Original Article

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Prothrombotic State and Cardiovascular Risk in Hyperuricaemic Individuals: Does Hyperglycaemia Play a Role? A Case-control Study

Biochemistry Section

SHILPA BHARDWAJ[1], ASHOK AHIRWAR[2], BHAWESH MISHRA[3], GAURAV SINGLA[4], ANJU JAIN[5]

ABSTRACT

Introduction: The role of Hyperuricaemia (HU) in pathogenesis of cardiovascular disorders is debated. A number of hypothetical mechanisms that link HU to increased cardiovascular risk are researched.

Aim: To evaluate the hypercoagulable state and cardiovascular risk in diabetic hyperuricaemics, nondiabetic hyperuricaemics in comparison to healthy controls, and to analyse whether chronic hyperglycaemia has a causal role in HU associated cardiovascular risk.

Materials and Methods: A case-control study was conducted in which 60 known hyperuricaemic cases and 30 healthy controls were included in the study. Cases were further divided into nondiabetic hyperuricaemic and diabetic-hyperuricaemic subgroups. Routine blood biochemistry including high sensitivity C-Reactive Protein (hs-CRP), Plasminogen Activator Inhibitor-1 (PAI-1) and lipid profile was performed to assess endothelial function and hypercoagulability. Data were expressed as Mean±SEM.

Results: Levels of hs-CRP and PAI-1 were significantly higher in diabetic hyperuricaemics ($p<0.001$) and nondiabetic hyperuricaemics ($p<0.001$) compared to controls. The difference was not significant between the hyperuricaemic subgroups. Serum Uric Acid (UA) levels showed a significantly positive correlation with hs-CRP ($r=0.554$, $p<0.001$) and PAI-1 ($r=0.525$, $p=0.003$) levels among the cases. Association between UA and glucose was not significant in diabetic hyperuricaemics ($r=0.270$, $p=0.15$).

Conclusion: HU is associated with endothelial dysfunction and prothrombotic state leading to increased cardiovascular risk. Hyperglycaemia does not have a direct causal role in HU associated cardiovascular risk.

Keywords: Cardiovascular morbidity, Endothelial dysfunction, Hyperglycaemics

INTRODUCTION

Uric Acid (UA) is the terminal product of purine degradation. Altered serum UA levels have been associated with many disease states. Low UA level is a risk factor for neurodegenerative diseases such as multiple sclerosis, Parkinson's disease and Alzheimer's disease whereas, high UA level is associated with gout, hypertension, diabetes, metabolic syndrome, obesity and chronic renal disease [1]. It has been debated whether high levels of UA in the blood increases cardiovascular risk. Some studies have revealed that HU has a significant association with increased cardiovascular morbidity while others claim that any apparent association with cardiovascular morbidity is probably due to the association of UA level with other risk factors [2,3]. A number of proposed mechanisms that can lead to increased cardiovascular risk in hyperuricaemic subjects are being researched [4,5]. Cardiovascular risk assessment can be done by established markers of endothelial dysfunction like high sensitivity C-Reactive Protein (hs-CRP) and Plasminogen Activator Inhibitor-1 (PAI-1) or by conventional markers like blood lipids. hs-CRP is associated with low-grade sustained vascular inflammation whereas PAI-1 is a prothrombotic adipokine associated with plaque proliferation and atherosclerosis progression [6,7].

Chronic hyperglycaemic conditions are known to predispose individuals to increased cardiovascular risk. The mechanisms are understood and well established [8]. Though purine metabolism and carbohydrate (fructose and glucose) metabolism are linked through the pentose phosphate pathway, but whether they actually effect each other's concentrations and consequences is unclear [9]. Whether a chronic hyperglycaemic state increases cardiovascular risk in a previously hyperuricaemic individual is questionable. The hypothesis of present study was that HU is a risk factor for developing complications in diabetic individuals. Hence, the present study was conducted with an aim to determine whether diabetic-hyperuricaemic subjects are at an increased cardiovascular risk as compared to nondiabetic hyperuricaemics and to see if chronic hyperglycaemia has a causal role in HU associated cardiovascular risk.

