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ASSOCIATION OF C-REACTIVE PROTEIN AND URIC ACID WITH TYPE 2 DIABETES

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Abstract

Diabetes mellitus is a group of metabolic diseases characterized by high blood glucose levels that result from defects in insulin secretion or action or both. Some recent studies had shown that elevated level of C-reactive protein (CRP) and uric acid are involved in the pathogenesis of type 2 diabetes, regardless of other characteristics of subjects. Our aim was to investigate the association between CRP and uric acid levels with diabetes in Bangladeshi population. Two hundred type 2 diabetic subjects (male 110, female 90) and 60 non diabetic subjects (30 male, 30 female) were included in the study over six months. CRP and uric acid were measured by Latex Agglutination method and enzymatic colorimetric method, respectively. CRP was significantly higher ($p < 0.01$) in diabetic compared to non-diabetic subjects for both male and female subjects. The level of uric acid was also significantly higher in male and female diabetic subjects ($p < 0.05$ and $p < 0.01$) compared to non-diabetic subjects, respectively. In multiple regression analysis, CRP showed a positive association with uric acid and BMI in both diabetic and non-diabetic subjects. On the other hand, uric acid showed positive association with fasting blood sugar (FBS), diastolic blood pressure, HbA1c and CRP in case of diabetic subjects, but with age, BMI, CRP, HbA1c and creatinine in non-diabetic subject. Uric acid also shows a significantly ($p < 0.001$) positive Pearson correlation with CRP. These data strongly suggest that compared to non-diabetic subjects, diabetic subjects have significantly higher level of CRP and uric acid.

Introduction

Identifying risk factors for the development of type 2 diabetes is essential for its early screening and prevention. Previous studies have suggested that serum CRP and uric acid levels are positively associated with the development of type 2 diabetes.[1,2] Pradhan *et al*.[3] suggested that patients with elevated basal levels of CRP are at an increased risk of type 2 diabetes. It is well-known that cytokines operate as a network in stimulating the production of acute-phase proteins like CRP. *In vivo* studies have shown that adipose tissue secretes IL-6, which regulates CRP production and could, potentially, induce

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chronic systemic inflammation in subjects with excess body fat.[4] Therefore, patients with type 2 diabetes who are, usually obese, could potentially have high IL-6 and CRP. On the other hand, it was shown in a prospective follow-up study that high serum uric acid is associated with higher risk of type 2 diabetes independent of obesity, dyslipidemia and hypertension.[5] Further potentially important biological effects of uric acid relate to endothelial dysfunction by inducing antiproliferative effects on endothelium and impairing nitric oxide production and inflammation, e.g., through increased CRP expression, although these issues are considered controversial.[6,7]

Recent evidence suggests that uric acid plays a role in cytokine secretion.[8] Moreover, uric acid has been identified as a mediator of endothelial dysfunction and systemic inflammation.[6] Chien *et al.*[9] reported a positive association between the plasma concentration of uric acid and the incidence of type 2 diabetes in Chinese individuals. This association was somewhat attenuated after adjustment for metabolic syndrome, suggesting that the association between hyperuricemia and diabetes was partly mediated through the metabolic syndrome, in particular insulin resistance. Such results may not be contrary to the suggested protective effects of uric acid**.** It is quite conceivable, in the context of the complex cellular environment of the metabolic syndrome which is clearly associated with oxidative stress, antioxidant properties of uric acid might convert to a pro-oxidant state owing to reactive oxygen species (ROS) accumulation.[10] This may also lead to adverse effects on endothelial function and a proinflammatory response, both of which are known to be associated with new onset of type 2 diabetes.[11] However, it remains controversial whether CRP and uric acid are associated with the development of type 2 diabetes. As limited information is available about the relationship of diabetes with CRP and uric acid in Asia, we examined the relationship of pre existing type 2 diabetes with the levels of CRP and uric acid in Bangladeshi population.

Research design and Methods

Two hundred type 2 diabetic subjects (male 110, female 90) and 60 non-diabetic subjects (30 male, 30 female) with age ≥40 were included in the study. Subjects were in fasting condition for at least eight hours and in the subsequent morning venous blood was drawn at fasting and 2 hrs after breakfast respectively. Blood samples were allowed to clot for 30 min then centrifuged for 10 min at 3000 rpm and serum samples were collected for the estimation of fasting glucose, serum lipid profile (Total cholesterol, HDL-C, LDL-C and TG), creatinine, CRP and uric acid. For HbA1c estimation, 2 ml blood was separately collected along with fasting and transferred to a test tube containing heparin as anticoagulant. Blood pressure (BP) was measured via a sphygmomanometer. Body mass index (BMI) of the subjects were calculated using standard formula, BMI = weight (kg)/ [Height (m)]^2. Hepatic complication like jaundice, hepatitis B or C virus

positive or chronic liver failure and cirrhosis patient, patient with abnormal serum creatinine that means chronic renal failure were excluded from the study.

