Interrelationship of serum uric acid levels and cardiovascular disease risk factors in Bangladeshi patients treated with antihypertensive drugs

***Ishtiaq Mahmud[1], Dip Bhowmik[1], Shahdat Hossain[2], Md. Mesbah Uddin[3], Sharif Neaz[4], Arun Das[4], Nuruzzaman Masum[4], Shahjalal Hussain[2], Sohrab Alam[5]**

[1]Dept. of Biochemistry & Molecular Biology, University of Dhaka, Dhaka-1000, Bangladesh, [2]Dept. of Biochemistry & Molecular Biology, Jahangirnagar University, Savar, Dhaka -1342, Bangladesh, [3]Clinical Pathology Department, Dhaka Medical College and Hospital,Dhaka-1000, 12 Bangladesh; [4]Dept. of Biochemistry & Molecular Biology,Tejgaon college, Dhaka-13, Bangladesh; [5]Dept. of Immunology, Bangladesh Institute of Research & Rehabilitation in Diabetes, Endocrine and Metabolic Disorders (BIRDEM), Dhaka, Bangladesh.

.

ABSTRACT

Aims: To explore the association between serum uric acid levels and cardiovascular disease (CVD) risk factors in hypertensive subjects treated with (WD) or without lipid-lowering and antihypertensive drugs (WOD). ***Study design*****:** Three groups of subjects with age range 50-70 y were included in the investigation: *i) Normotensive* healthy control subjects; ii) *hypertensive* subjects who did not start 'taking' lipid-lowering-/antihypertensive drugs and had cardiovascular-risk factors such as high blood pressure and high blood cholesterol; and iii) *hypertensive* subjects, who were already on lipid-lowering-/antihypertensive drugs at least for 3-months. ***Place and Duration of Study*****:** Dept. of Biochemistry & Molecular Biology, University of Dhaka, Jahangirnagar University and Tejgaon college; Dhaka Medical College Hospital and Institute of Research & Rehabilitation in Diabetes, Endocrine and Metabolic Disorders (BIRDEM), Dhaka, between April 2014 and May 2015. ***Methods:*** We included 197 subjects ((40 controls, 59 hypertensive subjects without drugs (WOD) and 98 subjects with drugs (WD)). Anthropometric as well as measurements blood pressure, weight/height and laboratory tests, such as lipid profile, electrolytes, zinc, uric were done. **Results:** The hypertensive subjects without drugs (WOD) had significantly (*P<.05*) higher levels of CVD risk factors, including blood pressure, serum Total cholesterol (TC) and uric acid (UA) [Hypertensive WOD vs. Control subjects: SBP: 169±1.30 vs. 125±2.75 and DBP: 92.3±1.50 vs. 78.5±1.50 mmHg; TC: 378±9.60 vs. 176±3.20 mg/dL; UA: 12.0 ±0.10 vs. 4.10±0.20 mg/dL). Antihypertensive drugs significantly (P<.05) ameliorated the blood pressure, TC, HDL-C levels, LDL-C/HDL-C and TG/HDL-C ratios. Multiple regression analysis showed serum uric acid levels were positively but independently correlated with LDL-C. ***Conclusion.*** Elevated serum uric acid and LDL-C levels were positively correlated independently of other measured confounders such as body mass index, high blood pressure, triacyglycerol/total cholesterol, electrolytes and zinc. Our results suggest that corrective measures to control hyperuricemia might be one of the approaches to manage damaging effects of uric acid on cardiovascular diseases during hypertension. These predictors, however, need further work to validate reliability on a large number of sample sizes.

Keywords*: Uric acid, LDL-C, Zn, K, Cardiovascular disease risk factors, Epidemiology*

* Tel.: +xx xx 265xxxxx; fax: +xx aa 462xxxxx.
E-mail address: xyz@abc.com.

1. INTRODUCTION

The excessive accumulation of uric acid, the metabolic end product of purine, leads to various diseases [1], including gout, in humans. However, hyperuricemia is a risk factor not only for gout, but also for cardiovascular diseases [2, 3]. Hyperuricemia is closely related to obesity, hypertension [4] and dyslipidaemia [5]. Previous studies have demonstrated a strong relationship between serum uric acid levels and coronary heart disease (CHD), with some studies suggesting that uric acid may be an independent risk factor for cardiovascular diseases [4,6-8]. Moreover, a recent meta-analysis showed that hyperuricemia may increase the risk of CHD events, independently of traditional CHD risk factors [9]. However, the nature of the relationship between uric acid and cardiovascular disease remains a subject of debate [10-12]. Recently, a series of controversial and conflicting findings from epidemiological studies have been reported [4-12]. Bangladesh is one of the developing countries, where both the incidence and prevalence of cardiovascular diseases are increasing in an alarming rate [13-15]. Because of an impressive track record for growth and development during the past decades, Bangladesh has been experiencing an increased prevalence of the CVDs. Despite recent advances in treatment for hyperlipidemia and diabetes as well as availability of sophisticated clinical methods, there is an increase in mortality rates for cardiovascular diseases (CVD) every year, demonstrating that cardiovascular risk factors are very high. Therefore, both diagnostic and additional therapeutic strategies are highly needed to evaluate CVDs, while, on the other hand, prompt and continuous efforts should also be exerted to develop new biomarkers for achieving high diagnostic accuracy in the prediction of risks and treatment of CVDs. Since uric acid has been considered an indicator of other CVD risk factors such as hypertension, dyslipidemia, obesity, glucose intolerance, and renal disease [16-19], and multiple studies provide strong evidence that an elevated uric acid may also bear independent risk factor association with total and/or CV mortality [20-23]. Therefore, in the present investigation on the Bangladeshi population, we have examined whether the serum uric acid could act as an independent risk factor for CVDs. In addition, patients with diabetes have lower serum levels of zinc [24]. There are studies on non-diabetic subjects, which suggest that low serum level of zinc is associated with increased incidence of cardiovascular diseases [25-27]. In this study with CVD patients, we mainly examined the association between serum uric acid level and cardiovascular disease risk factors.

2. MATERIALS AND METHODS

A total number of 200 subjects were included in his study irrespectively of race, religion and socioeconomic status. Of the total, 40 subjects were healthy control, 59 were cardiovascular subjects (taking blood pressure-, and lipid-lowering drugs), and 98 were cardiovascular subjects (without taking blood pressure, and lipid-lowering drugs).

