DOI: 10.7860/JCDR/2018/31649.11745

Original Article

Biochemistry Section

Association of Plasma Uric Acid with Inflammatory and Oxidative Stress Markers in Diabetic Nephropathy in North Indian Population: A Case Control Study

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ABSTRACT

Introduction: Uric acid (UA), despite being a major antioxidant in human plasma, is also associated with development of diseases associated with oxidative stress. There have been few studies exploring the relationship of Plasma Uric Acid (PUA) with oxidative stress and inflammation.

Aim: To analyse the association between UA and markers of oxidative stress and inflammation in diabetic nephropathy.

Materials and Methods: The present case control study enrolled 100 participants and were categorized into two Groups (50 each) i.e., Type 2 Diabetes Mellitus without complication (T2DM) and Type 2 Diabetes Mellitus with Nephropathy (DN). Markers of oxidative stress like reduced Glutathione (GSH), Ferric Reducing Ability of Plasma (FRAP), Glutathione-S-Transferase (GST) and Malondialdehyde (MDA) were measured spectrophotometrically. Plasma TNF-α, hsCRP, urinary MCP-1 as markers of inflammation were estimated by ELISA. PUA was measured by uricase-PAP method. Student's t-test, pearson correlation and, linear regression were used for statistical analysis.

Results: Plasma TNF-α, hsCRP, urinary MCP-1 were significantly ($p<0.001$) higher in DN as compared to patients with T2DM. GSH, FRAP and GST were lower ($p<0.001$) in DN as compared to T2DM group. However, plasma MDA was significantly higher in DN group as compared to T2DM. PUA significantly correlated negatively with GSH($r=-0.937$, $p<0.001$), FRAP ($r=-0.649$, $p<0.01$), GST ($r=-0.905$, $p<0.01$) and positively with MDA ($r=0.931$, $p<0.01$), TNF-α ($r=0.552$, $p<0.01$), hsCRP ($r=0.815$, $p<0.01$), uMCP-1 ($r=0.811$, $p< 0.001$). In multivariate analysis, PUA was associated negatively with FRAP (Model 3:$p=0.045$) and GST (Model 3:$p=0.44$) but lost significance with GSH (Model 3:$p=0.741$), MDA (Model 3:$p=0.884$). However, PUA was associated with positively with TNF-α (Model 3:$p=0.038$), hsCRP (Model 3:$p=0.036$) and uMCP-1 (Model 3:$p=0.040$).

Conclusion: PUA was associated negatively with FRAP, GST and positively with TNF-α, hsCRP, uMCP-1 in diabetic patients. These results suggest that UA contributes to oxidative stress and systemic inflammation.

Keywords: Chronic kidney disease, Diabetes mellitus, Inflammatory markers, Pro-oxidant

INTRODUCTION

Diabetic Nephropathy (DN) is increasingly acknowledged as a global public health problem [1,2]. Despite marked improvement in diabetes care in recent years, a number of diabetic patients developing nephropathy is still increasing [3]. The role of UA in the pathogenesis of DN is still debatable [4,5]. Therefore it is essential to study possible association of UA and diabetic nephropathy.

UA is an end product of degradation of purine nucleotides. In majority of mammals, urate oxidase enzyme converts urate into allantoin, significantly reducing PUA levels. However, in humans gene for urate oxidase is nonfunctional [6] resulting in PUA levels that are both higher and more fluctuating than lower mammals [7]. In last decade, studies performed in vitro and in experimental animals have reported that increased PUA levels may contribute to the development of Cardiovascular Disease (CVD), hypertension [8], T2DM [9] and renal disorders [10]. The pathogenesis of these diseases is not fully understood and seems to be extremely complex. However, it is clear that there is involvement of oxidative stress and inflammation is common in the development of these diseases [10-12].

