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EFFECT OF OXIDATIVE STRESS ON LIPID PROFILE IN TYPE 2 DIABETES MELLITUS

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Abstract

Background: Diabetes mellitus is a group of metabolic diseases characterized by hyperglycemia resulting from defects in insulin secretion and insulin action or both. The chronic hyperglycemia is associated with long-term damage, dysfunction, and failure of normal functioning of various organs. Hyperglycaemia generates oxidative stress and progressive inflammation due to organ damage. In this study, we have analysed the serum levels of some oxidative and inflammatory markers and Lipid profile in newly diagnosed type 2 diabetes patients.

Methods: Case-control study comprising of aged-sex matched subjects: newly diagnosed T2DM cases (n=30) and controls (n=30). The serum samples of subjects were analysed for levels of CRP by turbidimetry, MDA by Buege and Aust method, while NO levels by Cortas and Wakid's kinetic cadmium reduction method using spectrophotometer. Lipid profile was analysed using agarose gel electrophoresis. Statistical analysis was done using Mini-tab 17 software with 95% confidence interval.

Results: In comparison to healthy controls, serum levels of MDA and NO in T2DM patients were significantly increased. Serum CRP was significantly increased. The lipid profile was significantly affected with decrease in VLDL and Chylomicron, while increase in LDL levels. Levels of HDL and Lipoprotein (a) were unaffected.

Conclusion: There is increased inflammatory and oxidative stress in type 2 diabetes which affects the lipid profile and dysfunction of body organs causing disease progression. Lipid profile and lipoprotein (a) analysis with antioxidant supplements might help control and keep check on disease status.

Keywords: Newly diagnosed Type 2 Diabetes Mellitus, MDA, Nitric Oxide, Lipoprotein (a), Lipid profile

INTRODUCTION:

Diabetes is a group of metabolic disorders which is a major source of morbidity, mortality and economic cost to the society. It is characterized by hyperglycemia and insufficiency in the production or action of insulin. Long term elevation in hyperglycemia is associated with macro- and micro-vascular complications leading to heart diseases, stroke, blindness kidney diseases and various other complications. Along with hyperglycemia, there are several other factors that play role in pathogenesis of diabetes such as inflammation oxidative stress and hyperlipidemia. [1, 2] T2DM is associated with chronic low grade inflammation, possibly through a pathway involving a cytokine-mediated acute-phase response to infection and other inflammatory processes. C-reactive protein (CRP) is an acute-phase reactant produced primarily in the liver hepatocytes under the stimulation of adipocyte-derived pro-inflammatory cytokines, including IL-6 and TNF-α. [3] Novel data further suggest that chronic adipose tissue inflammation and β-cell stress cause an activation of the adaptive immune system, which may also participate in the progression of the inflammatory response. Autoimmune and inflammatory mechanisms during hyperglycemia induced glucotoxicity could favor an increased expression of

several b-cell antigens, thus increasing b-cell apoptosis through autoantibodies. [4]

T2DM is a major risk factor for cardiovascular diseases and acute oxidative stress by high production of reactive oxygen species (ROS) related to the lipotoxicity and glucotoxicity. [1, 2] Increased oxidative stress appears to be a deleterious factor leading to insulin resistance, β-cell dysfunction, impaired glucose tolerance, and, ultimately, T2DM. Chronic oxidative stress is particularly dangerous for β-cells because pancreatic islets are among those tissues that have the lowest levels of antioxidant enzyme expression, and β-cells have high oxidative energy requirements. In addition, there is considerable evidence that increased free radicals impair glucose stimulated insulin secretion, decrease the gene expression of key β-cell genes, and induce cell death. Obesity may play a role in the relationship between systemic oxidative stress and these conditions. [5]

