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High serum uric acid is associated with oxidation of nucleosides in patients with type 2 diabetes

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ABSTRACT

Uric acid presents different roles in an organism, since it can act as an antioxidant or a pro-oxidant molecule. High serum uric acid levels may cause damage to several structures, including nucleic acids and its components. Therefore, in this study the association between increased serum uric acid concentrations and oxidation of nucleosides was investigated by assessment of urinary 8-hydroxydeoxyguanosine (8-OHdG) in patients with type 2 diabetes (T2D) and in healthy individuals. Urinary 8-OHdG and biochemical parameters were assessed in 61 patients who were initially grouped into 2 groups based on the median serum uric acid levels (< 5.3 mg/dL and ≥ 5.3 mg/dL). Urinary 8-OHdG was higher in patients with T2D and serum uric acid levels ≥ 5.3 mg/dL, when compared with the patients with serum uric acid levels < 5.3 mg/dL; however, co-occurrence of high serum uric acid with high urinary 8-OHdG was not observed in healthy individuals. A significant positive correlation between 8-OHdG and uric acid ($r = 0.40$, $P < 0.01$) was observed in patients with T2D. High serum uric acid levels were associated with high urinary 8-OHdG levels in patients with T2D, and this association was independent of gender, hypertension, body mass index, and serum creatinine.

1. Introduction

Hyperuricemia is a condition that has been associated with certain pathological conditions, such as gout [1], diabetes mellitus (DM) [2], stroke [3], and hypertension [4]. Uric acid is a molecule that exhibits different activities in an organism [5], since it can act as an antioxidant [6] or a pro-oxidant molecule [7,8]. Uric acid at high levels in the serum can cause damage to several structures and cells [9–12], mainly by activating oxidative pathways mediated by the renin-angiotensin system (RAS) [11,13] and nicotinamide adenine dinucleotide phosphate (NADPH) oxidase [9,12,14,15]. Interestingly, the paradoxical pattern of uric acid manifests itself with respect to DNA damage, in which it can protect [16] or induce injury [17,18] to the DNA. Under certain conditions where an increase in pro-oxidant mechanisms is demonstrated (as observed in some pathologies), 8-hydroxydeoxyguanosine (8-OHdG) is formed, which indicates oxidative damage to guanine nucleoside [19].

Although evidence shows that uric acid may act as an antioxidant or as a pro-oxidant under certain circumstances, it is still not fully understood whether elevated serum uric acid concentrations are capable of promoting increased nucleoside oxidation in patients with type 2 diabetes (T2D). Therefore, the aim of the present study was to investigate the association between increased serum uric acid concentrations and oxidation of nucleosides assessed *via* estimation of urinary 8-OHdG in patients with T2D and in healthy individuals.

2. Materials and methods

2.1. Study population

Overall, 61 individuals were examined in this study, which included 46 patients with T2D enrolled at the University Hospital of Santa Maria

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(Rio Grande do Sul, Brazil) and 15 healthy individuals. The individuals were grouped into 2 groups based on the median serum uric acid levels of this population *i.e.*, < 5.3 mg/dL and ≥5.3 mg/dL. Clinical characteristics and medical histories of the patients were collected *via* a clinical and epidemiological assessment questionnaire or from the hospital's medical register. Height and weight were used to calculate the body mass index (BMI) by dividing the weight in kilograms with the square of the height in meters. Exclusion criteria included pregnancy, infectious diseases, liver diseases, fever, acute or chronic inflammatory diseases, and medical history of malignancy. The study protocol was approved by the Institutional Ethics Committee (12303113.0.0000.5346), and written informed consent was obtained from all patients.

2.2. Sample collection and laboratory assays

Blood samples were collected from all patients, after an overnight fast period of at least 8 h, *via* the venous puncture technique into Vacutainer® tubes (BD Diagnostics, Plymouth, UK) containing EDTA, sodium fluoride plus EDTA, or no anticoagulants. The samples were centrifuged at 2500 × *g* for 15 min. Fasting glucose was measured using plasma, while serum was used to assess uric acid, total cholesterol, HDL cholesterol, LDL cholesterol, triglycerides, and high-sensitivity C-reactive protein (hs-CRP). These measurements were performed using standard methods *via* the Dimension RxL Max® automated analyzer (Siemens Healthcare Diagnostics Inc., Malvern, Pennsylvania, USA). Pro-inflammatory interleukin-6 (IL-6) in the serum was measured using commercial ELISA kits (R&D Systems Inc, Minneapolis, Minnesota, USA). The EDTA containing whole blood samples were used to measure glycated hemoglobin (HbA_{1c}) *via* the D-10® analyzer (Bio-Rad, California, USA), and the EDTA containing plasma was used to measure advanced oxidation protein products (AOPPs) *via* the Cobas Mira® automated analyzer (Roche Diagnostics, Mannheim, Germany). First-morning urine samples were obtained from the patients and centrifuged at 1000 × *g* for 5 min, and the supernatants were used to measure urinary albumin and 8-OHdG levels. Urinary 8-OHdG was measured using ELISA kits (Trevigen, Gaithersburg, USA), as per the manufacturer's instructions. The estimated glomerular filtration rate (eGFR) was calculated using the creatinine equation obtained from the Chronic Kidney Disease Epidemiology Collaboration (CKD-EPI) [20].

