SERUM OXIDATIVE STRESS MARKERS ARE NOT ASSOCIATED WITH RENAL AND COMMON CAROTID ARTERIES ARTERIOSCLEROTIC VASCULAR CHANGES IN PATIENTS WITH GOUT

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Abstract. Objective: To establish the association between serum levels of reactive oxygen species (ROS) products, nitric oxide (NO) radicals and ascorbate radicals with renal resistive index (RRI), common carotid artery resistive index (CCARI) and intima-media thickness (IMT) in gout patients, and to find out whether the connection is more pronounced when tophi are present. **Methods:** A cross-sectional study including 71 consecutive gout patients, divided into two groups according to the presence of subcutaneous tophi. Serum concentrations of ROS products, NO radicals and ascorbate radicals were determined by ex vivo electron paramagnetic resonance (EPR) study. RRI was measured in both kidneys at the level of interlobar arteries with 3.5 MHz transducer. By applying ultrasound of the common carotid arteries, conducted with 10 MHz linear transducer CCARI and IMT were measured. **Results:** Gouty arthritis without tophi and gouty tophi subjects were age-matched. Serum uric acid and distribution of conventional cardiovascular risk factors was equal in the groups. However, in tophi patients CRP and the number of individuals who had suffered a cardiovascular event were higher. In the two stages of the disease serum levels of ROS products, NO radicals, ascorbate radicals, as well as RRI and CCARI were comparable but intima-media was thicker in gouty tophi. Serum concentrations of ROS products, NO radicals and ascorbate radicals did not correlate with RRI, CCARI and IMT. Among untreated and treated with Allopurinol or Febuxostat patients the means of ROS products, NO radicals, ascorbate radicals, RRI, CCARI and IMT were similar. **Conclusions:** In the earlier and advanced stage of the disease we found no difference in oxidative stress level but the degree of inflammation was higher in tophi subjects. No connection was established between serum ROS products, NO radicals and ascorbate radicals with renal and carotid arteries arteriosclerotic vascular changes. We suggest that in gout individuals intrinsic inflammation has a leading role in the process of atherogenesis.

Key words: gout, serum oxidative stress markers, arteriosclerotic vascular alterations

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INTRODUCTION

Numerous studies – cross-sectional, prospective, retrospective have demonstrated the relationship of gout with cardiovascular pathology. It is complex and is determined by genetic factors, the level of hyperuricemia, urate body stores, the high level of oxidative stress and the chronic pro-inflammatory state in the organism. Compared to asymptomatic hyperuricemia subjects, oxidative stress and chronic inflammation are higher in patients with gout [1]. Further, oxidative stress and chronic inflammation are interrelated and interacting processes. They both lead to the development of arteriosclerosis [2] and could be used as its markers [3]. Animal models as well as human investigations support the causal role of oxidative stress in the evolution of atherosclerosis [2, 4]. Evidence in the literature demonstrates that chronic and acute overproduction of reactive oxygen species (ROS) is an integral part for the progression of cardiovascular disease. ROS mediate various signaling pathways that underlie vascular inflammation in atherogenesis. Although uric acid is known as one of the major antioxidants in the human plasma, it predicts the development of obesity, arterial hypertension and cardiovascular disease, which are tightly connected to oxidative stress. Uric acid may function either as an antioxidant or pro-oxidant, depending on whether it is in the plasma or within the cell. Pro-oxidative effects of uric acid occur in cardiovascular disorders and contribute to their progression [5]. There are not many studies to examine the relationship between target organ changes in patients with gout and serum concentrations of ROS products, NO radicals and ascorbate radicals. In our study we aimed to establish the association between serum levels of reactive oxygen species (ROS) products, nitric oxide (NO) radicals and ascorbate radicals with ultrasound parameters reflecting distensibility and rigidity of the renal vessels and common carotid arteries in gout patients, divided according to the presence of subcutaneous tophi. In this way we tried to get a notion for the overall atherosclerotic process and the degree of oxidative stress in the earlier and advanced stage of the disease.

PATIENTS AND METHODS

This was a single center cross-sectional study, including 71 consecutive gout patients, fulfilling the 2015 American College Classification criteria [6]. Subjects were divided into two groups – 45 patients with gouty arthritis without tophi and 26 patients with gouty tophi (demographic, clinical and laboratory details are summarized in table 1). At enrollment pa-

tients were physically examined by a rheumatologist from the Rheumatology Clinic of the University Hospital “St. Iv. Rilski” in Sofia. The enrolled patients were subjects who had experienced a first flare, who have been managing a prolonged attack or patients with chronic tophaceous gout in whom the choice of therapy needed to be evaluated. The diagnosis of gouty tophi was made in individuals with subcutaneous tophi. Study protocol has been approved by the ethics committee of the institution. All participants gave informed consent in accordance with the Declaration of Helsinki.

