

## EVALUATION OF DYSLIPIDEMIA AND OXIDATIVE STRESS IN TYPE II DIABETES PATIENTS: A CASE CONTROL STUDY

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### Abstract

**Background:** Oxidative stress is induced in type II Diabetes Mellitus which is a type of metabolic disorder that causes hyperglycaemia and alteration in carbohydrate, protein and fat metabolism. Type II Diabetes Mellitus (DM) is the most common endocrine disease prevailing in the present world. Elevated level of oxidant appears to be a favourable factor leading to impaired glucose tolerance, insulin resistance, dyslipidemia and ultimately leading to T2DM, hyperglycemia and dyslipidemia and this appears to be one of the trigger for the development of CVD. **Materials and Methods:** This observational, case-control study was conducted in the Department of Biochemistry, Central Research lab and Central clinical Lab of LNCT University Bhopal. A total of 200 subjects (Cases-100 and 100 were control) between the age group of 20-60 yrs were enrolled in the study. Blood sample (5 mL) was collected from each subject & Serum was separated by centrifuging blood at 2000 rpm for 15 min. HbA1c was measured by kit based method, Estimation of blood glucose was done by GOD POD method, Lipid profile and other routine investigations were done by auto analyser. assay of lipid peroxidation i.e amount of malondialdehyde (MDA) formed was calculated, Superoxide dismutase (SOD) activity was assayed and calculated. All of the statistical analysis was done by using the Windows based Statistical Package for Social Sciences (SPSS) version 27.0. Student's t-test and Pearson correlation analysis was applied and p-values <0.05 were considered as significant. **Result:** Significant changes have been observed in fasting blood sugar, Glycated Haemoglobin (HbA1c) and total cholesterol (TC) along with triglycerides. Low-density lipoprotein cholesterol (LDL-C) was elevated in case group, along with very low-density lipoprotein cholesterol (VLDL-C), MDA and SOD. **Conclusion:** Increase level of blood glucose leads to the generation of free radicals and decreased levels of antioxidants. Therefore for the prevention of vascular complications problems in Type II Diabetes subjects, early detection of dyslipidemia and oxidative stress may decrease the oxidative stress induced complications of type II diabetes mellitus.

## INTRODUCTION

Diabetes mellitus is a congregation of metabolic syndrome that is described by raised degrees of glucose in blood (hyperglycemia) and deficiency in progress or activity of insulin created by the pancreas inside the body.<sup>[1]</sup> Insulin is a protein orchestrated in beta cells of pancreas because of different improvements like glucose, sulphonylureas, and arginine besides glucose is the significant determinant. An increase blood glucose level is related with large scale and small vascular difficulties prompting heart diseases, stroke, visual impairment and kidney disease.<sup>[2]</sup> Sidewise to hyperglycemia,

there are a few different elements that assume extraordinary part in pathogenesis of diabetes, for example, hyperlipidemia and oxidative stress prompting high risk of diabetes.<sup>[3]</sup>

Type-2 Diabetes (T2DM) is the most widespread and clinically vital metabolic disease which has end up a global pandemic in recent years and a main healthcare burden globally. In 2013, there were an expected 382 million patients with diabetes around the world. Concern has been arise as T2DM occurrence continues to amplify, and it is projected that there may be greater than 590 million patients recognized with this disorder till 2035.<sup>[4]</sup>

Evidence demonstrates that adult males are at higher chance of T2DM than females, which is at the least in part propose to be due to differential adiposity garage styles in adults.<sup>[5]</sup> Studies have proven that adults with T2DM are much more likely to develop CVD; however females with T2DM who do develop CVD are much more likely to have a worse diagnosis, which is concept to be at least partially due to males being more likely to attain medical goals in T2DM.<sup>[5]</sup> Glycated hemoglobin (HbA1c) is a commonly used predictor for sugar control in the long-term. In conjunction with its role as a predictor for the mean blood glucose level, HbA1c showed the risk for the occurrence of diabetic complications in diabetes patients.<sup>[6,7]</sup> Apart from traditional risk factors like dyslipidemia, exceedingly high HbA1c has now been regarded as an autonomous threat for vascular disease in cardiac patients.<sup>[8]</sup>

