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NEGATIVE ASSOCIATION OF SERUM URIC ACID WITH PERIPHERAL BLOOD CELLULAR AGING MARKERS

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Abstract: *Objectives:* We aimed to explore the association between serum UA and cellular aging markers. *Design:* The current cross-sectional analysis was based on data collected within a type 2 diabetes project. *Settings:* Serum uric acid (UA), which has both antioxidant and pro-oxidant properties, is thought to be involved in cellular aging processes. *Participants:* There are 536 participants included in total, 65.3% of which are women. The average serum UA in women was 267.8 umol/l, lower than in men of 337.7 umol/l (P<0.001). *Measurements:* Serum UA, blood lipid profile, HbA1c, plasma glucose and insulin were determined. The peripheral blood leukocyte telomere length (LTL) and mitochondrial DNA copy number (mtDNAcn) were assessed using a real-time PCR assay. Logistic regressions were used to analyze the associations between serum UA and cellular aging markers. *Results:* In Spearman's correlation analysis, there were significantly negative correlations between serum UA and LTL in both women and men (r=-0.162, P=0.006; and r=-0.232, P=0.004, respectively). The logistic regression adjusted for age, BMI, WC, daily energy intake, HbA1c, TG, and LDL-C revealed that the ORs of shorter LTL comparing the extreme serum UA quintiles was 5.52 (95% CI 1.69-18.02; P for trend =0.025) in women and 6.49 (95% CI 1.38-30.45; P for trend =0.108) in men. Furthermore, the OR (95% CI) for shorter LTL per 1 SD increment in serum UA was 1.51(1.10-2.07) in women and 1.64(1.01-2.65) in men. In regard to mtDNAcn, the association between elevated serum UA and lower mtDNAcn only reached significance in men when comparing the second and fifth quintiles with reference quintile (OR=3.73(1.07-13.04) and 3.76(1.01-14.09) , separately, and P for trend=0.066). *Conclusions:* Our results indicate a significant negative association between serum UA and peripheral blood cellular aging markers. Serum UA might play a role in promoting cellular aging.

Key words: Uric acid, telomere length, mitochondrial DNA, oxidative stress, cellular aging.

Introduction

With advancing age, telomere length (TL) and mitochondrial DNA copy number (mtDNAcn) both decrease, making them two important markers of cellular aging (1, 2). Shorter TL and decrease in mtDNAcn have been associated with aging and aging-related disorders.

Of note, UA is thought to be a natural antioxidant but also have pro-oxidant properties leading to the increased production of reactive oxygen species (ROS), lipid peroxidation, DNA damage, and the expression of inflammatory cytokines (3). Exposure to oxidative stress and proinflammatory mediators are thought to accelerate TL attrition and mtDNA damage (4). Whether UA contributes to TL attrition and mtDNA damage is not known, and few studies have explored such relationships, particularly in large populations.

Dei Cas A et al. found a nonsignificant inverse correlation between serum UA and LTL in a cross-sectional study with 88 healthy young people at very low cardiovascular risk (5). However, de Vos-Houben J M J et al. found that UA was positively associated with LTL in 143 elderly Dutch men (mean age 83.9 years) and 109 elderly Greek men (mean age 84.6 years) (6). Because of the small study populations and heterogeneity between studies, it is hard to draw conclusions. The relationship between UA and peripheral blood mtDNAcn has not yet been explored.

With this cross-sectional analysis, we evaluated peripheral blood LTL, mtDNAcn and serum UA to explore the correlations between them. Our hypothesis was that increased serum UA is associated with decreased markers of cellular aging markers.

Materials and Methods

Subjects

The current analysis is based on data collected within a T2DM project in a Beijing suburb in China between March 2014 and January 2015 (7). A total of 599 participants completed a questionnaire given by well-trained interviewers about basic demographic characteristics, including age, sex, diet, medical history and time since the diagnosis of diabetes (years). All individuals underwent a physical examination, and fasting blood samples were taken. All individuals underwent an

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oral glucose tolerance test (OGTT) after an overnight fast (>10 h). Blood samples were collected at 0, 30, 60 and 120 min. The glucose tolerance status of each subject was classified based on the 1999 WHO criteria. Normal glucose tolerance (NGT) was indicated by FPG<6.1 mmol/L and 2-h postprandial glucose (2-h PG)<7.8 mmol/L. Prediabetes was indicated by impaired fasting glucose (IFG; 6.1 mmol/L≤FPG<7.0 mmol/L and 2-h PG<7.8 mmol/L) and/or impaired glucose tolerance (IGT; FPG<6.1 mmol/L and 7.8≤2-h PG<11.1 mmol/L). Diabetes was indicated by FPG≥7.0 mmol/L or 2-h PG≥11.1 mmol/L.