Fasting blood sugar, glucose level 2 hrs after breakfast, serum total cholesterol, HDL-C and triglycerides was measured by enzymatic colorimetric method. The LDL-C level in serum was calculated by using Friedewald formula.[12] The percentage of HbA1c was measured on the basis of Boronate affinity assay by spectrophotometric method. Estimation of serum creatinine was done by alkaline picrate method. CRP and uric acid was measured by Latex Agglutination method and enzymatic colorimetric method, respectively.

Statistical analysis was performed using SPSS software for Windows version 16 (SPSS Inc., USA). All the data were expressed as mean ± SD and percentage as appropriate. To see the statistical significance, paired sample tests, Pearson correlation coefficient test and multiple regression analysis were done. A p value of <0.05 was considered statistically significant.

Results and Discussion

Table 1 summarizes age, duration of diabetes, blood pressure, family history of diabetes, medication and BMI of the diabetic subjects. This table also shows biochemical characteristic of the diabetic subjects. Similarly Table 2 summarizes age, BMI and biochemical characteristics of non-diabetic subjects.

There was no significant age and blood pressure difference between non-diabetic, and diabetic subjects. Although compare to all non-diabetic subjects systolic and diastolic blood pressure were slightly higher in diabetic subjects. BMI was significantly higher in diabetic subjects as compared to non-diabetic subjects in both male ($p < 0.05$) and female ($p < 0.001$), respectively. Fasting blood sugar and HbA1c were significantly higher in diabetic subjects as compared to non-diabetic subjects in both gender ($p < 0.001$). Although the level of creatinine for both diabetes and non-diabetes subjects are in the normal range, but the level was significantly higher in diabetic compared to non-diabetic subjects ($p < 0.05$).

Serum HDL-cholesterol was found to be significantly higher in non-diabetic subjects as compared to diabetic subjects ($p < 0.01$). Male diabetic and non-diabetic subjects had significantly lower serum LDL-cholesterol levels than female diabetic and non-diabetic subjects, respectively. Diabetic male had significantly higher serum triglyceride levels than diabetic female ($p < 0.01$).

As shown in Fig. 1, CRP was significantly higher ($p < 0.01$) in male and female diabetic subjects as compared to non-diabetic subjects, respectively. On the other hand, the level of uric acid was also significantly higher ($p < 0.05$ and $p < 0.01$) in diabetic as compared to non-diabetic subjects in both male and female (Fig. 2).

Table 1. Clinical and biochemical characteristics of the diabetic subjects.

Variables	Male (n = 110)	Female (n = 90)	All (n = 200)
Age (years)	54.3 ± 9.8	54.0 ± 9.3	54.0 ± 9.6
Duration of diabetes (years)	11.1 ± 6.2	9.9 ± 5.0	10.6 ± 5.7
Systolic blood pressure (mmHg)	78.8 ± 10.4	81.0 ± 5.8	79.8 ± 8.7
Diastolic blood pressure (mmHg)	127.2 ± 15.8	128.4 ± 13.2	127.8 ±14.7
Family history of diabetes	82 (74.6)	74 (82.2)	156 (78.0)
Smoking status	36 (32.7)	Nil	36 (18.0)
Oral hypoglycemic drug	64 (58.2)	54 (60.0)	118 (59.0)
Insulin	49 (44.6)	38 (42.2)	87 (43.5)
Anti-hypertensive drug	86 (78.8)	79 (87.8)	165 (82.5)
Lipid lowering drug	92 (83.6)	81 (90.0)	173 (86.5)
Body mass index (kg/m^2)	24.4 ± 3.3	26.4 ± 3.9	25.9 ± 3.7
Fasting blood sugar (mmol/l)	7.6 ± 2.7	7.0 ± 1.8	7.3 ± 2.4
After breakfast sugar "	11.5 ± 3.8	10.0 ± 2.9	10.8 ± 3.5
HbA1c (%)	8.2 ± 1.3	7.8 ± 1.3	8.1 ± 1.3
CRP (mg/l)	11.7 ± 8.8	13.2 ± 9.8	12.4 ± 9.3
Uric acid (mg/dl)	5.6 ± 1.6	5.2 ± 1.5	5.4 ± 1.6
Creatinine "	1.1 ± 0.3	0.9 ± 0.3	1.0 ± 0.3
Triglyceride "	153.5 ± 28.1	141.5 ± 28.7	148.0 ± 28.9
Cholesterol "	172.3 ± 26.0	179.5 ± 29.5	175.5 ± 27.8
HDL-Cholesterol "	32.6 ± 3.6	33.5 ± 4.6	33.0 ± 4.1
LDL-Cholesterol "	109.7 ± 25.4	117.1 ± 25.9	113.0 ± 25.8

