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Association between xanthinoxidase activity and parameters of glucose homeostasis in patients with type 2 diabetes mellitus

Abstract. ***Background.*** *The purpose of the study is to determine the associations between clinical and anthropometric parameters, glucose homeostasis and serum xanthinoxidase (XO) activity in patients with type 2 diabetes mellitus (T2DM) taking into account gender, glycemic control and serum XO activity.* ***Materials and methods.*** *One hundred and twenty-five T2DM patients aged 34 to 81 years were examined, with an average age of 58.9 ± 9.4 years, disease duration from 1 month to 29 years (average of 8.9 ± 6.6 years). The age of patients at the time of the disease manifestation in the general sample was from 29 to 71 years, on average 50.6 ± 9.1 years.* ***Results.*** *The authors have found a nonlinear dependence of serum XO activity on fasting insulin concentration in patients with T2DM in the total sample, described by the multiplicative model (r = 0.45; p = 0.001). Serum XO activity in patients with T2DM in the general sample increases with adaptive increase in secretory activity of β-cells on an empty stomach according to the HOMA_β%. Serum XO activity was highest in T2DM patients with low fasting insulin sensitivity (HOMA_S% < 50 %). In addition, it has been determined that the serum XO activity in the subjects is nonlinearly associated with the QUICKI (r = –0.35; p = 0.016) and Caro indices (r = –0.40; p = 0.007). We have found a nonlinear dependence of serum XO activity on fasting insulin (r = 0.50; p = 0.08), HOMA_β% (r = –0.53; p = 0.06), HOMA_S% (r = –0.48; p = 0.09), HOMA-IR (r = –0.48; p = 0.09) in men with optimal glycemic control (HbA1c < 7.5 %) at the trend level and Caro (r = –0.64; p = 0.02). In women of this group, there was a nonlinear dependence of serum XO activity on fasting insulin (r = 0.56; p = 0.004), HOMA_β% (r = 0.56; p = 0.003), HOMA_S% (r = –0.54; p = 0.005), HOMA-IR (r = 0.54; p = 0.005), QUICKI (r = –0.50; p = 0.01) and Caro (r = –0.61; p = 0.003).* ***Conclusions.*** *In patients with T2DM, the serum uric acid is linearly associated with the level of serum XO activity, which determines 34 % of its variability. In patients with T2DM, regardless of the state of glycemic control, serum XO activity is nonlinearly associated with parameters characterizing the state of glucose homeostasis (fasting insulin, HOMA_S%, HOMA_β%, QUICKI and Caro indices). Predictors of high serum XO activity in patients with T2DM are the level of postprandial blood glucose (t = –3.53; p = 0.004) and serum uric acid (t = 4.73; p = 0.0005).*
Keywords: *xanthine oxidase; hyperuricemia; type 2 diabetes mellitus; metabolic syndrome*

Introduction

Worldwide, type 2 diabetes mellitus (T2DM) is a major public health challenge due to its high prevalence and increasing trend, associated morbidity and mortality, as well as huge economic burdens [1, 2]. Hyperuricemia (HU) is increasingly being considered a potential pathogenic factor for T2DM, metabolic syndrome, hypertension and chronic kidney disease, as well as atherosclerosis, and several adverse consequences of vascular disease (stroke, myocardial infarction, and cardiovascular death) [3, 4]. HU is the result of increase in 16 % of all mortality causes and 39 % of total cardiovascular disease cases [5]. We define HU as ≥ 420 μmol/l for men and ≥ 360 μmol/l for women [6, 7]. Furthermore, many factors can influence the concentrations of serum uric acid (UA), e.g. diet, obesity, and metabolic syndrome [8, 9].

HU pathogenicity is associated with its low solubility in the extracellular environment leading to crystal formation, low affinity (and deposition) to certain tissues and antigenicity (after crystal phagocytosis). This mixture of quantitative and qualitative etiological HU factors is confounding because

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normouricemic individuals may show symptoms while others with HU may not. In the clinical context, HU is seen as a prognostic indicator of renal disease, T2DM, cardiovascular disease and inflammation [7, 10], thus being a (modest) risk factor for mortality [11].

