Original Article

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

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Abstract:

Objective: To evaluate the correlation of uric acid with oxidative stress and endothelial dysfunction in type 2 diabetic subjects.

Material and Methods: The study included 120 subjects, among when 60 were type 2 diabetes subjects and the remaining 60 were age and gender matched healthy controls. The biochemical parameters, blood glucose, lipid profile, uric acid and homocysteine, were measured by standard kits in an autoanalyzer. Oxidative stress was evaluated by measuring malondialdehyde (MDA) and total antioxidant power by manual methods such as thio-barbituric acid reactive substances and ferric reducing ability of plasma (FRAP). Endothelial dysfunction was assessed by measuring nitric oxide (NO) by the kinetic cadmium method.

Results: A significant elevation of triglycerides, low density lipoprotein (LDL), and MDA were observed in the type 2 diabetes mellitus patients while FRAP and NO were significantly reduced compared to the healthy controls. In addition, the uric acid levels had a highly significant correlation with FRAP (r=0.212, p-value=0.020), and moderately significant correlation with triglycerides (r=0.173, p-value=0.057) and homocysteine (r=0.178, p-value=0.051). Uric acid was negatively correlated with MDA and positively correlated with NO, but not statistically significant.

Conclusion: Our findings suggest that uric acid may have antioxidant properties since it had a significant positive correlation with FRAP.

Keywords: ferric reducing ability of plasma, malondialdehyde, nitric oxide, type 2 diabetes mellitus, uric acid

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Introduction

The prevalence of hyperuricemia has rarely been observed and investigated in developing countries.[1] In the 1950s uric acid (UA) was recognized as a factor contributing to the pathogenesis of various cardiovascular diseases such as stroke and ischemic heart disease.[2] Since there has been extensive epidemiological and experimental evidence suggesting that UA is a relevant and independent risk factor for cardiovascular disease and renal disease, mostly in patients with hypertension, heart failure or diabetes.[3] In addition, high concentrations of UA have been associated with glucose intolerance, dyslipidaemia, renal dysfunction, and involved in the mechanisms that induce oxidative stress, inflammation, endothelial dysfunction, hypertension and metabolic syndrome.[4]

However, UA has both pro-oxidant and antioxidant activity. The antioxidant activity of UA reduces oxygen radicals and protects erythrocyte membranes from lipid peroxidation. The effect of UA occurs under specific conditions in which exogenously added UA protects cells from oxidants.[5] Hence, UA is a potent endogenous antioxidant partially enhanced by its interaction with other powerful antioxidants.[4] This antioxidant property of plasma UA prevents lipid peroxidation only as long as ascorbic acid is present in the plasma.[5]

On the other hand, endothelial dysfunction occurs in the preliminary stage of atherosclerosis, which is also caused by hyperuricemia. Elevated levels of UA are also considered to be a risk factor for hypertension and renal disease, and improvement in endothelial function was observed by lowering UA levels in hyperuricemia subjects.[6] However, radicles are formed when a noncrystalline molecule reacts with soluble UA, which increases lipid oxidation and induces various pro-oxidant effects on vascular cells. In-vitro and in-vivo study findings suggest that UA may contribute to endothelial dysfunction by inducing an anti-proliferative effect on the endothelium and reducing nitric oxide production. This helps us understand the role of elevated UA in the pathogenesis of vascular complications.[7]

However, the above data indicates some controversy concerning the role of UA as protective or harmful in various diseases. Hence, this study was designed to evaluate the role of UA and its correlation with oxidative stress and endothelial dysfunction in type 2 diabetes subjects.

Material and Methods

Study design: The cross-sectional study enrolled 120 subjects in the age group of 39-60 years. Among these patients 60 had type 2 diabetes and were undergoing regular treatment at the Government Hospital or the Vinayaka Missions Kirupanada Variyar Medical College, Salem, Tamil Nadu, India. The remaining 60 subjects were age- and gender-matched healthy controls. The study was conducted in the period of 2011 to 2015.

Selection criteria: Type 2 diabetes subjects who were undergoing regular anti-diabetic treatment were included in the study. Type 2 diabetes subjects with lifestyle characteristics such as smoking and alcohol drinking, certain diseases such as kidney disease, liver disease, hypertension and cardiovascular complications and subjects currently taking lipid-lowering drugs were excluded from the study.

Ethical clearance: Ethical clearance was obtained from the institutional ethics committee at Vinayaka Missions Kirupananda Variyar Medical College, Salem, Tamil Nadu, India.