Clinical and laboratory assessments included collecting detailed information related to current smoking habit, presence of arterial hypertension defined as systolic blood pressure ≥ 140 mm Hg, diastolic blood pressure ≥ 90 mm Hg or use of antihypertensive medication; diabetes mellitus, based on documented use of insulin and/or oral hypoglycemic agents; dyslipidemia, based on elevated fasting lipid levels or low HDL and/or documented use of lipid-lowering agents; reduced glomerular filtration rate taken at $\text{eGFR} < 90$ ml/min calculated by the Cockcroft-Gault formula. The serum levels of ROS products, NO radicals and ascorbate radicals were determined by *ex vivo* electron paramagnetic resonance (EPR) study. All EPR measurements were performed at room temperature on an X-band EMXmicro, spectrometer Bruker, Germany, equipped with Standard Resonator. Spectral processing was done by using Bruker WIN-EPR and Sinfonia software. The concentrations of ROS products, NO radicals and ascorbate radicals were calculated as double integrated plots of EPR spectra and results were expressed in arbitrary units [7, 8, 9]. The other laboratory parameters considered in the analysis were CRP, ESR, creatinine, blood urea nitrogen (BUN) and serum uric acid. Patients were defined as having suffered a cardiovascular event if they had coronary artery disease, cerebrovascular disease and/or peripheral artery disease. Exclusion criteria were exacerbated cardiac or renal failure, dilated or hypertrophic cardiomyopathy, severe valvulopathy, arterial blood pressure $> 140/90$ mm Hg and heart rate < 50 bpm or > 90 bpm during the ultrasonographic measurement.

Subjects underwent multimodal ultrasound examination. Measurements were performed by a single operator, who was unaware with the cases clinical data. By means of the value of renal resistive index (RRI) we judged for resistance and compliance of renal vessels [10]. The parameter was determined in both kidneys at the level of interlobar arteries with 3.5 MHz transducer, working with pulse Doppler frequency of 2.5 MHz. The Doppler probe vol-

ume was 2 mm with pulse repetition frequency from 1.5 to 2 kHz and angle of orientation < 60°. Carotid arteries were examined with 10 MHz linear transducer working with pulse Doppler frequency of 5 MHz. Intima-media thickness (IMT) was bilaterally recorded in the far wall of the common carotid arteries 1-2 cm proximal to the bifurcation. Common

carotid artery resistive index (CCARI) reflected the rigidity and distensibility of the common carotid arteries. It was determined with a probe volume of pulse Doppler 3 mm and angle of orientation between 30° and 60° [11, 12]. The mean values of the clinical, laboratory and ultrasound parameters were analyzed.

