Antioxidant Activity of Uric Acid and its Correlation with Oxidative Stress and Endothelial Dysfunction in Type 2 Diabetes Mellitus

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Research Article

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Abstract

Objective: Uric acid is an end product of purine metabolism and it has two different functions such as pro-oxidant and anti-oxidant. Where, pro-oxidant and anti-oxidants are opposite in action. Oxidative stress and endothelial dysfunction are a foremost cause of complications in diabetes mellitus, where uric acid may play a major role in this process. Hence, the present study has been designed to evaluate antioxidant activity of uric acid and its correlation with oxidative stress and endothelial dysfunction in type 2 diabetic subjects.

Methods: We included 120 subjects in this study with age group of 39 -60 years. Among these 60 were type 2 diabetic subjects and 60 were healthy controls. The estimation of biochemical parameters such as blood sugar, lipid profile, uric acid, and homocysteine are measured in fully auto-analyzer with well recognized methods. MDA measured by TBARS method, total antioxidant capacity as FRAP and NO estimated by Kinetic cadmium method in spectrophotometer.

Results: The study was found significant elevation of triglyceride, LDL and MDA and significant lower level of FRAP and NO in T2DM than healthy control. Uric acid was insignificant in T2DM compared to healthy control. However, uric acid has significant correlation with FRAP (r=0.2116, p=0.02) and moderate correlation with triglyceride (r=0.1736, p=0.0579) and homocysteine (r=0.1779, p=0.0519). MDA was negatively and NO was positively correlated with uric acid but statistically insignificant.

Conclusion: We have found antioxidant activity of uric acid where it was determined by significant positive correlation with FRAP in type 2 diabetes mellitus.

Introduction

Uric acid (UA) is an end product of purine metabolism. The prevalence of hyperuricemia has rarely seen and investigated in developing countries. Uric acid has two major functions include pro-oxidant and anti-oxidant. Where pro-oxidant action elevates oxidative stress and antioxidant activity reduces oxidants [1]. The antioxidant activity of uric acid reduces oxygen radicals and protects erythrocyte membrane from lipid peroxidation. The effect of uric acid is under specific conditions in which exogenously added uric acid protected cells from oxidants. However, plasma uric acid can prevent lipid peroxidation only as long as ascorbic acid is present in plasma [2].

Endothelial dysfunction is a preliminary stage of atherosclerosis, which is also caused by hyperuricemia. Elevated level of uric acid also a risk factor for hypertension and renal disease, and improvement in endothelial function was seen by lowering UA level in hyperuricemia subjects [3]. Soluble uric acid can react to form radicals, which increase lipid oxidation and induce various pro-oxidant effects on vascular cells. In vitro and in vivo study findings advise that uric acid may contribute to endothelial dysfunction by inducing anti-proliferative effect on endothelium and reducing nitric oxide production. This makes us to comprehend the role of elevated uric acid in pathogenesis of vascular complications [4].
Uric acid is also a potent endogenous antioxidant, it partially established by interaction with another powerful antioxidant. Experimental in vitro study suggested that uric acid acts as other antioxidants which can shift from protective function of antioxidant to pro-oxidant effect according to their level [5]. However, data is controversial on protective or harmful effect of uric acid in biological system in various diseases. Hence, the study has been designed to evaluate the effect of uric acid and its correlation with oxidative stress in type 2 diabetic subjects.

**Materials & Methods**

The present study was designed as cross-sectional study. One hundred twenty subjects are enrolled into the study with age and gender group of 39 – 60 years. Among these 60 were type 2 diabetic subjects who were undergone regular treatment at Govt. hospital and VMKV medical College, Salem, Tamil Nadu, India. The remaining 60 subjects are age and gender matched healthy controls.

