Original Article

Biochemistry

Lp (a), Uric Acid, Oxidants and Antioxidant Vitamins in Type 2 Diabetic Patients without Cardiovascular Complications

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ABSTRACT

Introduction: Type 2 diabetes mellitus patients have increased morbidity and mortality as compared to the general population, particularly with respect to coronary heart disease (CHD), possibly due to increased oxidative stress and dyslipidaemia, and recent data suggest that elevated levels of lipoprotein (Lp) (a) and uric acid (UA).

Methods: The present study included 60 type 2 diabetic patients without any cardiovascular complications and 60 age and sex matched healthy subjects as the controls. The serum levels of lipids, lipoproteins, lipoprotein (a), oxidants and antioxidant vitamins in the type 2 diabetic patients and in the healthy controls were estimated.

Results: The Lp (a), UA, lipid peroxide (LPO), total cholesterol, triglyceride and low-density lipoprotein cholesterol (LDL-C) levels were significantly elevated, while the vitamin C and E levels were significantly lowered in the diabetic patients as compared to those in the controls ($p<0.05$). In the correlation analysis, lipid peroxide was found to be negatively correlated with vitamin C and E ($r = 0.52$, $p=0.0010$, $r = -0.49$, $p=0.0019$) and to be positively correlated with uric acid ($r = +0.31$, $p=0.0246$) in the diabetic patients. In the ROC curve analysis, significant areas under the curve (AUC) were obtained for lipoprotein (a) ($p<0.01$), uric acid ($p<0.05$) and lipid peroxide ($p<0.05$).

Conclusion: The study showed significantly elevated Lp (a) and uric acid levels in the type 2 diabetic patients without any vascular complications, thus indicating that their measurement, along with the other routine investigations in the type 2 diabetics, may facilitate the early identification and the interventions for patients who were prone to cardiovascular complications.

Key Words: Type 2 diabetes mellitus; Oxidative stress; serum uric acid; Lp (a); antioxidants; CVD risk factors

INTRODUCTION

The incidence of diabetes mellitus has been rapidly increasing in most of the industrialized and many developing countries during the last thirty years and the trend is continuing. Currently, India has 40.9 million people with diabetes and the projected estimate for the year 2025 is 69.9 million [1]. The morbidity and mortality is higher in patients with type 2 diabetes due to the cardiovascular complications and the risk of atherosclerotic coronary artery disease (CAD) is increased by 2-4 fold in the type 2 diabetics (T2DM) [2].

Oxidative stress, as well as defects in the antioxidant defense systems, have been recognized as the causative factors for the development of the major diabetic complications [3, 4]. An enhanced oxidative stress has been observed in the T2DM patients, as has been indicated by the increased lipid peroxidation [5] and the diminished antioxidant status [6]. Similarly, diabetes induced disturbances in the lipid profile is responsible for the increased incidence of atherosclerosis; a major complication of diabetes mellitus [5]. Though diabetic dyslipidaemia and the oxidant and the antioxidant status in the diabetic patients were characterized extensively; the data on the mean levels of Lp (a) in these patients are quite contradictory [7, 8] and conflicting reports are available regarding the oxidant and antioxidant status in patients of T2DM without any cardiovascular complications.

Similarly, this has been a matter of debate for a few decades, since hyperuricaemia has been presumed to be a consequence of insulin resistance rather than its precursor and has been presumed to be associated with oxidative stress to be related to the development of the complications in diabetes (9). Although, much of the literature addresses the association of hyperuricaemia, hypertension, and diabetes, the levels of uric acid (UA) in the serum of the T2DM patients still are under scrutiny and their relationship with other cardiovascular risk factors and oxidant/antioxidant indicators remains unclear.

Hence, this study was designed to determine the Lp (a) and the uric acid levels along with the lipid profile and the oxidative and the antioxidant status in diabetes mellitus patients without any vascular complications as compared to the subjects in the normoglycemic group. Further, this study evaluates their association with each other and the diagnostic utility of Lp (a), LPO and uric acid in the T2DM patients.

MATERIALS AND METHODS

Subjects: The study was conducted on successive patients after an informed consent was obtained from them and after it was approved by the Institutional Human Ethics Committee. The study group consisted of sixty (60) patients with type 2 diabetes mellitus; who were aged 39-76 years, without any history of angina or myocardial infarction. All had normal electro cardiograms and cardiac stress tests. The diagnosis of NIDDM was based on the criteria of the World Health Organization study group on diabetes i.e., fasting plasma glucose ≥140 mg/dl, 2-h post-glucose load ≥200 mg/dl, or two random plasma glucose values >200 mg/dl. The diabetic patients were normotensive, without a secondary cause of hyperglycaemia and on treatment with only insulin. They did not have any other complications of diabetes. The control group consisted of sixty (60) age and sex matched healthy individuals with normal glucose tolerance tests and the absence of the history of any vascular disease (myocardial infarction, stroke, or intermittent claudication). All the patients and the healthy controls

were non-smokers and non-alcoholic. Diabetic patients who received allopurinol, nutritional supplements, oral hypoglycaemic agents or other antioxidant therapy were excluded from the study.