Table 1. Demographic, clinical and laboratory characteristics of the patients

Index	Gouty arthritis without tophi patients (n = 45)			Gouty tophi patients (n = 26)			p-value
	N (%)	Mean (SD)	Median (Min; Max)	N (%)	Mean (SD)	Median (Min; Max)	
Age (years)	45	55.76 (13.47)	57.0 (20.0;81.0)	26	58.77 (8.62)	60.0 (38.0;77.0)	0.477
Sex (males)	37 (82.2)			24 (92.3)			0.307
Disease duration (years)	44	16.96 (41.8)	2.0 (0.1;21.6)	26	36.26 (68.36)	11.0 (0.2;25.2)	0.001
Patients with gout flare	9 (20.0)			9 (34.6)			0.173
BMI (kg/m ²)	45	30.57 (6.24)	29.2 (20.2;48.3)	26	28.81 (3.56)	28.6 (22.2;34.6)	0.352
Hb (g/l)	45	147.71 (15.29)	148.0 (102.0;169.0)	26	134.54 (15.63)	136.0 (99.0;167.0)	0.001
Ht (g/l)	45	0.43 (0.04)	0.4 (0.3;0.5)	25	0.40 (0.04)	0.4 (0.3;0.5)	0.018
Serum level of uric acid (μmol/l)	45	479.42 (115.62)	480.0 (235.0;745.0)	26	496.04 (122.13)	496.5 (252.0;767.0)	0.555
BUN (mmol/l)	31	6.33 (3.46)	4.9 (2.9;18.2)	17	6.61 (2.96)	7.21 (1.6;13.7)	0.490
eGFR (ml/min)	45	108.87 (46.26)	100.7 (29.7;209.6)	26	92.62 (38.51)	90.3 (18.8;169.3)	0.185
ESR (mm/h)	43	21.91 (16.5)	18.0 (2.0;72.0)	20	32.00 (29.81)	21.0 (2.0;105.0)	0.387
CRP (mg/l)	43	11.49 (15.36)	5.3 (0.2;64.3)	21	29.40 (41.51)	12.6 (0.7;141.8)	0.048
TC (mmol/l)	38	5.42 (1.23)	5.3 (3.2;8.5)	22	5.43 (1.21)	5.2 (3.6;8.5)	0.896
HDL (mmol/l)	33	1.26 (0.43)	1.1 (0.8;2.6)	19	1.26 (0.44)	1.1 (0.6;2.3)	0.842
LDL (mmol/l)	29	3.48 (1.13)	3.6 (1.8;6.1)	18	3.30 (1.09)	3.5 (1.5;5.2)	0.584
VLDL (mmol/l)	28	0.82 (0.41)	0.6 (0.3;2.0)	17	0.84 (0.46)	0.8 (0.2;2.2)	0.779
TG (mmol/l)	39	1.81 (0.96)	1.5 (0.5;4.5)	21	1.86 (1.08)	1.7 (0.5;4.8)	0.774
Serum level of ROS products	45	0.52 (0.27)	0.5 (0.2;1.3)	26	0.50 (0.28)	0.4 (0.2;1.5)	0.830
Serum level of NO radicals	45	3.94 (2.8)	3.7 (0.2;11.5)	26	4.40 (3.04)	3.9 (0.3;12.3)	0.543
Serum level of ascorbate radicals	45	6.15 (2.04)	6.1 (1.7;10.6)	26	6.40 (2.47)	6,3 (2.5;10.9)	0.650
Dyslipidemia	28 (87.5)			16 (94.1)			0.646
BMI (> 30 kg/m ²)	20 (44.4)			10 (38.5)			0.623
Arterial hypertension	37 (82.2)			23 (88.5)			0.735
Patients suffered a cardiovascular event	3 (6.7)			7 (26.9)			0.031
Patients with diabetes mellitus	2 (4.4)			3 (11.5)			0.348
Chronic kidney failure	16 (35.6)			13 (50.0)			0.233
Smokers	16 (37.2)			9 (34.6)			0.828
Patients treated with diuretics	15 (33.3)			7 (26.9)			0.574

STATISTICAL ANALYSES

Statistical analyses were done by using SPSS version 13.0 (SPSS Inc., Chicago, IL, USA). Means with SDs, Medians, Min, Max and percentages were calculated to describe the clinical and ultrasound characteristics of the patients. The test of Kolmogorov-Smirnov was used to verify the distribution of normality. Intergroup comparisons were performed by t-test or Mann-Whitney test. Proportional differences were checked by applying the chi-square and Fisher's exact test. All tests were two tailed, and p values less than 0.05 were considered statistically significant.

RESULTS

Gouty arthritis without tophi and gouty tophi patients were age-matched. The serum levels of uric acid, the proportion of subjects with flare as well as the distribution of known cardiovascular risk factors was equal in the groups. However, in the tophi group CRP and the number of individuals who had suffered a cardiovascular event were higher ($p = 0.048$; $p = 0.031$) (table 1). Serum concentrations of ROS products, NO radicals and ascorbate radicals were comparable in the two stages of gout ($p = 0.783$; $p = 0.521$; $p = 0.651$), (fig. 1 a, b, c). In the groups no difference was registered in renal and common carotid arteries resistance ($p = 0.483$; $p = 0.273$) but intima-media was thicker in the presence of tophi, ($p = 0.027$), (fig. 1 d, e, f). We did not establish any correlation between the serum levels of ROS products, NO radicals and ascorbate radicals with RRI, CCARI and IMT (fig. 2 a, b, c, d, e, f, g, h, i). A weak negative correlation existed between the serum concentration of NO radicals and age ($r = -0.248$, $p = 0.037$), (fig. 2 j). Among untreated and treated with Allopurinol subjects no difference was found in the mean values of ROS products, NO radicals, ascorbate radicals, RRI, CCARI and IMT, (fig. 3 a, b, c, d, e, f). The same applies to untreated and treated with Febuxostat individuals in whom the mean serum concentrations of ROS products, ascorbate radicals, RRI, CCARI and IMT were comparable, (fig. 3 g, i, j, k, l). A tendency of higher serum NO radicals was detected in individuals taking Febuxostat in comparison to those not treated with it (mean $\pm$ SD; 5.18 ± 2.28 vs 3.92 ± 2.95 , $p = 0.076$), (fig. 3 h).