Oxidative stress is one of the main causes of cellular function impairment. It is a condition of disproportionate production of free radicals and Reactive Oxygen Species (ROS), as well as reduced antioxidative mechanism either due to decreased intake of antioxidants or their excessive consumption [13]. Acute elevation of UA may act as antioxidant only in hydrophilic environment by scavenging singlet oxygen, peroxyl radical, hydroxyl radicals. However, chronic hyperuricaemia is associated with increased risk of stroke [14]. Despite of anticipated beneficial role of UA, elevated UA in later stages loses its antioxidant activity. It has been observed that UA degradation in later stages leads to the generation of ROS like peroxynitrite, aminocarbonyl [15]. UA also oxidizes Low Density Lipoprotein (LDL) [15]. These free radicals are predominantly harmful to lipids than any other cellular components. Kanellis J et al., further strengthened the concept that UA acts as a pro-oxidant by increasing oxygen radicals in circulation which may promote lipid oxidation and hence also lead to inflammation and vascular endothelial dysfunction [16]. The mechanism of synthesis of the ROS is very intricate and remains a topic for debate.

An experimental study has documented that circulatory UA stimulates systemic inflammation and hence leads to the development of CVD, hypertension and renal failure [16]. UA is associated with the release of various pro-inflammatory cytokines like Tumour Necrosis Factor-Alpha (TNF-α) [17], Monocyte Chemoattractant Protein-1 (MCP-1) [18] and C-Reactive Protein (CRP) [19]. This observation was however further established by a study which acknowledged that exposure of human mononuclear cells to UA produced cytokines viz., TNF-α, IL-1β, IL-6 and neutrophils produced various pro-inflammatory proteins [17]. Few experimental studies have observed

that UA is a culprit for the development of human vascular diseases via pro-inflammatory mechanism [14,17]. However, the role of UA as a causative factor of inflammation or as an indicator of pro-inflammatory state 'per se' remain unclear.

Despite our improved knowledge about pathogenesis of DN, the debate still continues whether elevated serum UA may be hazardous either by increasing oxidative stress or by stimulating inflammation leading to renal injury. Studies have not reported the association of UA with both oxidant-antioxidant parameters and inflammatory biomarkers in a single setting. Therefore, our current study was planned to explore any possible association between PUA levels and markers of oxidative stress, as well as inflammation.

MATERIALS AND METHODS

Study design: This was an age and sex matched case control study comprising of total 100 participants with T2DM. The participants were enrolled into the study while attending medicine/nephrology outpatient clinic of University College of Medical Sciences and Guru Teg Bahadur Hospital between October 2010 and April 2012. They were divided into two groups of 50 each viz., Group I: patients with Type 2 diabetes mellitus without vascular complications (T2DM), Group II: patients with Type 2 diabetes mellitus with nephropathy (DN, Stage 2 and Stage 3). According to American Diabetes Association (ADA), Stages of Chronic Kidney Disease (CKD) with respect to Glomerular Filtration Rate (GFR) (mL/min/1.73 m^2 body surface area): (a) Stage 1: kidney damage with normal or increased GFR ≥90; (b) Stage 2: kidney damage with mildly decreased GFR; 60–89; (c) Stage 3: moderately decreased GFR; 30–59; (d) Stage 4: severely decreased GFR; 15–29; (e) Stage 5: kidney failure with GFR; ≤15 as per Kidney Disease: Improving Global Outcomes, KDIGO [20]. The plan of this study was approved by Institutional Ethics Committee for Human Research. Informed and written consent were taken from all the participants earlier to the inclusion into the study. Keeping 80% probability of detecting an effect using a two-tailed test with conventional levels of alpha (0.05), a quick calculation reveals that sample size will be at least 50.

Participant's selection: The participants underwent health examination which included a detailed health questionnaire, physical examination with anthropometric measurements and necessary laboratory tests. Diabetes was diagnosed on the basis of revised ADA criteria, 2015 [21]. Nephropathy in diabetic patients was identified by qualitative analysis in early morning random midstream urine sample by dipstick method (Urine Test 11 MAU, Piramal Diagnostic, India). DN is a generic term referring to any deleterious effect on kidney structure and/or function caused by diabetes mellitus, the first being characterized by microalbuminuria and later by decreased GFR. Urinary albumin to creatinine ratio (µg/mg creatinine): (a) less than 30 is considered normal; (b) 30-300 is considered microalbuminuria; and (c) greater than 300 is considered as macroalbuminuria (overt proteinuria) [22]. It was further confirmed by measuring Urinary Albumin: Creatnine Ratio (UACR). Subjects having T2DM without any vascular complications served as a control and subjects having T2DM with nephropathy was designated as cases. All diabetic subjects with retinopathy and dipstick positive proteinuria and microalbuminuria were clubbed in Group II; however, all the patients were in pre-dialysis stage.

