RELEVANCE OF URIC ACID IN PROGRESSION OF TYPE 2 DIABETES MELLITUS

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Abstract

Recent studies have introduced serum uric acid (UA) as a potential risk factor for developing diabetes, hypertension, stroke, and cardiovascular diseases. The value of elevated levels of UA in serum as a risk factor for diabetes development is still under scrutiny. Recent data suggest that clearance of UA is being reduced with increase in insulin resistance and UA as a marker of prediabetes period. However, conflicting data related to UA in serum of patients with Type 2 diabetes prompted us to study the urine/serum ratio of UA levels (USRUA) in these patients and healthy controls. All subjects included in the study were free of evidence of hepatitis B or C viral infection or active liver and kidney damage. Patients receiving drugs known to influence UA levels were also excluded from this study. Analysis of glucose and uric acid were performed on Dade Behring analyzer using standard IFCC protocols. Interestingly, our data demonstrated about 2.5 fold higher USRUA values in diabetic patients as compared to control subjects. Furthermore, there was a trend of correlation of USRUA value with the blood glucose levels in diabetic patients, which was more prominent in diabetic men than in women. With aging, levels of uric acid increased in serum of diabetic patients, and this effect was also more profound in male than in female diabetics. In conclusion, this study showed significantly elevated USRUA levels in patients with Type 2 diabetes, a negative USRUA correlation with the blood glucose levels in diabetic patients, and an effect of sex and age on the uric acid levels. Since literature data suggest a strong genetic effect on UA levels, it would be pertinent to perform further, possibly genetic studies, in order to clarify gender and ethnic differences in UA concentrations.

KEY WORDS: Diabetes mellitus, uric acid, insulin resistance, biomarker

Introduction

For some time, it has been recognized that serum uric acid (UA) is positively associated with serum glucose levels in healthy subjects (1). Recent studies have demonstrated that UA levels are higher in subjects with prediabetes and early Type 2 diabetes then in healthy controls (2,3). Furthermore, an elevated serum UA level was found to increase chances for developing Type 2 diabetes in individuals with impaired glucose tolerance (4). Hyperuricemia has been also added to the set of metabolic abnormalities associated with insulin resistance and/or hyperinsulinemia in metabolic syndrome (5-8). An elevated UA levels, as reported, often precedes the development of obesity (9), hyperinsulinemia (10-12), and diabetes (13-15). In addition, uric acid has been implicated in the development of metabolic syndrome (16) and hypertension (17). However, hyperuricemia is not always found in diabetic individuals. Conflicting data exist about UA levels in Type 2 diabetes, as low levels were found in diabetic patients, while elevated serum UA is a feature of hyperinsulinemia and impaired glucose tolerance (18). Although several studies have implicated the role of UA in progression of prediabetes to diabetes, studies related to UA levels in diabetes development are controversial and deserve further analysis. Therefore, in this study we have analyzed and examined potential role of UA as a biomarker for impaired glucose metabolism and diabetes progression by analyzing serum and urine levels of UA in Type 2 diabetic patients in Bosnia and Herzegovina (BH). In addition, here we also examined effects of glucose control, gender, and age on USRUA levels in diabetic patients.

Materials and Methods

Patients

In this study we have analyzed UA levels in a group of 23 patients diagnosed with Type 2 diabetes mellitus and 20 healthy controls. All human subjects involved in this study were patients of General hospital in Sarajevo, BH. All research involving human subjects and material derived from human subjects in this study was done in accordance with the ethical recommendations and practices of the Sarajevo General Hospital and complied with ethical principles outlined in World Medical Association Declaration of Helsinki – Ethical Principles for Medical Research Involving Human Subjects (initiated in June 1964, last amendment in October 2000). Subjects included in this study were free of evidence of hepatitis B or C viral infection or active liver and kidney damage.