Sample collection: Informed consent was obtained from each subject prior to beginning the study. Five milliliters of venous blood was collected from each subject after 12 hours of overnight fasting. One milliliter of blood was transferred to a fluoride tube for estimation of glucose, 1 ml to a sodium citrate tube for the ferric reducing ability of plasma (FRAP) and nitric oxide (NO) assays, and the remaining 3 ml in a plain tube for lipid profile, malondialdehyde (MDA) and HCY.

Serum and plasma samples were separated from blood by centrifugation at 3,000 rpm for 15 minutes. Blood glucose, lipid profile, and UA were estimated on the same day of sample collection, and the remaining serum/plasma samples were transferred to labelled cuvettes and stored at -20 °C in a deep freezer until the remaining parameters (MDA, FRAP, NO & homocysteine) were measured.

Methods: Fasting and postprandial blood glucose, total cholesterol (T. Chol), triglycerides (TGL) and high-density lipoproteins (HDL) were measured using commercially available standard kits, UA by uricase/PAP (4-aminoantipyrine) and HCY by the UV (ultraviolet-visible) 2-point kinetic reaction method. The above parameters were measured in a fully automated process. Low density lipoproteins (LDL) and very-low-density lipoproteins (VLDL) were calculated by using the standard Friedewald formula. BMI (body mass index) was calculated by using the formula = weight (kg)/height^2 (meters). Oxidative stress was evaluated by measuring malondialdehyde (MDA) and total antioxidant power by manual methods such as thio-barbituric acid reactive substances (TBARS) and FRAP tests. Endothelial dysfunction was assessed by measuring NO by the kinetic cadmium method. All the above parameters were measured in a spectrophoto-meter.

Statistical analysis: The research data were statistically analyzed using SPSS software version 24; mean and standard deviations were determined by Microsoft Excel. Statistical significance was assessed by the Kruskal Wallis test. A p-value<0.050 was considered statistically significant. Pearson correlation was used to evaluate the correlation of UA with other variables and scatter graphs were constructed (with Microsoft Excel) to help visualize the correlations of UA with other parameters in the study.

Table 1 Biochemical parameters between the study groups

Parameter	Cases (T2DM) (n=60) Mean±S.D	Control (n=60) Mean±S.D	p-value
Age (years)	50.85±10.23	49.3±10.27	0.365
BMI (kg/m^2)	25.12±6.41	20.48±0.81	<0.001**
FBS (mg/dl)	159.85±68.49	87.62±9.95	<0.001**
PPBS (mg/dl)	277.98±85.90	119.65±6.07	<0.001**
Lipid profile (mg/dl)			
Total cholesterol	194.76±40.30	183.05±36.59	0.066
Triglycerides	161.86±66.87	121.30±67.57	<0.001**
HDL-c	41.05±8.05	41.65±9.28	0.876
LDL-c	186.20±42.21	117.14±35.52	<0.001**
Oxidative stress			
MDA (µmol/l)	2.48±1.70	0.72±0.17	<0.001**
FRAP (µmol/l)	0.70±0.10	0.91±0.30	<0.001**
Endothelial dysfunction			
NO (µmol/l)	13.83±8.00	18.56±9.69	<0.05*
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<0.001 indicates highly significant, *p-value<0.050 indicates significance.

S.D.=standard deviation, T2DM=type 2 diabetes mellitus, BMI=body mass index, FBS=fasting blood sugar, PPBS=post-prandial blood sugar, HDL-c=high density lipoprotein cholesterol, LDL-c=low density lipoprotein cholesterol, MDA=malondialdehyde, FRAP=ferric reducing ability of plasma, NO=nitric oxide, HCY=homocysteine, UA=uric acid

Results

Table 1 illustrates the significant elevations of BMI and fasting and postprandial blood glucose in type 2 diabetes subjects compared to healthy controls found in this study. Table 1 also shows significantly higher levels of Tgl and LDL in type 2 diabetes subjects than healthy controls, but non-significant differences were found in the levels of total cholesterol and HDL among the groups.

Our study found oxidative stress and endothelial dysfunction in our type 2 diabetes subjects due to significant elevation of MDA and decreased levels of FRAP and NO compared to the healthy controls (Table 1). The study also found estimated UA and HCY levels were statistically nonsignificant between the groups (Table 1).