To avoid potential confounding factors, the patients having acute and chronic infections, fever, malignancy, other renal disorders, cirrhosis of liver and congestive heart failure, alcoholism and smoking were excluded. All the patients in Group II had retinopathy however patients with macrovascular complications like stroke and CAD were excluded. The dietary sources rich in purine like meat, seafood and sprouts were restricted from the diet of subjects for a week before the enrollment. Patients who were on inhibitors of renin– angiotensin aldosterone system, aspirin and vitamin D analogues were advised to stop these drugs for one week before inclusion in the study because these are reported to affect oxidative stress and inflammatory markers. Patients using UA lowering drugs or diuretics were also excluded.

Anthropometric Measurements and Biochemical Analysis

On inclusion into the study arterial blood pressure was measured three times on the left arm in sitting position using a clinically validated automatic device (Omron, HEM-907, Matsusaka, Japan) after applying the appropriate cuff size and after a period of 10 minutes rest with the subject in the sitting position, the average of the all three values was used for analysis. Body Mass Index (BMI) was calculated as weight (in kilogram) divided by height (in meter square). Blood samples were collected in the morning after the participants had been fasting for at least 8 hours-12 hours. Blood was collected into vacutainers coated with salts of fluoride and oxalate for plasma glucose, EDTA vials for various other biochemical parameters. Blood sample collected in EDTA vials was subjected to centrifugation at 3000 rpm for 10 minute to separate the plasma at room temperature. For estimation of TNF-α and hsCRP, plasma was stored in aliquots at –80°C. Urine samples for evaluation of uMCP-1 were also stored at –80°C till further estimation. All these investigations were carried out once at the time of entry into the study.

Plasma glutathione-S-transferase; GST [23], antioxidant capacity of blood measured as Ferric Reducing Ability of Plasma; FRAP [24], malondialdehyde; MDA [25] and reduced blood glutathione; GSH [26] were estimated immediately after collection. Plasma TNF-α (Diaclone, France), hsCRP (Calbiotech, USA) and uMCP-1 (Weldon, California) were measured by sandwich enzyme linked immunosorbent assay. The sensitivity for TNF-α, hsCRP, uMCP-1 was less than 8 pg/mL, 0.005 mg/mL and 7.8125 pg/Ml respectively. Glycosylated haemoglobin (HbA1c) was estimated by ion-exchange resin chromatography using commercially available kits (Fortress Diagnostics, UK). The detection limit was < than 4.3%.

Fasting and postprandial glucose, urea, creatinine, UA, total cholesterol, TAG, HDL, LDL, VLDL as routine biochemical parameters were assayed in automated analyser (Olympus AU 400) using commercial kits.

STATISTICAL ANALYSIS

Continuous variables were reported as mean and Standard Deviation (SD). Demographic and clinical data between two groups were analysed by Student's t-test. Relationship between studied parameters was determined by Pearson correlation analysis. We examined the association of UA (independent variable) levels with oxidative stress parameters and inflammatory markers as dependent variables using linear regression analysis. Since PUA concentration depends on various co-variables therefore analysis was conducted without adjustment (Model 1) followed by analysis with age, sex adjustment (Model 2) and further adjustment which include age, sex along with BMI, arterial blood pressure, HbA1c, total cholesterol (Model 3) and $p<0.05$ was considered significant.

