ISSN: 2395-6429

INTERNATIONAL JOURNAL OF CURRENT MEDICAL AND PHARMACEUTICAL RESEARCH

IJCMPR

Available Online at http://www.journalcmpr.com

DOI: http://dx.doi.org/10.24327/23956429.ijcmpr20170236

CASE STUDY

HYPERURICEMIA IS IMPLICATED IN VASCULAR COMPLICATIONS OF TYPE 2 DIABETES MELLITUS

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ARTICLE INFO

Article History:

Received 20th June, 2017
Received in revised form 2nd July, 2017
Accepted 27th August, 2017
Published online 28th September, 2017

Key words:

uric acid, type 2 diabetes mellitus, vascular complications

ABSTRACT

Background: Complications due to type 2 diabetes mellitus (T2DM) are a major cause of disability, reduced quality of life and death. Serum uric acid (SUA) may endanger organ damage in DM through endothelial dysfunction. But the data suggestive of whether SUA is also associated with the development of vascular complications in T2DM is scarce, and controversial. The aim of our study is to ascertain the role of elevated SUA in the development of complications in T2DM by evaluating endothelial dysfunction by analysing serum nitric oxide (NO) and high senstivity-CRP (hs-CRP) levels and correlating them with SUA levels in patients of T2DM.

Materials and methods: 100 T2DM patients diagnosed by ADA criteria were taken (group I). Group II (n=100) consisted of healthy controls. Group I was further divided into 2 subgroups, subgroup IA were diabetics with complications and subgroup IB were diabetics without complications. Fasting blood sugar (FBS), serum lipid, serum NO, serum hs-CRP and SUA were determined in all.

Results: Mean SUA in diabetics with complication was higher than that of diabetics without complications and that of controls. UA level in diabetics without complication was significantly higher than that of controls but in normal reference range. Diabetics with complications had decreased level of NO and increased level of hs-CRP as compared to diabetics without complication and controls. There was significant correlation between SUA concentration and hs-CRP. Decrease in serum NO level is associated with increase in SUA, but no direct correlation was found between these parameters. However hs-CRP shows negative correlation with NO.

Conclusion: Diabetics with complications have hyperuricemia and have low levels of serum NO & high levels of hs-CRP. This indicates hyperuricemia inactivates serum NO and decreases its bioavailability, increasing endothelial dysfunction. Thus hyperuricemia should alert clinicians to take therapeutic measures which will help in reducing risk of life threatening complications of T2DM.

INTRODUCTION

Type 2 diabetes mellitus (T2DM) is undoubtedly one of the most challenging health problems in the 21st century and the number of diabetic patients diagnosed has reached 366 million in 2011.[1] Complications due to diabetes are a major cause of disability, reduced quality of life and death. The number of patients diagnosed each year with macrovascular and microvascular complications attributed to T2DM is rising.[2,3] Cardiovascular diseases are the leading causes of death for people with T2DM. Therefore, much epidemiologic evidence was committed to risk factors related to development of T2DM and its complications.

Despite significant progress in diagnosis, treatment and prevention, diabetes particularly continues to remain the leading cause of hyperlipidemia, endothelial dysfunction, coronary artery diseases, metabolic syndrome and represents global socioeconomic burden.[4] Serum uric acid (SUA), the product of purine metabolism, used to be thought predominantly as a predictor of gouty diathesis.[5] However, as a member of metabolic syndrome, uric acid (UA) could worsen insulin resistance by disturbing insulin-stimulated glucose uptake.[6] It is also implicated as one of the potential risk factors associated with the complications above mentioned. The probable mechanism by which UA may

endanger organ damage is through endothelial dysfunction whereby it may affect cardiovascular function and structure.[7]
UA is the most abundant antioxidant in plasma, reacts directly with nitric oxide (NO) in a rapid irreversible reaction resulting in the formation of 6-aminouracil and depletion of NO, which is an endothelial cell derived relaxing factor. The reduction in endothelial NO level leads to endothelial dysfunction preceding the development of hypertension, arterial stiffness and cardiovascular diseases.[8] C- reactive protein (CRP) is an acute phase protein produced by liver in response to interleukin -6 (IL-6) stimulation and is raised in most conditions associated with infection, inflammation, or tissue damage, for which it is a sensitive marker.[9] Studies have also shown that CRP is an independent determinant of endothelium-dependent vascular function in both diseased and healthy subjects.[10,11]

