Original Article

Effect of Hyperuricemia on serum nitric oxide levels in diabetic patients with hyperlipidemia

Parineeta Samant[*a], Z.G. Badade[a], Dr. Sandeep Rai[b]

[*a]Dept. of Biochemistry, Dept. of Medicine[*b], MGM Medical College, Kamothe, Navi-Mumbai

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ABSTRACT

Introduction: Recent studies have shown that elevated serum uric acid is associated with insulin resistance, hyperlipidemia, obesity, hypertension & cardiac diseases. The elevated level of uric acid is a risk factor in diabetes mellitus. Hyperuricemia if not corrected can inactivate nitric oxide (NO) which leads to hyperlipidemia & increases the risk of cardiac diseases by affecting endothelial function & arterial stiffness. **Objectives:** The aim of our study was to determine whether hyperuricemia depletes serum NO level in diabetic patients and to find out correlation between the levels of NO in study group. Methods Total ninety patients were selected and divided in to three groups. Group I (n=30) - Controls; Group II (n=30) - Diabetic patients with hyperlipidemia & hyperuricemia; Group III (n=30) - Diabetic patients with hyperlipidemia & normouricemia. The values of fasting sugar, lipid profile, serum uric acid were estimated by auto analyzer using transasia kits. Serum NO is estimated by Cortas & Wakid method. **Result:** It is observed that Group II showed highly significant decreased levels of serum NO (p< 0.0001) than Group I. Group III also showed decreased NO level but not statistically significant when compared with Group I (p>0.05). Comparison between Group II & Group III showed significantly declined NO level in Group II (p< 0.0001). Conclusion Our results suggests that diabetic patients with hyperlipidemia & hyperuricemia shows low levels of serum NO & indicates that hyperuricemia inactivates serum NO and decreases it s bioavailability.

1. Introduction

Diabetes mellitus is considered to be the major public health concern throughout the world, including India. 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. Serum uric acid (UA) is implicated as one of the potential risk factors associated with all above [1]. The probable mechanism by which UA may endanger organ damage is through endothelial dysfunction whereby it may affect cardiovascular function and structure [2] .

It is the most abundant antioxidant in plasma, reacts directly with 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 [3]. The study was undertaken to determine whether hyperuricemia depletes serum NO level in diabetic patients and to find out correlation between the levels of NO in diabetic subjects with hyperuricemia, normouricemia having high lipid profile and healthy controls.

* Corresponding Author : Parineeta Samant
Dept. of Biochemistry
MGM Medical College, Kamothe, Navi-Mumbai
E.mail: parineeta.samant13@gmail.com

1.Material & methods

A total 90 patients with type 2 Diabetes mellitus from the outpatient Diabetic clinic at the MGM medical college & Hospital were included in this study. Type 2 DM was diagnosed based on the World Health Organization 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 MGM Medical college. All the subjects were divide in to three groups; Group I (n=30) - Controls; Group II (n=30) - Diabetic patients with hyperlipidemia & hyperuricemia; Group III (n=30) - Diabetic patients with hyperlipidemia & normouricemia.

The three groups were comparable for age & gender. The following patients were excluded from study,the pregnant women and those with current illness (such as hepatic, cardiac or renal disease), those with HIV positive status.

2.1 Biochemical analysis: After an overnight fast, blood was taken from a forearm vein. The blood was clotted and immediately centrifuged to separate serum which was used for the determination of FBS and serum uric acid concentrations. FBS was measured using a glucose oxidase method [4] which is available as a kit manufactured by Transasia. Serum uric acid was assessed by uricase enzymatic method [5] using Transasia uric acid kit. Serum Lipid profile was assessed by enzymatic method [5] using Transasia kit. The Serum NO was estimated by Cortas & Wakid method. The body mass index was calculated as the weight in kilograms divided by the squared height inmeters (Kgm^2) [6].

2.2 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 were considered to be statistically significant.