Diabetes Mellitus patient experiences dyslipidaemia which is a significant clinical anomaly, featured by group of metabolically consistent plasma lipid and lipoprotein irregularity that involves high-density lipoprotein cholesterol (HDL-C) and low-density lipoprotein cholesterol (LDL-C), triglyceride (TG), and total cholesterol (TC) levels.<sup>[9,10]</sup> Abnormal lipoprotein patterns are absorbed separately or in grouping. Dyslipidemia has been recognized as the variable risk factor for cardiovascular disorders, which is a common basis of illness and death rates in maximum evolving nations.<sup>[11]</sup>

Lipid profile as well as diabetes has been demonstrated to be significant interpreters for metabolic disorders like dyslipidaemia, hypertension, and cardiovascular diseases.<sup>[12]</sup> Lipids have a significant role in the pathogenesis of diabetes mellitus. Various features may disturb blood lipid levels due to correlation between carbohydrates and lipid metabolism. Thus, any disturbance in the metabolism of carbohydrates leads to disorder in metabolism of lipid and vice versa. In the majority of patients with T2DM, Insulin resistance is a primary defect. In non-diabetic people resistance of insulin in amalgamation with hyper-insulinemia has a robust extrapolative value for the development of future for type 2 diabetes.<sup>[13]</sup>

Through the production of reactive oxygen species (ROS), oxidative stress (OS), has been anticipated as the primary cause for the essential progress of insulin resistance, beta-cell dysfunction, reduced glucose tolerance, T2DM and HPTN, it has also been concerned in the development of enduring complications of diabetes.<sup>[14]</sup> Additional nutrition and a sedentary existence led to glucose and fatty acid burden, results in production of ROS. Moreover, plasma proteins with reaction of glucose forms advanced glycation end products (AGEs), activating reactive oxygen species (ROS) production.

Oxidative stress yielded by free radicals is one of the possible causes of the degenerative diseases. Biochemical changes and damages in various components of living cells, such as proteins, lipids, carbohydrates and nucleic acids are caused by free

radicals. Lipid peroxidation reaction is caused by free radical attacks against lipid constituent in the cell membrane, which produce toxic elements to the cells like malondialdehyde (MDA).<sup>[15]</sup>

Several studies have identified high oxidative stress and antioxidant defense in people who metabolize glucose differently.<sup>[16,17]</sup>

We also investigated the relationship between fasting blood glucose (FBG), glycated hemoglobin, serum lipid profile, and MDA in people with Type II diabetes in our study.

## MATERIALS AND METHODS

It is a Case Control study conducted in the Department of Biochemistry, Central Research lab and Central clinical Lab of LNCT university Bhopal.

### Selection of Cases

Type 2 DM Patient will be selected who attending OPD, of LNCT Medical & Jk Hospital, and willing to participate in the study on the basis of inclusion or exclusion criteria.

As per WHO norms: Case having fasting blood sugar ( $\geq 126$ mg/dl) and 2 hour post-prandial blood sugar ( $\geq 200$ mg/dl).

### Inclusion Criteria

#### CASE

- Patients of age between 20-60
- Patient having sign and symptoms of T2DM
- Subject or subject's representative has signed the consent form.

### Exclusion Criteria

#### CASE

- Subject is suffering from any chronic disease other than T2DM.
- Patients of above the age of 60 years & less than 20 years
- Patients having T1D

#### Control

Healthy normal individuals/ volunteers between the age group of 20-60 years of age.

### Collection Of Sample

Around 5.0 ml blood is collected after 12 hour fasting for lipid profile, blood sugar fasting under aseptic condition.