**Selection criteria:** Type 2 diabetic subjects who were undergone regular anti-diabetic treatment are included in the study. Type 2 diabetic subjects with lifestyle modification (such as smoking and alcohol), complications (like kidney disease, liver disease, hypertension and cardiovascular complications) and subjects with lipid lowering drugs were excluded from the study.

**Ethical clearance:** The ethical clearance was obtained from institutional ethical committee at VMKV Medical College, Salem, Tamil Nadu, India.

**Sample collection:** Five milliliters of venous blood sample was collected after 12 hours overnight fasting and obtaining inform consent from each subject. 1ml of blood sample was transferred to fluoride tube for estimation of glucose, 1ml in sodium citrate tube for ferric reducing antioxidant power (FRAP) and nitric oxide (NO) and 2ml in plane tube for lipid profile, malondialdehyde (MDA) and homocysteine (HCY).

Serum and plasma samples are separated from blood by centrifugation at 3000rpm for 15 minutes. Blood glucose, lipid profile, and uric acid were estimated on the same day of sample collection, and remaining serum/plasma samples are transferred to labelled cuvettes and stored at -20°C deep freezer until remaining parameters (MDA, FRAP, NO & homocysteine) are measured.

**Methods:** Fasting and post-prandial blood glucose, total cholesterol (T. Chol), Triglyceride (Tgl) and high-density lipoprotein (HDL) were measured by using commercially available standard kits. Uric acid estimated by uricase/PAP and homocysteine by UV 2-point kinetic reaction method. The above parameters are measured in fully-autoanalyzer. Where low density lipoprotein (LDL) and very low-density lipoprotein (VLDL) are calculated by using standard Friedewald's formula. BMI is calculated by using formula = weight (kg)/Height² (meters). MDA by thio-barbituric acid reactive substances (TBARS) method, total antioxidant as FRAP and NO by kinetic cadmium reduction method and these parameters are measured in spectrophotometer.
Statistical analysis: Research data was statistically analyzed by using SPSS software version 24; Mean and standard deviation was done by Microsoft excel. Statistical significance of data variables between the groups was performed by “Kruskal Wallis” test. ‘p’ value <0.05 was considered to be statistically significant. Pearson correlation was performed to evaluate the correlation of variables in the group with uric acid. Scatter graphs are used to discuss the correlation of uric acid with other parameters in the study, which were done by Microsoft excel.

Result

In our present study, Table 1 illustrates significant elevation of BMI, fasting and post-prandial glucose in type 2 diabetic subjects than healthy controls. Table 1 also shows significant higher level of Tgl and LDL in type 2 diabetic subjects than healthy controls, but insignificant difference was observed in the level of total cholesterol and HDL among the groups.

Our study was observed oxidative stress and endothelial dysfunction in type 2 diabetic subjects by significant elevation level of MDA and decreased level of FRAP and NO compared to healthy controls (Table 1). The study also estimated uric acid and homocysteine, but no significant was identified among the groups (Table 1).

The study was primarily designed to perceive a correlation of uric acid with oxidative stress and endothelial function. We have found significant positive correlation of uric acid with FRAP (r=0.21, p=0.02) (Table 2). Though uric acid was negatively correlated with MDA and positively with NO, but statistically insignificant (MDA r=-0.08, p=0.35& NO r=0.05, p=0.54) (Table 2). In additional to this we also found moderate significant correlation of uric acid with triglyceride (r=0.17, p=0.057) and homocysteine (r=0.17, p=0.051), but statistically insignificant.

Different scattering graphs were expressed to demonstrate the correlation of uric acid with Triglyceride, MDA, and FRAP, NO and homocysteine in type 2 diabetic subjects (Graph 3 to Graph 7).