UA significantly affects all types of metabolism. HU promotes the development of insulin resistance (IR), accompanied by an increase in blood glucose levels, which stimulates mental activity, ensures the formation of glycogen and lipid reserves. It affects the metabolism of catecholamines, causing an increase in blood pressure, which in conditions of sodium deficiency is an important mechanism for maintaining vital processes. However, the factors leading to the development of hypertension, atherosclerosis and chronic kidney disease. Accordingly, HU has been added to the IR a cluster of metabolic abnormalities that are addressed clinically as metabolic syndrome.

UA metabolism is closely related to glucose and fructose metabolism and obesity [12]. However, not all studies support the association between UA and T2DM. In a large representative sample of the U.S. population, P. Bandaru and A. Shankar reported that higher serum UA levels were inversely associated with DM [13]. Such inconsistency raises doubts regarding the causal relationship between serum UA concentration and T2DM. Further, evidence from genetic studies does not support a causal relationship between serum UA levels and risk of T2DM [14, 15]. It has been suggested that xanthine oxidase (XO) may underlie the UA — T2DM association [13].

XO is a metalloflavoenzyme that catalyzes oxidation of hypoxanthine to xanthine and then to UA. Apart from its role in UA production, XO also generates oxidants, which are key players in the T2DM development process [16–18]. Although XO activity has been linked to cardiometabolic risk factors [19] and inhibition of XO activity leads to an improved cardiometabolic risk profile [20, 21], no studies have examined the associations between serum XO activity and the risk of developing T2DM.

However, we still do not know if serum UA is a protective factor for the moderate oxidative stress in these situations or if it is a risk factor. Increased serum UA levels may be an indicator of up-regulated activity of XO, a powerful oxygen radical — generating system in human physiology. Increased reactive oxygen species accumulation contributes to endothelium dysfunction, metabolic and functional impairment, inflammatory activation, and other features of cardiovascular pathophysiology [22]. Hydrogen peroxide is one of the reactive oxygen species, the excess of which is toxic to individual cells. It also has links to aging and a multitude of conditions such as DM and neurodegenerative disorders such as Alzheimer disease [23, 24].

The purpose of the study is to determine the associations between clinical and anthropometric parameters and parameters of glucose homeostasis and serum XO activity in patients with T2DM taking into account gender, glycemic control and serum XO activity.

Materials and methods

The authors conducted the study by following the legislation of Ukraine and the principles of the Helsinki Declaration of Human Rights. The Ethics Commission of the State Institution "V. Danilevsky Institute for Endocrine Pathology Problems of the National Academy of Medical Sciences of Ukraine" considered and approved the design of the study, information for the patient, and the form of the informed consent to participate in the study.

One hundred and twenty-five T2DM patients aged 34 to 81 years, average age 58.9 ± 9.4 years with disease duration from 1 month to 29 years, average 8.9 ± 6.6 years were examined. The age of patients at the time of the disease manifestation ($age_{manif.}$) in the general sample was from 29 to 71 years, on average 50.6 ± 9.1 years. According to gender, the subjects were distributed as follows: 55 men aged 34 to 77 years, average age 57.3 ± 9.2 years with disease duration from 1 month to 25 years, on average 8.49 ± 6.55 years, 70 women aged 43 to 81 years, average age 60.10 ± 9.49 years with disease duration from 2 months to 29 years, average 9.25 ± 6.59 years. Subjects received oral hypoglycemic therapy.

According to the state of glycemic control, the subjects were divided into two groups (Table 1).

Anthropometric data: height (m) was measured using a medical mechanical Harpenden stadiometer, weight (kg) — on electronic scales Beurer GS 20 Summer Sky with a measurement accuracy of up to 100 g (maximum weight 180 kg), waist circumference (WC, cm) was measured at the midpoint between the lower margin of the last palpable rib and the top of the iliac crest using a stretch-resistant tape, hip circumference (HC, cm) was measured with a centimeter tape at the level of a large spit. Calculation of anthropometric indices: determined the ratio of WC to HC (WC/HC); body mass