### Assay for Glucose

Glucose estimated by enzymatic, colorimetric, end point, glucose oxidase-peroxidase method (GOD-POD) in serum samples. The values are reported in mg/dl.<sup>[17]</sup>

### Assay for Lipid Profile

Lipid profile was estimated- Total Cholesterol (TC) Triglyceride (TAG) by enzymatic method and High-Density Lipoprotein (HDL) measured by non HDL precipitation method followed by enzymatic method in serum samples.<sup>[19,20]</sup> The low-density lipoprotein (LDL) cholesterol concentration was calculated by friedwald's formula using (TC, HDL, TAG) (LDL cholesterol=  $TC - HDL - TAG/5$  (mg/dL) by Vitros-250 auto analyser Johnson & Johnson, USA.<sup>[18]</sup>

### Assay for Glycosylated hemoglobin (HbA1c %)

Glycosylated hemoglobin measured by kit-based method where anticoagulated whole blood used as sample. Measuring range= 3-8% HbA1c, measuring interval 0.1% HbA1c, with precision of <5% (coefficient of variation). For subjects without diabetes mellitus, the normal reference range for the HbA1c is between 4% and 5.5%, between 5.6% and 6.5% pre-diabetic. A level of 6.6% or higher means patient is diabetic.

### Assay for Lipid peroxidation

The assay of lipid peroxidation was done according to the method of Wright et al. The reaction mixture consisted of 0.58 ml phosphate buffer (0.1M, pH 7.4), 0.2 ml microsome, 0.2 ml ascorbic acid (100 mM) and 0.02 ml ferric chloride (100 mM) in a total of 1ml. This reaction mixture was then incubated at 37°C in a shaking water bath for 1h. The reaction was stopped by the addition of 1ml of TCA (10%). Following addition of 1.0ml TBA (0.67%), all the tubes were placed in a boiling water bath for a period of 20min. The tubes were shifted to ice bath and then centrifuged at 2500×g for 10 min. The amount of malondialdehyde (MDA) formed in each of the samples was assessed by measuring the optical density of the supernatant at 535nm. The results were expressed as the nmol MDA formed/h/g tissue at 37°C by using a molar extinction coefficient of  $1.56 \times 10^5 \text{ M}^{-1} \text{ cm}^{-1}$ .<sup>[21]</sup>

### Assay for Superoxide dismutase

Superoxide dismutase (SOD) activity was assayed by the method of Stevens et al. The assay mixture consisted of 50 mM, pH 10.4 glycine buffer, 20 mg/ml epinephrine solution, and cytosolic fraction (10% w/v) in a total volume of 1.0 ml. Enzyme activity was recorded at 480 nm and the activity was calculated as uM epinephrine oxidized /min/mg protein.<sup>[22]</sup>

## RESULTS

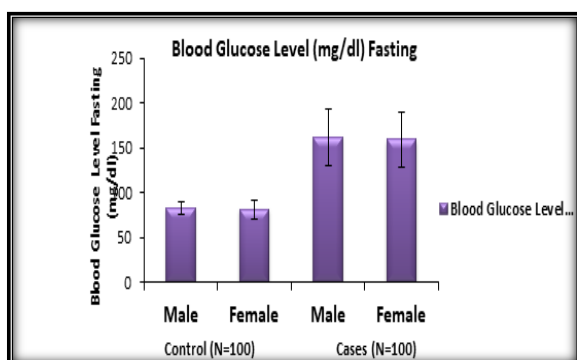


Figure 1: Blood Glucose Level (mg/dl) Fasting control (Group I) & Type-2 Diabetes Mellitus cases (Group II)

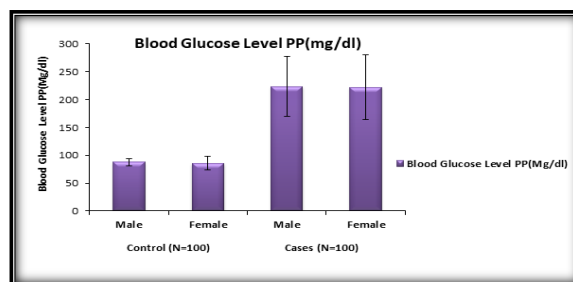


Figure 2: Blood Glucose Level (mg/dl) pp control (Group I) & Type-2 Diabetes Mellitus cases (Group II)