Lipids are reported as one of the primary targets of ROS. Hydroperoxides have toxic effects on cells both directly and through degradation to highly toxic hydroxyl radicals. They may also react with transition metals like iron or copper to form stable aldehydes, such as malondialdehyde (MDA), that damage cell membranes. MDA has been documented as a primary biomarker of free radical mediated lipid damage and oxidative stress. [1] MDA reacts both irreversibly and reversibly with proteins and phospholipids with profound effects. In particular, the collagen of the cardiovascular system is not only stiffened by cross – links, mediated by MDA but then becomes increasingly resistant to remodelling. It is significant in T2DM because the initial modification of collagen by sugar adducts forms a series of glycation products which then stimulate breakdown of lipids to MDA and hence further cross- linking by MDA of the further stiffening of modified collagen. [6]

Nitric oxide is a gaseous molecule secreted by the endothelium and a major modulator of endothelial function. [7] It is a key regulatory molecule with extensive metabolic, vascular, and cellular effects. The regulation of NO metabolism is particularly important in type 2 diabetes, because activation of NO synthase (NOS) is under insulin control through the Akt pathway. Thus, disturbances of NO generation may be a consequence of insulin resistance affecting also the vascular response. An impaired NO metabolism is found in type 2 diabetes, particular in the presence of nephropathy. [8]

Lipoprotein (a) [Lp (a)] is an LDL-like particle that contains an apolipoprotein B100 molecule covalently bound to a plasminogen-like glycoprotein, apolipoprotein (a) [apo(a)]. Epidemiological evidence supports a direct and causal association between Lp(a) levels and coronary risk. On the contrary, a few prospective findings demonstrate inverse association of Lp(a) levels with risk of type 2 diabetes (T2DM). [9] The aim of our study was to analyse serum of newly diagnosed type 2 diabetic patients and evaluate the association of lipid profile with indicators of oxidative stress and inflammation which are linked to the development of T2DM.

MATERIAL AND METHODS

Randomized case control study was undertaken in Department of Biochemistry, Grant medical college and Sir J. J. groups of Hospitals, Mumbai. The subjects recruited in study groups were 30 healthy controls and 30 cases of newly diagnosed type 2 diabetes. Subjects of both the sex in age group of 30 to 60 years and willing to participate in the study were recruited. Subjects with HIV/AIDS infected, diagnosed for malignancies, neurological or psychiatric disorders and tuberculosis were excluded from the study. Informed consent was taken from subjects. Ethical approval was taken from Institutional Ethics Committee of Sir J. J. Group of Hospitals & GGMC, Mumbai.

Serum Analysis: Blood samples of the subjects were collected in plain vacutainers and serum was separated for analysis of CRP, MDA, NO, HDL, VLDL, Lipoprotein (a), LDL and Chylomicron. The serum levels of CRP was analysed by turbidimetry, MDA by Buege and Aust method, while that of NO was analysed by Cortas and Wakid's kinetic cadmium reduction method. The results were read colorimetrically on Spectrophotometer at 530nm. The lipid profile (HDL, VLDL, Lipoprotein (a), LDL and Chylomicron) was estimated by agarose gel electrophoresis using Sebia Hydragel K20 test kit.

Statistical analysis: Statistical analysis (Mean and Standard Deviation) was done using Mini-tab 17 software with 95% confidence interval

RESULTS:

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Table 1: Age and Sex wise distribution of recruited subjects

Groups	Males (n=15)	Females (n=15)	Total (n=30)
Control (Mean ± SD)	51.0 ± 4.23	48.8 ± 3.57	49.9 ± 4.0
T2DM (Mean ± SD)	52.86 ± 7.88	45.93 ± 10.15	49.4 ± 9.6

Table 2: Biochemical parameters in control and T2DM

Groups (n = 30)	CRP (mg/l)	MDA (nmol/ml)	NO (µmol/l)	HDL (mg/dl)	Lipo (a) (mg/dl)	VLDL (mg/dl)	LDL (mg/dl)	Chyl (mg/dl)
Control (Mean ± SD)	2.15 ± 0.70	1.46 ± 0.35	32.09 ± 4.10	22.97 ± 1.40	2.87 ± 1.4	35.79 ± 16.44	28.64 ± 10.38	9.76 ± 6.21
T2DM (Mean ± SD)	5.95 ± 1.46	3.19 ± 0.54	70.45 ± 116.87	22.88 ± 6.15	2.86 ± 1.75	20.29 ± 12.49	47.97 ± 11.97	5.11 ± 4.32

