

Original Research Article**TITLE: ROLE OF OXIDATIVE STRESS MARKER MDA IN TYPE 2 DIABETES MELLITUS ASSOCIATED WITH HYPERLIPIDEMIA****ABSTRACT**

Aim : The aim of the study was to evaluate the role of Oxidative stress(OS) marker Malondialdehyde (MDA) and abnormal lipid levels in the pathogenesis of micro and macrovascular complications of Type 2 DM, thus ,the purpose of the study was to analyse the metabolic deregulation associated with DM which causes secondary pathophysiologic changes in multiple organ systems. This imposes a tremendous burden on the individual with diabetes and on the health care system.

Study Design: Case-control study.

Place and the Duration of the Study: : The present study was conducted in Department of Clinical Biochemistry S.B.K.S. MI.& R.C. Waghodiya, Gujarat between September 2014 and May 2015.

Methodology :60 Healthy controls and 60 Type 2 DM subjects (with & without complications) were included in the study. All subjects were age; body mass index and sex matched. Biochemical analytes measured were MDA and Lipid Profile.

Results: Mean values of MDA in type 2 diabetic subjects with complications and without complications were significantly higher ($P < 0.001$) than values observed in healthy controls . Values obtained for the lipids in type 2 diabetic subjects with complications were significantly high ($P \leq 0.001$) compared to type 2 diabetic subjects without complications.

Conclusion: The findings of the study showed a significant correlation between MDA , an OS marker and various lipid parameters in diabetic subjects with complications compared to diabetic subjects without complications. Thus, dyslipidemia accompanied with OS appears to play a critical role in the development and progression of diabetic complications.

Key Words :Lipid Profile, Diabetes Mellitus, Oxidative Stress , MDA

INTRODUCTION

Diabetes Mellitus (DM) comprises a group of common metabolic disorders that share the phenotype of hyperglycemia. Several distinct types of DM exist and are caused by a complex interaction of genetic, environmental factors and life-style choices. Depending on the etiology of the DM, factors contributing to hyperglycemia may include reduced insulin secretion, decreased glucose utilization and increased glucose production. The metabolic deregulation associated with DM causes secondary pathophysiologic changes in multiple organ systems that impose a tremendous burden on the individual with diabetes and on the health care system.^[1] DM has emerged as a major health care problem in India. According to the Diabetes Atlas published by the International Diabetes Federation (IDF), there were an estimated 40 million persons with DM in India in 2007 and this number is predicted to rise to almost 70 million people by 2025 by which time every fifth diabetic subject in the world would be an Indian .^[2]

Type 2 DM is a combination of resistance to insulin action and an inadequate compensatory insulin secretory response. This form of DM, accounts for approximately 90-95% of those with DM.^[3]

In type 2 DM Increased hepatic glucose production occurs early in the course of DM, though likely after the onset of insulin secretory abnormalities in adipose tissue and obesity. Free fatty acid (FFA) flux from adipocytes is increased, leading to increased lipid [VLDL and triglyceride (TG)] synthesis in hepatocytes. This is responsible for the dyslipidemia found in type 2 DM [elevated TG, reduced high-density lipoprotein (HDL), and increased small dense low density lipoprotein particles (LDL) particles].^[1]

According to Stanislaw et al., good glycemic control (defined as near normoglycemia) as well as effective treatment of high blood pressure and dyslipidemia delay development and progression of microangiopathy.

DM is typically associated with increased generation of free radicals and / or impaired antioxidant defence mechanism, representing a central contribution for reactive oxygen species (ROS) in the onset, progression, and pathological consequences of DM. Increased free radical generation leads to a condition of oxidative stress. Oxidative stress leads to generation of MDA which is formed by both lipid oxidation and as a by-product of prostaglandin and thromboxane synthesis. Oxidation of complex lipids in vivo is largely caused by oxygen derived free radicals such as $\text{OH}^\bullet$.^[4] The major targets of these damaging species are the long chain polyunsaturated fatty acids (PUFAs) of cellular phospholipids, which are particularly prone to attack because of the arrangement of double and single bonds. The resultant lipid peroxide frequently decomposes to a radical.^[5] which reacts with most biological molecules, including proteins and lipids. Further decomposition of these lipid peroxides produces toxic aldehydes, in particular MDA (mainly from arachidonic acid).^[6]