RESULTS

The demographic and clinical characteristics of the study population are depicted in [Table/Fig-1]. The participant were age (p=0.686) and sex matched (p=0.974). No significant difference was observed with respect to blood pressure i.e., Systolic Blood Pressure (SBP, p=0.077) and Diastolic Blood Pressure (DBP) (p= 0.247) and BMI (p=0.336) between both the study groups. PUA was significantly higher in Group II subjects (8.55±0.7, p<0.001) than in patients of Group I (3.40±0.64). Plasma urea and creatinine levels were higher in Group II when compared to Group I. Fasting, postprandial plasma glucose and HbA1c were significantly higher (p< 0.001) in Group II than Group I suggestive of poorer glycaemic control in later Group.

Lipid profile depicted by total cholesterol, TAG, LDL-C, VLDL-C, total cholesterol to HDL-C ratio and LDL-C to HDL-C ratio were significantly higher in Group II (p<0.001) patients as compared to Group I. However, HDL-C levels were lower in Group II patients as compared to patients with Group I. Estimated Glomerular Filtration Rate (eGFR) was significantly lower in patients of Group II (p<0.001) as compared to Group I.

Variables	Group I (n=50)	Group II (n=50)	p-value
Age (years)	56.46±8.5	55.78±8.3	0.686
Sex ratio (Male/Female)	26/24	28/22	0.974
BMI (Kg/m^2)	22.10±2.1	22.65±1.4	0.336
SBP (mm/Hg)	136.02±2.3	136.76±1.9	0.077
DBP (mm/Hg)	82.64±0.9	82.84±0.8	0.247
Urea (mg/dL)	25.79±5.9	91.52±4.8	<0.001
Creatinine (mg/dL)	0.95±0.2	3.66±1.5	<0.001
Uric acid (mg/dL)	3.40±0.64	8.55±0.7	<0.001
Fasting glucose (mg/dL)	143.58±4.5	187.30±16.25	<0.001
Postprandial glucose (mg/dL)	186.74±11.15	258.94±31.3	<0.001
HbA1c (%)	7.27±0.2	8.13±0.2	<0.001
Total Cholesterol (mg/dL)	177.00±16.5	257.16±26.6	<0.001
TAG (mg/dL)	142.46±13.04	199.32±25.03	<0.001
HDL-C (mg/dL)	34.66±4.9	32.40±4.9	<0.001
LDL-C (mg/dL)	114.25±17.7	185.79±29.0	<0.001
VLDL-C (mg/dL)	28.09±2.8	39.86±5.0	<0.001
Total Cholesterol/ HDL-C ratio	5.23±1.0	8.15±1.7	<0.001
LDL-C/HDL-C ratio	3.41±0.9	5.92±1.6	<0.001
eGFR (mL/min/1.73 m^2)	97.4±0.6	56.2±0.91	<0.001
UACR (mg/g of creatinine)	-	183.56±15.2	<0.001

[Table/Fig-1]: Characteristics of the overall study population.
Data are expressed as mean+SD.
Group I: Control (T2DM); Group II: Cases (DN)
BMI: Body Mass Index; SBP and DBP: Systolic and Diastolic Blood Pressure; TAG: Triacylglycerol; HDL-C: High Density Lipoprotein- Cholesterol; LDL-C: Low Density Lipoprotein- Cholesterol; VLDL-C: Very Low Density Lipoprotein-Cholesterol; eGFR: Estimated Glomerular Filteration Rate; UACR: Urinary Albumin Creatinine Ratio; p<0.05 is considered significant
Test employed: Student's t test

The comparison of mean levels of estimated oxidative stress and inflammatory markers is presented in [Table/Fig-2]. Whole blood reduced GSH and plasma FRAP as antioxidant markers are significantly reduced in Group II patients in comparison to patients of Group I. In contrast, plasma MDA levels were significantly raised in Group II as compared to Group I patients. However, plasma GST levels showed a different behaviour, it was significantly raised in Group I as compared to Group II. Mean plasma levels of TNF-α, hsCRP and uMCP-1 as markers of inflammation were significantly raised in Group II patients when compared to Group I subjects. [Table/Fig-3,4] describes the Pearson correlations of different oxidative stress markers and inflammatory cytokines with PUA in overall study population and individual study groups. With the exception of MDA which had an inverse association with uric acid, all oxidative stress markers showed a highly significant (p<0.001) negative correlation with UA. On studying the association of inflammatory markers with UA, it was observed that all markers of inflammation i.e., TNF-α (p<0.001, r = 0.552), hsCRP (p<0.001, r = 0.815), uMCP-1 (p<0.001, r = 0.811) showed a significant positive correlation with UA. These parameters when studied in individual study group, it was noticed that they follow the similar trend but lost their significance.