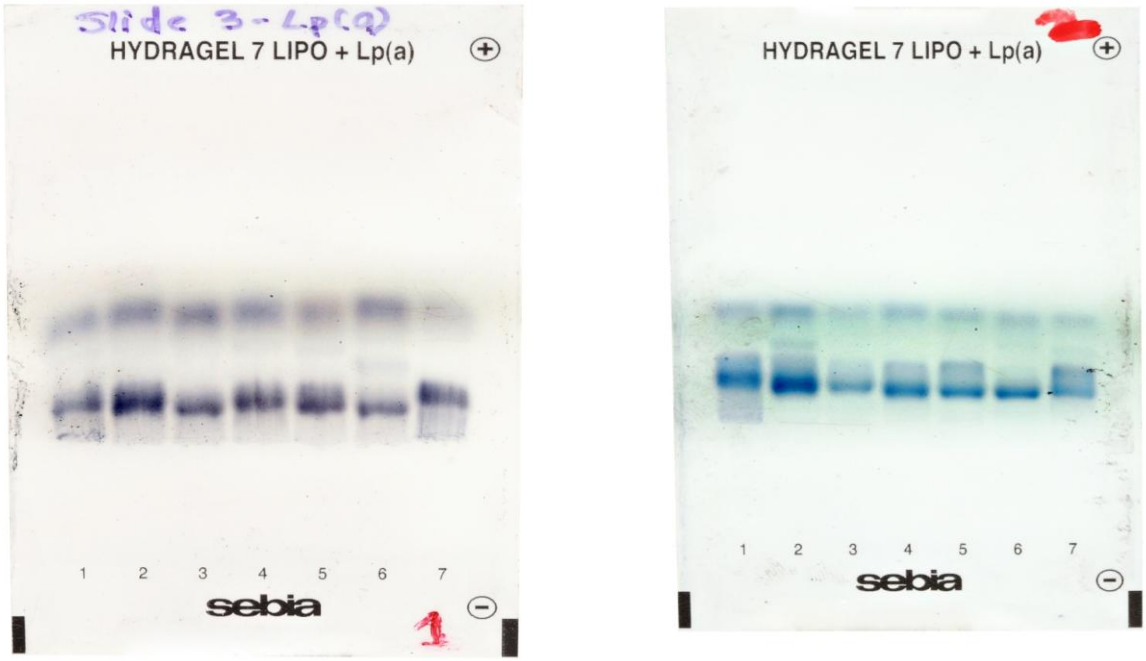

Figure 1: Electrophoretic analysis of serum Lipo (a) in T2DM patients.

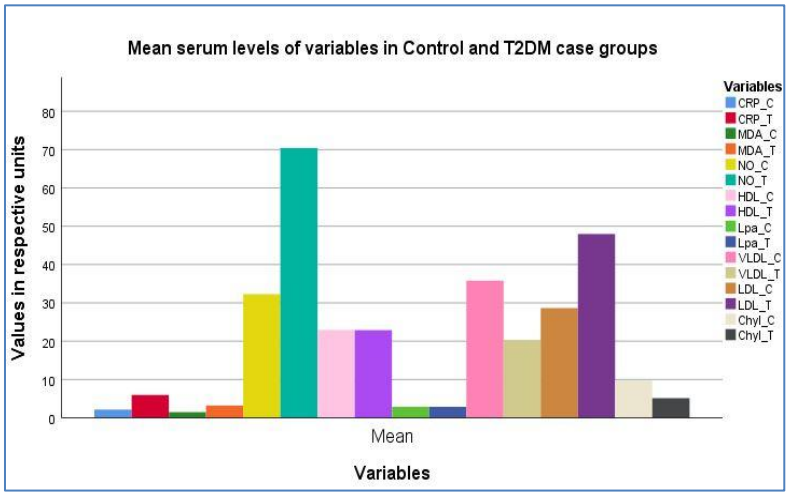

Figure 2: Comparison of mean serum levels of variables in Control and T2DM case groups

Table 3A: Correlation of CRP and MDA in Control group with CRP, MDA, NO, HDL, Lipo (a), VLDL, LDL and Chyl in T2DM group.

