VIA MEDICA

ORIGINAL PAPER

Acta Angiol
Vol. 24, No. 1 pp. 1–8
Doi: 10.5603/AA.2018.0001

ISSN 1234–950X
www.journals.viamedica.pl/acta_angiologica

Systemic oxidoreductive balance in patients without clinical manifestation of atherosclerosis

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Abstract

Introduction. *Oxidative stress plays an important role in atherosclerosis, but numerous clinical trials have not confirmed a favourable effect of antioxidant supplementation. We aimed to determine the oxidative stress parameters in patients without clinical manifestation of vascular disease.*

Material and methods. *Forty-eight patients were divided into two groups in relation to the presence or absence of clinically silent signs of atherosclerosis (ankle-brachial index < 0.9, intima-media thickness ≥ 0.9 mm, the presence of carotid atherosclerotic plaques, silent ischaemia in a treadmill stress test or focal myocardial contractility found in echocardiography). Plasma concentrations of: retinol, ascorbic acid, alpha-tocopherol and uric acid, as well as the products of oxidative DNA damage repair: 8-oxo-7,8-dihydro-2'-deoxyguanosine (8-oxodG) in blood leucocytes and urine, and 8-oxo-7,8-dihydroguanine (8-oxo-Gua) in urine.*

Results. *Patients with signs of subclinical atherosclerosis had lower blood concentration of alpha- tocopherol, and a non-significantly greater urine concentration of 8-oxoGua. Women had significantly greater blood concentration of ascorbic acid and alpha-tocopherol, as well as lower level of retinol and uric acid. They also had greater leucocyte concentration of 8-oxodG. Plasma concentration of alpha-tocopherol 30.34 μM distinguished patients with and without signs of subclinical atherosclerosis.*

Conclusions. *Oxidative stress has clinical importance in the early stages of atherosclerosis and may be helpful in predicting its subclinical stage. Women had higher level of antioxidant defence, which explains their natural protection against early atherosclerosis development. Further studies are needed to determine the usefulness of tocopherol determination as a biomarker for atherosclerosis risk evaluation.*

Key words: oxidative stress, biomarkers, atherosclerosis, intima-media thickness, vitamin E

Acta Angiol 2018; 24, 1: 1–8

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Introduction

Oxidative stress is a result of an imbalance between the production of reactive oxygen species (ROS) and the effectiveness of ROS scavenging in antioxidant defence and plays a part in the pathogenesis of several diseases, one of which is atherosclerosis [1, 2]. It is the final path though which all of known atherogenesis risk factors (e.g. smoking, diabetes mellitus, hypertension, dyslipidaemia) lead to endothelial dysfunction. Oxidative stress also affects atherosclerosis plaque formation through the oxidation of low-density lipoprotein (LDL). Oxidised LDL (ox-LDL) penetrates cell membranes, not only via LDL receptors, but also through scavenger receptors and phagocytosis, which leads to the uncontrolled accumulation of cholesterol in macrophages [1–4]. This process leads to the transformation of macrophages into foam cells. When cholesterol accumulation exceeds a critical value, foam cell disintegration occurs. Cholesterol released into the vascular wall provokes an inflammatory reaction associated with ROS overproduction in a vicious circle with atherosclerosis progression, the final outcome of which is plaque instability, rupture and arterial thrombosis with unfavourable clinical implications [2]. Oxidative stress is also an important pathomechanism of ischaemia/reperfusion damage of vessel's wall, which leads to oxidative DNA damage, lipid peroxidation, activation of inflammatory and cytotoxic processes, activation of matrix-metalloproteinases (MMPs), recruitment of endothelial progenitor cells, affection of endothelial and vascular smooth muscle cells apoptosis, proliferation and differentiation, and finally to disturbance in vessel wall remodelling, angiogenesis and neovascularisation. Chronic oxidative stress interferes also with physiological redox signalling during intracellular metabolic processes, including ATP synthesis [5]. Therefore, treating oxidative stress is a target for the prevention of atherosclerosis development and progression. However, the results of laboratory experiments on animals and data obtained in observational studies that showed negative associations between antioxidant intake and all-cause and cardiovascular mortality have not been confirmed in several randomised controlled trials (RCTs) [2–7]. The majority of the trials failed to find a favourable cardiovascular outcome for retinol (vitamin A), ascorbic acid (vitamin C), or tocopherol (vitamin E) supplementation [8–14]. Only a few studies revealed a favourable effect for vitamin E supplementation, especially in low doses [3]. Similarly, conflicting results of the influence of antioxidant-reach food consumption on surrogate outcomes (endothelial function, carotid intima-media thickness [cIMT], C-reactive protein [CRP], interleukin-17 [IL-17]) have been reported [3, 8, 9, 15, 16].