Subjects who had positive T1DM-related antibodies, who were on hypoglycemic therapy or steroids, or who had missing serum UA or cellular aging marker data were excluded. As a result, 536 participants were included in this study. The study protocol was approved by the Ethics Committee of Peking Union Medical College Hospital. The subjects voluntarily signed informed consent forms.

Anthropometric Measurements

All subjects underwent a thorough physical examination, including measurements of body height, weight, waist circumference (WC), hip circumference (HC), and blood pressure (BP). Weight and height were measured without shoes in light clothing, and body mass index (BMI) was calculated by dividing the body weight in kilograms by the square of the height in meters.

WC was measured to the nearest 0.1 cm midway between the iliac crest and the costal margin. HC was measured to the nearest 0.1 cm at the level of the trochanters. Each measure was performed twice by the same observer, and the mean value was recorded.

BP was measured on the left arm with a standard mercury sphygmomanometer with the subjects in a seated position at rest. BP was measured twice, and the mean value was calculated.

Biochemical Measurements

Participants fasted for more than 10 h overnight before their blood samples were collected in the morning. Blood samples were prepared for immediate analysis or stored at -80°C for further analysis. Plasma glucose was measured in a glucose oxidase assay. Hemoglobin (HbA1c) analysis was performed by high-performance liquid chromatography (intra-assay coefficient of variation (CV)<3%, interassay CV<10%). UA, creatinine (Cr), cholesterol (TC), triglyceride (TG), high-density lipoprotein cholesterol (HDL-C) and low-density lipoprotein cholesterol (LDL-C) were determined using an automated analyzer. Serum insulin was measured by a chemiluminescence enzyme immunoassay. Here, eGFR was calculated according to the CKD-EPI creatinine equation (8).

Measurement of Peripheral Blood mtDNAcn

Peripheral blood mtDNAcn measurement has been described in detail before (7). Briefly, genomic DNA was extracted from leukocytes in peripheral blood samples using the QIAamp DNA blood midi kit (Qiagen, Hilden, Germany). Purified DNA samples were diluted and quantified using a NanoDrop 1000 spectrophotometer (Thermo Fisher Scientific, Wilmington, DE, USA). The relative mtDNAcn was measured by real-time PCR and corrected by the simultaneous measurement of nuclear DNA. The average intra-assay and interassay CVs were 4.2% (range, 1.6%–9.8%) and 4.6% (range, 0.9%–7.8%), respectively.

Measurement of LTL

The measurement of peripheral blood LTL has also been described in detail before (9). In short, TL was determined as the relative ratio of telomere repeat copy number to single copy number (T/S) using the novel monochrome multiplex quantitative PCR protocol described by Cawthon (10). The within-plate and between-plate CVs were 18% and 7%, respectively.

Statistical Analysis

Quantitative variables with a normal distribution are presented as the means ± standard deviations (SDs). Non-normally distributed parameters were transformed, and categorical data are presented as ratios and percentages. Descriptive statistics of population characteristics were generated by sexes. The chi-square test was used to compare categorical variables, and continuous variables were compared using t test. The mtDNAcn and LTL were adjusted for age and sex. Spearman's correlation analysis was performed for UA and biomarkers related to cellular aging.

Serum UA was modeled as quintiles, and the lowest quintile served as the reference group. LTL and mtDNAcn were categorized according to quartiles, and the lowest quartile was considered a decreased value. Logistic regression was used to analyze the association between the dichotomized levels of cellular aging markers (lowest quartile vs. upper quartiles) across the quintiles of serum UA. Tests of linear trends across quintiles were conducted by including the quintiles in the models as a continuous variable. We also calculated the risk of decreased cellular aging markers associated with a 1 SD change in serum UA. All analyses were also performed separately by sexes.

All p-values are two-sided, and P<0.05 indicated statistical significance. All statistical analyses were performed using SPSS, version 22.0 (IBM Corp., Chicago, IL, USA).

Results

Clinical and demographic characteristics in women and men

The clinical and demographic characteristics of women and men are presented in Table 1. There are 536 participants in total, 65.3% of which are women. Women had lower WC and total energy intake(all P<0.001). The average serum UA in

women was 267.8 umol/l, which was also lower than in men of 337.7 umol/l (P<0.001). For cellular aging markers, peripheral blood mtNDAcn was significantly lower in men than women (P=0.004), and the LTL had no significant difference in them (P=0.602).Both sexes had similar glucose status and lipid profile.

