VOLUME - 11, ISSUE - 06, JUNE - 2022 • PRINT ISSN No. 2277 - 8160 • DOI : 10.36106/gjra

Original Research Paper

Biochemistry

"CORRELATION OF OXIDATIVE STRESS AND DYSLIPIDEMIA IN TYPE-2 DIABETIC PATIENTS"

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ABSTRACT

Background: Diabetes mellitus (DM) is one of the main chronic metabolic disease of the 21st century. DM is a group of metabolic diseases characterized by hyperglycemia resulting from defects in insulin secretion and insulin action or both. Prediabetes or impaired glucose in fasting is halfway between normal glucose levels and diabetes diagnostic levels. **Aims and objectives:** To correlation of oxidative stress and dyslipidemia in type-2 diabetic patients. **Methods:** The prospective case-control study was conducted at the tertiary teaching in Department of Biochemistry, Index medical college, Indore, M.P. on 400 patients of Type 2 DM attending OPD who were 30-70 years of age. Patients with chronic diseases like Chronic Kidney Disease, COPD, cancer, Metabolic disorders like Hypothyroidism, Infectious diseases like TB, HIV, Hepatitis, Pregnant and breastfeeding women, etc, were excluded from the study. **Results:** In our study, The Pearson Correlation of lipid profile markers with the dyslipidemia profile observed a significant association ($P<0.001$). The Oxidative stress markers like Lipid hydroperoxide (LPO), Malondialdehyde (MDA), Superoxide Dismutase (SOD) and Catalase showed significant correlation with dyslipidemia parameters like Fasting blood sugar, Post prandial blood sugar, HbA1c Level and Duration of diabetes ($P<0.001$). In our study, correlation between dyslipidemia parameters with inflammatory markers was significant ($P<0.001$) **Conclusion:** In our study, These findings concluded that the levels of inflammatory cytokines and oxidative stress were higher in dyslipidemia individuals compared to controls.

KEYWORDS : Diabetes mellitus, oxidative stress marker, dyslipidemia, inflammatory.

INTRODUCTION

DM is a group of metabolic diseases characterized by hyperglycemia resulting from defects in insulin secretion and insulin action or both. Prediabetes or impaired glucose in fasting is midway between normal glucose levels and diabetes diagnostic levels. Hyperglycemia increases the risk of microvascular complications, while dyslipidemia is a major risk factor for macrovascular complications like oxidative stress in patients with type 2 diabetes[1].

Oxidative stress is the equilibrium between the generation and elimination of reactive oxygen species (ROS). In healthy conditions, cellular antioxidant enzymes are responsible for the regulation of ROS productions. Because of the unique molecular structure, lipids are more vulnerable to oxidation. Oxidative stress occurs when the concentrations of ROS exceed those of antioxidant neutralizing species, such as nicotinamide adenine dinucleotide phosphate (NADPH) and glutathione (GSH).

Oxidative stress, secondary to persistent hyperglycemia and dyslipidemia plays a key role in the pathogenesis of T2DM and its complications by excess ROS generation, auto-oxidation of glucose, non-enzymatic protein glycosylation, lipid peroxides formation, impaired glutathione metabolism, impaired activities of antioxidant defense enzymes and decreased concentrations of low molecular weight antioxidants such as ceruloplasmin and uric acid.

Type 2 diabetics often have increased risk of cardiovascular disease (CVD) development. This risk in part is due to dyslipidemia, which is generally characterized by high plasma triglyceride (TG) concentrations, reduced high-density lipoprotein (HDL)-cholesterol (HDL-C), and high or relatively normal low-density lipoprotein (LDL)-cholesterol (LDL-C) concentrations. In addition, oxidative stress plays a significant role in the pathogenesis of atherosclerosis-related conditions including CVD, stroke and diabetes mellitus (DM).

HDL particles from diabetics are known to be compositionally abnormal, most likely due to the fact that they are susceptible to structural modifications mediated by mechanisms which are characteristic of DM including dyslipidemia, glucose homeostasis and redox balance. Several studies have identified high oxidative stress and antioxidant defense in people who metabolize glucose differently.[2] In spite of such an extensive research work and international data available with regards to the relationship between oxidative stress in type 2 diabetic patients in the recent past from our geographical region that emphasizes the significance of such a novel.

So, the present study was aimed to study the hyperglycemia induced oxidative stress and status of dyslipidemia in type 2 diabetic patients.

MATERIALS AND METHODS

The above prospective case-control study was conducted out at a tertiary care teaching hospital, Indore on 400 critically Type 2 DM patients after taking the consent. The study was approved from institutional Ethical committee.