But the data suggestive of whether SUA is also associated with the development of vascular complications in T2DM is scarce, and controversial. Recently, Kim *et al* suggested that elevated SUA was associated with diabetic nephropathy.[12] Zoppini *et al* found that elevated SUA concentrations independently predicted cardiovascular mortality in T2DM.[13] However, Ong *et al* revealed that SUA did not predict cardiovascular complications in T2DM.[14] Various types of study populations and study designs might have contribution to these disparate findings. Thus, the aim of our study is to ascertain the role of elevated SUA in the development of complications in T2DM by evaluating endothelial dysfunction by analysing NO and high senstivity-CRP (hs-CRP) levels and correlating them with SUA levels in patients of T2DM.

MATERIALS AND METHODS

A total 100 patients with T2DM from the outpatient Endocrinology clinic at the Pt. B.D.S. PGIMS, Rohtak were included in this study. T2DM was diagnosed based on the American Diabetes Association (2011) criteria. All of them were being treated by antidiabetic oral agents or insulin at the time of the study. This study was carried out after obtaining approval from ethical committee of Pt. B.D.S. PGIMS, Rohtak. All the subjects were divide in two groups; Group I (n=100) was type 2 diabetic patients, Group II (n=100) consisted of age and sex matched healthy controls. Group I was further divided into 2 subgroups, subgroup IA were diabetics with complications and subgroup IB were diabetics without complications. The diabetic complications primarily analysed were: nephropathy (microalbunimia), neuropathy, retinopathy (ophthalmic examination), cardiovascular disease (angiography), cerebrovascular diseases.

The individuals excluded were, the pregnant women, those with current illness (such as hepatic, cardiac, COPD, immunological disorder or renal disease), those taking drugs like diuretics, antihypertensive or, lipid-lowering agents, hyper or hypouricemic agents, excess vitamin supplements and hormone replacement for menopause, those with hypo or hyperthyroidism. Patients with cerebrovascular disease (defined as angina, myocardial infarction, coronary or peripheral revascularization procedures, and stroke), kidney disease, peripheral and autonomic nervous tissue disease and eye changes due to causes other than diabetes, those with HIV positive status were also excluded. For biochemical analysis, after an overnight fast, blood was taken from a forearm vein. The blood was clotted and centrifuged after half an hour to separate serum which was used for the determination of fasting blood sugar (FBS), serum lipid, serum NO, serum hs-CRP and SUA concentrations. FBS was measured using a glucose oxidase method[15] which is available as a kit manufactured by Transasia. SUA was assessed by uricase enzymatic method [16] using Transasia uric acid kit. Serum lipid profile was assessed by enzymatic method. Serum NO was estimated by Griess reagent.[17] Hs –CRP was estimated using ELISA method.[18]

Statistical Methods: Paired t-test was used to compare the results of various parameters among the studied groups. Linear regression analysis (Person correlation coefficient, r) was performed for determining the degree of association between different parameters. All values expressed as mean±SD, and p values of ≤ 0.05 was considered to be statistically significant.

RESULTS

100 diabetic patients were analysed for all the required parameters after assessing their anthropometric measurements. The mean age of the subjects included was 55.82 $\pm$ 1.29 yrs. Clinical and biological data of diabetic subjects are summarized in Table-I.

The mean SUA in diabetics with complication was 9.24 $\pm$ 0.33 mg/dl, which was significantly higher than 4.43 $\pm$ 0.03 mg/dl of diabetics without complications and that of controls. The UA level in diabetics without complication was significantly higher than that of controls but in normal reference range, but in diabetics with complications it was much outside reference range (hyperuricemia). The relationship between SUA concentration & endothelial dysfunction parameters is shown in table II, fig1. In our study, we found diabetics with complications had decreased level of NO (40.65 $\pm$ 2.51 µmol/l) and increased level of hs-CRP (3.47 $\pm$ 0.18 mg/l) as compared to diabetics without complication and controls. This is depicted in table 2, figure 2 and 3. SUA in diabetics without complications were significantly high than the controls but still in normal range. The hs-CRP in diabetics without complication was significantly higher as compared to controls (table 2, fig 3).