3. Result

Clinical and biological data of healthy and diabetic subjects are summarized in Table-I. Diabetic patients and control have matched ages as shown by the non-significant differences between groups when compared with control group (P > 0.05). The number of male and female was identical. BMI was statistically high when compared with control group (P< 0.005). FBS was high in the diabetic group as compared with the control group (P < 0.001). Serum uric acid concentration was higher than control. Relationship between serum uric acid concentration & other variables are shown in table 2. There was significant correlation between serum uric acid concentration and serum nitric oxide. Group II shows highly significantly decreased level of serum NO (p<0.0001) than Group I. Group III also showed decreased level of NO but not statistically significant when compared with Group I (p > 0.05). There was significant correlation between serum uric acid concentration & serum Triglycerides, FBS, HDL, and LDL.

Table–1 Showing age, BMI, Fasting blood sugar in Control and Study groups

Parameters	Group-I Control (Mean ± SD)	Group-II Diabetic, Hyperlipidemic & Hyperuricemia (Mean ± SD)	Group-III Diabetic, Hyperlipidemic & Normouricemia (Mean ± SD)
Age	51.96±4.11	51.8±6.19	50.8±4.89
Sex			
BMI(k/m2)	29.18±0.98	32.33±1.12	31.40±1.08
FBS (mg/dl)	89.48± 10.46	155.60±19.18**	148.53±21.25**

Table–2 Showing values of Fasting blood sugar, Lipid profile, Uric acid & Nitric oxide in Control and Study groups

Parameters	Group-I Control (Mean ± SD)	Group-II Diabetic, Hyperlipidemic & Hyperuricemia (Mean ± SD)	Group-III Diabetic, Hyperlipidemic & Normouricemia (Mean ± SD)
FBS mg/dl	89.48± 10.46	155.60±19.18**	148.53±21.25**
Cholesterol mg/dl	130.166± 23.70	260.06±21.21**	252.76±18.64**
TG mg/dl	90.51±13.70	179.83±14.08**	166.63±20.74**
HDL mg/dl	46.65±7.48	37.86±4.02	40.83±4.16*
LDL mg/dl	65.41±22.56	188.20±27.35**	169.07±26.07**
VLDL mg/dl	18.10±2.74	36.02±2.85**	33.06±4.22**
Nitric oxide µmol/L	92.30±4.97	49.89±5.44**	89.31±3.06*
Uric acid mg/dl	3.94±0.45	8.25±1.54**	4.123±0.74

**p< 0.0001 as compared to control
*p>0.05 as compared to control

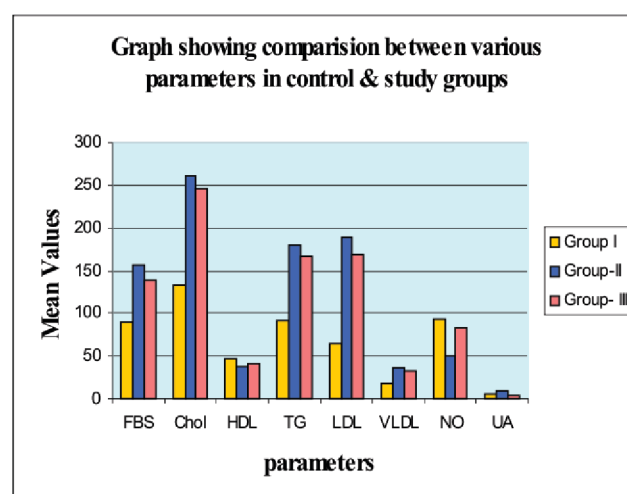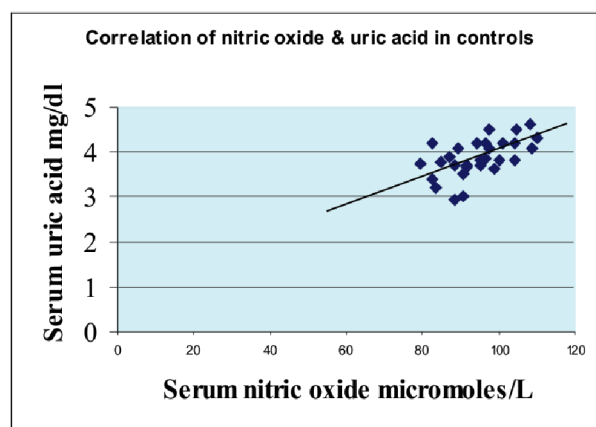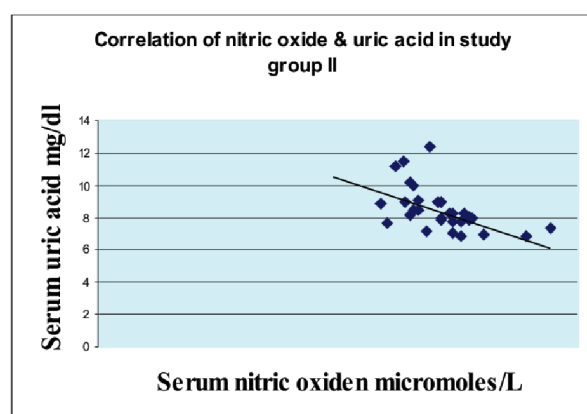**Figure 1: Graph showing comparison between FBS, Lipid profile, SUA & NO**