According to DeZwart, ; Mahboob et al, Increased free radical production is said to mediate tissue injury in a wide range of diseases and DM is no exception. Free radicals are formed disproportionately in DM by glucose degradation, which may play an important role in the development of complications in diabetic patients. The generation of free radicals may lead to lipid peroxidation and cause severe damage in DM patients OS is increased in DM owing to an increase in the production of oxygen free radicals, such as superoxide ($\text{O}_2^{\bullet-}$), hydrogen peroxide (H_2O_2) and hydroxide ($\text{OH}^\bullet$), radicals and deficiency of antioxidant defence mechanisms. Increased nonenzymatic and auto-oxidative glycosylation is one of the possible mechanisms that contribute to the formation of free radicals and free radical – induced lipid peroxidation in DM.^[7]

The purpose of the present study was to evaluate the role of oxidative stress and abnormal lipid levels in pathogenesis of various complications in type 2 DM

METHODOLOGY:

The present study was conducted in Department of Clinical Biochemistry Dhiraj General Hospital, S.B.K.S. MI.& R.C. Piparia- Waghodiya, Gujarat. The subjects included in the study were:

- A) 60 Healthy controls –Group 1 selected from volunteers such as doctors, resident doctors, paramedical staff and healthy relatives / attendants of patients.
- B) 60 Type 2 DM patients (30 Type 2 DM without complications – Group 2 & 30 Type 2 DM with complications - Group 3) attending the outpatient clinics or admitted in wards of Department of Medicine Dhiraj General Hospital, S.B.K.S. MI.& R.C. Piparia. The duration of DM in diabetic subjects ranged from 5 to 16 years.

All subjects were age; body mass index and sex matched. Approval was taken from research ethics committee of S.B.K.S medical college, waghodia Vadodara, before starting the study. They were informed and written consent were taken during study.

We excluded the subjects who were habitual smokers, alcoholics, hypertensive and having active inflammatory diseases, nutritional deficiencies, estrogen therapy, malignancy and active immunological diseases. Blood samples were drawn from patients and controls, after overnight fast. Blood was allowed

to clot for 30 minutes at room temperature and then centrifuged at 3000 rotations per minute (rpm) for 10 minutes to obtain clear unhemolysed serum (Haemolysed serum samples were excluded from analysis). Aliquots of serum samples were prepared by transferring them into separate plain vials. These were labeled properly and stored at -20°C until assayed.

Biochemical analytes measured were Fasting plasma glucose by Glucose Oxidase-Peroxidase method, serum total cholesterol by Cholesterol Oxidase-Peroxidase method, triglycerides by Glycerol phosphokinase-Peroxidase method, high density lipoprotein-cholesterol Phosphotungstic Acid, End point method, (very low density lipoprotein cholesterol and low density lipoprotein cholesterol were calculated by Friedwald's equation), Malondialdehyde-Colorimetric method of measurement.^[8]

Statistical Analysis:

Differences in the parameters between the groups were analyzed by means of the student's t test. Variables were presented as mean $\pm$ standard deviation (S.D). Correlations between variables were tested using the Pearson rho (r: correlation coefficient) correlation test. Chi-square (χ^2) analysis was used for comparison of groups. The accepted level of significance for all statistical analyses used in the study was $P = 0.05$. In correlation analysis, $P = 0.02$ (one tailed P value) was also considered.

RESULTS AND DISCUSSION

Elevated plasma MDA and FPG levels were observed in the Type 2 diabetic subjects. Mean values of MDA in type 2 diabetic subjects with complications (5.90 nmol/ml) and without complications (4.87 nmol/ml) were significantly higher ($P < .001$; $P = .003$) than values observed in healthy controls (4.06 nmol/ml) (Table 1).

Ozdemir et al., (2005) observed a significant increase in MDA in patients with type 2 DM compared with control group. One of the study suggested that permanent structural membrane alterations occur in DM, due to increased production of reactive oxygen species and decreased antioxidants in the circulation.^[8]

In our study we observed a significant difference ($p < .001$) in the mean FPG values in Type 2 diabetic subjects with complications [Mean FPG: 185.0 mg/dl] compared to type 2 diabetic subjects without complications [Mean FPG: 136.0 mg/dl]. The findings of our study showed significantly high MDA levels ($P = .002$) in type 2 diabetic subjects with complications compared to type 2 diabetic subjects without complications (Table 1).