Discussion

Prevalence of diabetic complications is frequently increasing in world wide. There are many factors which are involved to cause complications in type 2 diabetes mellitus. Hyperuricemia is considered to be one of the risk factors to cause type 2 diabetes mellitus and also cardiovascular disease. Moreover, uric acid is commonly associated with glucose intolerance, hypertension, dyslipidaemia and metabolic syndrome. Since 1950s uric acid was recognized as factor to contribute in the pathogenesis of cardiovascular diseases such as stroke and ischemic heart disease [6]. Extensive epidemiological and experimental evidence suggests that uric acid is a relevant and independent risk factor for cardiovascular disease and renal disease, mostly in patients with hypertension, heart failure or diabetes [7].

The present study was recognized that type 2 diabetic subjects have pathological changes, which was stated by the investigation of parameters related to dyslipidaemia (increased Tgl & LDL levels), oxidative
stress (increased MDA and decreased FRAP) and endothelial dysfunction (reduced NO availability). Since uric acid is an independent risk factor to cause type 2 diabetes and cardiovascular disease, our study focused to elucidate the correlation of uric acid with other risk factors include triglyceride, MDA, FRAP, NO and homocysteine are as follows.

**Correlated of Uric acid with Triglyceride**

Graph 3 shows slight uphill linear pattern, but few values are scattered in a wide band showing linear relationship between uric acid and triglyceride, statistically insignificant. As we know dyslipidaemia and uric acid are individual risk factors to cause complications in diabetes. But uric acid is strongly correlated with triglyceride than MDA and NO. An earlier cross-sectional study has found that uric acid levels are associated with HDL-c and triglycerides than insulin resistance in dyslipidaemia subjects. Where, atherogenic index of plasma was found to be significantly correlated with uric acid [8]. Moreover, Post-hoc analysis of GREACE and the life trials changed interest to focus on serum uric acid level and its contribution in atherosclerotic cardiovascular disease [9]. Sharma S et al has identified dyslipidaemia in type 2 diabetic subjects with reduced level of uric acid. It shows strong benefit effect of uric acid in diabetes [10]. But still it is uncertain that whether uric acid has strong beneficial or harmful effect associated with other parameters.

**Correlation of Uric acid with Malondialdehyde (MDA)**

The major function of uric acid is either an antioxidant or pro-oxidant depends on variety of factors. UA is a powerful signalling molecule that can affect intracellular signal transduction leads to generation of oxidants through nicotinamide adenine dinucleotide phosphate (NADPH) oxidase and expression of pro-inflammatory mediators [11]. Additionally, increased UA level through impaired renal excretion was observed in subjects with obese, insulin resistant and hypertension. Where in local ischemia condition uric acid production increases parallel to reactive oxygen species (ROS). However, clinical and experimental evidence suggest that uric acid has antioxidant property. This antioxidant activity of uric acid is overcome by pro-oxidant and pro-inflammatory effects of ROS [1,9]. In our study Graph 4 shows slight downhill, values of upper linear line scattered in a wide band represents negative relationship of uric acid with MDA (p=0.056), but statistically insignificant. This clarifies that uric acid has modest pro-oxidant property to cause oxidative stress in type 2 diabetic subjects. Anyhow a greater number of clinical studies are required to establish and confirm the pro-oxidant activity of uric acid.

**Correlation of Uric acid with FRAP**

Uric acid is a powerful scavenger of free radicals and provides 60% of free-radical scavenging capacity in plasma [12]. The present study Graph 5 displays slight uphill linear pattern and few values are scattered in a wide distribution, positive relationship was found between uric acid and FRAP and statistically significant. An earlier study was observed higher level of FRAP in subjects with hyperuricemia and also found decreased uric acid level with reduced antioxidant capacity [12]. Glantzounis G.K observed lowering uric acid by allopurinol has protective effect in situation associated with oxidative stress (e.g.
ischaemia reperfusion injury, cardiovascular disease) [1]. Nieto F.J et al evaluated that higher level of uric acid was associated with elevated antioxidant capacity among individuals with atherosclerosis [13].