There are contradictory results concerning the effect on survival between natural antioxidant intake in experimental and observational studies and vitamin supplementation in RCTs. Therefore, we assumed that, apart from the potential importance of providing balanced and complex natural vitamins, this discrepancy may also result both from interpersonal differences in ROS production, i.a. depends on variable proinflammatory phenotype expression in inflammatory cells [5] as well as in the bioavailability of antioxidants and individual potential for their storage and the maintenance of adequate and protective blood concentrations [17, 18]. We hypothesised that patients with high levels of oxidative stress or a depletion in natural antioxidant defence may be the most likely to benefit from antioxidant therapy or e.g. exercise training. Therefore, the natural or interventional intake of antioxidants might not be a good parameter for the evaluation of the importance of oxidative stress in the course of cardiovascular diseases. As a result, we based our study on plasma and urine determination of oxidative stress parameters, such as products of DNA damage, e.g. 8-oxo-7,8-dihydro-2'-deoxyguanosine (8-oxo-dG) in leucocyte DNA, and the urinary excretion of the products of oxidative DNA damage repair, e.g. 8-oxoguanine (8-oxo-Gua) and 8-oxo-dG, as well as on blood determination of parameters of antioxidant defence, such as retinol (vitamin A), ascorbic acid (vitamin C), tocopherol (vitamin E), uric acid, glutathione, antioxidative enzymes, etc. [19–25]. The aim of this study, which was performed with adult patients without clinical manifestation of vascular diseases, was to compare the levels of these substances between individuals with and without signs of atherosclerosis.

Material and methods

Forty-eight patients with a mean age of 56.4 ± 4.8 years were studied. Twenty eight (58%) of them were males and 20 (42%) were females (Table 1). The inclusion criteria were as follows: lack of any acute and chronic disorders which could affect determinations of oxidoreductive balance, lack of history of acute coronary syndrome, myocardial infarction, stroke, transient ischaemic attack, intermittent claudication, aortic aneurysm or other vascular diseases, over the age of 18 and able to give informed consent for participation in the study. The exclusion criteria were as follows: symptoms of any vascular diseases, diabetes mellitus (as an equivalent of vascular diseases), intake of diet supplements which could affect oxidoreductive balance, the inability to perform a treadmill stress test, and history or physical signs of inflammatory diseases or neoplasm. In all the patients who qualified for the

study, medical histories (symptoms, past history of diseases, use of stimulants, intake of vegetables and fruit in everyday diet, and use of diet supplements and vitamins) were obtained, and physical examination and basic laboratory investigations (blood morphology, determinations of creatinine, sodium, potassium glucose, alanine aminotransferase, C-reactive protein, total, LDL and high-density lipoprotein [HDL] cholesterol, and triglycerides) were performed. Moreover, the following were determined in all the subjects: ankle-brachial index (ABI), duplex ultrasound of carotid arteries with an estimation of cIMT, electrocardiogram (ECG), treadmill stress test on a running track in accordance with the Bruce protocol, and echocardiography readings. Markers of subclinical vascular injury were arbitrarily established: an ABI cut-off value for lower limb ischaemia was established at < 0.9 [26], a diagnosis of atherosclerotic plaques in the carotid arteries was indicated by a cIMT > 0.9 mm, signs of silent ischaemia during a treadmill stress test (a decrease of ST interval of more than 1 mm in at least two leads) or the presence of a regional reduction in myocardial contractility in echocardiography. On the basis of these criteria, the patients were divided into two groups: with and without at least one of the mentioned signs of subclinical atherosclerosis.