Control subjects definition

Healthy control subjects' health status was evaluated by the physicians after measurements of blood pressure, anthropometrics and laboratory parameters, including serum lipid profile, electrolyte elements such as Na, K, Cl, and micronutrient zinc (Zn) and uric acid. Healthy control subjects also were with no serious disease.

Case definition

High blood pressure (hypertension) is by far the most important risk factor for cardiovascular disease (CVD). Therefore, case subjects, who had cardiovascular-risk factors such as high blood pressure and high blood cholesterol, were defined by the presence of symptoms consistent with cardiac disease, such as, self-reporting complaints of persistent high pressure. Physicians re-evaluated the subjects' complaints by determining relevant parameters, as were done for control subjects. The participants were asked for whether they

had already visited the doctors and started 'taking' of lipid-lowering- and anti-hypertensive drugs. Responders with 'no' were included and assigned as hypertensive subjects without drugs (WOD). On the other hand, if the subjects, with hypertension and high lipid profile, were already taking antihypertensive and lipid-lowering drugs, for at least 3-months, were included in the study and classified as hypertensive subjects with drugs, WD.

Inclusion criteria

The inclusion criteria for the control and hypertensive subjects was that the adult subjects must be aged ranging from 50 to 70 years.

Exclusion criteria

Subjects with diseases, such as infection, major surgery, renal failure, renal disease, liver malfunction and diabetes, history of using specific steroidal drugs and other pre-existing medical conditions or history of illegal drug use and crossing the age limit (40 to 70) were excluded from the study.

Sampling and analysis

Body weight and height were measured with minimal clothing and bare feet. BMI was calculated as the weight in kilograms per the square of height in meters, and blood pressure was measured while the person was in the sitting position after a 5-min rest. A patient was defined as having hypertension if systolic blood pressure was ≥160 mmHg, if diastolic pressure was ≥95 mmHg, or if the patient was receiving drugs for treatment of hypertension. Blood samples were allowed to clot for thirty minutes and then centrifuged for 10 min at 3000 rpm and serum samples were collected for the estimation of serum lipid profile [Total cholesterol, HDL-C, LDL-C, TG (Semi-auto analyzer, BSA 3000, Tamil Nadu, India], serum electrolytes [(Na^+, Cl^-, K^+), Diestro 103 AP Electrolyte Analyzer, Buenos Aires, Argentina], micronutrient Zn^{2+} (Atomic absorption spectrophotometry, GF-AAS, 6650 Shimadzu, Japan) and uric acid (Semi-auto analyzer, BSA 3000, Tamil Nadu, India).

2.1 Statistical analyses

To investigate the relationship between different parameters, we calculated Pearson correlation coefficients; it is shown as correlation matrix diagonal table. To find out independent (from other confounding factors) correlation, data were subjected to multiple regression analysis. To analyze the differences in the values of parameters among different subject groups, we performed one-way ANOVA test. We then used Fisher's PLSD test for multiple comparisons. Statistical software used was GraphPad prism v.4 and StatView v.4.

3. RESULTS

The clinical characteristics of the subjects are summarized in Table 1 and Table 2. The age of the control subjects was significantly (*P<.05*) lower than those of the hypertensive subjects with (WD) or without drugs (WOD). The age was significantly higher (*P<.05*) in the female subjects than that of the male subjects in WOD group (Female vs. Male: 66.9±1.3 vs. 57.6±1.3 y), while the age of the female subjects was lower than that of the male subjects in the WD group (Female vs. Male: 62.4±1.3 vs. 67.8±0.93 y). However, the average age of the subjects, irrespective of gender, was not statistically different between WOD versus WD group (WOD vs. WD subjects: 62.3±1.1 vs. 65.1±0.80). The body weight of the control subjects also was not significantly different with that of the cardiovascular disease groups (WD or WOD). Irrespective of gender, the average body mass indices (BMI) were significantly (P<.05) higher in the hypertensive WOD or WD groups, the highest values being in the subjects with drugs (WD) group (Control:WOD:WD=20.9±0.13: 27.4±0.10: 28.1±0.12).The average (of male+female) blood pressure (both systolic/diastolic) was the highest (*P<.05*) in the subjects without drugs (WOD), as compared to that in the subjects

with drugs (WD) or control subjects (Control:WOD:WD; SBP, 125±2.75: 167±01.30: 164±1.30; DBP, 79.1±1.8: 94.4±01.5: 89.8±0.80;). Both systolic and diastolic blood pressure decreased siginificantly (*P<.05*) in the subjects with drugs (WD) (Table 1)

The levels of serum total cholesterol (TC) and triacylglycerol (TG) were significantly (*P<.05*) higher in the subjects without drugs (WOD), as compared to those in the subjects with drugs (WD) or control subjects. However, the levels of TC and TG were significantly (*P<.05*) lower in the subjects who took drugs (WD) (Control:WOD:WD subjects, TC:176±3.2: 378±9.6: 253±2.10; TG: 200±4.40: 359±16.3: 260±10.5). The average levels of HDL-C significantly increased (*P<.05*) in the subjects who took drugs (WD) (Control:WOD:WD subjects=22.7±0.60: 21.7±0.10:33.2±1.0). The levels of LDL-C were not reduced significantly; the TG/HDL-C and LDLC/HDL-C ratios were, however, significantly (*P<.05*) reduced in the subjects with drugs (WD) (Table 2). When compared to those of the control subjects, the levels of Na or Cl were not altered either in the subjects with (WD) or without drugs (WOD) (Table 2). The levels of K were significantly decreased (*P<.05*) in the subjects with drugs or without drug groups. The levels of Zn were significantly lower (*P<.05)* both in the subjects with (WD) or without drugs (WOD), when compared with those of the control subjects (Control:WOD:WD subjects=52.4±1.70: 11.8±0.10: 10.2±0.17). Finally, the levels of serum uric acid were higher (*P<.05*) both in the subjects with or without drugs (193% higher in the WOD subjects and 178% higher in the WD subjects). Considering the serum uric acid concentrations >7 mg/dL in men and >6 mg/dL in women as hyperuricemia ; and ≤7 mg/dL in men and ≤6 mg/dL as normouricemia, 25.38% male subjects with drugs were hyperuricemic and 14.72% male subjects without drugs were hyperuricemic in our investigation. Correspondingly, 24.36% female subjects with drugs (WD) were hyperuricemic, while 15.22% female subjects without drugs (WOD) were hyperuricemic. The (minor) differences in age, body weight and/or blood pressure between male vs. female were not reflected in the biochemical parameters.