**Figure 2: Correlation between serum uric acid & nitric oxide in control**

Correlation of nitric oxide & uric acid in controls

r = 0.363

Figure 3: Correlation between serum uric acid & nitric oxide in Group II

Correlation of nitric oxide & uric acid in study group II

r = -0.53

Figure 4: Correlation between in serum uric acid & nitric oxide Group III

Correlation of nitric oxide & uric acid in study group III
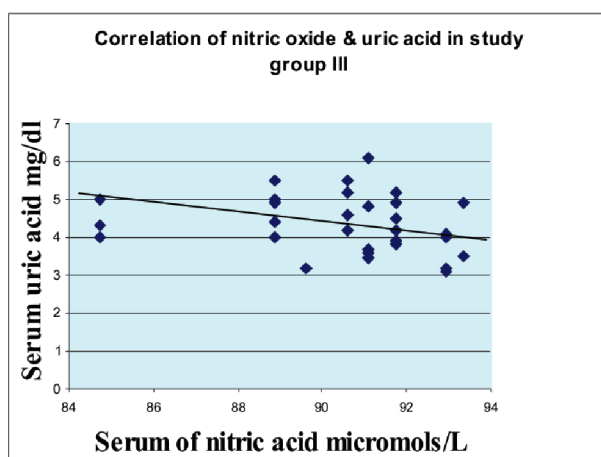
r = -0.158

4.Discussion

Variations in uric acid levels have been increasingly associated with insulin resistance, hyperinsulinemia, and type-2 diabetes [7,8,9,10]. In Type 2 diabetes, hyperuricemia seems to be associated with the insulin-resistance syndrome, endothelial dysfunction, coronary artery disease, arterial stiffness.

In our study we found decreased level of NO in Diabeteic hyperuricemic group (Group II). This gourp also showed increase in LDL level on comparision with control group (Group I). 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 & type 2 diabetes. Here we also found negative co-orelation between NO and uric acid in diabetes patients (Group II & group III) . It is important to note that diabetic & normouricemic group shows decrease in NO as compared to control (Group I) but the difference is not statistically significant. This proves that hyperuricemia plays a significant role in depleting NO level.

There are multiple causes for depletion of NO, like uncoupling of endothelial nitric oxide synthase enzyme system. When this enzyme system uncouples the endothelium becomes a net producer of superoxide & ROS instead of net production of the protective antioxidant properties of eNO.