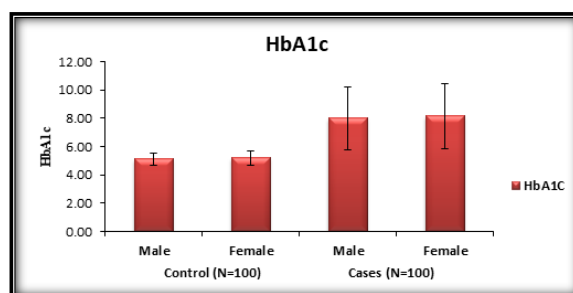


Figure 3: HbA1c control (Group I) & Type-2 Diabetes cases (Group II)

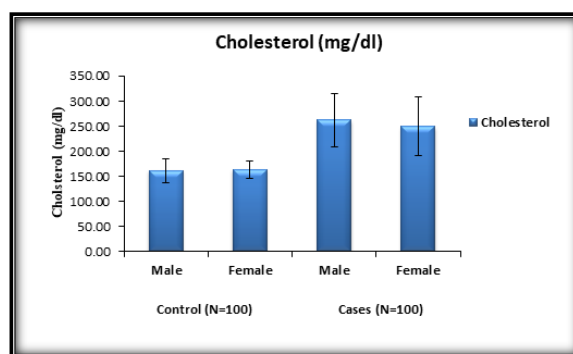


Figure 4: Cholesterol control (Group I) & Type-2 Diabetes Mellitus cases (Group II)

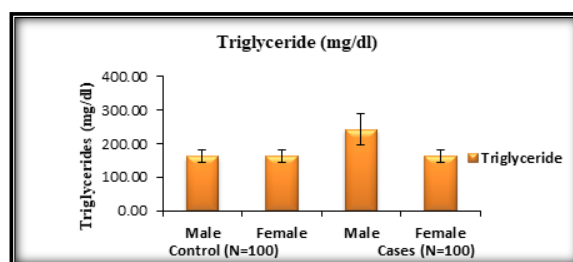


Figure 5: Triglycerides mg/dl control (Group I) & Type-2 Diabetes Mellitus cases (Group II)

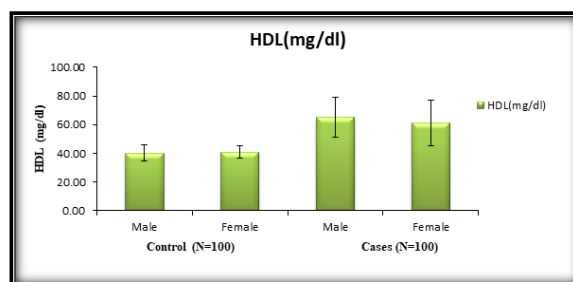
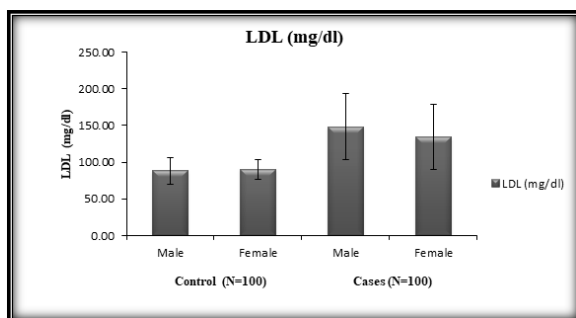
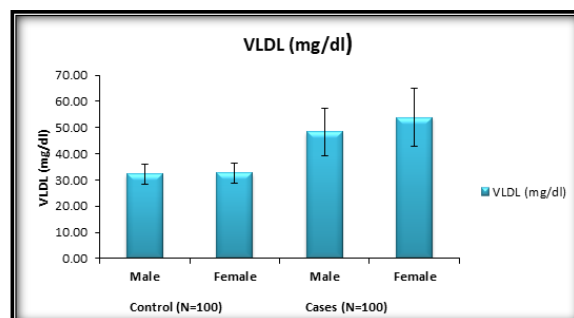


Figure 6: HDL (mg/dl) control (Group I) & Type-2 Diabetes cases (Group II)