Control / T2DM	r	P	Control / T2DM	r	P
CRP / CRP	-0.068	0.721	MDA / CRP	0.055	0.773
CRP / MDA	-0.339	0.067	MDA / MDA	-0.113	0.554
CRP / NO	0.208	0.269	MDA / NO	0.279	0.136
CRP / HDL	0.011	0.955	MDA / HDL	0.019	0.921
CRP/ Lipo(a)	0.089	0.639	MDA / Lipo(a)	0.126	0.505
CRP / VLDL	-0.201	0.288	MDA / VLDL	0.165	0.383
CRP / LDL	0.233	0.215	MDA / LDL	-0.105	0.581
CRP / Chyl	0.103	0.588	MDA / Chyl	0.090	0.635

Table 3B: Correlation of NO and HDL in Control group with CRP, MDA, NO, HDL, Lipo(a), VLDL, LDL and Chyl in T2DM group.

Control / T2DM	r	P	Control / T2DM	r	P
NO / CRP	-0.075	0.693	HDL / CRP	-0.046	0.808
NO / MDA	0.044	0.818	HDL / MDA	-0.163	0.390
NO / NO	0.017	0.928	HDL / NO	-0.042	0.825
NO / HDL	-0.013	0.946	HDL / HDL	0.110	0.563
NO / Lipo(a)	-0.310	0.096	HDL / Lipo(a)	-0.138	0.469
NO / VLDL	-0.040	0.835	HDL / VLDL	0.123	0.516
NO / LDL	0.279	0.136	HDL / LDL	-0.121	0.524
NO / Chyl	0.156	0.409	HDL / Chyl	-0.176	0.353

Table 3C: Correlation of Lipo(a) and VLDL in Control group with CRP, MDA, NO, HDL, Lipo(a), VLDL, LDL and Chyl in T2DM groups.

Control / T2DM	r	P	Control / T2DM	r	P
Lipo(a) / CRP	0.184	0.330	VLDL / CRP	0.054	0.778
Lipo(a) / MDA	0.080	0.675	VLDL / MDA	0.298	0.110
Lipo(a) / NO	0.065	0.732	VLDL / NO	-0.161	0.395
Lipo(a) / HDL	-0.121	0.523	VLDL / HDL	-0.090	0.636
Lipo(a) / Lipo(a)	-0.116	0.541	VLDL / Lipo(a)	0.283	0.129
Lipo(a) / VLDL	-0.220	0.244	VLDL / VLDL	-0.272	0.145
Lipo(a) / LDL	0.182	0.337	VLDL / LDL	0.094	0.621
Lipo(a) / Chyl	0.282	0.132	VLDL / Chyl	0.166	0.380

Table 3D: Correlation of LDL and Chyl in Control group with CRP, MDA, NO, HDL, Lipo(a), VLDL, LDL and Chyl in T2DM group.

Control / T2DM	r	P	Control / T2DM	r	P
LDL / CRP	-0.057	0.767	Chyl / CRP	-0.034	0.857
LDL / MDA	-0.207	0.271	Chyl / MDA	-0.268	0.152
LDL / NO	0.306	0.100	Chyl / NO	-0.054	0.778
LDL / HDL	0.053	0.781	Chyl / HDL	0.053	0.780
LDL / Lipo(a)	-0.148	0.435	Chyl / Lipo(a)	-0.329	0.076
LDL / VLDL	0.497	0.005	Chyl / VLDL	-0.194	0.304
LDL / LDL	-0.263	0.160	Chyl / LDL	0.283	0.129
LDL / Chyl	-0.245	0.191	Chyl / Chyl	0.102	0.593

DISCUSSION:

Type 2 diabetes and its complications constitute a major worldwide public health problem, with high rates of morbidity and mortality. [1, 2] T2DM is strongly associated with both microvascular (retinopathy, nephropathy, and neuropathy) and macrovascular complications, including ischemic heart disease, peripheral vascular disease, and stroke.