The study was primarily designed to look for correlations of UA with oxidative stress and endothelial function. We found a significant positive correlation of UA with FRAP (r=0.212, p-value=0.020) (Table 2). UA was negatively correlated with MDA and positively correlated with NO, but both were statistically nonsignificant (MDA r=-0.086, p-value=0.350 & NO r=0.056, p-value=0.541) (Table 2). We also found a moderately significant correlation of UA with triglyceride (r=0.173, p=0.057) and HCY (r=0.178, p-value=0.051), but again statistically nonsignificant. Different 'scatterplots' were constructed to visually demonstrate the correlations of UA with triglycerides, MDA, FRAP, NO and HCY in type 2 diabetes subjects.

Discussion

Type 2 diabetes mellitus is a complicated disease, with long-term complications caused by dyslipidemia, oxidative stress and endothelial dysfunction. Dyslipidaemia is caused by increased triglycerides and LDL-c, while oxidative stress is induced by increased oxidants (MDA) and decreased antioxidants (FRAP), and endothelial dysfunction caused by reduced availability of NO. Since UA is an independent risk factor for type 2 diabetes and cardiovascular disease, our study focused on elucidating the correlations of UA with other risk factors, namely triglycerides, MDA, FRAP, NO and HCY, discussed following.

Correlation of UA with triglycerides

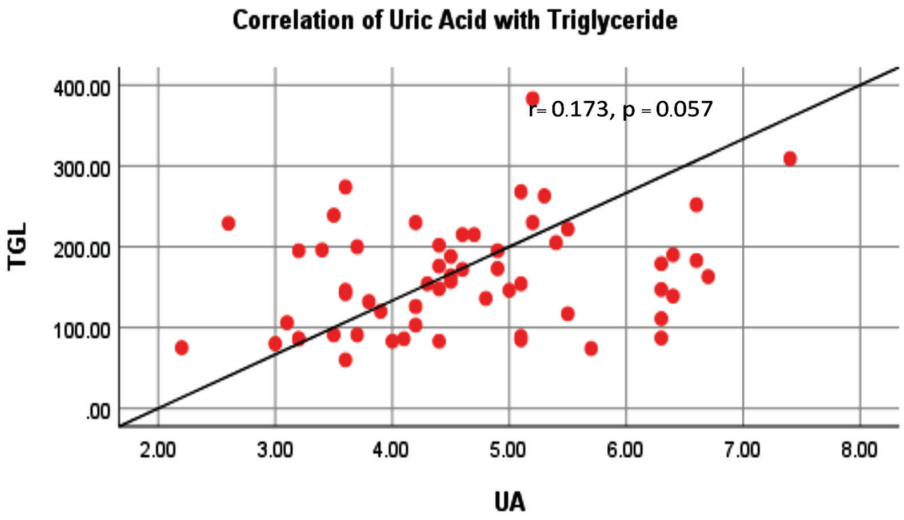
Figure 1 shows a slight uphill linear pattern, with a few values scattered in a wide band showing a linear relationship between UA and triglycerides, moderately significant correlation with a p-value of 0.057. Dyslipidemia and UA are individual risk factors that cause complications in diabetes. However, UA more strongly correlated with triglycerides than with MDA and NO. An earlier cross-sectional study found that UA levels were associated with HDL-c and triglycerides, insulin resistance in dyslipidaemia subjects. The atherogenic index of plasma was also found to be significantly correlated with UA.[8] Additionally, post-hoc analysis found that serum UA levels and their contribution to atherosclerotic cardiovascular disease.[9] Sharma et al.

Table 2 Correlation of uric acid with lipid profile, oxidative stress, endothelial dysfunction

	Uric acid								
	BMI	**T. Chol**	**Tgl**	**HDL**	**LDL**	**MDA**	**FRAP**	**NO**	**HCY**
r-value	0.151	0.135	0.173	-0.090	0.105	-0.086	0.212	0.056	0.178
p-value	0.099	0.141	0.057@	0.328	0.254	0.350	0.020*	0.541	0.051@

*"p-value" <0.050 indicates significance @p=0.057 indicates moderate significance.