MATERIALS AND METHODS

A case-control study was conducted after ethical approval from the Institutional Committee, LHMC, New Delhi. The present study is according to declaration of Helsinki. It was conducted in a Tertiary Care Hospital of New Delhi between January 2012 to December 2015. A total of 90 subjects were enrolled in the study after a bilingual voluntary informed written consent. Sixty known hyperuricaemic cases aged between 20 to 60 years were selected and further divided into two subgroups based on patient history and biochemical findings: nondiabetic hyperuricaemic and diabetic hyperuricaemic. All the patients were of type 2 Diabetes Mellitus on oral hypoglycaemics with duration more than five years. Thirty age and sex matched controls were also included in the study. Hence, group 1 consisted of healthy control and group 2 consisted of hyperuricaemic individuals. Serum UA concentration ≥7.0 mg/dL was considered as HU. Fasting plasma glucose ≥126 mg/dL was used to define diabetes.

Inclusion criteria of cases: Hyperuricaemic patients with and without type 2 diabetes mellitus of more than 5 years.

Exclusion criteria of cases: Pregnant women and those with current illness such as cancers, thrombosis, sepsis, hepatic,

cardiac or renal disease. Patients in postperiod and in acute inflammatory states were also excluded. Also, none of the patients were on any drugs which could increase UA levels. All possible causes of secondary HU were also ruled out. Individuals with history of tuberculosis and other chronic diseases which may affect HU were excluded from study. Individuals on drugs such as thiazides, frusemide, pyrazinamide, ethambutol etc., were also excluded from study.

Inclusion criteria of control: Healthy volunteers were included as controls.

Exclusion criteria of control: The exclusion criteria for controls was same as that of cases.

Morning fasting blood samples were collected from the antecubital vein into evacuated tubes (BD Vacutainer® systems). Routine blood chemistry including blood glucose and lipid profile were performed on Beckman Coulter's Synchron CX® Chemistry Analysers. hs-CRP and PAI-1 levels were estimated by sandwich ELISA method.

STATISTICAL ANALYSIS

All Statistical analyses were performed using IBM SPSS Statistics (Version 20.0, IBM SPSS, IL, USA) and GraphPad Prism (Version 5, GraphPad Software, CA, USA). Data were presented as mean±SEM. Comparison of means between the study groups was done by analysis of variance (ANOVA) followed by Tukey's post-hoc test. Statistical significance was set as $p<0.05$ (two-tailed). Pearson correlation analysis was done to assess the strength of association of various cardiometabolic risk factors (hs-CRP, PAI-1, blood glucose and lipids) with UA levels. Regression analysis was performed in the hyperuricaemic subgroups using hs-CRP and PAI-1 as the dependent variable, and UA and blood glucose as independent variables.

RESULTS

The serum UA concentration was 9.18±0.59 mg/dL in the nondiabetic hyperuricaemic (group 2A), 9.95±0.59 mg/dL in diabetic hyperuricaemic (group 2B) and 4.33±0.24 mg/dL in the controls (group 1).

hs-CRP levels were significantly higher in nondiabetic hyperuricaemics and diabetic hyperuricaemics as compared to controls: 7.05±0.30 mg/L (group 2A) vs 2.87±0.21 mg/L (group 1), $p<0.001$ and 7.77±0.59 mg/L (group 2B) vs 2.87±0.21 mg/L (group 1), $p<0.001$. The difference was not found to be significant when the two hyperuricaemic subgroups were compared, $p=0.42$ [Table/Fig-1].

A significantly positive correlation was found between PAI-1 and UA levels among the cases (pearson r=0.525, R square=0.298, $p=0.003$). A similarly positive correlation was found between hs-CRP and UA levels (pearson r=0.554, R square=0.332, $p<0.001$).

Pearson correlation analysis performed between UA and blood glucose among diabetic hyperuricaemics (group 2B) showed a non significant association between the two (r=0.270, $p=0.15$). In the same subgroup we found a significantly positive correlation between blood glucose and PAI-1(r =0.703, $p<0.001$). Similar positive correlation was seen between blood glucose and hs-CRP (r=0.573, $p=0.001$) [Table/Fig-2].