Table 2. Blood parameters of the subjects

Variables	Control Subjects (Con)			Patients without drugs (WOD)			Patients with drugs (WD)		
Sex	Male (n=23)	Female (n=17)	All (n=40)	Male (n=29)	Female (n=30)	All (n=59)	Male (n=50)	Female (n=48)	All (n=98)
TC (mg/dL)	178[a] ±4.50	173[a] ±4.50	176[a] ±3.2	377[b] ±14.0	378[b] ±13.4	378[b] ±9.60	256[c] ±3.0	251[c] ±2.90	253[c] ±2.10
TG (mg/dL)	207[a] ± 3.70	192[a] ±8.70	200[a] ±4.40	339[b] ±23.3	379[b] ±22.6	359[b] ±16.3	258[c] ±14.4	262[c] ±15.6	260[c] ±10.5
LDL-C (mg/dL)	133[a] ±3.6	133[a] ±2.70	133[a] ±2.30	169[b] ±1.80	167[b] ±1.90	168[b] ±1.30	171[b] ±1.40	169[b] ±1.40	170[b] ±1.0
HDL-C (mg/dL)	23.2[a] ±.80	22.1[a] ±0.90	22.7[a] ±0.60	22.1[a] ±1.10	21.2[a] ±1.30	21.7[a] ±0.80	32.0[b] ±1.10	35.0[b] ±1.50	33.2[b] ±1.0
TG/HDLC	9.15[a] ±0.51	8.88[a] ±0.34	9.04[a] ±0.23	16.18[b] ±1.18	20.21[c] ±1.88	18.23[b,c] ±0.80	8.78[a] ±0.63	8.82[a] ±0.86	8.80[a] ±0.50
LDL/HDL	5.96[a] ±0.26	6.17[a] ±0.26	6.05[a] ±0.20	8.28[b] ±0.48	8.80[b] ±0.48	8.54[b] ±0.37	5.65[a] ±0.19	5.33[a] ±0.24	5.50[a] ±0.15
Na (mmol/L)	137[a] ±.20	136[a] ±0.40	137[a] ±0.20	138[a] ±0.80	137[a] ±0.60	138[a] ±.40	138[a] ±0.40	137[a] ±0.40	138[a] ±0.40
K (mmol/L)	5.56[a] ±0.14	5.76[a] ±0.20	5.65[a] ±0.13	4.32 ±0.14	4.24[b] ±0.18	4.30[b] ±0.10	4.49[b] ±0.14	4.37[b] ±0.15	4.40[b] ± 0.10
Cl (mmol/L)	104[a] ±0.40	103[a] ±0.40	104[a] ±0.30	103[a] ±0.4	103[a] ±0.40	103[a] ±0.30	103[a] ±0.30	103 ±0.30	103[a] ±0.20
Zn (µg/dL)	51.0[a] ±2.1	55.2[a] ±2.8	52.4[a] ±1.70	11.8[b] ±0.20	11.7[b] ±0.20	11.8[b] ±.10	10.0[b] ±0.2	10.4[b] ±0.30	10.2[b] ± 0.17
Uric acid (mg/dL)	4.40[a] ±0.30	3.70[a] ±0.40	4.10[a] ±0.20	11.7[b] ±0.14	12.0[b] ±0.14	12.0[b] ±0.10	11.4[b] ±0.08	11.3[b] ±0.08	11.4[b] ±0.60

Results are mean ± SEM. Data were analyzed by one-way ANOVA, followed by Fisher's PLSD for post hoc comparisons. Values in the same row those share the common superscript are not significantly different at P<0.05.

Pearson's correlation coefficient (r) was calculated to reveal the strength of the association between the two variables. Serum uric acid levels were positively associated with age, BW, BMI, SBP, DBP,TC, TG, LDL-C, HDL-C and negatively associated with K and Zn. Subjects with the highest uric acid levels exhibited a higher prevalence of hypertension (as indicated by the increased SBP/DBP), central obesity (as indicated by the increased BMI, TC,TG and LDL-C). As expected, other cardiovascular risk factors including age, BW, SBD, DBP, TC,TG, LDL-C, HDL-C, K or Zn were also correlated at different extents (see the correlation matrix Table 3).

The Pearson's correlation, which is performed by bivariate regression analysis, however, does not assure about the two-variables whether they are actually dependent on each other and/or independent from each other. In multiple regression analysis, we thus included all the independent variables into the model and analyzed which ones are statistically significant. In multiple correlation analysis (Table 4), the serum uric acid was correlated with LDL-C significantly (*P<.05*). In other words, all 14 parameters (except Na and Cl) were correlated with serum uric acid (Table 3), but not all 14 parameters add on collectively to predict better the dependent variable *i.e*. serum uric acid. Multiple regression analysis thus revealed that serum LDL-C only had "add independent information" about serum uric acid. In other ways, "the relationship between serum uric acid and LDL-C" was independent from the 'confounding effects' of other cardiovascular risk factors (age to Zn) (Table 4).

Table 3. Correlation coefficient matrix analysis among different variables measured.