In the linear regression Model unadjusted for covariates, plasma UA expressed as a continuous variable was found to have a significantly negative association with levels of GST and FRAP ([Table/Fig-5], Model 1). However PUA was found to have negatively correlated with GSH and positively with MDA but were not statistically significant (p=0.935, p=0.984). After adjustment for the first set of covariates, the beta coefficients for PUA were slightly increased and they remained statistically significant except for GSH and MDA ([Table/Fig-5], Model 2). After further adjustment, the results were virtually similar ([Table/Fig-5], Model 3).

While exploring the relationship between PUA and markers of inflammation, linear unadjusted models, UA was a significant positive predictor of TNF-α, hs-CRP except uMCP-1 ([Table/Fig-6], Model 1). On adjustment for age and sex, the beta coefficients for PUA were slightly reduced and they remained statistically significant ([Table/Fig-6], Model 2). After additional adjustment for (BMI, Total cholesterol, Blood pressure) the association remains unchanged ([Table/Fig-6], Model 3).

DISCUSSION

The present case-control study was designed to find out the role of UA in the pathogenesis of DN. The main strength of this study is that we address the association of PUA levels with oxidative stress and inflammatory markers taking into consideration of all confounding factors under one umbrella. Data of the present study suggests that the levels of antioxidant were further reduced and pro-oxidant were raised in patients with DN in comparison to patients with T2DM without nephropathy, which was reported in our previous study [11]. Our results were supported by a study [27] which reported that there was a significant decrease in reduced GSH and increase in MDA levels in diabetic patients with micro-vascular complications. Our results were further confirmed by a related recent study [28] that showed that levels of MDA were increased and FRAP levels were decreased in patients with DN. However, GST behaved in a subtle dissimilar manner. Various studies have reported ambiguity

	Group I (n=50)	Group II (n=50)			p value
Oxidative stress markers		Total	Stage 2	Stage 3	
GSH (mg/g Hb)	1.89±0.06	0.90±0.01[a]	0.96±0.02	0.91±0.01	<0.001
GST (nmol/min)	8.26±0.37	7.38±0.10[a]	6.91±0.05	4.22±0.39	<0.001
FRAP (µmol/L)	409.0±55.11	170.7±14.3[a]	200.7±14.2	165.3±8.12	<0.001
MDA (nmol/mL)	2.60±0.35	5.14±0.39[a]	5.01±0.10	6.20±0.04	<0.001
Inflammatory markers					
TNF-α (pg/mL)	15.3±3.7	20.6±3.9[a]	17.1±0.2	19.7±0.8	<0.001
hsCRP (mg/L)	3.6±1.5	8.5±1.7[a]	5.9±0.5	7.3±0.1	<0.001
uMCP-1 (pg/mg creatinine)	278.5±125.0	5632.7±2275.8[a]	3172.7.3±311.2	5099.2±222.7	<0.001

[Table/Fig-2]: Mean plasma levels of oxidative stress and inflammatory markers in study population.
Group I: Control (T2DM); Group II: Cases (DN)
[a]Significantly different from Group I: Control (T2DM) at p < 0.001
GSH: Reduced Glutathione, GST: Glutathione-S-Transferase, FRAP: Ferric Reducing Ability of Plasma, MDA: Malondialdehyde
TNF- α: Tumor Necrosis Factor-Alpha, hsCRP: High Sensitive C-Reactive Protein, uMCP-1: Urinary Monocyte Chemoattractant Protein-1
Date are expressed as mean±SD
Test employed: Student's t test