The present study findings show significantly high mean values of total cholesterol (TC), triglycerides (TG), high density lipoprotein-C (HDL-C), very low density lipoprotein-C (VLDL-C) and low density lipoprotein-C (LDL-C) respectively ($P = .001$, $P < .0001$, $P = .02$, $P < .0001$, $P = .004$) in type 2 diabetic subjects without complications (Mean values in mg/dl: TC: 199.0; TG: 160.0; HDL-C: 49.1, VLDL-C: 31.9 and LDL-C: 117.0) compared to healthy controls (Mean values in mg/dl: TC: 175.0 ; TG: 95.5; HDL-C: 52.2; VLDL-C: 19.1 and LDL-C: 105.0). Also, significantly high values ($P < .0001$) for various lipid parameters were observed in type 2 diabetic subjects with complications (Mean values in mg/dl: TC: 235.0; TG: 218.0; HDL-C: 42.4; VLDL-C: 43.7 and LDL-C: 148.0) compared to values obtained in healthy controls (Table 1).

124 **Table 1: Mean Values of FPG, MDA and Lipids in Study Subjects**

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S. No	Parameters	Groups Studied		
		(values as Mean $\pm$ SD)		
		Group 1 Healthy Controls	Group 2 Type 2 DM without complications	Group 3 Type 2 DM With complications
1	FPG (mg/dl)	82.2 $\pm$ 13.4	136.0 $\pm$ 29.3	185.0 $\pm$ 47.6
2	MDA (nmol/ml)	4.06 $\pm$ 1.51	4.87 $\pm$ 1.65	5.90 $\pm$ 1.61
3	Total C (mg/dl)	175.0 $\pm$ 24.0	199.0 $\pm$ 22.5	235.0 $\pm$ 36.6
4	Triglyceride (mg/dl)	95.5 $\pm$ 20.7	160.0 $\pm$ 55.3	218.0 $\pm$ 110.0
5	High density lipoprotein-C (mg/dl)	52.2 $\pm$ 7.1	49.1 $\pm$ 8.06	42.4 $\pm$ 8.68
6	Very low density lipoprotein-C(mg/dl)	19.1 $\pm$ 4.1	31.9 $\pm$ 11.0	43.7 $\pm$ 21.9
7	Low density lipoprotein-C(mg/dl)	105.0 $\pm$ 24.1	117.0 $\pm$ 27.4	148.0 $\pm$ 32.7

126 There is much evidence that OS is involved in the etiology of several diabetic complications. During
127 diabetes or insulin resistance, failure of insulin stimulated glucose uptake by fat and muscle causes

glucose concentrations in blood to remain high. Consequently, glucose uptake by insulin independent tissues increases. Increased glucose flux enhances oxidant production.^[9,10,11]

According to the Slatter et al., (2000) in their study stated that MDA is a highly toxic by-product formed by lipid oxidation derived free radicals. MDA reacts both irreversibly and reversibly with proteins and phospholipids with profound effects. In DM the initial modification of collagen by sugar adducts forms a series of glycation products which then stimulates breakdown of the lipids to MDA and hence further cross linking by MDA of the already modified collagen. According to Kume et.al. considerably high values of MDA have been observed in DM with complications.

Values obtained for the lipids that is TC, TG, HDL-C, VLDL-C and LDL-C in type 2 diabetic subjects with complications were significantly high (P <.001) (Table2) compared to type 2 diabetic subjects without complications.

Table 2: Comparison Of Mean Values of MDA & Various Lipid Levels Amongst The Study Subjects

S. No	Groups Compared		Parameters					
			MDA	Total C	TG	HDL-C	VLDL-C	LDL-C
1	Healthy Controls	t	3.01	5.96	10.3	2.36	10.3	2.90
	vs							
	Type 2 DM							
	subjects without complications	P	.003 S	. 001 S	< .0001 HS	. 02 S	< .0001 HS	. 004 S
2	Healthy Controls	t	6.88	12.1	10.9	7.35	10.9	7.50
	vs							
	Type 2 DM							
	subjects with complications	P	< .001 HS	< .0001 HS	< .0001 HS	< .0001 HS	< .0001 HS	< .0001 HS
3	Type 2 DM	t	3.14	5.96	3.38	3.68	3.38	4.32

subjects without
complications

vs	<i>P</i>	.002	.001	.001	.0004	< .0001	< .0001
Type 2 DM		HS	S	S	S	HS	HS

subjects with
complications

HS: Highly Significant S: Significant

- The accepted level of significance for all statistical analyses used in the study was $P = 0.05$.

A positive correlation was observed between TC and MDA ($r=0.36$; $P=0.009$) and also between LDL-C and MDA ($r=0.27$; $P=0.05$) in type 2 diabetic subjects with complications (Table 3). However, significant positive correlation was not observed between TC and MDA ($r = 0.12$, $P = 0.39$) and LDL-C and MDA ($r = 0.03$; $P = 0.81$) in type 2 diabetic subjects without complications. A significant positive correlation was observed between TG and MDA ($r = 0.31$; $P = 0.02$) in type 2 diabetic subjects without complications as well as type 2 diabetic subjects with complications ($r = 0.35$; $P = 0.01$) (Table 3).