It was recently shown that uric acid activates NADPH oxidase resulting in increased production of reactive oxygen species, leading to decreased bioavailabilty of NO and increased protein nitration [11] . In hyperuricemia NO may also get converted to 6-aminouracil by an irreversible reaction. Thus uric acid although one of the major antioxidant [12] 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, antithromogenic properties, regulation of vascular smooth muscle cell tonicity [13]. The disturbance in regular physiology of endothelium is linked to the inadequate capacity of Ca+2/ calmudulin sensitive nitric oxide synthase to generate adequate quantities of NO [14].

The endothelium plays a major role in maintaining vascular tone and modulating blood flow and pressure. Central to these functions is the generation of nitric oxide (NO), which is a potent vasodilator [15] but excess of uric acid plays a major role in quenching NO availability.

Thus uric acid, an antioxidant becomes a pro-oxidant & can induce oxidative in cells, as soluble uric acid is capable of entering smooth muscle cells with deleterious effects. After uptake through a nonenzymatic organic anion transport system, uric acid activates vascular smooth muscle mitogens, which result in the cellular proliferation that is characteristic of atherosclerosis [16].

 In diabetic patients hyperuricemia 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 [5,17,18]. This redox stress consumes the natural occurring local antioxidants such as: SOD, GPX, and catalase. Once these local intimal antioxidants are depleted uric acid can undergo the paradoxical antioxidant – prooxidant switch or the urate redox shuttle [19,20].

Figure:5
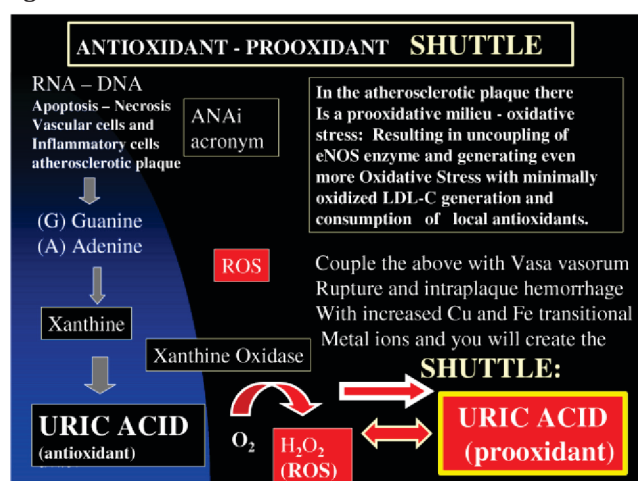
Increased level of serum uric acid definitely should be considered as one of the injurious stimuli to arterial cell wall & endothelial dysfunction. It accelerates the atherosclerotic condition & makes the intima acidic [21]. This is associated with uncoupling of the eNOS enzyme and a decreases locally produced NO and depletes bioavailability.

The cumulative effect of hyperuricemia, hyperglycemia is endothelial dysfunction. Changes in endothelial function may affect the coronary artery circulation being unable to cope with the increased metabolism of myocardial muscles.

5. Conclusion

Our results suggests that diabetic patients with hyperlipidemia & hyperuricemia shows low levels of serum NO & indicates that hyperuricemia inactivates serum NO and decreases it's 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 nitric oxide synthase 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 uric acid measurement has diagnostic and prognostic importance in Type 2 diabetes mellitus in order to maintain normal endothelial function.

6. References

[1]   1.ArnerP. Insulin resistance in type 2 diabetes & Role of fatty acids. Diabetes Metab. Res. Rev 2002;18(2): S5-S9

[2]   Johnson RJ, Kang DH, Feig D, Kivlighn S, Kanellis J, Watanabe S, Tuttle KR, Rodriguez-Iturbe B, Herrera-Acosta J, Mazzali M. Is there a pathogenetic role for uric acid in hypertension and cardiovascular and renal disease? Hypertension. 2003;41: 1183–1190

[3]   Gersch C. Palli,kim KM, AngerhoferA, Johnson RJ Henderson GN. Inactivation of nitric oxide by uric acid. Nucleosides nucleotides Nucleic acids. 2008;27(8)pp 967-78.