SAMPLE COLLECTION

Venous blood samples were collected from both the patients and the control subjects in the fasting state (for 12 hours) into three sterile plastic tubes; the first was treated with EDTA, the second with heparin and the third was left to clot, to separate the serum, which was collected by centrifuging the blood at 4000 rpm for 15 minutes at room temperature. Both the plasma and serum was stored at –80°C until the assays were performed.

Laboratory analysis: Plasma glucose was estimated by the glucose oxidase-peroxidase method by using a kit. Serum total cholesterol, triglycerides (Tgl) and high density lipoprotein cholesterol (HDL-C) were estimated by using commercially available kits and a Beckman Synchron CX9 auto analyzer. The low density lipoprotein cholesterol (LDL-C) and very low density lipoprotein cholesterol (VLDL-C) concentrations were calculated by using Friedewald's Formula. Vitamin E was measured by the method of Baker et al (1980) [10] and vitamin C was measured by the method of Roe and Kuether (1943) [11]. Lipid peroxide was assayed by using thiobarbituricacid acid reactive substances (TBARS) by a spectrophotometric method [12]. Lp (a) and uric acid were assayed by using a Beckman Synchron CX9 auto analyzer by using commercially available kits which were based on an immunotrubidimetric method (Daiichi Pure chemicals, Japan) for Lp (a) and uricase and on the peroxidase principle for uric acid.

Statistical Analysis: All the values which were obtained were expressed as mean and standard deviation (SD). The Mann Whitney U test was applied to compare the difference in the means between the controls and the study group. The correlation between the variables was studied by using Spearman's rank sum test. The differences were considered as significant if the p value was <0.05. All the analysis was carried out by using the SPSS statistical software package (Version 11.5).

Receiver Operating Characteristics (ROC) Curves: Receiver operating characteristics (ROC) were used to discriminate the positive from the negative results. The area under the curve (AUC) can range from 0.5 to 1 and the diagnostic test that approaches 1 indicates a perfect discriminator.

RESULTS

The clinical characteristics of the study group are shown in [Table/Fig-1]. The study groups were age and sex matched. Fifty three (53) % of the diabetic patients had a family history of diabetes. In comparison with the control subjects, the diabetic patients had higher BMIs ($p<0.05$). As expected, the diabetic patients had higher blood glucose and HbA_{1c} values as compared to the controls ($p<0.05$). The increased HbA_{1c} reflected the poor metabolic control of the patients.

[Table/Fig-2] summarizes the lipid profile of the controls and the type 2 diabetes mellitus patients. The levels of total cholesterol and triglycerides and the LDL-C levels were significantly increased in the diabetic patients than in the controls ($p<0.05$). On the other hand, the levels of HDL-C were significantly decreased in the diabetic patients as compared to the controls. The Lp (a) levels were significantly higher in the diabetic patients without cardiovascular complications than in the healthy controls ($p<0.05$).

[Table/Fig-3] illustrates the levels of lipid peroxide and uric acid and the antioxidant status in the controls and in the diabetic subjects. The extent of the lipid peroxidation was significantly increased in the diabetic patients as compared to the healthy controls ($p<0.05$). The levels of vitamin C and vitamin E were significantly lowered in diabetics as compared to the healthy controls ($p<0.001$).

[Table/Fig-4] shows the correlation between the variables. In the correlation analysis, a significant positive correlation was found between LPO and uric acid ($r= +0.31$, $p= 0.0246$), while a significant

Parameter	Controls	Type 2 diabetics
Number (n)	60	60
Sex (M/F)	35/25	33/27
Age group (yrs)	55 ± 11	54 ± 8
BMI (kg/m^2)	23.0 ± 3.4	26.2 ± 4.1*
Duration of Diabetes (yrs)	–	7.2 ± 5.9
Fasting blood glucose (mg/dL)	76 ± 19	154 ± 22*
HbA_{1c} (%)	5.9 ± 1.5	9.9 ± 1.7*
Urea (mg/dL)	23.3 ± 6.4	6.4 ± 4.6
Creatinine (mg/dL)	0.9 ± 0.2	1.0 ± 0.2

[Table/Fig-1]: Clinical and Biochemical characteristics of controls and type 2 diabetics
Values are Mean ± SD; *p<0.05 (compared to controls)