Table 1. Characteristics of patients in groups by glycemic control, years

Parameter	HbA1c < 7.5 %		HbA1c ≥ 7.5 %	
	Men (n = 31)	Women (n = 34)	Men (n = 31)	Women (n = 31)
Age	54.22 ± 13.43		56.50 ± 11.65	
	53.92 ± 13.54	54.43 ± 13.42	52.83 ± 10.79	59.10 ± 11.62 p_1 = 0.03
$Age_{manif.}$	40.74 ± 15.56		43.15 ± 13.63	
	41.73 ± 15.93	40.00 ± 15.34	39.65 ± 13.48	45.64 ± 13.30 p_1 = 0.028
Duration	13.04 ± 9.93		13.63 ± 9.43	
	12.64 ± 10.92	13.36 ± 9.14	12.93 ± 7.77	14.11 ± 10.49

Note: p_1 — the probability of differences between one group depending on the glycemic control.

index (BMI, kg/m^2) was assessed by dividing the person's weight by height squared. According to the World Health Organization, BMI of 18.5 to 24.9 kg/m^2 defines normal weight, 25 to 29.9 kg/m^2 defines overweight and greater than or equal to 30 — obesity [25].

We assessed glucose homeostasis according to the recommendations of the RSSDI-ESI Consensus group 2020 [26]. The level of fasting glucose (FBG) in capillary and venous blood and 2-h blood glucose (PBG) in capillary blood (mmol/L) were determined by glucose oxidase method on a Biosen C-line analyzer (EKF, Germany).

Venous blood samples were obtained from the cubital vein after 8 hours of fasting. We determined the level of fasting insulin (μmol/L) by immunochemiluminescence method, using the Insulin Elisa kit (ELISA DRG Diagnostics, USA). The degree of IR was assessed by the HOMA-IR index, the functional activity of β-cells on an empty stomach by the HOMA_β% index, faster oral insulin sensitivity — by HOMA_S%, calculated using HOMA Calculator v2.2 for free use. The Caro index was calculated as the ratio of FBG level (mmol/l) to fasting insulin concentration (μmol/L) (normal < 0.33). The QUICKI index (normal > 0.45) was calculated using an online calculator for free access.

The level of glycated hemoglobin (HbA1c, %) was determined by photocolorimetric method using a commercial set of reagents JSC Reagent on a photoelectric photometer KFK-3.

Serum UA (μmol/L) was determined by colorimetric method using a set of reagents Spine Lab, Uricase-POD (Ukraine). The concentration of SC in women ≤ 360 μmol/L, in men — ≤ 420μmol/L was considered normal serum UA.

Serum XO activity (nmol/min/ml) was determined by peroxidase method according to the instructions of the Xanthine Oxidase Assay Kit (Sigma-Aldrich, USA). The control group consisted of 30 healthy individuals. There were no statistically significant differences between the main and control groups by sex (Table 2).

The results were statistically processed, using the software package Statistica (StatSoft, version 10.1, USA). The normality of variables distribution was determined using the Shapiro-Wilk test. One-way analysis of variance was used for comparison. We used a regression analysis to determine the relationship between the studied indicators and the normal distribution of variables. Associations between dependent and independent variables were analyzed by multiple regression analysis. The results are presented in a tabular form and include such model characteristics as regression coefficient (B), standardized regression coefficient (β), and determination coefficient (R^2). The null hypotheses were tested at the significance level $P \leq 0.05$. The obtained results are presented in the tables in the form of $\overline{X} \pm s$, where $\overline{X}$ is an arithmetic mean, s — is a standard deviation.

Results

It has been found that in the total group of examined patients with T2DM serum UA depends on serum XO activity ($r = 0.34$; $p = 0.007$) (Fig. 1).

This dependence is described by a linear model:

Serum UA (μmol/L) = 324.3 + 22.7 × serum XO activity (nmol/min/ml),

where serum XO activity determines 34% variability of serum UA.

The authors have established nonlinear dependence of serum XO activity on fasting insulin concentration in patients with T2DM in the total sample, described by the multiplicative model ($r = 0.45$; $p = 0.001$) (Fig. 2) and represented by the equation:

Serum XO activity (nmol/min/ml) = 1.5 × fasting insulin 0.27 (μmol/L).

This model shows 20.7 % of the variability serum XO activity.

We have determined that serum XO activity in patients with T2DM in the general sample increases with adaptive increase in secretory activity of β-cells on an empty stomach according to the HOMA_β% index (Fig. 3).