Moreover, detailed examinations of oxidoreductive balance were performed as previously described [19–22]:

- in plasma: the concentration of high-sensitivity CRP (hs-CRP), as well as retinol (vitamin A), ascorbic acid (vitamin C), tocopherol (vitamin E), and uric acid;
- in blood leucocytes' DNA: 8-oxo-7,8-dihydro-2'-deoxyguanosine (8-oxodG);
- in urine: the level of 8-oxo-7,8-dihydroguanine (8-oxoGua) and 8-oxo-7,8-dihydro-2'-deoxyguanosine (8-oxodG).

Bioethics

The study protocol was approved by the local Bioethical Committee, and the study was conducted in compliance with the Declaration of Helsinki for medical research. All the patients signed informed consent for participation in the study.

Statistics

Statistical analysis was conducted using licensed versions of statistical software STATISTICA (a data analysis software system), StatSoft, Inc. (2015), version 12. The normal distribution of the study variables was checked using the Shapiro-Wilk test. The results were mainly presented as the mean ± standard deviation, or n, %. The statistical significance of the differences between the groups was verified using the Student's t-test and chi-squared test. The statistical significance level was set at a p-value < 0.05. Logistic regression using a quasi-Newton and simplex estimation method was applied to check the relationships between the presence of subclinical signs of atherosclerotic lesions and the variables analysed. The receiver operating characteristic (ROC) curve for associations between the presence of signs of subclinical atherosclerosis and the level of respective oxidoreductive balance parameters were determined.

Results

Of the 48 study participants with a mean age of 56.4 ± 4.8 years, 28 (58%) were males. The established signs of subclinical atherosclerosis defined above were found in 14 (29%) subjects, 8 (57%) had cIMT greater than 0.9 mm, 4 (29%) had ABI < 0.9, and 2 (14%) had asymptomatic decrease in ST interval during treadmill stress test and the presence of a regional reduction in myocardial contractility in echocardiography. Patients with subclinical atherosclerosis were similar to their counterparts in relation to the majority of the characteristics, although they were older and had a greater pulse pressure (the difference between systolic and diastolic blood pressure) (Table 1). Patients with signs of subclinical atherosclerosis had lower blood concentration of alpha- tocopherol, and non-significantly greater urine concentration of 8-oxo-Gua (Table 2). The remaining levels of the other studied parameters of oxidoreductive balance were similar in both groups.

When we have compared female and male patients (Table 3), we have found that women had significantly greater blood concentrations of vitamins C and E, as well as lower levels of vitamin A and uric acid. Women also had higher level of 8-oxodG in leucocytes.

As the intake of natural or artificial antioxidants might have affected the results obtained and been a potential confounding factor, we have compared the levels of the studied parameters of oxidoreductive balance between the patients divided in relation to the number of days of vegetables and fruit in their diet per week. However, we have not found any significant differences in the values of the oxidoreductive balance biomarkers investigated between these groups (detailed data are not presented).