Inclusion Criteria

Indian origin patients of 30-70 years of both gender male and female, diabetic patients diagnosed with dyslipidemia on the basis of WHO norms and patients who have given consent were included in the study.

Exclusion Criteria

Patients who have not given consent, Pregnant and breastfeeding women, patients with chronic diseases like CKD, COPD, cancer etc., patients having infectious diseases like TB, HIV and Hepatitis etc. and patients with metabolic disorders like Hypothyroidism were excluded from the study.

METHODOLOGY

An informed consent of participation in the study was taken by the patients or their significant others where appropriate. A total 400 consecutive subjects of Type 2 DM patients were selected who were attending OPD. The subjects were studied as per the proforma enclosed. A detailed clinical history including age, sex, occupation, socio economic status and any associated risk factors contributing for the illness were elicited from the patients. Samples from diabetic patients were collected and processed in the Department of Biochemistry, Index medical college, Indore. The patients were categorized in two groups: Dyslipidemia Cases (n=259) and Controls (n=141). Diabetes mellitus was defined as HbA1C >6.5 gm% or history of receiving treatment for diabetes mellitus or previously diagnosed diabetes mellitus.

Statistical Analysis

Data was analysed using Statistical Package of Social Sciences (SPSS, version 23.0) software and expressed in form of mean ± standard deviation. Continuous data was analysed using Chi Square Test. Inter group and intra group comparisons were done using Independent Sample T test / Un-paired t test for comparison of mean values. Association between variables was considered statistically significant if p-value was <0.05.

RESULTS

In our study, 63.25% were male and rests were female in studied patients. There was comparable sex distribution in both groups (P>0.05). The majority of the studied patients were in age group 41-60 years. The duration of diabetes was 14.12±5.60 years in cases and 13.44±5.01 years and controls. The blood sugar level (fasting, post-prandial and HbA1c) were significantly higher in the case group in comparison to control group (P<0.001). Lipid profile parameters (Total cholesterol, Triacylglycerol, HDL cholesterol, LDL cholesterol, VLDL cholesterol) were significantly higher in the case group in comparison to control group (P<0.001) (Table No 1).

Oxidative stress markers level (Lipid hydroperoxide (LPO), Malondialdehyde (MDA), Superoxide Dismutase (SOD) and Catalase) were significantly higher in the case group in comparison to control group (P<0.001) (Table No 2). Inflammatory markers level (HsCRP, IL6 and Small dence LDL) were significantly higher in the case group in comparison to control group (P<0.001) (Table No. 3). The Pearson Correlation of lipid profile markers with the dyslipidemia profile observed a significant association (P<0.001) (Table No. 4). In our study, correlation between dyslipidemia parameters with oxidative stress makers was shown and it was observed that Oxidative stress markers like Lipid hydroperoxide (LPO), Malondialdehyde (MDA), Superoxide Dismutase (SOD) and Catalase showed significant correlation with dyslipidemia parameters like Fasting blood sugar, Post prandial blood sugar, HbA1c Level and Duration of diabetes (P<0.001) (Table No. 5).

In our study, correlation between dyslipidemia parameters with inflammatory markers was shown and it was observed that inflammatory markers like HsCRP, IL6 and Small dence LDL showed significant correlation with diabetes parameters like Fasting blood sugar, Post prandial blood sugar, HbA1c Level and Duration of diabetes (P<0.001) (Table No. 6).