Clinical Diagnosis of Type 2 Diabetes Mellitus

Type 2 DM was clinically diagnosed by standardized clinical examination conducted by specialist of Internal Medicine. All subjects underwent medical history and clinical examination. Criteria for selection of patients included in this study was that the history of Type 2 diabetes was present for more than five years. Under criteria of IDF, diabetes mellitus was diagnosed when fasting plasma glucose levels were higher than 7,0 mmol/l and postprandial serum glucose more than 11,1 mmol/. All patients had abdominal obesity (waist circumference more than 80 cm in women and 94 cm in men), following atherogenic dyslipidemia profile (triglycerides > 1,75 mmol/l, HDL < 1,0 mmol/l), and controlled blood pressure levels. More than half of diabetic patients had some diabetic complications, often polyneuropathy and microangiopathy, but renal function was normal. Diabetic patients were on standard drug therapy of 850 mg Metformine twice daily. Control subjects were of approximately same age (range of 50-75 years), but with normal glucose-tolerance test (fasting plasma glucose less than 6,2 mmol/l, and two hours postprandial glycaemia less than 7,8 mmol/l). They had no abdominal obesity as clinical criteria for insulin resistance.

Sample Analysis

Blood samples taken for the analysis were obtained from patients and subjects in fasting conditions from antecubital vein into siliconized tubes (BD Vacutainer Systems, Plymouth, UK). UA analyses were carried out in fresh samples. Analysis of glucose and UA in serum and urine were performed using glucose oxidase and uricase/peroxidase method, respectively, on Dade Behring analyzer. Standard IFCC protocols were used for all analyses. The USRUA ratio was calculated by dividing concentration of UA (μmol/L) in urine with its concentration in serum.

Statistical Analysis

Statistical analysis of the results from this study was done using SPSS 10.0 for Windows. Data are presented as mean ± SEM. Statistical significance was set as $p<0,05$.

Results

Our study analyzed the USRUA levels in a group of 23 patients diagnosed with Type 2 diabetes mellitus and 20 adequate controls. Strikingly, as shown in Figure 1, the USRUA values for diabetic patients were about 2,5 fold higher than in control subjects. Similar significant difference in USRUA values ($p<0,001$) was demonstrated in both, female and male diabetic patients, as compared to their corresponding controls (data not shown).

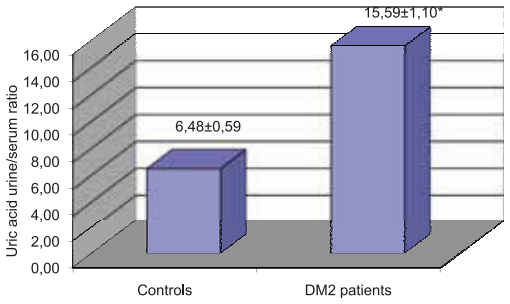

FIGURE 1. Average urine/serum ratios of uric acid in controls and patients with diagnosis of Type 2 diabetes mellitus.

Our data also demonstrated a trend in correlation of USRUA values with the blood glucose levels in diabetic patients, which was more prominent in male than in female patients (Figure 2).

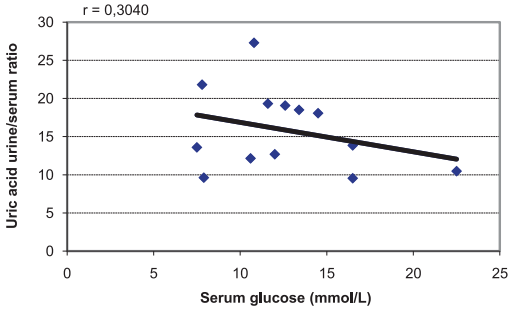

FIGURE 2. Correlation between USRUA and serum glucose levels in men diagnosed with Type 2 diabetes.

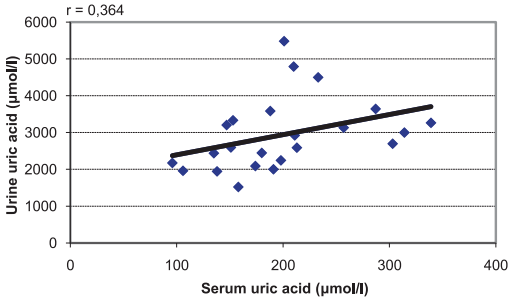

FIGURE 3. Correlation between serum and urinary concentration of uric acid in patients with Type 2 diabetes.