BMI=body mass index, T. Chol=total cholesterol, Tgl=triglycerides, HDL=high density lipoprotein, LDL=low density lipoprotein, MDA=malondialdehyde, FRAP=ferric reducing antioxidant power, NO=nitric oxide, HCY=homocysteine, UA=uric acid



TGL=triglycerides, UA=uric acid

Figure 1 Demonstrate the correlation of uric acid with triglycerides

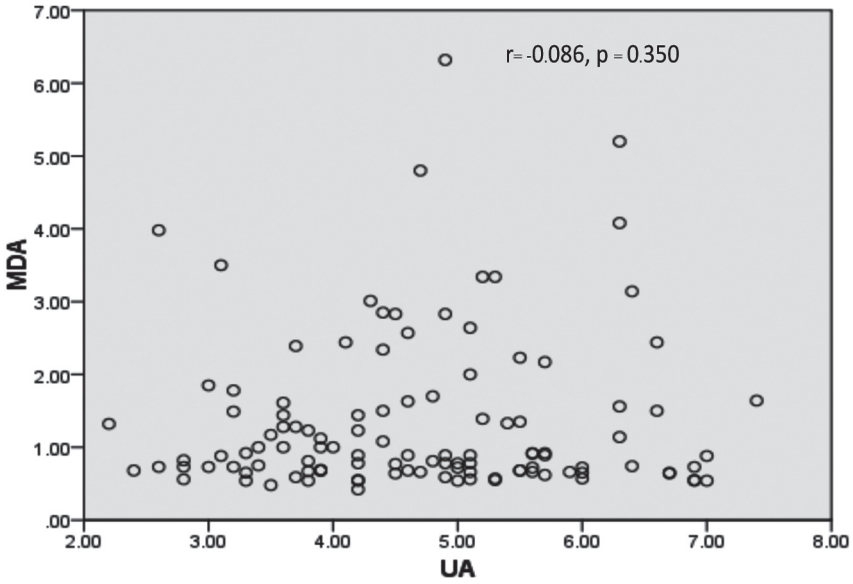
2018 identified reduced levels of UA with dyslipidaemia in type 2 diabetes subjects, and a strong negative correlation of UA with dyslipidaemia.[10] However, it is still uncertain whether UA has beneficial or harmful effects in association with other parameters in type 2 diabetes mellitus.

Correlation of UA with MDA

Malondialdehyde is an end product of lipid peroxidation by free radicals in the tissues of the body, with UA acting as either an antioxidant or pro-oxidant depending on a variety of factors. UA is a powerful signalling molecule that can affect intracellular signal transduction leading to the generation of oxidants through nicotinamide adenine dinucleotide phosphate (NADPH) oxidase and the expression of pro-inflammatory mediators.[11] In our study Figure 2 shows a slight downhill trend in UA and the values of the upper linear line scattered in a wide band represent a negative relationship of UA with MDA (r=-0.086). This indicates that UA has antioxidant properties, but without statistical significance. A study revealed increased UA levels through impaired renal excretion were observed in subjects with obesity, insulin resistance and hypertension. Another study found that under local ischemic conditions, UA production increased parallel to reactive oxygen species (ROS). However, clinical and experimental evidence suggest that antioxidant activity of UA can be overcome by the pro-oxidant and pro-inflammatory effects of ROS.[1,9] The present study found that UA was negatively correlated with MDA, indicating that UA has antioxidant properties, but our findings were not statistically significant (p-value=0.350). A greater number of clinical studies are required to establish and confirm the antioxidant activity or antioxidant properties of UA.

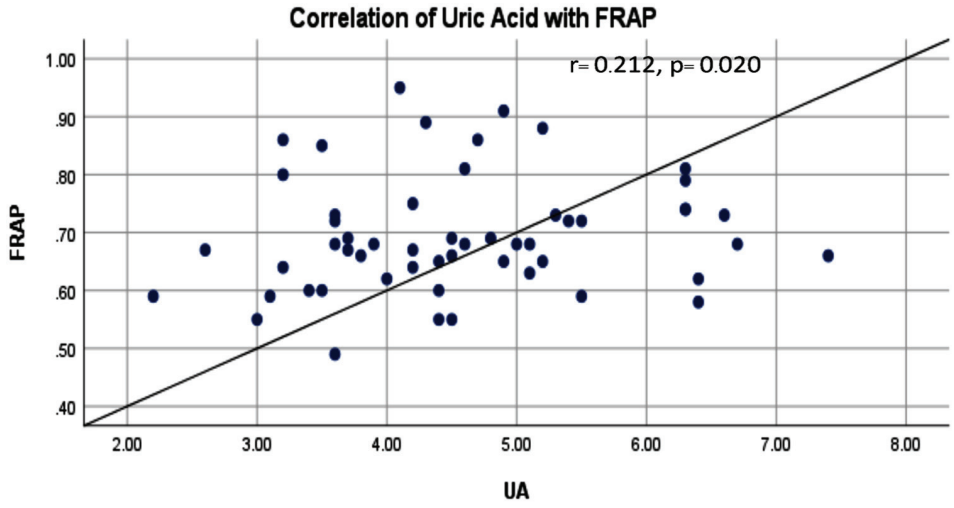
Correlation of UA with FRAP

UA is a powerful scavenger of free radicals and provides 60.0% of free radical scavenging capacity in plasma.[12] However, an experimental in-vitro study has suggested that UA acts as other antioxidants and can shift from a protective antioxidant function to a pro-oxidant effect



