels of lipids, lipoproteins and lipid peroxides in plasma may be due to the abnormal lipid metabolism [18]. Elevated levels of lipid peroxide in diabetes mellitus may be due to the alternate function of erythrocyte membrane. This inhibits the activity of superoxide dismutase enzyme leading to accumulation of superoxide radicals which cause the maximum lipid peroxidation and tissue damage in diabetes [19]. Increased lipid peroxidation may be due to the increased glycation of proteins in diabetes mellitus. The glycated protein might themselves act as a source of free radicals. There is a clear association between lipid peroxide and glucose concentration, which may also be thought to play a role in increased peroxidation in diabetes mellitus.

In poorly controlled diabetes mellitus, glucose oxidation through the pentose phosphate pathway leads to excessive formation of NADPH, which in turn can promote lipid peroxidation in the presence of cyt P-450 system. Oxy- hemoglobin in erythrocytes could act like cyt P-450 in the presence of NADPH and this could induce increased lipid peroxidation. Mitochondrial and microsomal membrane contain relatively large amount of polyunsaturated fatty acid in their phospholipid. These include fatty acid with 2,3,4,5 and 6 double bonds. Due to the presence of 3 or more double bonds, they are likely to be more sensitive to attack by free radicals resulting in high lipid peroxidation. Hence, the rate of peroxidation may be high causing the higher concentration of lipid peroxides and free radicals in diabetes mellitus [20,21].

In the present study, patients with diabetes mellitus showed a statistical increase in the levels of serum cholesterol, triglycerides, LDL and VLDL cholesterol and there is a significance decrease in HDL cholesterol when compared to normal controls. Similar findings were observed by Shankar Shetty et al., [22]. The possible causes for the change in their levels are followed; the serum cholesterol is increased in diabetic patients when compared with normal controls. Some of the possible reasons of high concentration of serum cholesterol in diabetes may be attributed to decreased muscular exercise or inhibition of cholesterol catabolism. Increased triglycerides may be due to insulin resistance which results in faulty glucose utilization, causes hyperglycemia and mobilization of fatty acids from adipose tissue. The fatty acid from adipose tissue is mobilized for energy purpose and excess fatty acids are accumulated in the liver, which are converted triglycerides [23]. Insulin increases the number of LDL – receptors, so chronic insulin deficiencies which occur in diabetes mellitus might be associated with diminished levels of LDL- cholesterol levels in diabetes mellitus. High levels of cholesterol, triglycerides, LDL, VLDL- cholesterol and low levels of HDL- cholesterol may be due to obesity, increased caloric intake and lack of muscular exercise in diabetes mellitus [24, 25]. In our study, we evaluated oxidative stress by estimating MDA and lipid profile among type 2 diabetic patients. However, our study involved in small sample size due to limited period and therefore the results inferred may not be considered as the reflection of larger population. Regular evaluation of MDA along with lipid profile is must in all type 2 diabetic cases to prevent the development of complications in type 2 diabetes mellitus.

V. Conclusion

The results of our present study concludes that poor glycemic control in diabetic subjects is the predominant cause for oxidative stress, leading to free radical- mediated lipid peroxidation, which possibly contribute to the development of diabetes and its complications.

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Conflict of interest: None to declare.
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Reference