	Age	BW	BMI	SBP	DBP	TC	TG	LDLC	HDLC	TG/HDL	LDL/HDL	Na	K	Cl	Zn	UA
Age	1.000															
BW	0.124	1.000														
BMI	0.560	-0.074	1.000													
SBP	0.422	-0.036	0.854	1.000												
DBP	0.191	-0.020	0.517	0.534	1.000											
TC	0.216	0.034	0.571	0.494	0.425	1.000										
TG	0.148	0.114	0.386	0.235	0.215	0.418	1.000									
LDLC	0.454	0.010	0.761	0.573	0.413	0.465	0.195	1.000								
HDLC	0.325	0.027	0.300	0.231	-0.043	-0.177	-0.273	0.234	1.000							
TG/HDL	0.003	0.039	0.218	0.128	0.174	0.428	0.797	0.073	-0.644	1.000						
LDL/HDL	-0.092	-0.038	0.116	0.035	0.200	0.390	0.313	0.160	-0.818	0.760	1.000					
Na	0.106	0.093	0.110	0.073	0.056	0.074	0.066	0.132	0.123	-0.043	-0.105	1.000				
K	-0.256	0.018	-0.482	-0.562	-0.226	-0.393	-0.120	-0.334	-0.203	-0.026	-0.004	-0.017	1.000			
Cl	-0.025	-0.054	-0.067	-0.089	-0.192	-0.129	0.020	0.019	0.110	-0.022	-0.076	-0.002	0.022	1.000		
Zn	-0.520	0.061	-0.943	-0.938	-0.513	-0.593	-0.377	-0.768	-0.195	-0.251	-0.181	-0.136	0.542	0.098	1.000	
UA	0.541	0.006	0.928	0.835	0.516	0.586	0.315	0.793	0.231	0.182	0.132	0.137	-0.511	-0.057	-0.943	1.000

Results were obtained from bivariate analyses. No correlation, r = 0 to ± 0.25; Poor correlation, r = ± 0.25 to ± 0.50; Moderate/good correlation, r = ±0.50 to ±0.75; Very good to excellent correlation r = ± 0.75 to ± 1.0 . *Ref: Dawson B, Trapp RG. Basic and Clinical Biostatistics. 4th Ed. New York: Lange Medical Books/McGraw-Hill; 2004.*

4. DISCUSSION

The results of the present investigation on Bangladeshi population clearly point to the following facts: (i.) the subjects with or without drugs were hypertensive; (ii.) the hypertensive subjects had higher body mass index (BMI), when compared to those of the control subjects; (iii.) the cardiovascular disease risk factors, including higher serum total cholesterol, LDL-C, TG, higher LDL-C/HD-LC or TG/HDL-C ratio, lower-serum HDL-C were accompanied with increased systolic and diastolic blood pressure *i.e*. hypertension. Most importantly, the CVD-risk factors were accompanied with the increases in the serum uric acid levels; (iv.) correlation coefficient matrix, as carried out by bivariate regression analyses, revealed significant positive relationships between uric acid versus age, BMI, SBP, DBP and dyslipidemia-related risk factors, namely, TC, TG, LDL-C, HDL-C, TG/HDL-C and LDL-C/HDL-C ratios, and significant negative relationship with K and Zn; (v.) the anti-lipidemic/hypertensive drugs ameliorated TC, TG, HDL-C, TG/HDL-C and LDLC/HDLA ratios, blood pressures of the hypertensive subjects; however, they did not have effects on the levels of electrolytes (Na, K, Cl), trace element Zn and serum uric acid. These results might suggest a critical role of uric acid in the regulation of dyslipidemia, in other words, hyperuricemia and dyslipidemia may share a common pathophysiology of cardiovascular diseases in hypertension. Our study corroborated well with the reports of Peng et al., (2015) [28], where they also noted the positive relation between dyslipidemia and serum uric acid. Nakagawa et al (2006) [29], Moriarity et al., (2000), [12] also reported that the relation between serum uric acid and TG is linear. Our results are also consistent with increased uric acid level and hypertriglyceridemia [30]. There is a debate on whether uric acid may exert an atherogenic effect independently of other known cardiovascular risk factors. It is possible for several independent variables to be individually correlated with a dependent variable (as seen after bivariate regression analyses), but all of them might not be statistically significant in the same multiple linear regression model. This led us to analyze the correlation of serum uric acid with all other measured parameters by multiple regression analysis, which can statistically infer about whether a given relationship is independent from the confounding effects of other cardiovascular risk factors. Interestingly, among all parameters, serum uric acid was found to significantly correlate independently from other confounding CVD risk factors (age, BW, BMI, SBP and DBP,TC, TG, HDL, Na/Cl/K/Zn) with serum LDL-C levels and the correlation was positive (Table 4). We are not sure as why serum uric acid was

independently correlated with LDL-C only. Correlation provides information on association rather than a cause- and-effect relationship between variables. Thus there is a possibility of a considerable effect of other uninvestigated confounding factors on the correlation between serum uric acid and LDL-C. Although it is very difficult to assume about these unknown factors, however, blood levels of antioxidants, oxidized LDL-C, kidney filtration rate and action of other pharmacologically active substances are believed to contribute to the independent relationship between uric acid versus LDL-C. LDL-C may modify the endothelial functions of the blood vessels of the cardiovascular systems [31].

In ischemia and/or hypoxia-reperfusion condition, which is typically seen during atherosclerosis, the production of uric acid is accelerated. Xanthine oxidase (XO) is actively present in the vascular endothelial cells. Production of uric acid by the xanthine oxidase may harvest free radicals. Moreover, the uric acid and xanthine oxidase have been found in greater concentration in atherosclerotic vessels than in healthy vascular tissues. This might be one of the underlying mechanisms for which LDL-C was positively (independent from other confounding factors) correlated with the uric acid levels in the present investigation. Ruggiero et al. (2007) reported that levels of serum uric acid are low in the presence of carotenoid antioxidants in the serum [32]. Holvoet et al., (2001, 2004) reported that oxidized LDL-C is associated with coronary heart disease and it (oxidized LDL-C) can act as a useful diagnostic marker for identifying patients with coronary artery disease [33,34] and is highly linked with the pathophysiology of the cardiovascular diseases [35]. The net consequence is that the high serum uric acid confers damage to endothelial integrity by over-production of reactive free radical species, which, in turn, are important contributors to vascular diseases. Besides anti-lipiemic drugs, diuretics and angiotensin II blockers were most prevalent drugs as medication for the drug taking cardiovascular subjects in our investigation. Subjects taking angiotensin receptor bolckers/diuretics had lower levels (~ 6%) of uric acid when compared to those of the subjects who did not start taking drugs, however, the difference did not rich significance (WOD: 11.3±0.06 vs WD: 12.0±0.10). Diuretics work with kidneys to excrete sodium from urinary system via urine. In turn, the sodium takes water from blood, and the water is also excreted. Diuretics are thus commonly used to treat hypertension because they lower blood pressure by helping our body eliminate sodium and water through our urine. However, some diuretics can also cause to eliminate more potassium in the urine. This can lead to low potassium levels in the blood (hypokalemia). Hypokalemia is present in patients with cardiovascular disease [36]. In our case, the levels of either Na or Cl were not altered significantly in the subjects with (WD) or without drugs (WOD). Hypokalemia were not observed in the subjects of WD group, as compared those of the WOD group. Still, the levels of K were, as compared to those of the controls, were higher ($P<.05$) in both of hypertensive subjects (WOD and WD). We speculate that it may relate to the impairments of kidney tubular functions in the hypertensive WOD and WD subjects. Angiotensin II type 1 receptor blockers (ARB) are a frequently used class of antihypertensive drugs. Nishida et al. (2013) [37] reported that the ARB losartan decreases the serum uric acid level. But in this investigation the angiotensin II blockers did not significantly affect the serum uric acid level in the subjects with drug group (WD). Serum uric acid was accompanied with CVD risk factors. No evidence exists that reducing hyperuricemia is harmful. So reducing the uric acid in the serum, as one of the independent markers of cardiovascular diseases, may help people to be free from cardiac problems as well as gout complications.