There was significant correlation between SUA concentration and hs-CRP but no correlation between SUA and serum NO, as shown in table- III. Decrease in serum NO level is associated with increase in SUA, but no direct correlation was found between these parameters. However hs-CRP shows negative correlation with NO (r=-.565, p<.001). Fig 4 shows correlation between serum uric acid and hs-CRP.

Table I Baseline characteristics of roup I (mean $\pm$ S.E.)

Age	55.82 + 1.29 years
Male : Female	53 : 47
BMI	26.58 $\pm$ 0.45 kg/m^2
Fasting Glucose	150.42 $\pm$ 5.68 mg/dL
Post Prandial Glucose	246.48 $\pm$ 8.30 mg/dL
HbA1c (%)	8.99 $\pm$ 0.23 %
Serum Triglycerides	186.97 $\pm$ 9.28 mg/dL
Serum Cholesterol	178.35 $\pm$ 5.27 mg/dL
HDL- Cholesterol	41.34 $\pm$ 3.10 mg/dL
LDL- Cholesterol	102.78 $\pm$ 3.94 mg/dL
VLDL- Cholesterol	38.18 $\pm$ 2.04 mg/dL
Blood urea	42.51 $\pm$ 3.50 mg/dL
Serum creatinine	1.07 $\pm$ 0.06 mg/dL

DISCUSSION

Variations in UA levels have been increasingly associated with insulin resistance, hyperinsulinemia, and T2DM.[19-22]

Table II Comparison of mean of serum uric acid levels and endothelial function parameters in patients with complications, without complications and controls (Mean ± S.E.)

parameters	subgroup ia	subgroup ib	controls
Serum uric acid (mg/dL)	9.24 ± 0.33*/**	4.43 ± 0.03*	3.41 ± 0.08
Serum NO (µmol/L)	40.65 ± 2.51*/**	63.16 ± 2.34	67.10 ± 1.54
Serum hs-CRP (mg/L)	3.47 ± 0.18*/**	1.13 ± 0.10*	0.93 ± 0.04

•* Significant with respect to controls
•** significant with respect to subgroup I B

Table III Pearson's correlation of serum uric acid and endothelial function parameters in diabetics

Parameters	Serum NO		Serum hs-CRP	
	r	p	r	p
Serum uric acid	-.092	.363	.208*	.038
Serum NO	-	-	-.565**	<.001

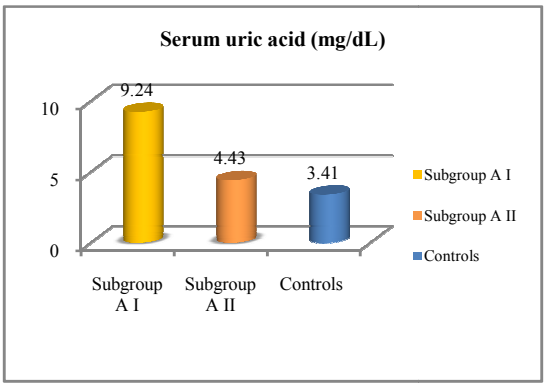

Fig1 Comparison of serum uric acid levels in patients with complications, without complications and controls

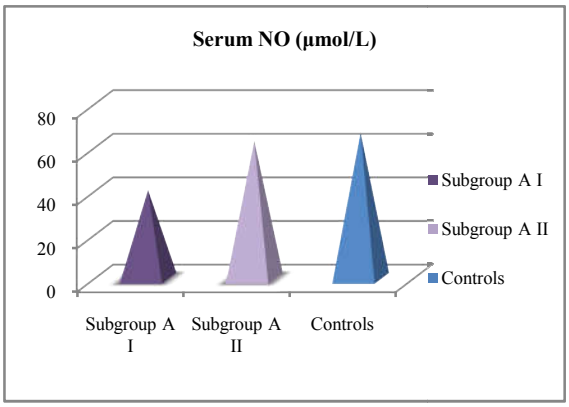

Fig 2 Comparison of serum nitric oxide levels in patients with complications, without complications and contols