Uric acid is a unique scavenger of peroxynitrite in the extracellular space. However, uric acid cannot scavenge superoxide, and presence of ascorbic acid thiols are required for complete scavenging of peroxynitrite [2]. Uric acid not only acts as scavenger, but also stabilizes ascorbate in biological fluids. Ascorbate stabilization is particularly evident in human and largely due to iron chelation by uric acid. Depletion of serum uric acid causes rapid oxidation of ascorbic acid where largely depends on iron [14]. Additionally, our study also supporting that uric acid has antioxidant property, since we observed significant Correlation uric acid with FRAP (‘p’=<0.05). This indicates that uric acid has high possibility of antioxidant property rather than pro-oxidant.

**Correlation of uric acid with NO**

In diabetes, hyperglycaemia may contribute to endothelial dysfunction in several ways. Reduced level of NO in diabetes maybe due to limited availability of NADPH (Nicotinamide adenine dinucleotide phosphate), a necessary cofactor for eNOS, may occur as a result of decreased activity of pentose phosphate pathway leads to decrease NO production. Oxidative stress promotes generation of superoxide (O2-), an anion generated by a number of pathways which can quench NO, reducing its bioavailability despite normal production leads to endothelial dysfunction [15]. Uric acid also plays a major role to decreases the bioavailability of NO in bovine aortic endothelial cells and adipocytes [2].

Still the mechanism of uric acid to damage organs is incompletely understood, but there is increasing evidence that endothelial dysfunction is a fundamental mechanism where uric acid may affect the cardiovascular and renal function. Graph 6 demonstrates slight uphill linear pattern where uric acid values are scattered in a wide band, showing positive relationship but statistically insignificant. Earlier studies have been observed that allopurinol lowers uric acid by inhibiting xanthine oxidase and improves endothelial function by interacting with anion superoxide production [7]. The experimental data directly implicate uric acid in endothelial dysfunction, but few other study reports in humans made controversial [16]. In our study uric acid was not correlated with endothelial dysfunction, the reason might be due to sample size.

**Correlation of Uric acid with Homocysteine**

Both uric acid and homocysteine are well known risk factors for cardiovascular disease [17]. Hyperhomocysteinemia is one of the important factors for cardiovascular disease. Graph 7 illustrates moderate uphill linear pattern where few uric acid values are scattered in a wide band, showing positive relationship but not significant as FRAP. However, relationship of uric acid with homocysteine is better than MDA, NO and triglyceride. An earlier study has observed that elevated level of homocysteine in male gout patients and hyperhomocysteinemia was not correlated with uric acid, but it was inversely correlated with renal dysfunction [18]. Where homocysteine triggers free radical production and impairs endothelial
function and initiates cardiovascular risk [19]. Our study was identified endothelial dysfunction, but it was not correlated with uric acid and homocysteine.

The present study was evaluated the correlation of uric acid with other risk factor to predict complications in type 2 diabetes mellitus. Uric acid is an individual risk factor to cause cardiovascular disease; it may be more predictable marker if correlates with other risk factors. Our study was found significant correlation of uric acid with total antioxidant capacity (FRAP) and moderate significant correlation with triglyceride and homocysteine. But statistically insignificant correlation of uric acid was found with total cholesterol, LDL, HDL, MDA and NO. The present study supports earlier reports that uric acid has strong predictive antioxidant property rather than pro-oxidant. Increasing uric acid may help to reduce oxidants and future complications in type 2 diabetes. However, we require large scale randomized study to get accurate report on antioxidant activity of uric acid because our study also found moderate significant correlation of uric acid with triglycerides and homocysteine.

**Conclusion**

Even though uric acid is insignificant in type 2 diabetic subjects, but it has significant correlation with total antioxidant capacity and moderate correlation with triglyceride and homocysteine. It confirms that uric acid contribution to antioxidant activity and reduces the risk of complication in type 2 diabetes mellitus. However, due to moderate significant relationship of uric acid with triglyceride and homocysteine, there is possibility to contribute uric acid in pathogenesis. Hence, it is still uncertain on what basis uric acid action is regulated.