**Figure 7: LDL (mg/dl) control (Group I) & Type-2 Diabetes Mellitus cases (Group II)**



**Figure 8: VLDL (mg/dl) control (Group I) & Type-2 Diabetes Mellitus cases (Group II)**

**Table 1: SOD (Protein/mg/min) control (Group I) & Type-2 Diabetes Mellitus cases (Group II).**

S.No.	Parameters	Group I Control		Group II Cases		t- Test
1.	SOD(Protein/mg/min)	M	0.81±0.16	M	3.02±0.73	19.57
		F	0.794±0.197	F	2.77±0.57	24.18
	Total (N)	Males & Females	100	Males & Females	100	

P Value < 0.001 recommended significant

SOD (Protein/mg/min) expressed mean ±SD

Level of SOD values mean ±SD in control & cases groups are shown in the table 13. Mean± SD Results of SOD in male cases 3.41±0.55 is high as compare to control group 2.09±0.72, the level of SOD in female cases are 3.165±0.624 as compared to control group 2.183±0.692 respectively. [Table 1] showed vital increment in the values of group II when compared to group I

**Table 2: MDA (nmol/mg) control (Group I) & Type-2 Diabetes Mellitus cases (Group II).**

S.No.	Parameters	Group I Control		Group II Cases		t- Test
1.	MDA(nmol/mg)	M	1.37±0.43	M	3.41±0.55	18.617
		F	2.183±0.692	F	3.165±0.624	6.776
	Total (N)	Males & Females	100	Males & Females	100	

P Value < 0.001 recommended significant

MDA (nmol/mg) expressed mean ±SD

Level of MDA values mean ±SD in control & cases groups are shown in the table 12. Mean± SD Results of MDA in male cases 3.41±0.55 is high as compare to control group 2.09±0.72, the level of MDA in female cases are 3.165±0.624 as compared to control group 2.183±0.692 respectively. Table 12 showed vital increment in the values of group II when compared to group I.

## DISCUSSION

Diabetes is a major health problem around the world. It produces serious health related and economic effect on population. Furthermore, the pandemic increase of diabetes is urged on by changing division like population maturing, financial, and nutritious and way of life style as well as a growth in obese adults and children.<sup>[23,24]</sup> Diabetes is a typical endocrine metabolic problem. Because of reduced insulin release or aversion or both, it is described by raised glucose levels from a different association of natural and epigenetic factors.<sup>[25]</sup> Hyperglycemia in DM is caused by both overproduction and underutilization of glucose. Moreover there is a general overabundance of glucagon levels. As an outcome, glucose formation increased instead of its utilization by the liver and furthermore there is an intense decrease of take-up of glucose into muscle and fat tissue, at long last adding to hyperglycemia.<sup>[26]</sup> As the measure of advancement of HbA1c is immediately connected to the concentration of sugar, glycated hemoglobin level reflects the integrated glucose levels over the intervening 6–8 weeks. The

association is notable among dyslipidemia and DM. We observed that the levels of Blood Glucose (mg/dl) Fasting in male cases 161.90±31.47mg/dl which is high as compare to control group 83.20±6.84. The levels of Blood Glucose (mg/dl) Fasting in female cases are 159.25±30.46 mg/dl as compared to control group 81.44±10.34 respectively in [Figure 1] showed vital increment in the values of group II when compared to group I. We observed that the level of Blood Glucose (mg/dl) PP in male cases 223.29±53.77 mg/dl which is high as compare to control group 87.84±6.48. The levels of Blood Glucose (mg/dl) PP in female cases are 222.07±58.29 mg/dl as compared to control group 85.8±11.94 mg/dl respectively in [Figure 2] showed vital increment in the values of group II when compared to group I. The values of HbA1c mean ±SD in control & cases groups are shown in the fig.3. The value of HbA1c in male cases 8.0±2.24 which is high as compare to control group 5.13±0.44. The value of HbA1c in female cases is 8.15±2.30 as compared to control group 5.19±0.48 respectively in fig.3 showed vital increment in the values of group II when compared to group I. The level of Cholesterol in