Table 4. Multiple correlation between uric acid (dependent variable) and 13 independent variables (X)

(X)	Coefficient	Std. Error	Std. Coeff.	t-Value	P-Value
Intercept	6.441	25.887	6.441	0.249	0.810
Age	0.027	0.034	0.066	0.776	0.460
Body	-0.036	0.035	-0.064	-1.028	0.334
BMI	0.358	0.254	0.321	1.411	0.196
Systolic	-0.010	0.036	-0.059	-0.291	0.778
Diastolic	0.000	0.049	0.001	0.007	0.994
TC	0.001	0.003	0.044	0.517	0.619
TG	-0.008	0.005	-0.335	-1.549	0.160
LDLC	0.044	0.014	0.334	3.128	0.014
HDLC	-0.004	0.086	-0.009	-0.044	0.966
TG/HDLC	0.218	0.111	0.481	1.960	0.086
LDL/HDLC	-0.297	0.323	-0.256	-0.917	0.386
Na	0.005	0.102	0.005	0.049	0.962
K	-0.353	0.285	-0.098	-1.241	0.250
Cl	-0.067	0.092	-0.048	-0.727	0.488
Zinc	-0.077	0.054	-0.390	-1.424	0.192

Data were subjected to multiple coreltion analysis.

The levels of zinc exhibited significantly negative correlation with age, BW, BMI, SBP/DBP, TC, TG, and LDL-C. Several studies indicate that zinc is vital to vascular endothelial cell integrity [38-39]. Zinc is inversely correlated with the atherosclerotic lesion formation [40]. Therefore, zinc can slow down the progression of atherosclerosis [41, 42]. The hypertensive subjects had zinc value of 11.8±0.10 ~10.2±0.17µg/dL (in WOD and WD subjects) compared to 52.4±1.7 µg/dL in the control subjects. There was a big difference between the values of the control versus hypertensive subjects of WD and WOD groups. Subjects with serum zinc concentration (11.8±0.10 ~10.2±0.17µg/dL) lower than the baseline of the controls (52.4.2± 1.7 µg/dL) had a higher risk for cardiovascular risk factors. In our study the deficiency of zinc levels caused uric acid to increase in a correlated manner (Table 3). The correlation of CVDs win zinc deficiency is still not clear. Hsieh et al. (2011) [43] have reported reduced serum zinc levels among the patients of Coronary Artery Disease. Other investigators have found zinc deficiency as a risk factor for ischaemic heart disease and its various clinical manifestations (Olsén et al., 2012) [44]. Zinc deficiency also leads to reduced survival in the patients of coronary artery disease (Pilz et al, 2009) [45]. The results of our investigation are thus consistent with these reports. A relevant study also was done in South Africa by a group of researchers. They stated that dietary zinc deficiency caused uric acid to increase by disturbing the glomerular filtration rate (Rasheed et al, 2012) [46]. Again, the serum zinc level exhibited negative correlation with the serum uric acid. The relationship of zinc and uric acid however was not independent from other confounding relationships (Table 4). The cause-effect relationship between serum uric acid and zinc is not clearly understood.

5. CONCLUSION

The debate is still ongoing on 'whether serum uric acid can act as an independent marker for cardiovascular disease or it simply results from the synergistic effects of other known cardiovascular risk factors'. The major finding of this study is that hypertensive hypercholesterolemic subjects had increased prevalence rate of elevated serum uric acid levels and that increased LDL-C is the strongest predictor of hyperuricemia in our

investigation. However, such a conclusion should be drawn on a large number of population sizes. The results are consistent with numerous published reports. However, the underlying pathophysiological mechanisms linking elevated LDL-C and hyperuricemia are currently unknown. The control of dyslipidemia by the lipid-lowering drugs did not correct or alter the uric acid levels in our investigation. This suggests that the relationship between LDL-C and uric acid is not simple as it is anticipated. Thus, it is urgent to develop appropriate treatment guidelines for hyperuricemia. Finally, understanding the mechanisms of the relevance of elevated serum uric acid levels in cardiovascular disease (CVD) and the biological basis of the link of LDL-C with elevated uric acid might help clinicians to identify and treat CVD patients, as well as help patients prevent these potentially devastating complications. Further research is essential to understand the relationship between serum uric acid and other cardiovascular risk factors.

ACKNOWLEDGEMENTS

Gratefully acknowledge the contribution of each of the technicians of the laboratories.