**Limitations**

The present study sample size was low due to excluding criteria where patients treated with antioxidants and lipid lowering drugs and complications are excluded. Hence, further study is required to elucidate the exact role of uric acid on oxidative stress and endothelial dysfunction in type 2 diabetes mellitus.

**Declarations**

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**Conflict of Interest:** No conflict

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**Tables**

Table/Graph 1: Basic Characteristics and Parameters in the study groups.

| Parameters                  | Cases (T2DM) (60N) | Control (60N) | ’p’ Value |
|-----------------------------|--------------------|---------------|-----------|
|                             | Mean               | SD            | Mean      | SD       |           |
| AGE                         | 50.85              | 10.23         | 49.3      | 10.27    | 0.365     |
| BMI (Kg/m²)                 | 25.12              | 6.41          | 20.48     | 0.81     | 0.000*    |
| FBS (mg/dl)                 | 159.85             | 68.49         | 87.62     | 9.95     | 0.000*    |
| PPBS (mg/dl)                | 277.98             | 85.90         | 119.65    | 6.07     | 0.000*    |
| Lipid profile (mg/dl)       |                    |               |           |          |           |
| T. Cholesterol              | 194.76             | 40.30         | 183.05    | 36.59    | 0.066     |
| Triglyceride                | 161.86             | 66.87         | 121.30    | 67.57    | 0.000*    |
| HDL-c                       | 41.05              | 8.05          | 41.65     | 9.28     | 0.876     |
| LDL-c                       | 186.20             | 42.21         | 117.14    | 35.52    | 0.000*    |
| Oxidative Stress            |                    |               |           |          |           |
| MDA (µmol/l)                | 2.48               | 1.70          | 0.72      | 0.17     | 0.000*    |
| FRAP (µmol/l)               | 0.70               | 0.10          | 0.91      | 0.30     | 0.000*    |
| NO (µmol/l)                 | 13.83              | 8.00          | 18.56     | 9.69     | 0.009*    |
| HCY (µmol/l)                | 15.84              | 7.32          | 16.38     | 5.63     | 0.984     |
| UA (mg/dl)                  | 4.64               | 1.15          | 4.73      | 1.25     | 0.570     |

*p value < 0.01 indicates highly significant

Table/Graph 2: Correlation of Uric Acid with lipid profile, oxidative stress, and endothelial dysfunction.

| Correlation of Uric Acid | BMI  | T. Chol | Tgl  | HDL  | LDL  | MDA  | FRAP | NO   | HCY  |
|--------------------------|------|---------|------|------|------|------|------|------|------|
| ’r’ Value                | 0.15 | 0.14    | 0.17 | -0.09| 0.10 | -0.08| 0.21 | 0.05 | 0.18 |
| ’p’ Value                | 0.09 | 0.14    | 0.057@| 0.33 | 0.25 | 0.35 | 0.02*| 0.54 | 0.051@|

*p value <0.05 indicates statistically significant. @ indicates moderate statistically significant.

**Figures**
UA = Uric Acid, TGL = Triglyceride.

Figure 1

Graph 3: Correlation of Uric Acid with Triglyceride in T2DM

$r = 0.17, p = 0.057$
Figure 2

Graph 4: Correlation of Uric Acid with MDA in T2DM

UA = Uric Acid, MDA Malondialdehyde

$r = -0.08, p = 0.35$
**Figure 3**

Graph 5: Correlation of Uric Acid with FRAP in T2DM

UA = Uric Acid, FRAP = Ferric Reducing Antioxidant Power

**Figure 4**

Graph 6: Correlation of Uric Acid with NO in T2DM

UA = Uric Acid, NO = Nitric Oxide
Figure 5

Graph 7: Correlation of Uric Acid with Homocysteine in T2DM

UA = Uric Acid, HCY = Homocysteine