In the nondiabetic hyperuricaemic subgroup, UA (β=0.556, $p=0.003$) was a stronger predictor of raised PAI-1 levels compared to blood glucose (β=0.017, $p=0.92$). In the same study group UA (β=0.691, $p<0.001$) was a stronger predictor of raised hs-CRP levels compared to blood glucose (β= -0.023, $p=0.88$). Contrarily, in the diabetic hyperuricaemic subgroup blood glucose (β=0.606, $p<0.001$) was a stronger predictor of raised PAI-1 levels compared to UA (β=0.361, $p=0.007$). In the same study, group blood glucose (β=0.456, $p=0.005$) was a stronger predictor of raised hs-CRP levels compared to UA (β=0.431, $p=0.003$) [Table/Fig-3].

DISCUSSION

Present study results showed that the levels of hs-CRP and PAI-1 were significantly raised in the two hyperuricaemic subgroups as compared to the control group [Table/Fig-1] suggesting an increased atherogenic risk in HU. A number of studies published over the last 60 years have supported the view that there is increased cardiac risk in HU [6,7]. Reports from prospective studies by Krishnan E et al., Moriarity JT et al., and Bos MJ et al., concluded that HU is independently associated with cardiovascular risk [9-11].

Several hypothetical mechanisms have been proposed to explain how high UA levels can contribute to the atherogenic disease process. High UA levels can stimulate the release of free radicals, which have been shown to be involved in adhesion molecule expression by inflammatory cells as well as in inflammatory cell activation and adherence to the damaged endothelium. This ultimately results in endothelial Injury [4]. Studies have also postulated that high UA leads to decreases Nitric Oxide (NO) levels. UA reacts directly with NO in a quick irretrievable reaction ensuing the formation of 6-aminouracil and depletion of NO [12-14].

Our results showed that lipid concentrations were not significantly different in the nondiabetic hyperuricaemic group as compared to the controls [Table/Fig-1]. This suggests that high UA levels do not affect the lipid profile and the endothelial dysfunction observed in the cases is possibly caused by the mechanisms discussed above.

Parameters \ Groups	Group 1 Healthy controls (n=30) Mean±SEM	Group 2				
		Sub-group 2A Nondiabetic hyperuricaemic (n=30) Mean±SEM	P_1	Sub-group 2B Diabetic hyperuricaemic (n=30) Mean±SEM	P_2	P_3
Uric acid (mg/dL)	4.33±0.24	9.18±0.59	<0.001	9.95±0.59	<0.001	0.52
Fasting blood glucose (mg/dL)	85.93±1.91	97.93±2.10	0.28	157.83±9.22	<0.001	<0.001
Total cholesterol (mg/dL)	130.47±5.15	144.13±9.52	0.53	163.33±11.24	0.03	0.29
Triglycerides (mg/dL)	96.13±5.08	125.87±11.48	0.11	174.33±12.64	<0.001	0.004
HDL-C (mg/dL)	45.90±1.54	43.43±1.57	0.52	36.93±1.69	<0.001	0.01
VLDL (mg/dL)	19.23±1.02	25.17±2.30	0.11	34.87±2.52	<0.001	0.004
LDL-C (mg/dL)	65.34±5.47	75.53±9.41	0.70	91.53±10.90	0.10	0.41
hs-CRP (mg/L)	2.87±0.21	7.05±0.30	<0.001	7.77±0.59	<0.001	0.42
PAI-1 (ng/mL)	62.61±4.33	102.9±6.45	<0.001	165.70±10.99	<0.001	0.74

[Table/Fig-1]: Biochemical characteristics of controls and cases.
P_1: p-value for Group 2A compared to Group 1; P_2: p-value for Group 2B compared to Group 1; P_3: p-value for Group 2B compared to Group 2A; SEM: Standard error of mean; Tukey's post-hoc test was used.

Correlation between serum uric acid concentration and other variables		
	Pearson r	p-value
Glucose	0.270	0.15
hs-CRP	0.554	**0.001**
PAI-1	0.525	**0.003**
Correlation between blood glucose concentration and other variables		
Uric acid	0.270	0.15
hs-CRP	0.573	**0.001**
PAI-1	0.703	**<0.001**

[Table/Fig-2]: Pearsons correlations in the diabetic hyperuricaemic subgroup.

Dependent variables / Independent variables	hs-CRP		PAI-1	
	β coefficient	p-value	β coefficient	p-value
In nondiabetic hyperuricaemic Sub-group 2A				
Uric Acid	0.691	**<0.001**	0.556	0.003
Glucose	-0.023	0.88	0.017	0.92
In diabetic hyperuricaemic Sub-group 2B				
Uric Acid	0.431	**0.003**	0.361	**0.007**
Glucose	0.456	**0.005**	0.606	**<0.001**

[Table/Fig-3]: Results of Regression Analysis in hyperuricaemic subgroups.