Parameter	Controls	Type 2 diabetics
Total cholesterol (mg/dL)	135 ± 20.5	167 ± 29.3*
Triglycerides (mg/dL)	110 ± 27.9	157 ± 76.4*
HDL-c (mg/dL)	45 ± 4.0	38 ± 5.1*
LDL-c (mg/dL)	98 ± 22.2	139 ± 7.5*
VLDL-c (mg/dL)	22 ± 5.5	29 ± 15.2
LP (a) (mg/dL)	14.9 ± 6.6	20.4 ± 11.1*

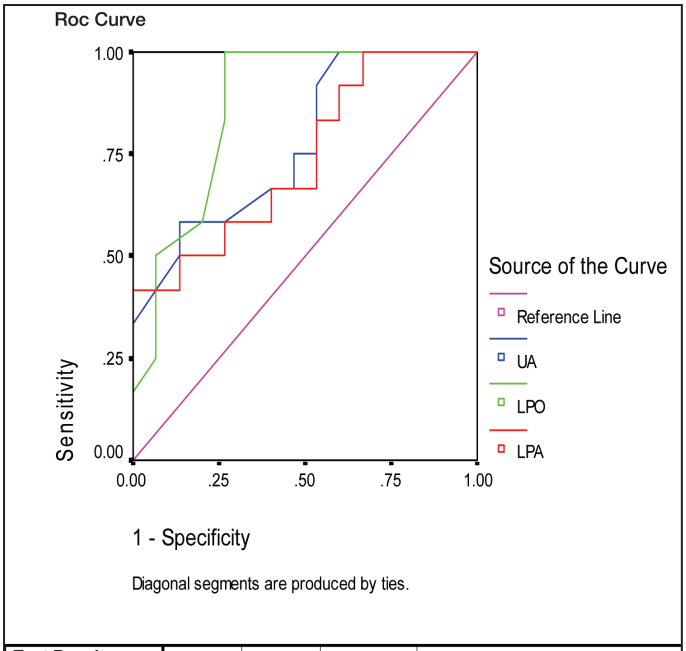
[Table/Fig-2]: Lipid Profile in controls and type 2 diabetes mellitus patients
Values are Mean ± SD; *p<0.05 (compared to controls).

Parameter	Controls	Type 2 diabetes
LPO (µmol/L)	1.76 ± 0.71	5.62 ± 0.42*
Uric acid (mg/dL)	4.24 ± 0.75	8.71 ± 0.83*
Vitamin C (mg/dL)	0.84 ± 0.23	0.34 ± 0.12**
Vitamin E (mg/dL)	1.17 ± 0.17	0.46 ± 0.13**

[Table/Fig-3]: Oxidant and Antioxidant status in controls and type 2 diabetes mellitus patients
Values are Mean ± SD; * p<0.05, ** p<0.01 (compared to controls).

Variables	r Value	p Value
LPO Vs Uric acid	0.31	0.024
LPO Vs Vitamin C	–0.52	0.001
LPO Vs Vitamin E	–0.49	0.001
Uric Acid Vs Vitamin C	–0.17	0.114 (NS)
Uric Acid Vs Vitamin E	–0.23	0.067 (NS)
LP (a) Vs Uric acid	0.22	0.070 (NS)
LP (a) Vs LPO	0.43	0.002
LP (a) Vs Vitamin C	–0.19	0.101 (NS)
LP (a) Vs BMI	0.39	0.010
LP (a) Vs HbA_{1c}	0.40	0.003

[Table/Fig-4]: Correlation among the variables in type 2 diabetes mellitus patients
*Statistically significant; NS – not significant.



Test Result Variable(s)	Area	SE	p value	95% Confidence Interval	
				Lower Bound	Upper Bound
LPO	.739	.097	.036	.549	.929
Lp (a)	.867	.071	.001	.728	1.006
Uric acid	.775	.090	.016	.598	.952

[Table/Fig-5]: Receiver Operating Characteristics Curve for Lp(a), LPO and Uric acid

negative correlation was found between LPO and vitamin C and vitamin E (r= –0.52, p=0.0010, r=–0.49, p=0.0019) in the study group. Further, a significant positive correlation was observed between Lp (a) and LPO (r=0.43, p= 0.0026) in the diabetic patients. Similarly, a significant positive correlation was observed between BMI and HbA_{1c} (r= 0.39, p=0.0102, r=0.40, p=0.0038) in the type 2 diabetics. There was no significant correlation of the antioxidants with uric acid and the other parameters in the study.