In our study a significant association ($P = 0.01$) was observed between MDA and TG in diabetic subjects with complications compared to diabetic subjects without complications ($P = 0.02$) (Table 3).

Table 3: Correlation of Serum MDA Values With Serum Lipids In Various Groups Of Subjects

Study Groups	TC			TG			HDL-C			VLDL-C			LDL-C		
	r	t	P	r	t	P	r	t	P	r	t	P	r	t	P
Group 1	+0.28	2.9	0.002 0.004	+0.10	0.96	0.16 0.33	-0.11	1.08	0.14 0.28	+0.05	0.48	0.31 0.63	+0.008	0.08	0.46 0.93
Group 2	+0.12	0.85	0.19 0.39	+0.31	2.24	0.01 0.02	-0.15	1.08	0.14 0.28	+0.31	2.24	0.01 0.02	+0.03	0.24	0.40 0.81
Group 3	+0.36	2.72	0.004 0.009	+0.35	2.66	0.005 0.01	+0.02	0.17	0.43 0.86	+0.35	2.66	0.005 0.01	+0.27	2.01	0.02 0.05

- r = correlation coefficient

TC: Total Cholesterol TG:Triglyceride HDL-C: High density lipoprotein-Cholesterol

LDL-C: Low density lipoprotein-Cholesterol

According to the study by Soliman (2008) hyperlipidemia is reported as one of the causative factors for increased lipid peroxidation in DM. Kesavulu et al., (2000) observed that the levels of LDL-C and TG were increased in diabetics with microvascular complications compared to those without these complications. Even levels of TBARS were much higher in diabetics with microvascular complications. The hyperglycemia in association with hyperlipidemia observed in diabetic patients could be the causative factor for the increased production of oxygen free radicals and lipid peroxides. The TBARS levels positively correlated with HbA_{1C} and total cholesterol in the study by Kesavulu et al., (2000). Thus, lipid peroxides may play a role in the pathogenesis of microvascular complications of DM.^[12]

Januszewski et al., (2003) proposed a variation on the advanced glycation end products hypothesis i.e. the hyperglycemia exacerbates the chemical modification of proteins by lipids and that lipids and advanced glycation end products, rather than carbohydrates and advanced glycation end products, may be the immediate and major source of chemical modifications leading to tissue damage, pro-inflammatory process and chronic complications in diabetes.^[13]

Thus, while severe hyperlipidemia may be sufficient to induce lipo- oxidative damage, hyperlipidemia compared with hyperglycemia and possibly an increase in OS in diabetes appears to exacerbate the chemical modifications of proteins in diabetes.

Gambhir et al., (2006) also found a significant positive correlation (r = 0.712) between MDA levels and TG in diabetics with complications. According to Gambhir et al., (2006) hypertriglyceridemia may lead to increased production of lipid derived free radicals. The resultant OS may be implicated as a causative factor for endothelial dysfunction, which may be a primary event in the pathogenesis of atherosclerotic vascular disease.^[14]

Chi-square analysis (χ^2) was performed (employing 2 X 2 contingency table) to compare the percentage distribution of study subjects in various risk ranges of lipids. Significant values were obtained for various lipids in diabetic subjects with complications within high risk range (as per NCEP ATP III Guidelines) compared to diabetic subjects without complications (Table 4).

Table 4:Comparative Analysis of Subjects With Lipid Levels As Per NCEP ATP III Criteria
Diabetic without complications

Lipid levels		vs			
		Diabetic with Complications			
Serum Cholesterol (mg/dl)		χ^2	P		Cramer's V
i)	161-199 and 200-239	4.36	0.04	S	0.26
ii)	161-199 and 240-279	12.17	.0005 HS		0.53
iii)	161-199 and $\geq$ 280	15.71	< .0001 HS		0.63
Serum Triglycerides (mg/dl)					
i)	< 150 and 150-199	0.05	0.82		0.05

ii) < 150 and 200-499 5.96 .01 S 0.30

HDL-C (mg/dl)