Values are mean ± SD for continuous variables and number (percentage) for categorical variables.

Table 2. Clinical and biochemical characteristics of the non-diabetic subjects.

Variables	Male (n = 30)	Female (n = 30)	All (n = 60)
Age (years)	55.8 ± 13.4	51.9 ± 9.8	53.9 ±11.8
BMI (kg/m^2)	22.6 ± 1.9	22.8 ± 1.9	22.7 ± 1.9
FBS (mmol/l)	5.2 ± 0.6	4.9 ± 0.6	5.1 ± 0.6
HbA1c (%)	5.1 ± 0.5	4.6 ± 0.6	4.9 ± 0.6
CRP (mg/l)	7.5 ± 4.6	8.0 ± 6.8	7.7 ± 5.8
Uric acid (mg/dl)	4.6 ± 1.3	4.3 ± 1.4	4.5 ±1.4
Creatinine "	0.9 ± 0.2	1.0 ± 0.2	0.9 ± 0.2
Triglyceride "	147.3 ± 24.7	146.8 ± 20.2	147.1 ± 22.4
Cholesterol "	167.3 ± 28.7	184.4 ± 26.2	175.9 ± 28.6
HDL-C "	35.5 ± 5.4	36.5 ± 5.6	36.0 ± 5.4
LDL-C "	102.3 ± 27.2	118.6 ± 22.2	110.5 ± 25.9

Values are mean ± SD. FBS, fasting blood sugar.

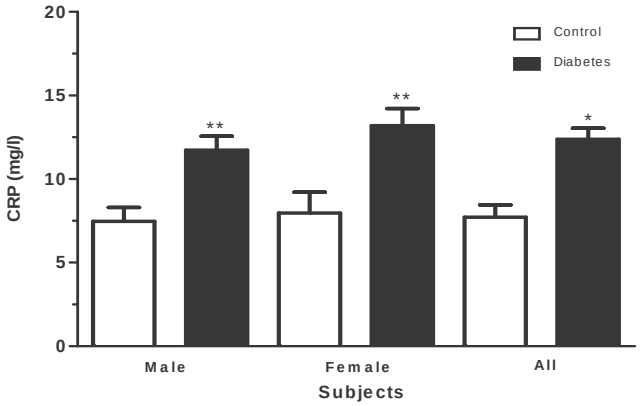

Fig. 1. CRP level of the study subjects. Values are mean ± SD. *p < 0.05, **p < 0.01 versus control subjects.

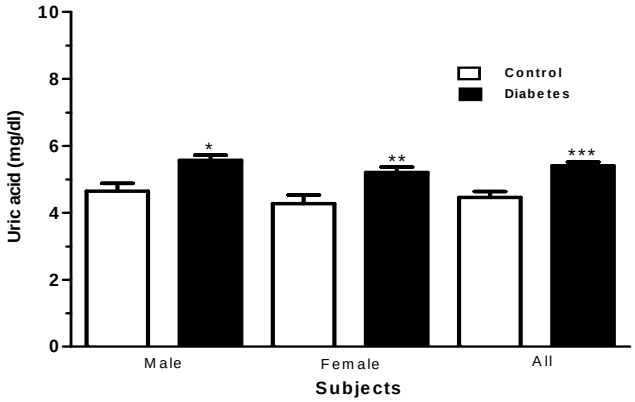

Fig. 2. Uric acid level of the study subjects. Values are mean ± SD. *p < 0.05, **p < 0.01, ***p < 0.01 versus control subjects.

In case of Pearson correlation among diabetic subject, CRP show significantly positive association with FBS ($p < 0.01$), ABF ($p < 0.01$), HbA1c ($p < 0.001$), BMI ($p < 0.01$) and uric acid ($p < 0.001$). But in non-diabetic subject CRP shows positive association only with age ($p < 0.01$) and uric acid ($p < 0.01$). On the other hand, uric acid shows Pearson correlation with FBS ($p < 0.001$), ABF ($p < 0.001$), diastolic blood pressure ($p < 0.01$) and CRP ($p < 0.001$) in diabetic subject but with only age ($p < 0.001$) in non-diabetic subject.