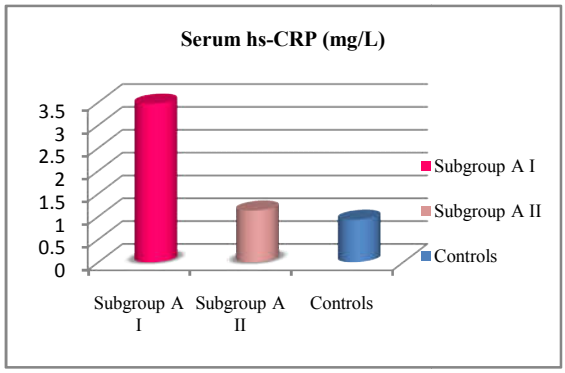

Fig 3 Comparison of serum hs-CRP levels in patients with complications, without complications and controls

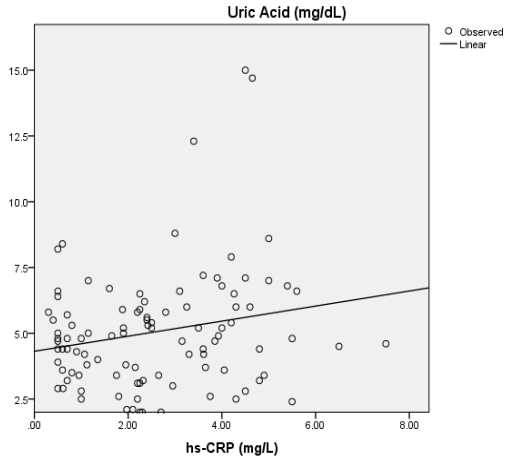

Fig 4 Relation between hs-CRP and serum uric acid in diabetics

In T2DM, hyperuricemia seems to be associated with the insulin-resistance syndrome, endothelial dysfunction, coronary artery disease, arterial stiffness. Our study showed the mean UA in patients was 5.05 ± 0.23 mg/dL as compared to the mean of 3.41 ± 0.08 mg/dL in controls. This is in surrogate with the findings of Masum *et al*[23] and Ogbera *et al*[24] in diabetics. Dehghan *et al*[25] demonstrated that SUA is a strong and independent risk factor for diabetes in a 10-year follow-up study. UA, an antioxidant becomes a pro-oxidant & can induce oxidative in cells, as soluble UA is capable of entering smooth muscle cells with deleterious effects. After uptake through a nonenzymatic organic anion transport system, UA activates vascular smooth muscle mitogens, which result in the cellular proliferation that is characteristic of atherosclerosis.[26]

In diabetic patients' higher UA levels becomes an additional risk as due to hyperglycemia, glucotoxicity places an additional burden of redox stress on the arterial vessel wall and capillary endothelium. Hyperglycemia induces both an oxidative stress and a reductive stress through pseudohypoxia with the accumulation of NADH and NAD(P)H in the vascular intima.[27,28] This redox stress consumes the natural occurring local antioxidants such as: SOD, GPX, and catalase. Once these local intimal antioxidants are depleted UA can undergo the paradoxical antioxidant – prooxidant switch or the urate redox shuttle.[29,30] Other possible mechanisms proposed to link association of UA to diabetes and endothelial dysfunction are as follows:

1. Abnormal β3 adrenergic receptor activity could result in increased insulin resistance by increasing delivery of non esterified fatty acid from intra-abdominal fat stores of the portal circulation. The presence of the Arg 64 allele in the β3-AR gene has also been shown to predispose patients to SUA level.[31]
2. UA alters the primary function of the beta cell by favouring arginine residue to combine with a critical site of the cell, which is a stimulator of insulin secretion.[32]
3. In addition, UA entering the adipocyte through URAT 1, may downregulate expression of XOR, which (xanthine dehydrogenase but not xanthine oxidase) is known as a crucial upstream regulator of activity of PPAR-γ, a master-regulator of adipogenesis, and involved in insulin resistance.[33]
4. Five novel loci have been shown to be associated with SUA at genome level. The novel loci include the glucokinase regulator protein (GCKR) having the same

single nucleotide polymorphism (SNP), as has previously been strongly associated with triglycerides, glucose and insulin levels. Glucose-6-phosphate thus formed is both a precursor for liver glycogen synthesis and a precursor for *de novo* purine synthesis. The GCKR SNP affects both SUA concentrations and insulin levels via some common, unmeasured mediator, linking diabetes and UA.[34]