Table 1
Clinical and demographic characteristics in women and men

	Women	Men	P
N (%)	350(65.3)	186(34.7)	-
Age(years)	52.2±11.3	53.8±11.0	0.114
BMI(kg/m2)	26.3±4.1	25.7±3.1	0.126
Waist circumference(cm)	86.1±9.9	89.4±9.1	< 0.001
Systolic pressure(mmHg)	127.2±18.4	129.1±17.5	0.243
Diastolic pressure(mmHg)	75.7±10.0	77.3±10.1	0.074
Total energy intake(kcal/d)	1461.0±592.9	1892.9±766.1	< 0.001
Glucose tolerance status			0.060
Normal, n (%)	128(36.6)	53(28.5)	-
Abnormal, n (%)	222(63.4)	133(71.5)	-
Medical history			
Hypertension, n (%)	7(2)	4(2)	0.907
Dyslipidemia, n (%)	6(2)	2(1)	0.561
HbA1c (%)	5.6 (5.3-6.1)	5.6 (5.3-6.0)	0.952
TG(mmol/L)	1.40 (0.99-1.98)	1.42 (0.94-2.21)	0.642
LDL-C(mmol/L)	2.85±0.72	2.82±0.71	0.615
eGFR (ml/min* 1.73m^2)	91.5±19.2	92.1±15.7	0.694
UA (umol/l)	267.8±65.7	337.7±89.4	< 0.001
LTL	28.69±0.81	28.74±0.86	0.602
MtDNAcn	6.64±0.69	6.46±0.63	0.004

Correlations of serum UA with cellular aging markers

Spearman's correlation coefficients of serum UA with cellular aging markers are shown in Table 2. The cellular aging markers LTL and mtDNAcn correlated positively with each other(r=0.086, P=0.145 in women; and r=0.212, P=0.008 in men, respectively).There were significantly negative correlations between serum UA and LTL in both women and men (r=-0.162, P=0.006; and r=-0.232, P=0.004, respectively). Neither of the negative associations between serum UA and mtDNAcn in women nor men reached significant (Table 2).

Associations between serum UA and cellular aging markers.

The risks of shorter LTL and lower mtDNAcn with serum UA increasing are shown in Table 3 and Table 4 by sexes. As for LTL, multivariate analysis after adjustment for age, BMI, WC, daily energy intake, HbA1c, TG, and LDL-C revealed that the odds ratio (OR) of shorter LTL increased with increasing serum UA quintile, and the OR comparing the extreme quintiles was 5.52 (95% CI 1.69-18.02; P for trend =0.025) in women and 6.49 (95% CI 1.38-30.45; P for trend =0.108) in men. Further more, the OR (95% CI) for shorter LTL per 1 SD increment in serum UA was 1.51(1.10-2.07) in women and 1.64(1.01-2.65) in men.

Table 2
Spearman's correlation coefficients of serum UA with cellular aging markers in women and men

In women			
	UA	**LTL**	**mtDNAcn**
UA	1		
LTL	-0.162 (0.006)	1	
mtDNAcn	-0.098 (0.081)	0.086 (0.145)	1
In men			
	UA	**LTL**	**mtDNAcn**
UA	1		
LTL	-0.232(0.004)	1	
mtDNAcn	-0.003(0.970)	0.212(0.008)	1

In regard to mtDNAcn, the association between lower mtDNAcn and elevated serum UA only reached significance in men when comparing the second and fifth quintiles with reference quintile (OR=3.73(1.07-13.04) and 3.76(1.01-14.09) , separately, and P for trend=0.066).

Discussion

This cross-sectional study have found negative associations between serum UA and peripheral blood cellular aging markers, including LTL and MtDNAcn.

Oxidative stress is a determining factor of cellular senescence and aging. In response to oxidative stress, cells activate different mechanisms, including repair pathways and those that inhibit cellular proliferation or induce apoptosis. UA is thought to have pro-oxidant properties, and we found that serum UA was significantly associated with peripheral blood cellular aging markers, which is theoretically consistent with the literature.