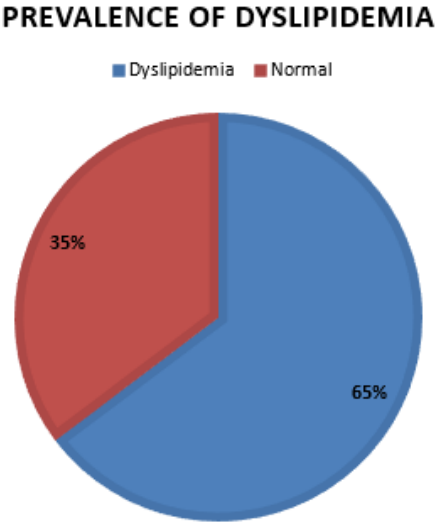

Table No.1: Patients Characteristics

		Dyslipidemia (n=259)	Control (n=141)	p-value
Age	≤40 Years	70 (27.0%)	38 (27.0%)	0.835
	41 – 50 Years	82 (31.7%)	41 (29.1%)	
	51 - 60 Years	85 (32.8%)	52 (36.9%)	
	>60 Years	22 (8.5%)	10 (7.1%)	
Sex	Male	159 (61.4%)	94 (66.7%)	0.329
	Female	100 (38.6%)	47 (33.3)	
Duration of diabetes (years)		14.12±5.60	13.44±5.01	0.340
Blood Sugar (mg/dl)	Fasting			
	Post-Prandial	265.46±89.44	214.89±69.63	<0.001
	HbA1c	9.17±2.45	7.13±2.07	<0.001
Lipid profile parameters	Total cholesterol (mg/dl)	209.57±55.12	146.69±33.99	<0.001
	Triacylglycerol (mg/dl)	194.81±110.86	104.63±40.65	<0.001
	HDL cholesterol (mg/dl)	36.54±13.37	53.08±11.00	<0.001
	LDL cholesterol (mg/dl)	113.65±39.43	103.24±29.82	0.009
	VLDL cholesterol (mg/dl)	40.92±19.08	32.30±15.86	<0.001

Table No. 2: Oxidative Stress Markers

Oxidative stress markers	Group		P value
	Dyslipidemia (n=259)	Control (n=141)	
Lipid hydroperoxide (LPO) (mol/g Hb)	6.06±0.86	4.22±0.85	<0.001
Malondialdehyde (MDA) (nmol/ml)	3.85±0.76	2.64±1.21	<0.001
Superoxide Dismutase (SOD) (U/ml)	203.61±48.02	126.46±37.89	<0.001
Catalase (mmol/min/g Hb)	159.92±30.42	120.08±35.09	<0.001

Table No. 3 Inflammatory Markers

Inflammatory markers	Group		P value
	Dyslipidemia (n=259)	Control (n=141)	

HsCRP [mg/l]	2.05±0.44	1.25±0.33	<0.001
IL6 (pg/mL)	35.17±14.77	23.46±11.37	<0.001
Small dence LDL (mmol/L)	36.28±14.48	23.87±7.36	<0.001

Table No 4: Correlation between Dyslipidemia Parameters with Lipid Profile

Lipid Profile	Total cholesterol	Triacylglycerol	HDL cholesterol	LDL cholesterol	VLDL cholesterol	
Duration of diabetes	**Pearson Correlation**	0.008	0.013	-0.034	0.056	0.093
	Sig. (2-tailed)	0.887	0.825	0.562	0.331	0.106
Fasting blood sugar	**Pearson Correlation**	0.091	0.177**	-0.211**	0.063	0.223**
	Sig. (2-tailed)	0.068	0.001	0.000	0.221	0.000
Postprandial blood sugar	**Pearson Correlation**	0.112*	0.152**	-0.233**	0.041	0.177**
	Sig. (2-tailed)	0.025	0.003	0.000	0.420	0.001
HbA1c_Level	**Pearson Correlation**	0.102*	0.118*	-0.329**	0.055	0.090
	Sig. (2-tailed)	0.041	0.022	0.000	0.283	0.080
**. Correlation is significant at the 0.01 level (2-tailed).						
*. Correlation is significant at the 0.05 level (2-tailed).						

Table No 5: Correlation Between Dyslipidemia Parameters With Oxidative Stress Makers.

	Total cholesterol	Triglycerides	HDL cholesterol	LDL cholesterol	VLDL cholesterol	
LPO	Pearson Correlation	.425**	0.358**	-0.482**	0.107*	0.198**
	Sig. (2-tailed)	0.000	0.000	0.000	0.036	0.000
MDA	Pearson Correlation	0.334**	0.215**	-0.359**	0.047	0.127*
	Sig. (2-tailed)	0.000	0.000	0.000	0.364	0.014
SOD	Pearson Correlation	0.304**	0.259**	-0.369**	0.083	0.180**
	Sig. (2-tailed)	0.000	0.000	0.000	0.108	0.000
Catalase	Pearson Correlation	0.352**	0.158**	-0.327**	0.086	0.144**
	Sig. (2-tailed)	0.000	0.002	0.000	0.093	0.005
**. Correlation is significant at the 0.01 level (2-tailed).						
*. Correlation is significant at the 0.05 level (2-tailed).						

Table No 6: Correlation between Dyslipidemia Parameters with Inflammatory Markers

Inflammatory markers	Total cholesterol	Triacylglycerol	HDL cholesterol	LDL cholesterol	VLDL cholesterol	
HsCRP	Pearson Correlation	.246**	.269**	-.470**	-.039	.161**
	Sig. (2-tailed)	.000	.000	.000	.443	.002
IL6	Pearson Correlation	.255**	.088	-.195**	.048	-.052
	Sig. (2-tailed)	.000	.085	.000	.350	.309
Small dence LDL	Pearson Correlation	.210**	.456**	-.323**	-.014	.538**
	Sig. (2-tailed)	.000	.000	.000	.784	.000
**. Correlation is significant at the 0.01 level (2-tailed).						
*. Correlation is significant at the 0.05 level (2-tailed).						