5. UA levels are also associated with a domain containing a scaffolding protein reported to interact with proteins thought to relate to uric acid handling (URAT1, NPT1). This domain is also related to inducible NO synthase (NOS) activity and several ion exchange proteins, linking endothelial function with UA.[34]

Comparing mean SUA levels in diabetics with complications and without complications, UA was significantly raised in diabetics with complications and was in hyperuricemic range (9.24 ± 0.33 mg/dL) as compared to 4.43 ± 0.03 mg /dL in diabetic without complications (table 14, fig 15). Causevic *et al*[35] showed diabetic patients who are hyperuricemic appear to be at increased risk for developing diabetic complications, especially renal and cardiovascular disease. Study done by Hayden and Tyagi reported that elevation of UA > 4 mg/dL should be considered a "red flag" in the patients who are at a risk for cardiovascular disease.[36] In a study done in Thai Chuengsamarna *et al*[37] found significant odds ratios between UA levels and several chronic vascular complications. Prevalence of chronic vascular complications in T2DM patients, namely coronary arterial disease, cerebrovascular disease, diabetic nephropathy, diabetic retinopathy, and diabetic peripheral neuropathy was significantly correlated with increase of UA level [2.29 (1.01–5.2), 16.01 (4.74–54.09), 9.99 (4.4–22.8), 4.43 (1.3–15.1), 4.37 (1.5–12.9)], and concluded elevated UA levels were significantly associated with diabetic chronic micro/macro-vascular complications.[37] Thus increased level of SUA definitely should be considered as one of the injurious stimuli to arterial cell wall and endothelial dysfunction. It accelerates the atherosclerotic condition & makes the intima acidic,[38] which is associated with uncoupling of the endothelial NOS (eNOS) enzyme and a decreases locally produced NO and depletes bioavailability.

It should be pointed out that not only observational but also experimental studies have been able to demonstrate an association between inflammation and endothelial dysfunction in humans. In addition to being a marker of inflammation, CRP may have direct detrimental effects on vascular tissues, which may partly explain the association of insulin resistance with inflammation and endothelial dysfunction.[39] Results presented by Cleland *et al* show a relationship between low-grade inflammation and basal endothelial NO synthesis.[39]

Additionally, intrinsic properties of the injured endothelium result in vasoconstriction, smooth cell proliferation, coagulation disorders, leukocyte aggregation, thrombosis, and vascular inflammation predisposing to atherosclerosis.[40] Here we have taken serum nitric oxide and serum hs-CRP as markers of endothelial dysfunction of DM. In our study we found diabetics with complications had decreased level of NO, increased level of hs-CRP. This is in accordance with data published in previous studies in which hyperuricemia has been associated with hyperglycemia & higher risk for developing impaired glucose tolerance & vascular complications in T2DM.[6]

The serum NO in our study was 49.20 ± 2.10 µmol/L in patients as compared to 67.10 ± 1.54 µmol/L in controls (p<.001). Similar result was shown by Ghosh *et al*[41]. The mean serum NO in diabetics with complications was significantly decreased as compared to diabetics without complications. A Turkish study compared the basal serum levels of NO in T2DM patients with different stages of diabetic retinopathy and compared them with the levels in non-diabetics using Griess reaction. The patients with T2DM had significantly higher levels of serum NO metabolites and decreased NO than the non-diabetics.[42] There are multiple causes for depletion of NO, like uncoupling of eNOS enzyme system. When this enzyme system uncouples the endothelium becomes a net producer of superoxide and reactive oxygen species (ROS) instead of net production of the protective antioxidant properties of eNO. It was recently shown that UA activates NADPH oxidase resulting in increased production of ROS, leading to decreased bioavailability of NO and increased protein nitration.[43] In hyperuricemia NO may also get converted to 6- aminouracil by an irreversible reaction. Thus UA although one of the major antioxidant[44] circulation can induce oxidative stress in variety of cells including vascular smooth muscle cells & thus mediate progression of cardiovascular diseases by disturbing several functions like barrier function of vascular endothelium, antithrombogenic properties, regulation of vascular smooth muscle cell tonicity.[45] The disturbance in regular physiology of endothelium is linked to the inadequate capacity of Ca+2/ calmadulin sensitive NOS to generate adequate quantities of NO.[46]

The endothelium plays a major role in maintaining vascular tone and modulating blood flow and pressure. Central to these functions is the generation of NO, which is a potent vasodilator,[47] but excess of uric acid plays a major role in quenching NO availability, which adds to the endothelial dysfunction.