The diagnostic accuracy of the individual risk factors were assessed by the receiver operating characteristic (ROC) curve analysis which revealed the significant area under the curve (AUC) for Lp(a), Uric acid and LPO, as depicted in [Table/Fig-5], with Lp(a) having a more significant AUC (0.867, p<0.01), followed by uric acid (0.775, p<0.05) and LPO (0.739, p<0.05).

DISCUSSION

Diabetes mellitus has been known to be a state of excess generation of free radicals which are contributed by several mechanisms, including hyperglycaemia and a defective antioxidant status, which causes oxidative stress. The data of the present study revealed a depleted level of the extracellular antioxidant status in the type 2 diabetics, regardless of any complications, in favour of an oxidative stress in such patients. These results were in agreement with those of previous studies [4, 13], which demonstrated a strong association between poor glycaemic control and the depletion of the protective antioxidant defence mechanisms in diabetes mellitus. In the present study, all the diabetic patients were poorly controlled, as was indicated by their high glucose and HbA_1C levels. In addition to this, dyslipidaemia, which we observed in the present study, was in agreement with the findings of other studies [5, 14]. These findings are not surprising, because long-term hyperglycaemia causes generalized vascular endothelial damage, which reduces functional lipoprotein lipase, leading to an increase in Tgl and a decrease in HDL-C [14].

The studies on Lp (a) are quite significant and show a higher incidence of coronary artery disease (CAD), with elevated Lp (a) levels in T2DM. Solfrizzi et al (2002) [15] suggested that the elevated Lp (a) levels did not appear to be an independent predictor of CAD, but that they were a risk factor only in the subjects with type 2 diabetes. In the present study, the Lp (a) levels were found to be significantly higher in the study group than in the controls. However, the mean plasma Lp (a) levels were still in the normal range in the study group. This observation was in agreement with the findings of Singla et al (2009) (16) who reported that there was a strong association between the Lp (a) levels and T2DM. Heller et al (1993) [17] suggested that hyperinsulinaemia could be a causal factor for the increase in the Lp (a) levels in T2DM. Similarly, Wolffenbuttel et al (1993) [18] also reported that the Lp (a) levels were elevated in diabetics as compared to the non-diabetic subjects of similar age. They also reported that the changes in insulin had no correlation with the degrees of metabolic control and the changes in the Lp (a) levels. However, it was not possible to draw a definite conclusion on this finding with our results, as we had not measured the effect of glycaemic control on Lp (a). However, higher Lp (a) levels were found to be associated with the diabetic complications, which could further enhance the cardiovascular complications (19) and hence, lower the Lp (a) levels should be considered in type 2 diabetic patients without cardiovascular complications.

So far, the studies on uric acid suggest that uric acid is a plasma antioxidant, followed by vitamin C which stabilizes it in plasma and protects it from oxidation [20]. However, the antioxidant property of uric acid has been questioned by recent studies in the exacerbated oxidative state of diabetes and they have demonstrated that uric acid could be related to the development of diabetes. In the present study, we found significantly elevated levels of serum uric acid in the T2DM patients without cardiovascular compilations. Nieto et al (2000) [21] reported that an increase in the serum uric acid in the T2DM patients might therefore reflect a compensatory mechanism to counter the oxidative stress that occurs in these conditions. However, a high level of uric acid does not confer protection and patients with elevated uric acid levels have a greater risk of developing cardiovascular events [22]. Similarly, Corry et al (2008) [23] also suggested that uric acid, although it is one of the major antioxidants in the circulation, can induce oxidative stress in a variety of cells, including the vascular smooth muscle cells and thus, mediate the progression of cardiovascular disease [24]. A positive association with lipid peroxide which was observed in the present study, may imply that though uric acid was a major plasma antioxidant in the circulation, followed by vitamin C, it could become a strong pro-oxidant in the diabetic state and could be associated with increased free radical formation and lipid peroxidation [20].

It is important to recognize the associations between these risk factors as these do not function in isolation. The association between the parameters which were assessed in this study showed a significant negative correlation of LPO with vitamins C and E, which indicated the depleted antioxidant status with an increased state of oxidative stress [13]. Correspondingly, a significant positive correlation of uric acid with LPO and its negative correlation with the antioxidant vitamins may predict a pro-oxidant activity of uric acid apart from its antioxidant property [20] and its association with oxidative stress [9].

From the present study, it may be concluded that type 2 diabetes mellitus without any cardiovascular complications shows significantly elevated Lp (a) and uric acid levels. Further, this study also revealed

the importance of assessing Lp (a) and uric acid in these patients, in addition to the markers of oxidative stress, the antioxidant status and the lipid profiles to enable the formulation of specific therapies for an early intervention and a better management of the disease. However, a major limitation of the present study was the small study population, which warrants further cross-sectional studies by using a larger sample size.

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