<40 7.68 .005 S 0.30

< 40 and $\geq$ 60 - - -

LDL-C (mg/dl)

i) < 100 and 100-129 0.83 0.36 0.16

ii) < 100 and 130-159 5.74 .01 S 0.40

iii) < 100 and 160-189 11.15 .0008 HS 0.61

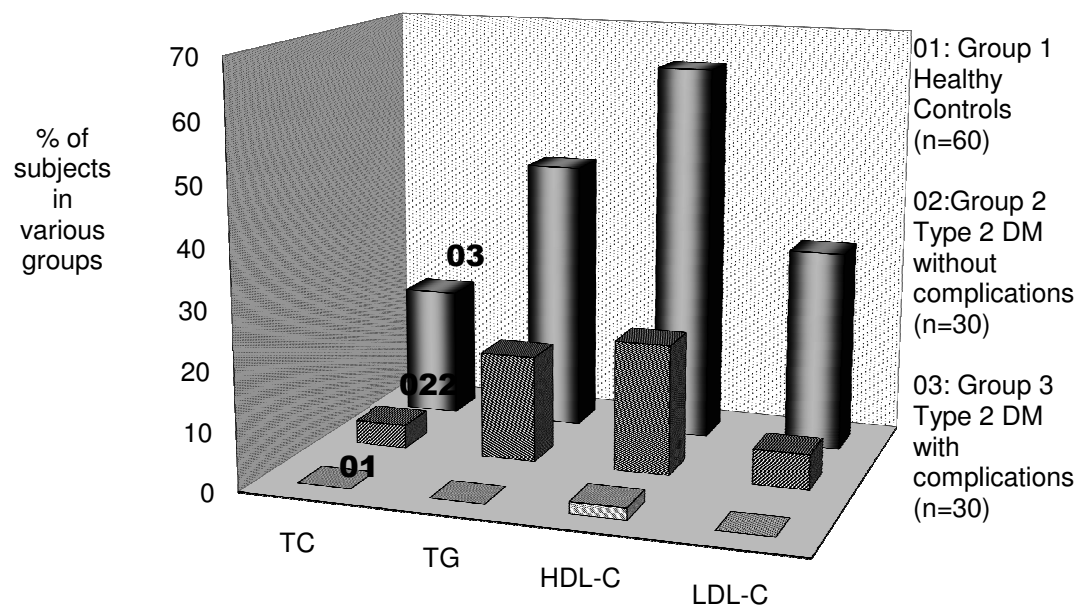
iv) < 100 and $\geq$ 190 4.25 .03 S 0.58

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- 192 • The accepted level of significance for all statistical analyses used in the study was $P= 0.05$.
- 193 • HS : Highly Significant
- 194 • S: significant
- 195

196 In the present study percentage distribution of subjects within various risk ranges of lipids (as per NCEP

197 III guidelines) was evaluated. It was observed that maximum number of subjects in borderline and high

198 risk ranges of lipids were among type 2 diabetics with complications (figure 1).



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Figure 1: Stratification of subjects (% distribution) in high risk ranges of TC: 240-279 mg/dl, TG: 200-499 mg/dl, HDL-C :< 40 mg/dl and LDL-C:160-189 mg/dl

TC: Total Cholesterol TG:Triglyceride HDL-C: High density lipoprotein-Cholesterol
LDL-C: Low density lipoprotein-Cholesterol

The results for lipids observed in the present study are in accordance to observations by Chapman (2007). He stated that type 2 DM is characterized by atherogenic dyslipidemic profile with mild to marked elevation of triglyceride-rich lipoprotein (VLDL and VLDL remnants) concentrations, an increase in small dense LDL and apolipoprotein B (apo B) and low levels of HDL. According to Chapman (2007) dyslipidemia is also characterized by a spectrum of qualitative lipid abnormalities reflecting perturbations in the structure, metabolism and biological activities of both atherogenic lipoproteins containing apo B [VLDL, intermediate density lipoprotein (IDL) and LDL] and anti-atherogenic HDL containing apo A-I and / or apo A-II.

According to Chapman (2007) lipid anomalies are closely associated with increased OS and endothelial dysfunction. Nacitarhan et al., (1995) reported high serum MDA levels in hyperlipidemic diabetic patients compared to normolipidemic diabetics.^[15] Thus, a positive association of MDA with TC, TG, VLDL and HDL-C observed in the present study indicates that hyperlipidemia may contribute to a significant elevation in serum MDA level.

CONCLUSION

The significant correlation of MDA, an OS marker and various lipid parameters observed in diabetic subjects with complications reflect the role of lipids in causing complications. Thus, dyslipidemia appears to play a critical role in the development and progression of diabetic complications.

ETHICAL APPROVAL

All experiments have been examined and approved by the appropriate ethics committee and have therefore been performed in accordance with the ethical standards laid down in the 1964 Declaration of Helsinki.

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