Next, we have performed multifactorial analysis using logistic regression (Table 4). We have found that the risk of the presence of subclinical signs of atherosclerosis increased significantly with the patient's age (odds ratio [OR]; 1.47 ± 95% confidence interval [CI]

Table 1. Comparison of clinical and biochemical data in individuals with and without subclinical signs of atherosclerosis

Parameter	Individuals without subclinical signs of atherosclerosis n = 34 (71%)	Individuals with subclinical signs of atherosclerosis n = 14 (29%)	P
Age (years)	55.4 ± 4.3	58.8 ± 5.1	0.02
Male gender (n, %)	19 (56%)	9 (64%)	0.83
Smoking (n, %)	5 (15%)	3 (21%)	0.88
Hypertension (n, %)	6 (18%)	6 (43%)	0.14
BMI (kg/m^2)	27.1 ± 3.8	28.6 ± 3.3	0.21
WHR	0.86 ± 0.08	0.88 ± 0.09	0.42
Systolic blood pressure (mm Hg)	122.2 ± 15.4	129.6 ± 15.7	0.14
Diastolic blood pressure (mm Hg)	75.6 ± 10.6	76.1 ± 10.1	0.89
Pulse pressure (mm Hg)	46.6 ± 9.7	53.6 ± 10.3	0.03
Leucocytes (G/l)	6.1 ± 1.7	6.6 ± 0.9	0.35
Erythrocytes (T/l)	4.9 ± 0.5	4.9 ± 0.3	0.99
Haemoglobin (g/dl)	14.6 ± 1.2	15.0 ± 0.7	0.24
Platelets (G/l)	244.7 ± 53.7	238.1 ± 40.6	0.68
Creatinine (mg/dl)	1.1 ± 0.2	1.1 ± 0.1	0.55
Blood glucose (mg/dl)	94.1 ± 14.2	102.8 ± 24.9	0.13
Alanine aminotransferase (U/l)	25.7 ± 8.3	32.6 ± 16.3	0.06
CRP (mg/l)	1.9 ± 4.0	1.3 ± 1.1	0.55
Total cholesterol (mg/dl)	226.9 ± 39.0	207.1 ± 31.6	0.09
HDL cholesterol (mg/dl)	58.7 ± 12.7	59.9 ± 13.4	0.77
LDL cholesterol (mg/dl)	139.6 ± 31.9	130.2 ± 26.7	0.33
Triglycerides (mg/dl)	122.4 ± 44.5	108.8 ± 33.9	0.30

Data presented as the mean ± standard deviation (SD) and number (n, %) for qualitative variables; BMI — body mass index; WHR — waist-to-hip circumference ratio; CRP — C-reactive protein; HDL — high-density lipoprotein; LDL — low-density lipoprotein

Table 2. Biomarkers of oxidative stress in individuals with and without subclinical signs of atherosclerosis

Parameter	Individuals without subclinical signs of atherosclerosis n = 34 (71%)	Individuals with subclinical signs of atherosclerosis n = 14 (29%)	P
Plasma hs-CRP (mg/l)	2.56 ± 5.7	1.60 ± 1.3	0.54
Plasma CRP (mg/l)	1.98 ± 4.0	1.33 ± 1.1	0.55
Plasma ascorbic acid (μM)	38.9 ± 20.7	36.6 ± 25.5	0.74
Plasma retinol (μM)	2.8 ± 0.6	2.8 ± 0.7	0.92
Plasma alpha-tocopherol (μM)	34.2 ± 6.01	29.95 ± 5.7	0.03
Plasma uric acid (μM)	345.4 ± 61.4	346.2 ± 65.3	0.97
Creatinine in urine (mmol/l)	11.2 ± 5.66	11.38 ± 6.22	0.92
8-oxo-Gua in urine (nmol/mmol creatinine)	7.4 ± 3.1	12.5 ± 19.9	0.18
8-oxo-dG in urine (nmol/mmol creatinine)	2.4 ± 1.6	2.7 ± 1.8	0.62
8-oxo-dG in leucocytes (8-oxo-dG/10^6 dG)	6.8 ± 2.1	6.2 ± 2.0	0.40

Data presented as the mean ± standard deviation (SD) and number (n, %) for qualitative variables. The statistical significance level was set at a p-value < 0.05; 8-oxo-Gua in urine — concentration of 8-oxoguanine in urine calculated in relation to the concentration of creatinine; 8-oxo-dG in urine — concentration of 8-oxo-7,8-dihydro-2'-deoxyguanosine in urine calculated in relation to the concentration of creatinine; 8-oxo-dG in leucocytes — 8-oxo-7,8-dihydro-2'-deoxyguanosine content in leucocytes; CRP — plasma concentration of C-reactive protein; hs-CRP — plasma concentration of C-reactive protein determined using the high-sensitivity method

1.05–2.05; p = 0.02) and decreased with an increase in tocopherol blood concentration (OR 0.67; 95% CI 0.45–1.00; p = 0.05).