Table 2. Index of serum XO activity in the control group, nmol/min/ml

Gender	Average value ($\overline{X} \pm s\overline{X}$)	Limits of the reference interval	P
Men	2.51 ± 0.29	1.79–3.40	< 0.001
Women	2.50 ± 0.19	1.69–3.31	< 0.001

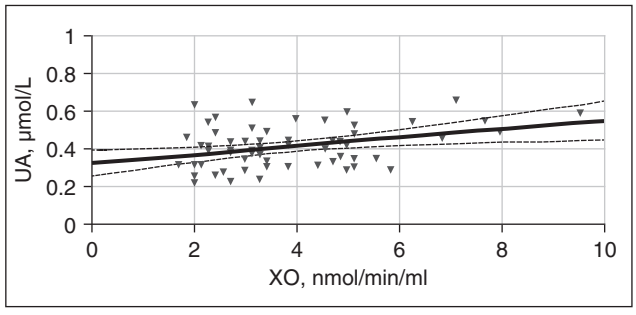

Figure 1. Linear dependence of serum UA on serum XO activity in patients with T2DM in the total sample

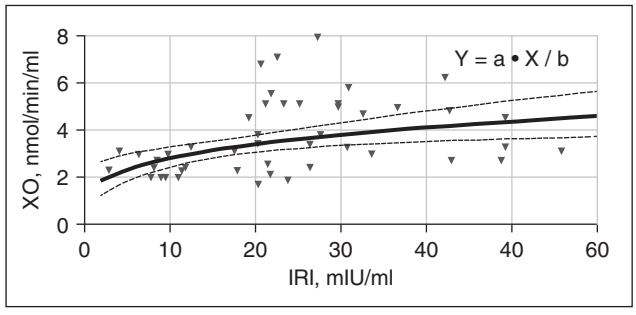

Figure 2. Non-linear dependence of serum XO activity on the concentration of fasting insulin in T2DM patients in the total sample

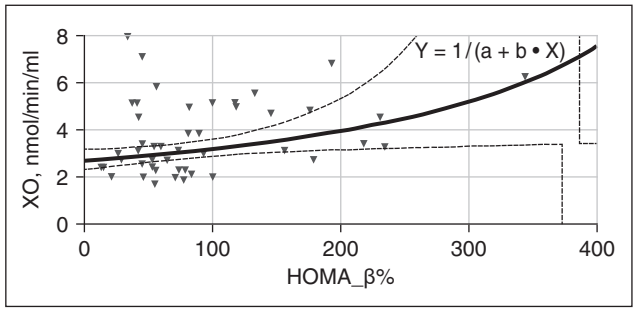

Figure 3. Non-linear dependence of serum XO activity on fasting secretory activity of β-cells by HOMA_β% index in patients with T2DM in the total sample

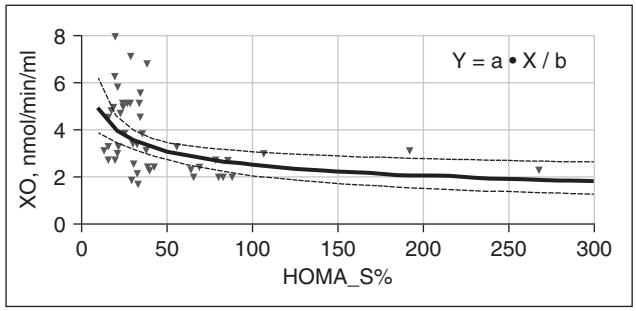

Figure 4. Non-linear dependence of serum XO activity on fasting insulin sensitivity according to the HOMA_S% index in T2DM patients in the total sample

Table 3. Anthropometric parameters and indicators of glucose homeostasis in patients with T2DM, taking into account gender and glycemic control