Telomeres are essential and dynamic regulators of cellular life span and chromosome integrity in eukaryocytes (11) and have been suggested as important markers of cellular aging (1). TL is regulated by proinflammatory cytokines and oxidative stress, which are primarily responsible for telomere loss and shortening (12, 13). However, few population studies have explored the association between serum UA and LTL. Early at 2007, a study (14) of 2524 subjects aged 35–55 years free of overt CVD found a significantly negative association between mean LTL and serum uric acid in men, and comparing normal

Table 3
ORs of lowest cellular aging markers with serum UA increasing in women

	Quintiles of UA							
	Q1	**Q2**	**Q3**	**Q4**	**Q5**	**P for trend**	**Per 1 SD increase**	**P value**
LTL								
	1	3.97(1.29-12.22)	3.70(1.17-11.73)	6.70(2.11-21.33)	5.52(1.69-18.02)	0.025	1.51(1.10-2.07)	0.010
MtDNAcn								
	1	1.21(0.53-2.77)	1.67(0.70-3.99)	1.47(0.60-3.60)	1.96(0.75-5.11)	0.654	1.25(0.91-1.73)	0.171

Table 4
ORs of lowest cellular aging markers with serum UA increasing in men

	Quintiles of UA							
	Q1	**Q2**	**Q3**	**Q4**	**Q5**	**P for trend**	**Per 1 SD increase**	**P value**
LTL								
	1	2.69(0.57-12.81)	2.31(0.47-11.30)	5.49(1.19-25.32)	6.49(1.38-30.45)	0.108	1.64(1.01-2.65)	0.044
mtDNAcn								
	1	3.73(1.07-13.04)	1.61(0.42-6.18)	0.83(0.19-3.53)	3.76(1.01-14.09)	0.066	1.22(0.79-1.88)	0.377

men and women with their hyperuricemic counterparts showed 260-bp (P = 0.002) and 119-bp (P = 0.004) longer telomeres in normal women and men. Moreover, Dei Cas A et al. also found an inverse correlation between serum UA and LTL in a cross-sectional study of 88 healthy young people at very low cardiovascular risk (5), although it was nonsignificant. However, de Vos-Houben J M J et al. found that UA was positively associated with LTL in 143 elderly Dutch men (mean age 83.9 years) and 109 elderly Greek men (mean age 84.6 years) (6).The disagreements may come from research heterogeneity, ethnic differences, and different research design, and more rigorous-designed cohort researches are needed to confirm the conclusion.

Mitochondria is key intracellular players in the aging process (15), and mtDNAcn decline has been thought to be another marker of cellular aging (2). The relationship between UA and peripheral blood mtDNAcn has not been explored thus far. In our study, we found a significant association between increased serum UA and decreased peripheral blood mtDNAcn. On one hand, mitochondria is an important source of ROS (15), mitochondrial dysfunction might cause excessive oxidative stress. On the other hand, telomere dysfunction activates P53 protein expression, which in turn inhibits the expression of peroxisome proliferator-activated receptor-C coactivator-1α (PGC-1α), decreases mitochondrial function, and promotes senescence (16). But this conclusion still needs to be verified in more studies.

Strengths and Limitations

The major strengths of this study are the well-characterized nature of the T2DM project cohort and the availability of OGTT measurements to allow for stratification by impaired glucose regulation. Thus, the study population is highly representative, with a sufficiently large number of study participants to conduct subgroup analyses and a large dataset on patient characteristics for the examination of potential confounders.

The study also has several limitations. The first limitation of this study is the cross-sectional design, which makes it difficult to generate causal interpretations of risk associations between serum UA levels and related biomarkers. Furthermore, no information on uric acid-lowering drugs was obtained, and it could be confounders in the analysis between UA and related biomarkers.

Conclusions

Our results indicate a significant negative association between serum UA and peripheral blood cellular aging markers, including LTL and MtDNAcn. Serum UA might play a role in promoting cellular aging, but more rigorous-designed cohort researches are needed to confirm the conclusion.

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Conflicts of interest: Dr. Yu has nothing to disclose; Dr. Liu has nothing to disclose; Dr. He has nothing to disclose;Dr. Li has nothing to disclose; Dr. Ma has nothing to disclose; Dr. Zhang has nothing to disclose; Dr. Ping has nothing to disclose; Dr. Li has nothing to disclose; Dr. Ma has nothing to disclose; Dr. Liu has nothing to disclose; Dr. Lv has nothing to disclose; Dr. Xu has nothing to disclose; Dr. Li has nothing to disclose.

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Ethical standard: The experiments complied with the current laws of China including ethics approval for the clinical study. All the phases of the study complied with the Ethical Principles for Medical Research Involving Human Subjects expressed in the Declaration of Helsinki.

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