COMPETING INTERESTS

Athe authors declare no conflict of interest

REFERENCES

1. So A, Thorens B. Uric acid transport and disease. J Clin Invest. 2010; 120(6): 1791–99.
2. Hall AP, Barry PE, Dawber TR, McNamara PM. Epidemiology of gout and hyperuricemia. A long-term population study. Am J Med. 1967*; 42(1):* 27-37.
3. Campio ED, Glynn RJ, DeLabry LO. Asymptomatic hyperuricemia. Risks and consequences in the normative aging study. Am J Med. 1967; 82(3): 421-26.
4. Neogi T, Ellison RC, Hunt S, Terkeltaub R, Felson DT. Serum uric acid is associated with carotid plaques: the National Heart, Lung, and Blood Institute Family Heart Study. J Rheumatol. 2009; 36(2):378–84.
5. Bos MJ, Koudstaal PJ, Hofman A, Witteman JC, Breteler MM.Uric acid is a risk factor for myocardial infarction and stroke: the Rotterdam study. Stroke. 2006; 37: 1503–1507.
6. Juraschek SP, Tunstall-Pedoe H, Woodward M, Serum uric acid and the risk of mortality during 23 years follow-up in the Scottish Heart Health Extended Cohort Study"Atherosclerosis. 2014; 233: 623–29.
7. Freedman DS, Williamson DF, Gunter EW, Byers T. Relation of serum uric acid to mortality and ischemic heart disease. The NHANES I Epidemiologic Follow-up Study", Am J Epidemeol. 1995; 141: 637-44.
8. Verdecchia P, Schillaci G, Reboldi G, Santeusanio F, Porcellati C, Brunetti P. Relation between serum uric acid and risk of cardiovascular disease in essential hypertension. The PIUMA study. Hypertension. 2000; 36: 1072-78.
9. Kim SY, Guevara JP, Kim KM, Choi HK, Heitjan DF, Albert DA. Hyperuricemia and coronary heart disease: a systematic review and meta-analysis. Arthritis Care Res. (Hoboken). 2010; 62: 170–180.
10. Culleton BF, Larson MG, Kannel WB, Levy D. Serum uric acid and risk for cardiovascular disease and death: the Framingham Heart Study. Annals of Intern. Med. 1999; 131: 7-13.
11. Brand FN, McGee DL, Kannel WB, Stokes J,. Castelli 3rd,WP. Hyperuricemia as a risk factor of coronary heart disease: The Framingham Study" Am J Epidemiol. 1985:121: 11-18.

12. Moriarity JT, Folsom AR, Iribarren C, Nieto FJ, Rosamond WD. Serum uric acid and risk of coronary heart disease: Atherosclerosis Risk in Communities (ARIC) Study, Ann. Epidemiol. 2000;10:136-143.
13. Zaman MA. Global scenario of cardiovascular risks and Bangladesh perspective. http://www.orion-group.net/journals/Journals/vol17_jan2004/130.htm.
14. http://www.nhf.org.bd/indexx.php
15. Sayeed MA., Mahtab H, Sayeed S, Begum T, Khanam PA, Banu A. Prevalence and risk factors of coronary heart disease in a rural population of Bangladesh", Ibrahim Med. Coll. J. 2010; 4(2): 37-43.
16. Facchini F, Chen YD, Hollenbeck CB, Reaven GM. Relationship between resistance to insulin-mediated glucose uptake, urinary uric acid clearance, and plasma uric acid concentration. JAMA 1991;266:3008-11.
17. Reaven GM. The kidney: an unwilling accomplice in syndrome X. Am J Kidney Dis 1997;30:928-31.
18. Timar O, Sestier F, Levy E. Metabolic syndrome X: a review. Can J Cardiol 2000;16:779-89.
19. Dincer HE, Dincer AP, Levinson DJ. Asymptomatic hyperuricemia: to treat or not to treat. Cleve Clin J Med 2002;69:594, 597, 600-594, 597, 602.
20. Alderman MH, Cohen H, Madhavan S, Kivlighn S. Serum uric acid and cardiovascular events in successfully treated hypertensive patients. Hypertension 1999;34:144-50
21. Bengtsson C, Lapidus L, Stendahl C, Waldenstrom J. Hyperuricaemia and risk of cardiovascular disease and overall death. A 12-year follow-up of participants in the population study of women in Gothenburg, Sweden. Acta Med Scand 1988;224:549-55
22. Freedman DS, Williamson DF, Gunter EW, Byers T. Relation of serum uric acid to mortality and ischemic heart disease. The NHANES I Epidemiologic Follow-up Study. Am J Epidemiol 1995;141:637-44
23. Klein R, Klein BE, Cornoni JC, Maready J, Cassel JC, Tyroler HA. Serum uric acid. Its relationship to coronary heart disease risk factors and cardiovascular disease, Evans County, Georgia. Arch Intern Med 1973;132:401-10

24. Kinlaw W, Levine A, Morley J, Silvis S, McClain C. Abnormal zinc metabolism in type II diabetes mellitus", Am J Med. 1993; 75: 273–277.
25. Reunanen A, Knekt , Marniemi J, Ma¨ki J, Maatela J. Aroma A. Serum calcium, magnesium, copper and zinc and risk of cardiovascular death A. Eur nJ Clin Nutr. 1996; 50: 431-437.
26. Singh R, Niaz M, Rastogi S, Bajaj S, Gaoli Z, Shoumin Z. Current zinc intake and risk of diabetes and coronary artery disease and factors associated with insulin resistance in rural and urban populations of North India., J Am Coll. Nutr. 1998; 17: 564–570.
27. HLee D, FolsomA, Jacobs D. Iron, zinc, and alcohol consumption and mortality from cardiovascular diseases: the Iowa Women's Health Study. Clin Nutr. 2005; 81: 787–791.
28. Peng T-C, Wang C-C, Kao T-W, Yi-H J, Yang Y-H, et al. Relationship between Hyperuricemia and Lipid Profiles in US Adults, BioMed Res. Intl. 2015; Article ID 127596, 7 pages.
29. Nakagawa T, Hu H, Zharikov S. et al. A causal role for uric acid in fructose-induced metabolic syndrome," The Am J Physiol.: Renal Physiol. 2006; 290(3): F625–F631.
30. Vuorinen-Markkola H, Yki-Järvinen H. Hyperuricemia and insulin resistance. J Clin Endocrinol. Metab. 1994;78(1) 25–29.