As shown in Figure 3, there was a correlation between serum and urinary UA levels in diabetic patients. Interestingly, with aging, levels of UA increased in serum of diabetic patients. As demonstrated in Figure 4, there was a correlation between diabetic patients' age and UA levels in serum, which was even more profound in male than in female diabetic patients (Figure 5).

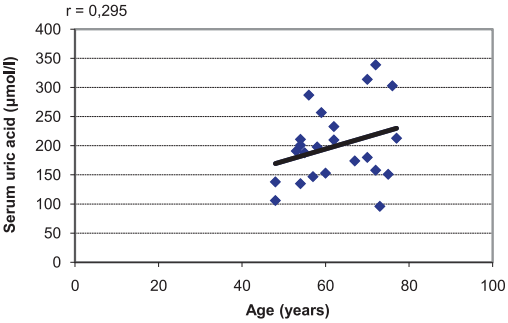

FIGURE 4. Correlation between age and serum uric acid level in patients with Type 2 diabetes.

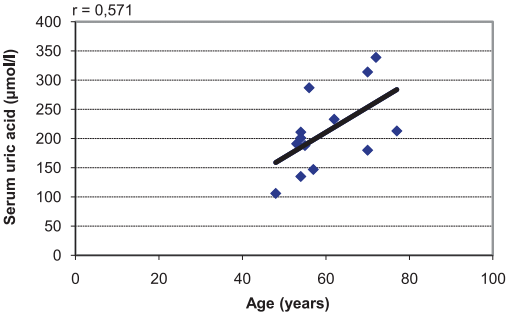

FIGURE 5. Correlation between age and serum uric acid levels in male patients with Type 2 diabetes.

Discussion

Variations in uric acid levels have been increasingly associated with insulin resistance, hyperinsulinemia, and diabetes (2-4, 12). Diabetic patients who are hyperuricemic appear to be at increased risk for developing diabetic complications, especially renal (18,19) and cardiovascular disease (20). In Type 2 diabetes, hyperuricemia seems to be associated with the insulin-resistance syndrome, impaired glucose tolerance, and an early onset of nephropathy, while hypouricemia is associated with nonadequate metabolic control, hyperfiltration, and a late onset of overt nephropathy (19, 21). Uric acid, although one of the major antioxidants in circulation (22), can induce oxidative stress in a variety of cells including vascular smooth muscle cells (23) and thus, mediate progression of cardiovascular disease (6, 17). The pathogenic mechanism appears to involve decreased nitric oxide (NO) bioavailability in vascular smooth muscle and endothelial cells and direct scavenging of

NO by uric acid (24). Decrease in endothelial NO production by uric acid, has been also associated with endothelial dysfunction and insulin resistance (25, 26). In addition, uric acid has been implicated in the development of hypertension (17) and elevated levels of uric acid has been reported particularly in newly diagnosed hypertension (27). Hyperuricemia is also closely linked to the various components of the metabolic syndrome (16) and represents a possible link between uric acid levels and cardiovascular morbidity and mortality (20). A possible link of UA levels and Type 2 diabetes incidence has been recently reported in different ethnic groups, including Austrian (28), Dutch (14), Chinese (25), and Japanese population (16). Until now, the uric acid levels have rarely been analyzed in diabetic patients in BH and this is one of the first studies in which uricemia was evaluated in these patients. In our study UA levels were measured in both, serum and urine samples, and then urine/serum ratio of uric acid levels was calculated and compared between Type 2 diabetic patients and control subjects. Patients receiving drugs known to influence UA levels were excluded from the analysis.