Parameters	Group I (r, p value) (n=50)	Group II (r, p value) (n=50)	Group I & II (r, p value) (n=100)
GSH (mg/g Hb)	-0.059, 0.686	-0.0.20, 0.892	- 0.937, <0.001
GST (nmol/min)	-0.192, 0.272	-0.304, 0.032	- 0.905, <0.001
FRAP (µmol/L)	-0.158, 0.282	-0.238, 0.056	- 0.649, <0.001
MDA (nmol/mL)	0.074, 0.610	0.051, 0.726	0.931, <0.001

[Table/Fig-3]: Correlation between uric acid and various oxidative stress parameters.
Group I: Control (T2DM); Group II: Cases (DN)
GSH: Reduced Glutathione, GST: Glutathione-S-Transferase, FRAP: Ferric Reducing Ability of Plasma, MDA: Malondialdehyde; p<0.05 is considered significant; Test employed: Pearson correlation

Parameters	Group I (r, p value) (n=50)	Group II (r, p value) (n=50)	Group I & II (r, p value) (n=100)
TNF-α (pg/mL)	0.161, 0.264	0.127, 0.378	0.552, <0.001
hsCRP (mg/L)	0.009, 0.949	0.125, 0.388	0.815, <0.001
uMCP-1 (pg/mg creatinine)	0.102, 0.482	0.206, 0.152	0.811, <0.001

[Table/Fig-4]: Correlation between uric acid and studied inflammatory markers.
Group I: Control (T2DM); Group II: Cases (DN)
TNF- α: Tumor Necrosis Factor-Alpha, hsCRP: High Sensitive C-Reactive Protein, uMCP-1: Urinary Monocyte Chemoattractant Protein-1; p<0.05 is considered significant
Test employed: Pearson correlation

Overall Population	GSH (mg/g Hb)	GST (nmol/min)	FRAP (µmol/L)	MDA (nmol/mL)
Model 1 β±SE p value	 -0.001±0.016 0.935	 -0.117±0.063 0.048	 -9.85±6.13 0.050	 0.001±0.051 0.984
Model 2 β±SE p value	 -0.003±0.017 0.834	 -0.123±0.065 0.045	 -10.65±6.10 0.045	 0.020±0.052 0.912
Model 3 β±SE p value	 -0.006±0.017 0.741	 -0.125±0.066 0.044	 -10.94±6.21 0.045	 0.001±0.051 0.884

[Table/Fig-5]: Linear regression models on the relationship between uric acid and oxidative stress markers.
Model 1:Unadjusted for various co variables; Model 2: Adjusted for age and sex
Model 3: Adjusted for age, sex, BMI, Total cholesterol, Blood pressure
GSH: Reduced Glutathione, GST: Glutathione-S-Transferase, FRAP: Ferric Reducing Ability of Plasma, MDA: Malondialdehyde;
β±SE: β Coefficients±Standard errors, p<0.05 is considered significant

Overall population	TNF-α (pg/mL)	hsCRP (mg/L)	uMCP-1 (pg/mg creatinine)
Model 1 β±SE p value	 0.071±0.016 0.044	 0.222±0.24 0.050	 454.23±35.10 0.049
Model 2 β±SE p value	 0.066±0.019 0.041	 0.210±0.24 0.036	 330.26±2.44 0.046
Model 3 β±SE p value	 0.053±0.017 0.038	 0.184±0.25 0.036	 207.66±4.72 0.040

[Table/Fig-6]: Linear regression models on the relationship between uric acid and inflammatory markers.
Model 1:Unadjusted for various co variables; Model 2: Adjusted for age and sex
Model 3: Adjusted for age, sex, BMI, Total cholesterol, Blood pressure
TNF- α: Tumor Necrosis Factor-Alpha, hsCRP: High Sensitive C-Reactive Protein, uMCP-1: Urinary Monocyte Chemoattractant Protein-1
β±SE: β Coefficients±Standard errors, p<0.05 is considered significant

in activity of GST in response to Oxidative Stress (OS) in diabetic patients [29,30]. Levels of GST is anticipated to increase with increased OS, however it seems that heightened levels of GST in T2DM is a compensatory mechanisms of antioxidants against OS in humans, which protects the progression from DM to DN. The study conducted by Yang Y et al., has reported that higher expression of GSTs in the endothelium shield against oxidative damage from aldehydes such as 4-hydroxynonenal (4-HNE) [31]. It has been reported in the previous study that there is inhibition of RBC-GST activity by uremic plasma [32]. Hence, there is a chance that the presence of uremic toxins may contribute to reduced GST activity in patients with DN most certainly by interfering with its induction.