DISCUSSION

In the present study we did not establish a difference in the level of oxidative stress between the earlier and advanced stage of gout. Judging by the serum value of CRP, the degree of intrinsic inflammation was greater in the presence of subcutaneous gouty tophi. We also did not establish any correlation between serum oxidative stress markers with renal and carotid arteries arteriosclerotic vascular alterations.

In the last decade, there is evidence that hyperuricemia alone is associated with a high common carotid artery resistive index and thickened intima-media [13, 14, 15]. The relationship between hyperuricemia and renal resistive index in hypertensive patients has been proven [16]. In investigation involving patients with gout, intima-media thickness and the frequency of atherosclerotic plaques compared to subjects with asymptomatic hyperuricemia was higher [17]. In one of our studies in which we examined subjects in the spectrum of gout we observed that arteriosclerotic

Fig. 1. Summary statistics of ROS products, NO radicals, ascorbate radicals, RRI, CCARI and IMT in gouty arthritis without tophi patients and gouty tophi patients

Fig. 2. Correlational analysis results

vascular changes are more advanced in gouty tophi individuals compared to gouty arthritis without tophi and patients with asymptomatic hyperuricemia [18]. Herein, we once again found that intima-media was thicker in the presence of tophi than in the earlier stage of the disease.

The production of uric acid is characterized by increased vascular oxidative stress [19]. Hyperuricemia correlates with the progression of cardiovascular diseases. It is thought that it could provoke the development of obesity, impaired glucose tolerance, arte-

rial hypertension, hyperlipidemia and chronic kidney disease [20, 21]. Uric acid possesses both oxidizing and scavenging properties which explains why it has not been identified as an independent cardiovascular risk factor [22, 23, 24]. It causes endothelial dysfunction, affects oxidative metabolism, platelet adhesion, haemoreology and aggregation, mechanisms that are related to the evolution of cardiovascular events [25]. High urate in gout is connected to increased oxidative stress. Early in the pathogenesis of gout along with inflammation, crystals trigger oxidative stress by

Fig. 3. Summary statistics of ROS products, NO radicals, ascorbate radicals, RRI, CCARI and IMT among untreated and treated with Allopurinol or Febuxostat patients

stimulating the production of ROS and NO radicals [26]. In turn ROS mediate various signaling pathways and induce apoptosis in endothelial and smooth muscle cells through ox-LDL [4]. Research conducted by Lisa Stamp showed that increased concentrations of urate in gout lead to the release of myeloperoxidase from neutrophils and its subsequent oxidation [27]. In the present study there was no difference in the serum values of uric acid and in the mean serum concentrations of ROS products, NO radicals and ascorbate radicals between gouty arthritis without tophi and gouty tophi subjects. Data exist that Allopurinol exerts protective effects against oxidative stress, because of decreasing of ROS [28]. However, we found that renal and carotid arteries stiffness were similar among untreated and treated with Allopurinol patients. The same applies to individuals untreated and treated with Febuxostat. In the tophi group CRP, the number of patients who had suffered a cardiovascular event and intima-media were greater. The

results led us to suggest that maintaining a higher level of chronic inflammatory process in this group of patients creates a complex environment in the body with a leading role in atherogenesis.

In conclusion, with the disease progression there was no difference in the level of oxidative stress. The degree of chronic inflammation was greater in patients with tophi and arteriosclerotic vascular alterations were more pronounced in this group. Ultrasound parameters reflecting renal and carotid arteries arteriosclerotic vascular changes did not correlate with serum oxidative stress markers. We suggest that in gout individuals intrinsic inflammation plays an important role in the process of atherogenesis. Several limitations deserve to be mentioned. This was a single center cross-sectional study, laboratory parameters, oxidative stress markers as well as ultrasound indices were measured at a fixed point of time. Future studies are needed to surpass the limitations.

Conflicts of interest: The authors declare no conflicts of interest

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