Our results demonstrated a profound increase in uric acid urine/serum ratios in Type 2 diabetic patients as compared to healthy controls. This is in line with data published in previous studies in which hyperuricemia has been associated with the higher risk for developing impaired glucose tolerance and Type 2 diabetes (10, 13, 14, 19). Here we also show a positive correlation between serum and urine UA levels in diabetic patients. Oral antidiabetic drug therapy used in this study had no effect on urine/serum ratio of UA in diabetic patients. Interestingly, our data suggest a trend of negative correlation between USRUA values and serum glucose levels, which was more profound in male than in female diabetic patients. These results complemented previous study in Austrian men in which the correlation between serum UA and fasting glucose levels was also negative (28), while study in Japanese men did not find a significant correlation between serum UA and glucose levels (16). Based on our results, it appears that in male patients with inadequate glucose control (higher blood glucose levels), the USRUA values were lower than in patients with lower blood glucose levels. This is in line with previous reports, where an effect of sex on UA levels in relation to glucose control was also observed in diabetic patients (21). However, the effect of gender on UA levels in diabetics is still controversial. Recently, Kramer and Muhlen (29) reported that although UA level predicted incident Type 2 diabetes in both sexes, their further analysis, stratified by glucose tolerance status, showed that UA predicted Type 2 diabetes in women with impaired fasting glucose but not in men. Here we demonstrated an effect of aging on serum UA in diabetic patients, and there was a positive correlation between diabetic patients' age and UA levels in serum, particularly in diabetic men. Serum UA levels were significantly higher in older male diabetic patients, while similar correlation was not observed in regards to urinary UA levels. Thus, our data suggest that with aging UA levels are increasing in serum of diabetic men, probably due to impaired UA clearance in these patients. Interestingly, in female diabetic patients, there was no effect of age or glucose control on UA levels in both, serum and urine. Previous studies reported that hyperglicemia was a risk factor for hyperuricemia (30, 31) and that an elevation of serum UA concentration increased the risk of Type 2 diabetes (10). This was complemented with our results, which demonstrated that the USRUA values were higher in diabetic patients than in controls. Insulin resistance may be the linking between elevated glucose and UA levels, but we (data not shown) and others (8) did not find a significant correlation between these parameters in patients with Type 2 diabetes. Interestingly, our data demonstrated significant positive correlation between USRUA and serum glucose levels in men diagnosed with Type 2 diabetes, as compared to diabetic women who participated in this study. This is in line with recent report, which has demonstrated that the inverse association between diabetes (or HbA1c level $\geq$ 7%) and serum UA levels was significantly stronger in men than in women (21). In general, our results demonstrated no effect of sex on uric acid levels in serum and urine in both control and diabetic patients. Although there was a trend of increased UA levels in males as compared to females (data not shown), this difference was not significant. This is in line with the recent report in which serum UA levels were analyzed in much higher number of people (about 2400), and found an increased incidence of hyperuricemia in males as compared to females (8). Since estrogen promotes uric acid excretion (32), this could explain higher incidence of hyperuricemia in men and increased levels of serum UA in postmenopausal women. This may also explain, at least partially, our finding that serum levels of UA in diabetic patients increased significantly with aging. In addition, there is evidence for a strong genetic influence on serum UA levels, with heritability estimates up to 73% (33). Furthermore, recent study by Kolz et al. (34) identified nine loci that are associated with serum UA levels, with profound

gender-specific effects of specific alleles on uric acid level regulation. Therefore, it appears that there is a strong genetic effect on UA levels and in order to clarify gender and ethnic differences in UA concentrations in diabetic patients, it would be pertinent to perform more studies of genetic variations that influence UA levels.

Conclusion

In summary, this study showed significantly elevated urine/serum ratio of uric acid in patients with Type 2 diabetes, as compared to healthy control subjects. This is in line with some previous studies, which suggest a possible link between UA levels and diabetes. Interestingly, serum UA levels were increased in Type 2 diabetic patients and this phenomenon seemed to be more profound in male diabetic patients, who also demonstrated more prominent effect of glucose control on UA clearance than their female counterparts. Since, to our knowledge, this is one of the first studies addressing prevalence of hyperuricemia in BH and its role in diabetes progression, more emphasis should be put on this risk factor for developing metabolic and cardiovascular disease in clinical practices in our country. Additional genetic studies addressing gender and ethno specific effects on UA levels in Type 2 diabetic patients appear also to be justified. Thus, considering the potential link of elevated urine/serum ratio of uric acid with insulin resistance, impaired glucose tolerance, and progression to diabetes, further research should attempt to determine whether it is effective to utilize USRUA levels as a predictor in prevention of Type 2 diabetes.

Acknowledgment

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List of Abbreviations

IFCC	-	International Federation for Clinical Chemistry
NO	-	nitric oxide
UA	-	uric acid
USRUA	-	urine/serum ratio of uric acid levels

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