To our best knowledge, this is the first case control study to assess the relationship between PUA and various oxidative stress markers. In the current case control study, as depicted in [Table/Fig-3] we found a significant negative association of PUA with anti-oxidant parameters i.e., GSH, FRAP, GST and significantly positive correlation pro-oxidant i.e., MDA. Indeed, GST and FRAP remained significantly negative correlated with PUA even after adjustment for important potential confounders. These result supported the idea that UA may be involved in the progression of micro-vascular complications in diabetes mellitus through production of ROS. Oxidative stress plays a key role in steatosis induced by UA. Cellular exposure to increased UA leads to mitochondrial oxidative stress with generation of ROS by Nicotinamide Adenine Dinucleotide Phosphate (NADPH) oxidase. As a result of which the activity of aconitase, an enzyme involved in the kreb's cycle was inhibited leading to accumulation of citrate. Since citrate is a substrate of de novo lipogenesis and therefore increased fat generation, hence more lipid peroxidation. Furthermore, ROS production increases endoplasmic reticulum stress, which is a determinant of fat accumulation [33]. Thus, elevated PUA contributes to oxidative stress within the glomerulus and tubulo-interstitium leading to endothelial dysfunction, thrombogenesis and remodeling fibrosis of kidney [34]. It has been reported in a previous study that UA activates MAPK pathway via oxidative stress, causes preglomerular arteriolar damage [35]. Correlation of PUA with FRAP is not in accordance with study done by Lyngdoh T et al., [36]. However, till date there is no study to support our findings of association of PUA with GSH, GST and MDA.

A recent study has reported that elevated UA levels upregulate Notch-1 expression and consequently leading to inflammatory reaction via Notch signalling pathway [37]. It has been established that PUA when entering the vascular smooth muscle cell stimulates the release of CRP and chemokine MCP-1. UA also stimulates human mononuclear cell to produce TNF-α, IL-1, IL-6. The finding from the present study also showed that PUA is positively associated with markers of inflammation viz., TNF-α, uMCP-1, hsCRP. This positive significant association was still maintained after adjustment for important confounding factors. Thus, these finding supports the hypothesis that UA is involved in inflammation by triggering the release of inflammatory mediators. Few studies have investigated the relationship between PUA and systemic inflammation. Our results were in accordance to the study conducted in patients of congestive heart failure by Leyva F et al., [12]. A study conducted on healthy people demonstrated that UA was associated positively with CRP [37]. Serum UA was positively associated with TNF-α, CRP in elderly people in Italy [37] whereas, UA levels was not associated with TNF-α and CRP in elderly men in Taiwan [3].

LIMITATION

The potential limitation of this study is small sample size and results need to be validating in larger sample size. Other limitation is that genetic factors influencing the levels of UA has not been considered in our study which may have an impact on the relationship between UA and progression of DN. Further experimental or interventional studies are needed to explore the relationship between UA and DN.

CONCLUSION

In conclusion, elevated PUA levels might contribute to the vicious cycle of inflammatory responses (chemokines) and oxidative stress (production of ROS). Our findings propose that PUA levels may be an important risk factor for further progression of renal dysfunction. Thus, our study provides new evidence that lowering the UA levels may represent a new therapeutic approach in the treatment of DN.

ACKNOWLEDGEMENTS

The present study was financially supported by Indian Council of Medical Research and Postgraduate Research Grant, University College of Medical Sciences, New Delhi. The authors are thankful to the Department of Biostatistics and Medical Informatics, University College of Medical Sciences, Delhi for statistical analysis.

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FINANCIAL OR OTHER COMPETING INTERESTS: As declared above.

Date of Submission: **Jul 14, 2017**
Date of Peer Review: **Sep 26, 2017**
Date of Acceptance: **May 11, 2018**
Date of Publishing: **Jul 01, 2018**