31. Mazzali M, Kanellis J, Han L, Feng L, Xia YY. et al. Hyperuricemia induces a primary renal arteriolopathy in rats by a blood pressure-independent mechanism. Am J Physiol Renal Physiol. 2002; 282(6):F991-7.
32. Ruggiero C, Cherubini A, Guralnik J, Semba RD, Maggio M, et al. The interplay between uric acid and antioxidants in relation to physical function in older persons" J Am Geriatr Soc. 2007; 55(8) 1206-1215.
33. Holvoet P. Oxidized LDL and coronary heart disease. Acta Cardiol. 2004; 59(5): 479-484.
34. Holvoet P, Mertens A, Verhamme P. et al. Circulating oxidized LDL is a useful marker for identifying patients with coronary artery disease," Arteriosclerosis, Thromb Vasc Biol. 2001; 21(5): 844–848.
35. Betsy BD. The Pathophysiology of Cardiovascular Disease and Diabetes: Beyond Blood Pressure and Lipids,' Diabetes Spectrum. 2008; 21(3):160-165.
36. Clausen T. Hormonal and pharmacological modification of plasma potassium homeostasis', Fundamen Clin Pharmacol. 2010; 24: 595-605.
37. Nishida Y, Takahashi Y, Susa N, Kanou N, Nakayama T, Asai S. Comparative effect of angiotensin II type I receptor blockers on serum uric acid in hypertensive patients with type 2 diabetes mellitus: a retrospective observational study", Cardiovasc Diabetol. 2013; 12:159.
38. Beattie JH, Kwun IS. Is zinc deficiency a risk factor for atherosclerosis? Br J Nutr 2004; 91(2): 177-181.
39. Clair J, Talwalkar R, McClain C J, Hennig B. Selective removal of zinc from cell culture media. J Trace Elemen. Exp Med. 1995; 7: 143–151.
40. Ren M, Watt F, Huat BTK, Halliwell B. Correlation of iron and zinc levels with lesion depth in newly formed atherosclerotic lesions. Free Rad Biol Med. 2003;34: 746–752.
41. Berger M, Rubinraut E, Barshack I, Roth A, Keren G, George J. Zinc reduces intimal hyperplasia in the rat carotid injury model", Atherosclerosis. 2004; 175: 229-234.
42. Reiterer G, Toborek M, Hennig B. Peroxisome proliferator activated receptors and γ require zinc for their anti-inflammatoryproperties in porcine vascular endothelial cells. J Nutr. 2004; 134: 1711-1715.
43. Hsieh BT, Chang CY, Chang YC, Cheng KY. Relationship between the level of essential metal elements in human hair and coronary heart disease. J Radioanal Nucl Chem 2011; 290: 165-169.
44. Olsén L, Lind, PM, Lind L. Gender differences for associations between circulating levels of metals and coronary risk in the elderly. Int J Hyg Environ Health 2012; 215 (3): 411-417.
45. Pilz S, Dobnig H, Winklhofer – Roob BM, Renner W Seelhorst U, Wellnitz B, et al. Low serum zinc concentrations predict mortality in patients referred to coronary angiography. Brit J NUtr 2009; 101: 1534-1540.
46. Rasheed N-Al, Nayira A, Baky A, Rasheed NAl, Shebly W, et al. Effect of vitamin E and α-lipoic acid on nano zinc oxide induced renal cytotoxicity in Rats, African J Pharm Pharmacol. 2012; 6: 2211-23.

Table 1. Demographic characteristics and blood pressures of all the subjects

Variables	**Control Subjects (CON)**			**Patients without drugs (WOD)**			**Patients with drugs (WD)**		
Sex	Male (n=23)	Female (n=17)	Average of male+female (n=40)	Male (n=29)	Female (n=30)	Average of male+female (n=59)	Male (n=50)	Female (n=48)	Average of male+female (n=98)
Age (y)	52.5^{a} ± 0.70	52.1^{a} ±0.90	52.3^{a} ±0.50	57.6^{b} ±1.3	66.9^{c} ±1.3	62.3^{d} ± 1.1	67.8^{c} ± 0.93	62.4^{d} ± 1.10	$65.1^{c,d}$ ± 0.80
BW (Kg)	66.0^{a} ± 1.3	61.4^{a} ±1.8	64.1^{a} ±1.10	64.0^{a} ±1.4	63.0^{a} ±1.1	63.5^{a} ±0.74	64.1^{a} ±1.12	63.1^{a} ±.95	63.6^{a} ±0.74
BMI (kg/m^2)	21.0^{a} ±0.14	20.8^{a} ±0.23	20.9^{a} ±0.13	27.2^{b} ±0.10	27.5^{b} ±0.18	27.4^{b} ±0.10	28.1^{c} ±0.25	28.1^{c} ±.14	28.1^{c} ±0.12
SBP (mmHg)	122^{a} ±1.97	128^{a} ± 6.5	125^{a} ± 2.75	169^{b} ±2.03	169^{b} ±1.70	169^{b} ±1.30	164^{c} ±1.05	164^{c} ±2.3	164^{c} ± 1.30
DBP (mmHg)	78.5^{a} ±1.5	80.0^{a} ±4.30	79.1^{a} ±1.80	97.3^{b} ±2.5	$92.3^{c,a}$ ±1.50	$94.7^{b,c}$ ±1.50	90.8^{a} ±0.98	88.97^{a} ±1.25	89.8^{a} ±.80

Results are mean ± SEM. Data were analyzed by one-way ANOVA, followed by Fisher's PLSD for post hoc comparison. Values in the same row those share the common superscript are not significantly different at $P<0.05$.