Using ROC curve analysis, we have found that plasma concentration of tocopherol at the level of 30.34 μM distinguished patients with and without signs of subclinical atherosclerosis (Fig. 1).

Discussion

In this study, we have investigated the levels of oxidoreductive balance parameters in adults without clinical manifestation of vascular diseases. We have found that the presence of subclinical signs of atherosclerosis (reduced ABI, increased cIMT, the presence

Table 3. Biomarkers of oxidative stress in female and male patients

Parameter	Females N = 20 (42%)	Males N = 28 (58%)	p
Plasma hs-CRP (mg/l)	3.36 ± 7.3	1.50 ± 1.57	0.19
Plasma CRP (mg/l)	2.59 ± 5.1	1.48 ± 1.09	0.17
Plasma ascorbic acid (μM)	49.5 ± 22.6	30.2 ± 17.8	< 0.01
Plasma retinol (μM)	2.6 ± 0.5	3.0 ± 0.7	0.04
Plasma alpha-tocopherol (μM)	35.4 ± 5.7	31.2 ± 6.0	0.02
Plasma uric acid (μM)	317.4 ± 42.4	365.9 ± 66.2	< 0.01
Creatinine in urine (mmol/l)	7.4 ± 4.1	14.1 ± 5.2	< 0.01
8-oxo-Gua in urine (nmol/mmol creatinine)	8.0 ± 3.6	9.2 ± 13.1	0.73
8-oxo-dG in urine (nmol/mmol creatinine)	2.9 ± 2.0	2.3 ± 1.3	0.24
8-oxo-dG in leucocytes (8-oxo-dG/10^6 dG)	7.5 ± 2.6	6.1 ± 1.4	0.03

Data presented as the mean ± standard deviation (SD) and number (n, %) for qualitative variables. The statistical significance level was set at a p-value < 0.05; 8-oxo--Gua in urine — concentration of 8-oxoguanine in urine calculated in relation to the concentration of creatinine; 8-oxo-dG in urine — concentration of 8-oxo-7,8--dihydro-2'-deoxyguanosine in urine calculated in relation to the concentration of creatinine; 8-oxo-dG in leucocytes — 8-oxo-7,8-dihydro-2'-deoxyguanosine content in leucocytes; CRP — plasma concentration of C-reactive protein; hs-CRP — plasma concentration of C-reactive protein determined using the high-sensitivity method

Table 4. Factors determining the risk of the presence of clinically silent atherosclerotic lesions; $Chi^2(5) = 24.50$; $p < 0.001$

Parameter	Constant	Age	Current smoker	Tocopherol	8-oxo-Gua_U	8-oxo-dG_L
Estimation	–9.62	0.39	0.26	–0.40	0.09	–0.40
Standard error	8.26	0.16	2.35	0.19	0.07	0.43
p	0.25	0.02	0.91	0.05	0.19	0.36
-95% CI	–26.45	0.05	–4.52	–0.79	–0.05	–1.27
+95% CI	7.21	0.72	5.04	–0.00	0.24	0.47
Wald test Chi square	1.36	5.55	0.01	4.21	1.83	0.87
P	0.24	**0.02**	0.91	**0.04**	0.18	0.35
OR for one unit	0.00	1.47	1.30	0.67	1.10	0.67
-95% CI	0.00	1.05	0.01	0.45	0.95	0.28
+95% CI	1350.50	2.05	154.12	1.00	1.27	1.60