Serum hs-CRP in patients is 2.58 ± 0.16 mg/L as compared to 0.93 ± 0.04 mg/L in controls (p<.001). In 2011, Azenabor *et al*[48] reported that increased level of acute phase reactants such as CRP are associated with number of microvascular complications in T2 diabetic subjects and suggested that they may play a role in pathogenesis of diabetic complications. Majid Khazaei[49] described CRP level to correlate positively with insulin resistance. Nystrom and co-workers indicated persistent endothelial dysfunction is related to elevated CRP levels in T2DM. A recent study demonstrated that one of the serum leptin interacting proteins is CRP, the expression of which can be stimulated by leptin in human hepatocytes. CRP has been shown to attenuate insulin signaling through regulation of spleen tyrosine kinase and RhoA in the phosphorylation of the insulin receptor like substrate-1 at the Ser307 site, Akt, and eNOS in vascular endothelial cells, leading to an imbalance between NO and endothelin-1 (ET-1) production.[50]
Our study demonstrates a positive correlation between SUA and hs-CRP (r=.208, p=.038). Similar results were shown by Anan *et al*[51] where they showed hs-CRP correlated with UA.

In vascular smooth muscle cells NADPH oxidase-dependent augmentation of ROS production by NO metabolites, is followed by ROS-dependent activation of the proinflammatory signaling via phosphorylation of p38 and ERF1/2 (MAP kinases). An activation of this mechanism in response to UA is

followed by an increase in the production of monocyte chemoattractant protein-1 which stimulates human mononuclear cells to produce IL-1 beta, IL-6, and tumor necrosis factor-α. Induction of nuclear transcription Nk-kappa β and a decrease in the production of adiponectin.[52] It has also been proposed that UA changes in the expression of ET-1. Moreover, UA can act as an endogenous danger signal capable of stimulating the innate immune response. Thus the effect of hyperuricemia might be partially responsible for the low-grade inflammation and insulin resistance in the adipose tissue.[53] Elevated SUA contributes to endothelial dysfunction and is pro-atherosclerotic and proinflammatory linking UA and CRP. UA also activates a inflammasome to process and secrete IL-1β, involved in mediating inflammation.[54] In particular, a combined elevation of IL-1β and IL-6, rather than the isolated elevation of IL-6 alone, independently increases the risk of T2DM and endothelial dysfunction associated with it.[55]

In our study, though there was decrease in serum NO levels associated with increase in SUA, but no direct correlation was found between these parameters. However hs-CRP shows negative correlation with NO (r=-.565, p<.001). Sarangi *et al*[56] showed significant ($p < 0.05$) positive correlation between hsCRP and NO metabolites among diabetics with and without complications. In endothelial cells, UA decreases NO bioavailability and inhibits cell migration and proliferation, which are mediated in part by the expression of CRP.[33]

Thus urate antioxidant –prooxidant switch causes endothelial uncoupling in addition to ROS, endothelin. XOR has been shown to localize immunohistochemically within atherosclerotic plaques allowing the endothelial cell to metabolise purine at the plasma membrane surface and within the cytoplasm. SUA elevation may indeed be a sensitive marker for underlying vascular inflammation and remodelling within the arterial vessel wall and capillary interstitium. It is possible that SUA levels could be as similarly predictive as hsCRP since it is a sensitive marker for underlying inflammation and remodeling within the arterial vessel wall.

CONCLUSION

The cumulative effect of hyperuricemia and hyperglycemia is endothelial dysfunction. Our results suggest that diabetic patients with complications have hyperuricemia and have low levels of serum NO and high levels of hs-CRP. This indicates hyperuricemia inactivates serum NO and decreases its bioavailability. This decrease may be due to inactivation of NO by an irreversible reaction resulting in formation of 6-aminouracil or due to uncoupling of NOS complex.

From a clinical stand point hyperuricemia should alert clinicians to take therapeutic measures which will help in reducing risk of life threatening complications. Thus UA measurement has diagnostic and prognostic importance in T2DM in order to maintain normal endothelial function.

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