Parameters	HbA1c < 7.5 %		HbA1c ≥ 7.5 %	
	Men (n = 31)	Women (n = 34)	Men (n = 31)	Women (n = 31)
BMI, kg/m²	30.79 ± 6.73		30.83 ± 5.78	
	32.34 ± 4.61	33.37 ± 5.92	30.49 ± 4.28	34.66 ± 6.09 p_1 = 0.017
WC, cm	100.50 ± 19.73		99.83 ± 18.92	
	108.11 ± 14.83	106.21 ± 14.77	104.00 ± 12.68	103.60 ± 18.25
WC/HC	0.97 ± 0.12		0.98 ± 0.10	
	1.05 ± 0.10	0.98 ± 0.09	1.03 ± 0.09	0.99 ± 0.08
FBG, mmol/L	7.98 ± 2.34		10.47 ± 3.12; p_3 = 0.00001	
	7.07 ± 1.68	7.54 ± 1.67	9.69 ± 2.88 p_2 = 0.0002	10.15 ± 2.95 p_2 = 0.0001
PBG, mmol/L	8.34 ± 2.48		10.71 ± 3.38; p_3 = 0.00001	
	8.22 ± 2.16	8.06 ± 2.13	10.27 ± 2.50 p_2 = 0.005	11.38 ± 3.04 p_2 = 0.00001
HbA1c, %	6.40 ± 0.66		9.18 ± 1.43; p_3 = 0.00001	
	6.19 ± 0.65	6.33 ± 0.88	9.57 ± 1.73 p_2 = 0.00001	9.09 ± 1.27 p_2 = 0.00001
Serum UA, μmol/L	356.13 ± 120.09		350.05 ± 125.06	
	405.20 ± 98.25	394.04 ± 116.66	406.14 ± 127.07	361.77 ± 121.24
Fasting insulin, μmol/L	25.79 ± 13.49		20.48 ± 10.92; p_3 = 0.09	
	24.28 ± 13.42	26.80 ± 13.69	17.37 ± 7.53	22.42 ± 12.42
HOMA_β%	128.24 ± 67.01		46.34 ± 26.11; p_3 = 0.00001	
	123.98 ± 67.04	131.07 ± 68.12	46.03 ± 27.05 p_2 = 0.007	46.56 ± 26.27 p_2 = 0.0001
HOMA_S%	46.06 ± 46.55		50.29 ± 32.35	
	52.34 ± 57.96	52.35 ± 30.15	41.87 ± 37.75	48.84 ± 34.66
HOMA-IR	3.40 ± 1.75		2.74 ± 1.38; p_3 = 0.09	
	3.12 ± 1.74	3.58 ± 1.77	2.48 ± 1.23	2.92 ± 1.50
QUICKI	0.30 ± 0.03		0.28 ± 0.02	
	0.30 ± 0.04	0.29 ± 0.03	0.29 ± 0.02	0.28 ± 0.02
Caro	0.38 ± 0.30		0.71 ± 0.50; p_3 = 0.009	
	0.41 ± 0.37	0.36 ± 0.20	0.79 ± 0.51 p_2 = 0.003	0.66 ± 0.49 p_2 = 0.05
Serum XO, nmol/min/ml	4.00 ± 1.70		3.74 ± 1.65	
	3.66 ± 1.54	4.17 ± 1.79	3.99 ± 1.77	3.59 ± 1.63

Notes: p_1 — probability of differences between genders within one group; p_2 — the probability of differences between one sex group in terms of glycemic control; p_3 — the probability of differences between groups depending on glycemic control.

This dependence is nonlinear, described by a reciprocal Y model (r = –0.34; p = 0.021) and is represented by the equation:

$$Serum\ XO\ activity\ (nmol/min/ml) = 1 / (0.37 - 0.001 \times HOMA\_\beta\%).$$

It has been found that the highest levels of serum XO activity are observed in patients with T2DM with low fasting insulin sensitivity (HOMA_S% < 50 %) (Fig. 4). This dependence is nonlinear, described by a multiplicative model (r = –0.52; p = 0.00001) and is represented by the equation:

$$Serum\ XO\ activity\ (nmol/min/ml) = 9.7 \times HOMA\_S\%^{-0.3}.$$

Fasting insulin sensitivity in this model determines 22.4% variability in serum XO activity in T2DM patients.

In addition, it has been determined that the serum XO activity in the subjects is nonlinearly associated with the indices QUICKI (r = –0.35; p = 0.016) and Caro (r = –0.40; p = 0.007).

There were no significant gender differences in serum UA and XO activity in groups of patients with glycemic control (Table 3). Indicators characterizing the state of glucose homeostasis differed significantly between the study groups and were characteristic of the state of glycemic control.