Inflammation is effective in disease progression which is monitored by analysing the levels of CRP. A number of prospective studies have described the association between circulating CRP levels and risk of incident type 2 diabetes. There is heterogeneity between studies, with some demonstrating an independently positive association of CRP with incident diabetes, while others show no association after adjustment for adiposity and insulin resistance. Differences in the association between CRP and diabetes by sex have also been reported. [3] In our study, since we have recruited the subjects which are age and sex matched, the confounding factors of age and sex has been ruled out [**Table 1].**

Oxidative stress, defined as an imbalance between the production of reactive oxygen species (ROS) and antioxidant defence systems, has been often associated with the development of diabetes and its complications. [10] MDA is highly toxic by-product formed in a part by lipid oxidation derived free radicals. Many studies have shown that its concentration is increased considerably in diabetes mellitus, MDA reacts both irreversibly and reversibly with proteins and phospholipids with profound effects. [11] Increased level of MDA in diabetics suggests that peroxidative injury may be involved in the development of diabetic complications. [1] EJ Ikekpeazu et al. 2011 and Kaefer M, et al. 2012 have shown in their study that the levels of MDA are significantly increased in the cases of type 2 diabetes. The similar results are observed in our study **[Table 2]**, which shows that the disease shows an increase in oxidative stress as it progresses. [11, 12]

While low levels of NO is beneficial for several physiological and cellular functions, high levels of NO may cause detrimental effects in the cells. High levels of NO may react with superoxide anion to generate peroxynitrite radical, which binds to proteins and thus affects their function. The serum NO data in T2DM patients that reported by different scientific literature is controversial. Some research articles reported increased NO levels in diabetes patients whereas others reported the opposite. [7, 13] E. Wright Jr., et al 2006 has stated that hyperglycaemic conditions result simultaneously in both increased NO production and decreased NO availability. [14] In our study, the level of NO in serum of diabetic patients was found to be increased as compared to the control group **[Table 2, Figure 2]**. So, we can state that the increased NO levels in the blood serum are a result of hyperglycemic condition in newly diagnosed type 2 diabetes patients without nephropathy. [8]

The study by Aclan Ozder 2014, suggested that common lipid abnormalities during diabetes induced dyslipidemia are hypercholesterolemia, hypertriglyceridemia and elevated LDL cholesterol. [15] In our study, the results show a significant increase in the serum levels of LDL, while a decrease in VLDL and Chylomicron levels. There was no change in HDL levels in T2DM group and compared to controls **[Table 2, Figure 2]**.

Lipoprotein (a) is believed to contribute to lipid induced atherogenesis similar to LDL particles. H. Vaverková et al. 2017 have shown in their study that the levels of lipoprotein (a) are inversely proportional to insulin resistance which indicates a decrease in levels as the disease progresses. [9] While a study carried out by Sunita Pujar et al. 2014 have shown an increase in the lipoprotein (a) levels in T2DM patients. [16] In contrast to these studies, we have found no significant change in the serum levels of lipoprotein (a) in newly diagnosed T2DM group as compared to the control group as shown in **Table 1, 2 and Figure 1, 2**. The correlation between the parameters in control and T2DM groups has been shown in **Table 3A, 3B, 3C and 3D**. From this result we can state that the since the subjects involved in our study were newly diagnosed with diabetes but not in state of developing severe disorders like atherosclerosis, nephropathy etc.

CONCLUSION:

In this study we conclude that there is a significant increase in lipid peroxidation and nitric oxide levels due to increased oxidative stress in diabetes, which may lead to serious tissue damage in body cells. Also, we see that there is no significant change in the lipoprotein (a) levels in newly diagnosed diabetic patients. Thus, the study of lipid profile in diabetes is necessary and we can distinguish the patients based on the disease severity depending on lipoprotein (a) levels. Since raised oxidative stress can worsen the disease conditions, care should be taken to provide

patients with anti-oxidants in therapy as well as diet. Antioxidant supplementation along with proper nutrition might help reduce severe damage of body organs in patients suffering from hyperglycemia.

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