8-oxo-Gua_U — 8-oxoguanine in urine; 8-oxo-dG_L — 8-oxo-7,8-dihydro-2'-deoxyguanosine in leucocytes; CI — confidence interval; OR — odds ratio

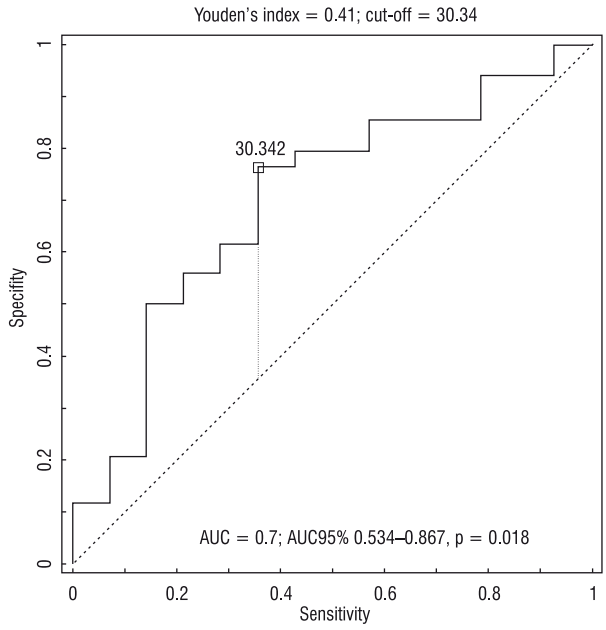

Figure 1. The ROC curve for associations between blood vitamin E concentration and the presence of clinically silent atherosclerotic lesions

of atherosclerotic plaques in the common carotid artery, signs of silent myocardial ischaemia during a treadmill stress test or in transthoracic echocardiography) were independently and positively related to the patient's age and negatively with blood tocopherol concentration (Table 2, Table 4). The presence of subclinical signs of atherosclerosis was also related to an increased stiffness of the arterial wall expressed by greater pulse pressure (the difference between systolic and diastolic blood pressure) (Table 1). This observation suggests that stronger antioxidant defence in blood may protect patients against the development of atherosclerotic plaque and arterial stiffness, which are recognised as independent risk factors of cardiovascular death. Moreover, we have found that female patients had greater blood concentrations of vitamins C and E, and lower blood vitamin A concentration (Table 3), which might be directly linked with the later development of atherosclerosis in women [27]. Blood concentration of vitamin E lower than 30.34 μM (Figure 1) was the only oxidoreductive parameter which was predictive of the presence of clinically silent

atherosclerotic lesions or arterial stiffness (greater pulse pressure).