We have found a nonlinear dependence of serum XO activity on fasting insulin (r = 0.50; p = 0.08) and HOMA_β% (r = –0.53; p = 0.06), HOMA_S% (r = –0.48; p = 0.09), HOMA-IR (r = –0.48; p = 0.09) in men with optimal glycemic control (HbA1c < 7.5 %) at the trend level and Caro (r = –0.64; p = 0.02). In women of this group, there was a nonlinear dependence of serum XO activity on fasting insulin (r = 0.56; p = 0.004), values of HOMA_β% (r = 0.56; p = 0.003), HOMA_S% (r = –0.54; p = 0.005), HOMA-IR (r = 0.54; p = 0.005), QUICKI (r = –0.50; p = 0.01) and Caro (r = –0.61; p = 0.003).

The authors have found a nonlinear dependence of serum XO activity on fasting insulin (r = –0.86; p = 0.007), HOMA_S% (r = 0.87); p = 0.005), HOMA-IR (r = 0.87; p = 0.005), QUICKI (r = 0.70; p < 0.05) and Caro (r = 0.76; p = 0.03) in men with suboptimal glycemic control and high risk (HbA1c ≥ 7.5 %). In women of this group, there was a nonlinear dependence of serum XO activity on fasting insulin (r = –0.69; p = 0.004), values of HOMA_β% (r = –0.62; p = 0.039), HOMA_S% (r = 0.57; p = 0.005), HOMA-IR (r = –0.59; p = 0.005), QUICKI (r = 0.58; p = 0.02) and Caro (r = 0.54; p = 0.03).

Analysis of the studied clinical, anthropometric parameters and indicators of glucose homeostasis in patients with T2DM in groups taking into account the level of serum XO activity showed that patients with high serum XO activity had significantly higher concentrations of fasting insulin, fasting β-cell secretory activity, low sensitivity fasting insulin and more pronounced manifestations of IR (HOMA-IR, Caro, QUICKI indices) (Table 4).

In order to assess the effect of the studied clinical, anthropometric and laboratory parameters on serum XO activity in patients with T2DM, we performed a step-by-step multifactor regression analysis, where the dependent variable was serum XO activity, and independent — age, BMI, WC/HC, FBG, PBG, HbA1c, serum UA, fasting insulin and HOMA-IR (model 1), age, duration, FBG, PBG, HbA1c, serum UA, fasting insulin and HOMA-IR (model 2, 3) and age, BMI, WC, FBG, PBG, HbA1c, serum UA, fasting insulin and HOMA-IR (model 4) (Table 5).

Thus, in the general sample of patients with T2DM serum XO activity is associated with serum UA and WC/HC (p = 0.001) (Table 5). In patients with T2DM with optimal

Table 4. Clinical, anthropometric parameters and indicators of glucose homeostasis in patients with T2DM, taking into account serum XO activity

Parameter	Normal level of XO (n = 30)	High level of XO (n = 31)	P
Age, years	55.96 ± 10.80	55.00 ± 9.49	0.64
$Age_{manif.}$, years	49.13 ± 8.93	48.63 ± 10.03	0.62
Duration, years	7.02 ± 7.18	7.01 ± 4.46	0.40
BMI, kg/m^2	32.73 ± 6.09	34.45 ± 4.98	0.27
WC, cm	104.44 ± 17.18	106.38 ± 14.35	0.79
WC/HC	1.02 ± 0.07	0.95 ± 0.05	0.002
FBG, mmol/L	8.76 ± 2.83	8.53 ± 3.03	0.56
PBG, mmol/L	10.15 ± 3.23	8.93 ± 3.23	0.16
HbA1c, %	8.07 ± 2.40	7.21 ± 1.95	0.21
Serum UA, μmol/L	392.42 ± 119.03	431.79 ± 109.06	0.15
Fasting insulin, μmol/L	19.66 ± 14.88	28.46 ± 8.50	0.022
HOMA_β%	71.48 ± 50.42	116.62 ± 81.77	0.03
HOMA_S%	62.75 ± 56.84	26.55 ± 6.67	0.0075
HOMA-IR	2.74 ± 1.92	3.96 ± 1.05	0.0137
QUICKI	0.30 ± 0.04	0.28 ± 0.02	0.033
Caro	0.71 ± 0.49	0.32 ± 0.15	0.0019
Serum XO, nmol/min/ml	2.59 ± 0.49	5.14 ± 1.41	0.00001