In multiple regression analysis, CRP showed a positive association with uric acid and BMI in both diabetic and non-diabetic subjects (Table 3). On the other hand, uric acid showed positive association with FBS, diastolic blood pressure, HbA1c and CRP in diabetic subjects, but with age, BMI, CRP and HbA1c in non-diabetic subjects (Table 4).

We have studied the associations of CRP and uric acid with type 2 diabetes in Bangladeshi adults. Our results are compatible with the hypothesis that CRP and uric acid may have a role in the pathogenesis of type 2 diabetes. CRP is produced by hepatocytes, and its gene expression is regulated by tumor necrosis factor-α (TNF-α) and interleukin-6 (IL-6), which are secreted by adipocytes.[13] As a result, obese individuals who have more and larger adipocytes also have higher baseline serum CRP. Because diabetes is more common in obese individuals, an association is expected between serum CRP and diabetes. However, some studies found that obesity does not explain the association of CRP with diabetes completely, suggesting an independent role for CRP in the development of diabetes.[14,15] It has been reported that CRP levels were significantly higher in both diabetic men and women as compared to their non-diabetic counterparts.[16] Increased CRP levels have been described in people with type 2

Table 3. Multiple regression analysis using CRP as the dependent variable.

Variables	Std. Error	EC(β)	t value	p value
Diabetes				
BMI	0.169	0.149	2.197	$p < 0.05$
Uric acid	0.469	0.345	4.354	$p < 0.001$
Non-diabetes				
BMI	0.425	0.335	2.399	$p < 0.05$
Uric acid	0.705	0.521	3.084	$p < 0.01$

EC = Estimate coefficient.

Table 4. Multiple regression analysis using uric acid as the dependent variable.

Variables	Std. Error	EC(β)	t value	p value
Diabetes				
FBS	0.071	–0.231	–2.181	$p < 0.05$
DBP	0.011	0.169	2.673	$p < 0.01$
HbA1c	0.131	0.428	3.954	$p < 0.001$
CRP	0.010	0.268	4.354	$p < 0.001$
Non-diabetes				
Age	0.013	0.456	4.163	$p < 0.001$
BMI	0.076	–0.325	–3.112	$p < 0.01$
CRP	0.024	0.312	3.084	$p < 0.01$
HbA1c	0.038	0.175	3.835	$p < 0.001$
Creatinine	0.610	–0.234	–2.507	$p < 0.05$

EC = Estimate coefficient.

diabetes[3] as well as in type 1 diabetes.[17] Our finding adds to the growing body of evidence that higher level of CRP may be related to the pathogenesis of type 2 diabetes. Potential mechanisms for this relationship may be direct or indirect. For example, cytokines such as elevated levels of IL-6, which is known to be a main stimulator of the production of most acute-phase proteins, were shown to increase the risk of diabetes.[18] 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 type 2 diabetes.[19] Alternatively, endothelial dysfunction may link inflammation to insulin resistance.[19,20] Some others explained the association through oxidative stress or innate immune system.[21] However, further studies are necessary to find a reasonable mechanism. King et al.[22] reported that inflammation might not only play a possible role in diabetogenesis but also in hyperglycemia after diabetes has been established.

In addition to CRP, some recent studies had shown that serum uric acid level is positively associated with type 2 diabetes regardless of other various characters.[2,5,9] It has also been reported that uric acid may be a useful predictor of type 2 diabetes in older adults with impaired fasting glucose.[23] Our study also shows similar results. Level of uric acid was significantly higher in diabetic subjects as compared to non-diabetic subjects in both genders. Chien *et al*.[9] reported that insulin resistance, which is closely related to metabolic syndrome and inflammation, may mediate the association between uric acid and diabetes risk. In our study, uric acid also shows positive correlation to CRP ($p < 0.001$). In multiple regressions analysis uric acid showed positive association with FBS, diastolic blood pressure, HbA1c and CRP in case of diabetic subjects.

In conclusion, the results of the present study suggests that compared to non-diabetic subjects diabetic subjects have significantly higher level of CRP and uric acid and positively associated with type 2 diabetes in Bangladeshi population. Further research should attempt to determine whether it is effective to utilize serum uric acid and CRP levels as a predictor of type 2 diabetes for its primary prevention.

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