[4]   Lott JA, Turner K. Evaluation of trinderõ s glucose oxidase method for measuring glucose in serum and urine. Clin Chem 1975 ;21(1):754-60.

[5]   5, Gochman N, Schmitz JM. Automated determination of uric acid with use of an uricase-peroxidase system. Clin Chem 1971; 17(11): 54-9.

[6]   Cantrill JA. Diabetes Mellitus. In: Walker R, Edwards C (Eds). CliniPharmacy and Therapeutics, 2nd edition. Churchill Livingstone, London 1999; 633-52.

[7]   Wun YT, Chan CS., Lui CS. Hyperuricaemia in Type W diabetes mellitus. Diabetes Nutr. Metab. 1998;138(1) : 63-69.

[8]   Kodama S, Saito K, Yachi Y, Asumi M, Sugawara A, Totsuka K, et al. Re - association between serum uric acid and development of type W diabetes mellitus. A Meta-Analysis. Diabetes Care. 2009;32(9) : 1737- 1742.

[9]   Niskanen L, Laaksonen DE, Lindstrom J, Eriksson JG, Keinanen-Kiukaanniemi S, Ilanne-Parikka P, et al. Serum uric acid as a harbinger of metabolic outcome in subjects with impaired glucose tolerance: the Finnish Diabetes Prevention Study. Diabetes Care. 2006; 29(3) : 709-711.

[10] Modan M, Halkin H, Karasik A, Lusky A. Elevated serum uric acid-a facet of hyperinsulinaemia. Diabetologia. 1987;30(9):713-718.

[11] Sautin YY, Nakagawa T, Zharikov S, Johnson RJ . Adverse effect of the classic antioxidant uric acid in dipocytes: NADPH oxidase mediated oxidative/nitrosative stress. Am J Physiol Cell Physiol 2007 ; 293: C584–C596.

[12] Ames B.N., Cathcart R., Schwiers E., Hochstein P. Uric acid provides an antioxidant defense in humans against oxidant - and radical-caused aging and cancer: a hypothesis. Proc. Natl. Acad. Sci. U S A 1981;78(11) : 6858-6862.

[13] Hunt BJ.The endothelium in atherogenesis. Lupus 2000;9 :189–193.

[14] Furchgott RF, ZawadzkiA JV The obligatory role of endothelial cells in the relaxation of arterial smooth muscles by acetylcholine. Nature 1980; 288 : 373–376.

[15] Erdogan D, Gullu H, Caliskan M, et al. Relationship of serum uric acid to measures of endothelial function and atherosclerosis in healthy adults. Int J Clin Pract 2005;59(11) : 1276-1282.

[16] Corry DB, Eslami P, Yamamoto K, Nyby MD, Makino H, Tuck ML. Uric acid stimulates vascular smooth muscle cell proliferation and oxidative stress via the vascular renin—angiotensin system. J Hypertens 2008;26 : 269-275.

[17] Williamson JR, Kilo C, Ido Y. The role of cytosolic reductive stress in oxidant formation and diabetic complications. Diabetes Res Clin Pract1999; 45 : 81-82.

[18] Aronson D, Rayfield EJ How hyperglycemia promotes atherosclerosis: molecular mechanisms. Cardiovasc Diabetol 2002; 1(1) pp1.

[19] Santos CX, Anjos EI, Augusto O. Uric acid oxidation by peroxynitrite: multiple reactions, free radical formation, and amplification of lipid oxidation. Arch Biochem Biophys 1999;372(2) : 285-294.

[20] Abuja PM: Ascorbate prevents prooxidant effects of urate in oxidation of human low density lipoprotein. FEBS Lett 1999;446(2–3) : 305-308.

[21] Naghavi M, John R, Naguib S, Siadaty MS, Grasu R, Kurian KC, van Winkle WB, Soller B, Litovsky S, Madjid M, Willerson JT, Casscells W: pH Heterogeneity of human and rabbit atherosclerotic plaques; a new insight into detection of vulnerable plaque.Atherosclerosis 2002; 164(1) :27-35.