DISCUSSION

Diabetes mellitus (DM) is one of the main chronic health conditions of the 21st century. Between the worldwide populations, 425 million people and 352.1 million people are estimated to have diabetes and impaired tolerance test for glucose, respectively. Pre-diabetes or impaired glucose in fasting is midway between normal glucose levels and diabetes diagnostic levels.

However, plenty of the populations could also bear unnoticed hyperlipidemia, marked by higher triglycerides (TG) levels, along with low density lipoprotein cholesterol (LDL-C) against lower high-density lipoprotein cholesterol (HDL-C). Dyslipidemia is often coupled with diabetes, and is really the primary cause for atherosclerosis. It is interpreted as a lipid triad, which involves the survival of small but dense LDL, lower HDL, and higher TG.

Apart from classical risk factors like dyslipidemia, exceedingly high HbA1c has now been regarded as an autonomous threat for vascular disease in cardiac patients. Studies in man have also demonstrated increased MDA with progressive hyperlipidemia.

The present study aimed to investigate the status of oxidative stress markers and inflammatory markers associated to diabetes mellitus type-2 and also study the association of oxidative stress in case of dyslipidemia, and established any correlation between inflammatory markers and cases. Siddiqui A et al conducted a study to investigate the association of oxidative stress and inflammatory markers with chronic stress and newly diagnosed type 2 diabetes and concluded that the chronic psychological stress and stress responses are associated significantly with inflammation and oxidative stress, which could act as mediating stress related risk factors for type 2 diabetes. Malik A et al conducted an observational analytical study of the Oxidative stress and inflammatory markers in type 2 diabetic patients and concluded the small intestinal bacterial over- growth (SIBO) in T2DM patients can cause oxidative stress and inflammation. Therefore, SIBO should be taken care to prevent further damage to intestine. Monnier L et al did a study of the activation of oxidative stress by acute glucose fluctuations compared with sustained chronic hyperglycemia in patients with type 2 diabetes and concluded the glucose fluctuations during postprandial periods and, more generally, during glucose swings exhibited a more specific triggering effect on oxidative stress than chronic sustained hyperglycemia., Yadav MK et al established an observational, case-control study of the evaluation of dyslipidemia and oxidative stress in type II diabetes patients and concluded that the increased blood glucose levels lead to the generation of oxygen free radicals and decreased levels of antioxidants, which causes erythrocyte fragility in type 2 diabetes.

In present study noted the 63.25% male and rest were female in studied patients. There was comparable sex distribution in both groups (P>0.05) (Table No. 1). Siddiqui A et al15 reported the both NDDM and NGT groups had similar gender distribution. Malik A et al16 reported the 52.0% were female patients and insignificant distribution in both groups. Monnier L et al17 reported the insignificant distribution of sex in both groups.

We noted the duration of diabetes was 14.12±5.60 years in

cases. We observed the blood sugar level (fasting, post-prandial and HbA1c) were significantly higher in the case group in comparison to control group (P<0.001). Yadav MK et all 8 reported that in Type II Diabetes subjects, the mean value of fasting serum glucose was higher compared to non-diabetic subjects. It was observed that the rise was highly noteworthy (P < 0.001), which is consistent with Amanullah et al, Mahajan et al and Meshram et al. Hyperglycemia in DM is caused by both overproduction and underutilization of glucose.

In our study the mean HbA1c in Dyslipidemia and normal individuals were 9.17±2.45 and 7.13±2.07 respectively. Yadav MK et all 8 reported the mean of HbA1c in diabetic type 2 and normal individuals were in the range 7.46±1.24 and 4.65±0.40%, respectively. Valued against Controls, the mean value of HbA1c was stronger in Diabetics. The increase was statistically highly significant (0.01). This is in accordance with Shetty et al, Sathiyapriya et al, Ren Y et al, Dalan et al and Singer et al. As the tempo of development of HbA1c is straightaway linked to the concentration of sugar, glycated hemoglobin level reflects the integrated glucose levels over the intervening 6–8 weeks.

Limitations of study

Our study has few limitations. Our study was single center and limited time duration so relatively small sample size. It was a case control study, and therefore we cannot say with certainty that it is the stress‐related oxidative stress pathway that has resulted in diabetes mellitus and not vice versa.

CONCLUSION

In conclusion, it could be concluded that the levels of inflammatory cytokines and oxidative stress were higher in dyslipidemia individuals compared to controls. These findings demonstrate a significant relationship between acute glucose swings and activation of oxidative stress.

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