MDA=malondialdehyde, UA=uric acid

Figure 2 Demonstrating the correlation of uric acid with malondialdehyde



FRAP=ferric reducing ability of plasma, UA=uric acid

Figure 3 Demonstrating the correlation of uric acid with ferric reducing ability of plasma

according to its level and the microenvironment conditions.[4] Figure 3 shows a slight uphill linear pattern, with a few values scattered in a wide distribution pattern indicating a statistically significant positive relationship between the UA and FRAP (p–value<0.020). An earlier study observed higher levels of FRAP in subjects with hyperuricemia and decreased UA levels with reduced antioxidant capacity.[12] In addition, Nieto et al. found that higher levels of UA were

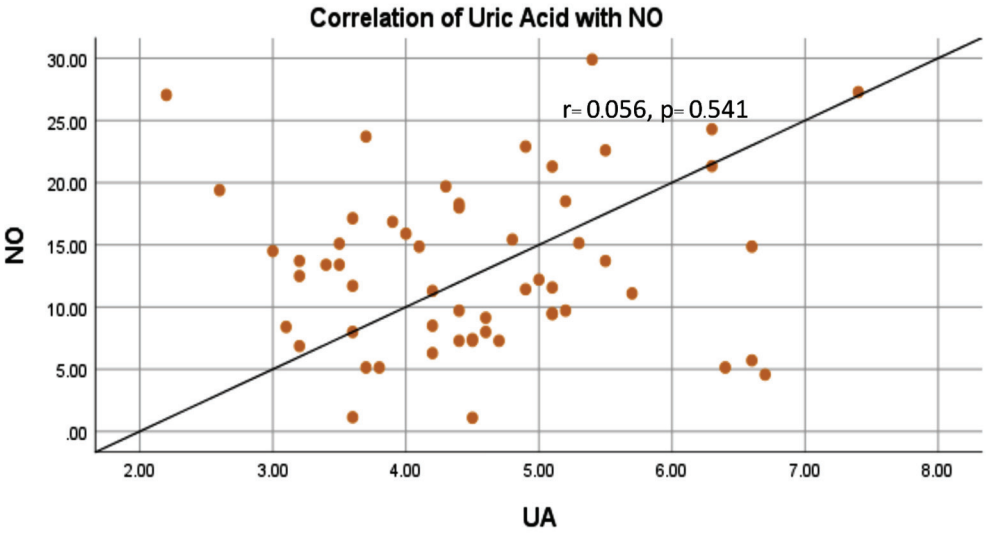
associated with elevated antioxidant capacity in individuals with atherosclerosis.[13] Glantzounis found that lowering UA with allopurinol had a protective effect in situations associated with oxidative stress (e.g. ischaemia reperfusion injury, cardiovascular disease).[1]

UA is a unique scavenger of peroxynitrite in the extracellular space. However, UA cannot scavenge superoxide, and the presence of ascorbic acid thiols is required for complete scavenging of peroxynitrite.[5] UA not only acts as a scavenger, but also stabilizes ascorbate in biological fluids. Ascorbate stabilization is particularly evident in humans and largely due to iron chelation by UA. Depletion of serum UA causes rapid oxidation of ascorbic acid, which largely depends on iron.[14] Our study found a significant positive correlation of UA with FRAP, and also a significant difference in the level of FRAP was observed in between the study groups (p-value<0.001). This indicates that UA has a higher possibility of antioxidant properties rather than pro-oxidant activity.