Table 2. Blood parameters of the subjects

Variables	Control Subjects (Con)			Subjects without drugs (WOD)			Subjects with drugs (WD)		
Sex	Male (n=23)	Female (n=17)	Average of male+female (n=40)	Male (n=29)	Female (n=30)	Average of male+female (n=59)	Male (n=50)	Female (n=48)	Average of male+female (n=98)
TC (mg/dL)	178[a] ±4.50	173[a] ±4.50	176[a] ±3.2	377[b] ±14.0	378[b] ±13.4	378[b] ±9.60	256[c] ±3.0	251[c] ±2.90	253[c] ±2.10
TG (mg/dL)	207[a] ± 3.70	192[a] ±8.70	200[a] ±4.40	339[b] ±23.3	379[b] ±22.6	359[b] ±16.3	258[c] ±14.4	262[c] ±15.6	260[c] ±10.5
LDL-C (mg/dL)	133[a] ±3.6	133[a] ±2.70	133[a] ±2.30	169[b] ±1.80	167[b] ±1.90	168[b] ±1.30	171[b] ±1.40	169[b] ±1.40	170[b] ±1.0
HDL-C (mg/dL)	23.2[a] ±.80	22.1[a] ±0.90	22.7[a] ±0.60	22.1[a] ±1.10	21.2[a] ±1.30	21.7[a] ±0.80	32.0[b] ±1.10	35.0[b] ±1.50	33.2[b] ±1.0
TG/HDLC	9.15[a] ±0.51	8.88[a] ±0.34	9.04[a] ±0.23	16.18[b] ±1.18	20.21[c] ±1.88	18.23[b,c] ±0.80	8.78[a] ±0.63	8.82[a] ±0.86	8.80[a] ±0.50
LDL/HDL	5.96[a] ±0.26	6.17[a] ±0.26	6.05[a] ±0.20	8.28[b] ±0.48	8.80[b] ±0.48	8.54[b] ±0.37	5.65[a] ±0.19	5.33[a] ±0.24	5.50[a] ±0.15
Na (mmol/L)	137[a] ±.20	136[a] ±0.40	137[a] ±0.20	138[a] ±0.80	137[a] ±0.60	138[a] ±.40	138[a] ±0.40	137[a] ±0.40	138[a] ±0.40
K (mmol/L)	5.56[a] ±0.14	5.76[a] ±0.20	5.65[a] ±0.13	4.32 ±0.14	4.24[b] ±0.18	4.30[b] ±0.10	4.49[b] ±0.14	4.37[b] ±0.15	4.40[b] ± 0.10
Cl (mmol/L)	104[a] ±0.40	103[a] ±0.40	104[a] ±0.30	103[a] ±0.4	103[a] ±0.40	103[a] ±0.30	103[a] ±0.30	103 ±0.30	103[a] ±0.20
Zn (μg/dL)	51.0[a] ±2.1	55.2[a] ±2.8	52.4[a] ±1.70	11.8[b] ±0.20	11.7[b] ±0.20	11.8[b] ±.10	10.0[b] ±0.2	10.4[b] ±0.30	10.2[b] ± 0.17
Uric acid (mg/dL)	4.40[a] ±0.30	3.70[a] ±0.40	4.10[a] ±0.20	11.7[b] ±0.14	12.0[b] ±0.14	12.0[b] ±0.10	11.4[b] ±0.08	11.3[b] ±0.08	11.4[b] ±0.60

Results are mean ± SEM. Data were analyzed by one-way ANOVA, followed by Fisher's PLSD for post hoc comparisons. Values in the same row those share the common superscript are not significantly different at P<0.05.

Table 3. Correlation coefficient matrix analysis among different variables measured.

	Age	BW	BMI	SBP	DBP	TC	TG	LDLC	HDLC	TG/HDL	LDL/HDL	Na	K	Cl	Zn	UA
Age	1.000															
BW	0.124	1.000														
BMI	0.560	-0.074	1.000													
SBP	0.422	-0.036	0.854	1.000												
DBP	0.191	-0.020	0.517	0.534	1.000											
TC	0.216	0.034	0.571	0.494	0.425	1.000										
TG	0.148	0.114	0.386	0.235	0.215	0.418	1.000									
LDLC	0.454	0.010	0.761	0.573	0.413	0.465	0.195	1.000								
HDLC	0.325	0.027	0.300	0.231	-0.043	-0.177	-0.273	0.234	1.000							
TG/HDL	0.003	0.039	0.218	0.128	0.174	0.428	0.797	0.073	-0.644	1.000						
LDL/HDL	-0.092	-0.038	0.116	0.035	0.200	0.390	0.313	0.160	-0.818	0.760	1.000					
Na	0.106	0.093	0.110	0.073	0.056	0.074	0.066	0.132	0.123	-0.043	-0.105	1.000				
K	-0.256	0.018	-0.482	-0.562	-0.226	-0.393	-0.120	-0.334	-0.203	-0.026	-0.004	-0.017	1.000			
Cl	-0.025	-0.054	-0.067	-0.089	-0.192	-0.129	0.020	0.019	0.110	-0.022	-0.076	-0.002	0.022	1.000		
Zn	-0.520	0.061	-0.943	-0.938	-0.513	-0.593	-0.377	-0.768	-0.195	-0.251	-0.181	-0.136	0.542	0.098	1.000	
UA	0.541	0.006	0.928	0.835	0.516	0.586	0.315	0.793	0.231	0.182	0.132	0.137	-0.498	-0.057	-0.943	1.000

Results were obtained from bivariate analyses. No correlation, r = 0 to ± 0.25; Poor correlation, r = ± 0.25 to ± 0.50; Moderate/good correlation, r = ±0.50 to ±0.75; Very good to excellent correlation r = ± 0.75 to ± 1.0 . *Ref: Dawson B, Trapp RG. Basic and Clinical Biostatistics. 4th Ed. New York: Lange Medical Books/McGraw-Hill; 2004.*

Table 4. Multiple correlation between uric acid (dependent variable) and 13 independent variables (X).

(X)	Coefficient	Std. Error	Std. Coeff.	t-Value	P-Value
Intercept	6.441	25.887	6.441	0.249	0.810
Age	0.027	0.034	0.066	0.776	0.460
Body weight	-0.036	0.035	-0.064	-1.028	0.334
BMI	0.358	0.254	0.321	1.411	0.196
Systolic	-0.010	0.036	-0.059	-0.291	0.778
Diastolic	0.000	0.049	0.001	0.007	0.994
TC	0.001	0.003	0.044	0.517	0.619
TG	-0.008	0.005	-0.335	-1.549	0.160
LDL-C	0.044	0.014	0.334	3.128	0.014
HDL-C	-0.004	0.086	-0.009	-0.044	0.966
TG/HDL-C	0.218	0.111	0.481	1.960	0.086
LDL/HDL-C	-0.297	0.323	-0.256	-0.917	0.386
Na	0.005	0.102	0.005	0.049	0.962
K	-0.353	0.285	-0.098	-1.241	0.250
Cl	-0.067	0.092	-0.048	-0.727	0.488
Zinc	-0.077	0.054	-0.390	-1.424	0.192

Data were subjected to multiple correlation analysis.