Our results concerning the potential anti-atherogenic properties of vitamin E are consistent with the results of a recent meta-analysis of observational studies made by Li et al. [28], in which blood concentration of total tocopherol was significantly lower in coronary artery disease (CAD) patients than that in controls. In this study, lower blood level of total tocopherols was also associated with early CAD onset. Similarly, in the Asymptomatic Carotid Atherosclerotic Disease in Manfredonia Study, patients with cIMT $\geq$ 0.8 mm had significantly lower blood concentration of vitamins A and E and beta-carotene than participants who did not show evidence of carotid atherosclerosis [29]. Our results also corroborate data reported in numerous observational and real-world studies, such as the Harvard Nurses' Health Study (NHS) and the Health Professional Study (HPS), which showed a cardioprotective effect of vitamin E supplementation [30]. Previously, Stephens et al. [31] in their Cambridge Heart Antioxidant Study (CHAOS), and, recently, Loffredo et al. [32], also found a reduced risk of myocardial infarction among patients on vitamin E supplementation. Cardioprotective properties of vitamin E supplementation were also revealed in a recent meta-analysis by Schwingshackl et al. [12]. However, some observational investigations found that the consumption of vitamin C, vitamin E and beta-carotene was not related to cardiovascular disease (CVD) mortality in women or to cancer mortality in either gender (the HAPIEE study) [14]. Large randomised placebo-controlled trial such as the HOPE Study [33, 34], and older [35] and more recent [9, 10, 36–38] meta-analyses have shown that vitamin E supplementation had no beneficial or adverse effect on cardiovascular outcomes, and may even have increased all-cause mortality [39]. Moreover, the above-cited recent meta-analysis by Schwingshackl et al. [12] showed that the intake of beta-carotene may be associated with increased risk of all-cause mortality. This observation may corroborate our observation in female patients (Table 3), who had a lower level of circulating vitamin A and were recognised as having reduced cardiovascular risk [27]. However, Koh et al. [40] and Gey et al. [41] showed a decreased risk of myocardial infarction in Chinese patients with high plasma levels of β-cryptoxanthin and lutein, and increased risk of coronary events in patients with low blood retinol concentration, respectively. Various attempts were made by the researchers to explain these contradictory outcomes as the effect of known and unknown drug-drug interactions or unexpected adverse drug reactions [30], because antioxidant vitamins in those RCTs were added to each patient's usual dietary regimen. One important factor in explaining the contrary findings of the effect of vitamin E on the course of vascular and neoplasmatic diseases might also be the different vitamin E doses given in respective investigations, which ranged from 300 to 1800 IU per day [42], and the absence of gamma-tocopherol in traditional preparations [43]. This member of tocopherol family exerts stronger cardioprotective, antioxidant and anti-inflammatory effects than alpha-tocopherol [43].

In our work, we have not only studied the concentration of antioxidant vitamins in blood, but also the level of oxidatively damaged DNA in urine and leucocytes. We have found a non-significantly greater urine concentration of 8-oxoGua in patients with clinically silent signs of atherosclerosis (Table 2). Różalski et al. [19] found that patients, who qualified for carotid endarterectomy and had echolucent carotid plaques (gray-scale median score $<$ 25), had also the highest antioxidant level and lowest excretion of DNA repair markers than remained patients and control. In a study by Sigala et al. [25], a higher level of oxidative stress expressed by the presence of ox-LDL in blood was associated with the presence of symptoms of carotid atherosclerotic plaque instability. No effect of antioxidants on cIMT was previously reported either [16]. These data confirm the clinical importance of disturbances in oxidoreductive balance in the pathogenesis of atherosclerosis, and suggest that serum antioxidant level, especially vitamin E, may act as a biomarker for the risk of atherosclerosis [19, 44].

Although we have found some statistically significant differences between the studied parameters, our results may have shortcomings which could decrease the strength of our deductions. Firstly, the studied group was small and potential confounding factors as age and gender were not balanced, but this authenticates the significance of the differences found. Secondly, a diagnosis of clinically silent atherosclerosis was based on non-invasive examinations and was graded only on two levels (present or absent). Thirdly, the consumption of fruit and vegetables was determined by a qualitative scale in a questionnaire only, and patients' diet before blood examination was not standardised. However, it was previously revealed that the level of some parameters of oxidative stress does not depend on diet [22]. Fourthly, we have determined only alpha-tocopherol from the tocopherol family, but currently gamma-tocopherol seems to be more important in cardioprotection [43].

In conclusion, our pilot study on a small number group of patients pointed out the potential importance of alpha-tocopherol blood concentration in the early stages of atherosclerosis and showed that its determination may be helpful in predicting its subclinical stage. Women had a higher level of alpha-tocopherol and

ascorbic acid, which may explain their natural protection against early atherosclerosis development, in spite of lower level of retinol and uric acid. Differences in the weekly intake of fruit or vegetables had no effect on the plasma level of the parameters of oxidoreductive balance studied, but, due to study limitations, this association needs to be evaluated in a larger sample size. Further studies in the large sample size are also needed to determine the usefulness of vitamin E determination as a biomarker of atherosclerosis risk.

Conflict of interest

None.

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