Correlation of UA with NO

In diabetes, hyperglycaemia may contribute to endothelial dysfunction in several ways. The reduced level of NO in diabetes is due to limited availability of NADPH (nicotinamide adenine dinucleotide phosphate), a necessary cofactor for endothelial nitric oxide synthase (eNOS), which may occur as a result of decreased activity of the pentose phosphate pathway. Oxidative stress promotes the generation of superoxide (O2-) by a number of pathways that can quench NO and reduce its bioavailability despite normal production, leading to endothelial dysfunction.[15] UA has been found to play a major role in reducing the bioavailability of NO in bovine aortic endothelial cells and adipocytes.[5]

The mechanism of how UA contributes to organ damage is still only partly understood, but there is increasing evidence that endothelial dysfunction is a fundamental mechanism through which UA may affect cardiovascular and renal function. Figure 4 shows a slight uphill linear



NO=nitric oxide, UA=uric acid

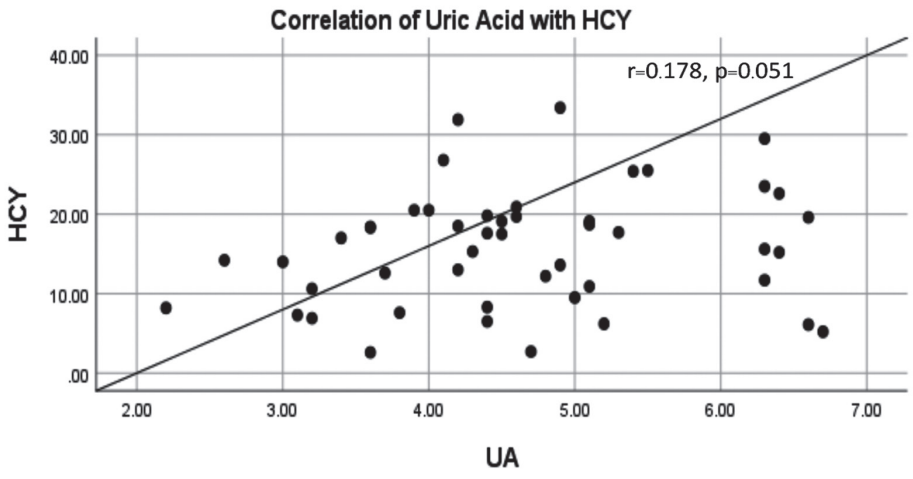
Figure 4 Demonstrating the correlation of uric acid with nitric oxide

pattern where UA values are scattered in a wide band, showing a positive relationship but statistically not significant (p-value=0.541), although earlier studies have suggested that allopurinol could lower UA by inhibiting xanthine oxidase and improve endothelial function by interacting with anion superoxide production.[3] One experimental study reported direct involvement of UA in endothelial dysfunction, but the finding remains controversial and have been contradicted by other studies.[16] Our study observed a significant difference in the level of NO between the groups (p-value<0.050), but the correlation of NO with UA was not significant. However, studies with larger sample sizes are required to further elucidate any correlations between UA and NO.

Correlation of UA with HCY

Both UA and HCY are well-known risk factors for cardiovascular disease.[17] Hyperhomocysteinemia is an important factor for cardiovascular disease. Figure 5 shows a moderate uphill linear pattern where a few UA values are scattered in a wide band, showing a positive relationship with HCY, statistically more likely significant (p-value<0.051). However, the relationship of UA with homocysteine better than those of MDA and NO. An earlier study observed that elevated levels of HCY in male gout patients and hyperhomocysteinemia were not correlated with UA, but they were inversely correlated with renal dysfunction.[18] Homocysteine triggers free radical production, impairs endothelial function and is associated with higher cardiovascular risk.[19] Our study identified endothelial dysfunction, but it was not correlated with UA or HCY.

Although UA was not significant in the study groups, it had a significant correlation with total antioxidant capacity and moderate correlation with triglycerides and homocysteine. This adds evidence to the theory that UA has antioxidant activity and reduces the risk of complications in type 2 diabetes mellitus. However, due to a moderately significant relationship of UA with triglycerides and homocysteine, UA may also contribute to pathogenesis. Hence, large-scale randomized studies are required to obtain an accurate report on the role of UA and its correlations with risk factors in type 2 diabetes.



HCY=homocysteine, UA=uric acid

Figure 5 Demonstrating the correlation of uric acid with HCY

The study sample size was low due to the exclusion criteria of excluding patients treated with antioxidants or lipid-lowering drugs or those with complications. Hence, further studies are required to elucidate the exact role of UA on oxidative stress and endothelial dysfunction in type 2 diabetes mellitus.

Conclusion

Based on our findings, we suggest that UA may have antioxidant properties since it had a significant positive correlation with FRAP, and in addition not a significant negative correlation with MDA. Our study indicates that estimation of UA in type 2 diabetes subjects may help to evaluate potential complications.

Conflict of interest

No

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