2.3. Statistical analysis

The variables were tested for normality using the D'Agostino-Pearson omnibus test. The parametric variables are presented as mean ± standard deviation (SD), and the non-parametric variables are presented in terms of median and interquartile range (IQR). Statistical differences between the groups were analyzed using Student's *t*-test or the Mann–Whitney test. The categorical data are expressed as percentages, and the groups were compared using Fisher's exact test. Spearman's correlation was performed to evaluate the relationship between the serum uric acid and urinary 8-OHdG values. Additionally, a multiple regression analysis was performed to investigate the influence of some variables on urinary 8-OHdG levels. Results were considered to be statistically significant when two-tailed P values were < 0.05. All results were analyzed using GraphPad Prism® version 6.01 (GraphPad Software, La Jolla, California, USA) and Statistica® version 9.1 (StatSoft Inc., Tulsa, Oklahoma, USA).

3. Results

The baseline characteristics of the participants included in the study are shown in Table 1. No differences in age, proportion of smokers, proportion of patients with T2D, diabetes duration, fasting glucose, HbA_{1c}, total cholesterol, HDL cholesterol, LDL cholesterol, triglycerides, hs-CRP, IL-6, AOPPs, and eGFR were detected between the groups.

Table 1
Baseline characteristics and biochemical parameters of the study participants stratified using serum uric acid values.

Parameters	Serum uric acid < 5.3 mg/dL	Serum uric acid ≥5.3 mg/dL	P-value
Age (y)	62.0 ± 9.4	58.3 ± 14.4	0.241
Male (%)	20.0	54.8	0.008
Smokers (%)	8.7	24.1	0.268
Hypertension (%)	48.3	76.7	0.033
Type 2 diabetes mellitus (%)	73.3	77.4	0.772
Diabetes duration (years)	13.1 ± 6.5	13.7 ± 9.4	0.844
BMI (kg/m^2)	28.7 (23.0–36.6)	30.9 (28.0–35.7)	0.032
Fasting glucose (mmol/L)	6.4 (5.3–8.2)	6.8 (5.6–8.4)	0.874
HbA_{1c} (mmol/mol)	42.6 (36.1–72.9)	44.8 (39.1–63.4)	0.894
HbA_{1c} (%)	6.4 (5.8–9.2)	6.6 (6.1–8.3)	0.892
Total cholesterol (mmol/L)	4.6 ± 0.7	4.4 ± 0.9	0.250
HDL cholesterol (mmol/L)	1.3 (1.1–1.6)	1.2 (1.0–1.5)	0.328
LDL cholesterol (mmol/L)	2.5 ± 0.6	2.2 ± 0.7	0.112
Triglycerides (mmol/L)	1.3 (1.0–1.9)	1.4 (1.1–1.7)	0.355
hs-CRP (mg/L)	0.4 (0.2–0.7)	0.8 (0.2–1.4)	0.225
IL-6 (pg/mL)	152.0 (83.0–197.3)	175.0 (137.5–257.0)	0.156
AOPPs (μmol/L)	65.0 (55.7–80.8)	63.6 (48.2–90.8)	0.969
eGFR (mL/min/1.73 m^2)	84.7 ± 17.8	79.7 ± 26.8	0.413
Serum creatinine (μmol/L)	76.0 (66.3–88.4)	88.4 (75.1–114.9)	0.008
Serum uric acid (mg/dL)	3.9 ± 0.8	6.6 ± 1.1	< 0.001
Urinary 8-OHdG (ng/mL)	16.6 (13.0–23.0)	20.0 (16.0–35.0)	0.014

Data are expressed as mean ± SD or median and IQR. AOPPs, advanced oxidation protein products; BMI, body mass index; HbA_{1c}, glycated hemoglobin; hs-CRP, high-sensitive C-reactive protein; eGFR, estimated glomerular filtration rate; IL-6, interleukin-6; 8-OHdG, 8-hydroxydeoxyguanosine.

However, significant differences were observed with respect to gender, hypertension, BMI, serum creatinine, serum uric acid, and 8-OHdG levels. The prevalence of hypertension was higher in patients with uric acid ≥5.3 mg/dL, when compared with patients with serum uric acid < 5.3 mg/dL (76.7% *versus* 48.3%, P = 0.033). The group with higher uric acid had slightly higher BMI values when compared with the group with lower uric acid (30.9 [28.0–35.7] *versus* 28.7 [23.0–36.6] kg/m^2, P = 0.032). Serum creatinine levels were also higher in the group with uric acid ≥5.3 mg/dL, when compared with the group with serum uric acid < 5.3 mg/dL (88.4 [75.1–114.9] *versus* 76.0 [66.3–88.4] μmol/L, P = 0.008).

Furthermore, urinary 8-OHdG levels were significantly higher in patients with high serum uric acid when compared with those with low serum uric acid (20.0 [16.0–35.0] *versus* 16.6 [13.0–23.0] ng/mL, P = 0.014). Urinary 8-OHdG levels were also analyzed separately in patients with T2D and in healthy individuals. Interestingly, the co-occurrence of high serum uric acid with high urinary 8-OHdG was only demonstrated in patients with T2D, as shown in Fig. 1. A significant positive correlation between serum uric acid and urinary 8-OHdG (r = 0.40, P < 0.01; Fig. 2) was also observed in patients with T2D. However, this correlation was not statistically significant in the healthy individuals (r = 0.24, P = 0.37). Furthermore, multiple linear regression analysis showed that the association between urinary 8-OHdG and serum uric acid concentrations was independent of other variables such as gender, hypertension, BMI, and serum creatinine, as shown in Table 2.

4. Discussion

The association between serum uric acid and urinary 8-OHdG in patients with T2D and healthy individuals was investigated in the present study. Interestingly, we observed the co-occurrence of high serum uric acid with high urinary 8-OHdG in patients with T2D only,