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Correlation of Oxidative Stress with Lipoprotein (a) 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 reactive oxygen species, which in turn cause damage to cells, ultimately resulting in secondary complications. Diabetic patients have a high risk of cardiovascular disease. Lipoprotein (a) is identified as a major risk factor of atherosclerosis in non-diabetic and diabetic patients.
Objective: In this study, we have analysed the serum levels of MDA, NO and lipoprotein (a) 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 MDA by Buege and Aust method, while NO levels by Cortas and Wakid's kinetic cadmium reduction method using spectrophotometer. Lipoprotein (a) levels were analysed using agarose gel electrophoresis. Statistical analysis was done using Mini-tab 17 software with 95% confidence interval.
Results: Serum levels of MDA and NO in T2DM patients were significantly increased as compared to healthy controls. There was no significant difference in lipoprotein (a) levels in diabetic patients.
Conclusion: There is increased oxidative stress in type 2 diabetes which affects the functioning of other body organs causing disease progression. Antioxidant supplements might help control and keep check on disease status.

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

INTRODUCTION

Diabetes is a major source of morbidity, mortality, and economic cost to the society.[1]
It is a group of metabolic disorders that is characterized by elevated levels of glucose in blood (hyperglycemia) and insufficiency in the production or action of insulin, a hormone synthesized in β cells of pancreas in response to glucose. Long term elevation in blood glucose levels 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 hyperlipidemia and oxidative stress. T2DM is a major risk factor for cardiovascular diseases and acute oxidative stress (OS) 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. If β-cell functioning is impaired, it results in an underproduction of insulin, fasting hyperglycemia, and, eventually, the development of T2DM. Obesity may play a role in the relationship between systemic oxidative stress and these conditions.[3]

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] Malondialdehyde 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 malondialdehyde 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.[4]

Nitric oxide is a gaseous molecule secreted by the endothelium and a major modulator of endothelial function.[5] 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.[6]

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).[7] The aim of our study was to analyse serum of newly diagnosed type 2 diabetic patients and evaluate the association of Lp(a) with indicators of oxidative stress, MDA and NO, which are linked to the development of T2DM.

MATERIALS 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 MDA, NO and Lipoprotein (a). The concentration of MDA was analysed by

Buege and Aust method, while that of NO was analysed by Cortas and Wakid's kinetic cadmium reduction method. The results of MDA and NO tests were read colorimetrically on Spectrophotometer at 530nm. The concentration of lipoprotein (a) 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

Table 1: Biochemical parameters in control and T2DM

Groups (n = 30)	Age (years)	MDA (nmol/ml)	NO (µmol/L)	Lipoprotein (a) (mg/dL)
Control (Mean ± SD)	49.9 ± 4.0	1.46 ± 0.35	32.09 ± 4.10	2.87 ± 1.4
Type 2 Diabetes (Mean ± SD)	49.4 ± 9.6	3.19 ± 0.54	70.45 ± 116.87	2.86 ± 1.75

Table 2: Correlation between Oxidative Stress markers and Lipoprotein (a)

Control / T2DM	r	P
MDA / MDA	-0.113	0.554
NO / NO	0.017	0.928
Lipoprotein (a) / Lipoprotein (a)	-0.116	0.541
MDA / NO	0.279	0.136
MDA / Lipoprotein (a)	0.126	0.505
NO / MDA	0.044	0.818
NO / Lipoprotein (a)	-0.310	0.096
Lipoprotein (a) / MDA	0.080	0.675
Lipoprotein (a) / NO	0.065	0.732

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. 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.[8]

Malondialdehyde (MDA) is highly toxic byproduct formed in a part by lipid oxidation derived free radicals. Many studies have shown that its concentration is increased considerably in diabetes mellitus, malondialdehyde reacts both irreversibly and reversibly with proteins and phospholipids with profound effects.[9] 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 1], which shows that the disease shows an increase in oxidative stress as it progresses.[9,10]

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. Altered serum NO levels in T2DM were reported by different investigators previously. 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.[5,11] E. Wright Jr., et al 2006 have stated in their review that hyperglycaemic conditions result simultaneously in both increased NO production and decreased NO availability.[12] In our study, the level of NO in serum of diabetic patients was found to be increased as compared to the control group [Table 1]. So, we can state that the increased NO levels in the blood serum are a result of hyperglycemic condition in type 2 diabetes. The correlation between MDA and NO

levels in type 2 diabetes is positive [Table 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.[7] While a study carried out by Sunita Pujar et al. 2014 have shown an increase in the lipoprotein (a) levels in T2DM patients.[13] 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 and 2. From this result we can state that the since the subjects involved in our study were newly diagnosed with diabetes but not prone to develop atherosclerosis, as indicated by lipoprotein (a) levels. Thus, the levels of lipoprotein (a) levels might help in distinguishing patients on the basis of disease stage.

CONCLUSION

In this study we conclude that there is a significant increase in lipid peroxidation 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, we can distinguish the patients based on the disease severity depending on lipoprotein (a) levels. We suggest that the study of lipid profile in diabetes is necessary. 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 might help reduce severe damage of body organs in patients suffering from hyperglycemia.

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