control & cases groups is shown in fig.4. The level of Cholesterol in male cases  $261.83 \pm 53.54$  which is high as compare to control group  $161.25 \pm 23.48$ , the level of Cholesterol in female cases are  $249.38 \pm 58.20$  as compared to control group  $163.80 \pm 16.72$  respectively in [Figure 4] showed vital increment in the values of group II when compared to group I. The level of Triglycerides values mean  $\pm$ SD in control & cases groups are shown in [Figure 5]. Mean  $\pm$  SD Results of Triglycerides in male cases  $261.83 \pm 53.54$  which is high as compare to control group  $161.25 \pm 23.48$ , the level of Triglycerides in female cases are  $249.38 \pm 58.20$  as compared to control group  $163.80 \pm 16.72$  respectively in [Figure 5] showed vital increment in the values of group II when compared to group I. The Level of HDL in control & cases groups is shown in [Figure 6]. The level of HDL in male cases  $40.28 \pm 5.87$  which is high as compare to control group  $161.25 \pm 23.48$ , the level of HDL in female cases are  $61.31 \pm 15.91$  as compared to control group  $40.96 \pm 4.18$  respectively in [Figure 6] showed vital increment in the values of group II when compared to group I. The level of LDL in control & cases groups is shown in [Figure 7]. The level of LDL in male cases  $148.48 \pm 44.64$  which is high as compare to control group  $88.63 \pm 18.10$ , the level of LDL in female cases are  $134.21 \pm 44.48$  as compared to control group  $90.17 \pm 13.00$  respectively in [Figure 7] showed vital increment in the values of group II when compared to group I. The level of VLDL in control & cases groups is shown in [Figure 8]. The level of VLDL in male cases  $48.30 \pm 9.16$  which is high as compare to control group  $32.22 \pm 3.79$ , the level of VLDL in female cases are  $53.89 \pm 11.16$  as compared to control group  $32.70 \pm 3.84$  respectively in [Figure 8] showed vital increment in the values of group II when compared to group I.

Lipid peroxide is formed from the process of lipid peroxidation because of the attack by free radicals. Biological membranes and lipoproteins are sensitive to lipid peroxide. It will decrease the stability of the cellular membrane, inducing oxidation of the thiol organizations by the enzyme inside the membrane and freeing product breakdown (along with malondialdehyde [MDA]) that results in cell injury. LDL oxidation by free radicals leads to tissue harm in blood vessels.<sup>[27]</sup> We observed that the level of MDA [Table 1] in male cases  $3.41 \pm 0.55$  is high as compare to control group  $2.09 \pm 0.72$ , the level of MDA in female cases are  $3.165 \pm 0.624$  as compared to control group  $2.183 \pm 0.692$  respectively. [Table 1] showed vital increment in the values of group II when compared to group I.

SOD is one of the antioxidant enzymes which catalyze dismutase reactions or disproportionation of superoxide into molecular oxygen and peroxide. Peroxide then undergoes catalytic response into water molecules by means of catalase and peroxidase. SOD plays an important function in shielding cells from toxic merchandise resulted from the system of cardio metabolism, and oxidative phosphorylation.<sup>[28]</sup> Plasma LDL, cholesterol, in

particular the oxidized cholesterol, contributes to generate free radicals on the endothelial cells of blood vessel walls. LDL oxidized into OxLDL stimulates the formation of superoxide anion inflicting mobile wall apoptosis within the vascular.<sup>[29]</sup> The present examine analyzed serum pastime of superoxide dismutase (SOD) in DM2 patients. We observed that the level of SOD (table 2) in male cases  $3.41 \pm 0.55$  is high as compare to control group is  $2.09 \pm 0.72$ , the level SOD in female cases are  $3.165 \pm 0.624$  as compared to control group  $2.183 \pm 0.692$  respectively. [Table 2] showed vital increment in the values of group II when compared to group I.

## CONCLUSION

Essential management of type-2 diabetes Mellitus involves routine involvement, such as dietary training and accomplishment of an exercise regime. If a patient fails to continue their limited lifestyle, chronic hyperglycemia directly disrupts equally insulin secretion and sensitivity, an episode of toxicity of Glucose which contributes to the progressive deterioration of hyperglycemia.

Type-2 diabetes has been recognized to be commonly associated with insulin resistance, obesity, hypertension, and lipid abnormalities, and our study showed that DM II is closely associated with hyperglycemia, dyslipidaemia and oxidative stress.

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