Some studies contradict our findings and support the view that UA does not have a causal role in the development of coronary heart disease and any apparent association is probably due to the association of UA level with other risk factors [3,15,16].

In the present study, a significant positive correlation between UA and PAI-1 levels among the hyperuricaemic subjects (r=0.525, p=0.003). A similar positive correlation was found between UA and hs-CRP concentrations (r=0.554, p<0.001). An association between UA concentrations and endothelial dysfunction has been reported by several authors, based on different methodologies. Ho WJ et al., reported endothelial dysfunction among hyperuricaemic subjects on the basis of significantly lower values for endothelium-dependent flow-mediated vasodilation [16]. Săvoiu G et al., observed that patients with HU had significantly higher carotid Intima-media thickness compared to normo uricaemics [17]. Hong Q et al., concluded that HU induces endothelial dysfunction via mitochondrial Na^{+}/Ca^{2+} exchanger-mediated mitochondrial calcium overload [18]. Zoccali C et al., studied endothelial dysfunction among hypertensive subjects and found a positive correlation between UA and CRP supporting a chronic inflammatory pathology [19].

The metabolism of glucose is linked to the purine metabolism through the pentose phosphate pathway. So there is a hypothetical possibility that higher blood glucose levels can increase UA concentrations and its consequences. Insulin resistance in type 2 diabetics leads to renal under excretion of UA whereas increased lactate in related disorders accelerates renal Urate Reabsorption Via Urate Transporter 1 (URAT1) [9,20]. Pearson correlation analysis done between UA and blood glucose concentrations among the diabetic hyperuricaemics revealed that the association between the two was not significant (r=0.270, p=0.15). On the other hand we found a strong positive correlation between blood glucose and PAI-1 (r =0.703, p<0.001), and similarly between blood glucose and hs-CRP (r=0.573, p=0.001) [Table/Fig-2]. These findings indicate that the increase in blood glucose does not significantly increase UA levels directly by the postulated mechanisms discussed above. Severe endothelial dysfunction in diabetic hyperuricaemic group could be caused independently by HU and hyperglycaemia through different mechanisms, without significantly affecting each other's concentrations directly. Comparison of lipid concentration between the hyperuricaemic diabetic and nondiabetic subgroups, showed significant differences for Triglycerides (p=0.004) and HDL (p=0.01) suggesting a typical metabolic syndrome like dyslipidemia leading to endothelial dysfunction and increased cardiovascular risk [20].

Limitation(s)

The limitation of the study was the small sample size. It does not allow the establishment of a direct causal role of HU in endothelial dysfunction and cardiovascular risk.

CONCLUSION(S)

Hyperuricaemia may be considered to be an independent risk factor for cardiovascular diseases in nondiabetic individuals. HU emulates a chronic systemic inflammatory condition associated with endothelial dysfunction and prothrombotic state leading to increased cardiovascular risk. HU in hyperglycaemic subjects does not cause significant increase in the cardiovascular risk compared to HU in normoglycaemics. Serum UA and blood glucose are independently associated with endothelial dysfunction. Large-scale clinical trials can help to clarify the exact mechanism underlying the role of UA in the development of cardiovascular diseases.

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PARTICULARS OF CONTRIBUTORS:
1. Assistant Professor, Department of Biochemistry, Rajiv Gandhi Super Speciality Hospital, Delhi, India.
2. Assistant Professor, Department of Biochemistry, AIIMS, Nagpur, Maharashtra, India.
3. Specialist, Department of Biochemistry, Deen Dayal Upadhyay Hospital, Delhi, India.
4. Assistant Professor, Department of Cardiology, Rajiv Gandhi Super Speciality Hospital, Delhi, India.
5. Director-Professor, Department of Biochemistry, Lady Harding Medical College and Associated Hospital, Delhi, India.

NAME, ADDRESS, E-MAIL ID OF THE CORRESPONDING AUTHOR:
Dr. Shilpa Bhardwaj,
186, Ground Floor, Sharda Niketan, Pitampura, Delhi-34, India.
E-mail: drshilpa2001@gmail.com

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