Koroner arter hastalığında plazma ve doku oksidatif durumu: Oksidatif stres ve koroner arter hastalığı

The plasma and tissue oxidative status in patients with coronary artery disease: oxidative stress and coronary artery disease

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Background: We compared the plasma and tissue total antioxidant capacity (TAC) and total oxidant status (TOS) in patients undergoing coronary artery bypass surgery.

Methods: The TAC and TOS levels were assessed in the plasma of 30 coronary artery endarterectomy patients and compared to 30 control samples. Atherosclerotic plaque TAC and TOS levels were also evaluated in the patients' group. The severity of coronary artery disease was calculated with the Gensini score index.

Results: The plasma TAC and TOS values were significantly lower in patients than in controls (p=0.008 and p=0.004 respectively). The TAC level was significantly lower in tissue than in plasma (0.11±0.03 mmol Trolox Equiv./L versus 0.65±0.25 mmol Trolox Equiv./L, p<0.001). The level of TOS was significantly higher in plasma than in tissue (0.80±0.46 μmol H_2O_2 equiv./L versus 10.9±3.7 μmol H_2O_2 equiv./L, p<0.001). Multiple linear regression analyses show that the Gensini score index was independently associated with plasma TAC levels (r=-0.898, p<0.001) and age (r=0.258, p=0.023).

Conclusion: The plasma TAC value, rather than tissue oxidative stress and antioxidant status, is related to atherosclerosis.

Key words: Atherosclerotic plaques; coronary artery disease; total antioxidant capacity; total oxidant status.

Amaç: Bu çalışmada koroner arter bypass cerrahisi uygulanan hastalarda doku ve plazma total antioksidan kapasite (TAK) ve total oksidan seviye (TOS) değerleri karşılaştırıldı.

Çalışma planı: Koroner endearterektomi uygulanan 30 hasta ve 30 sağlıklı kontrolde plazma TAK ve TOS seviyeleri değerlendirildi. Ayrıca hasta grubunda aterosklerotik plak TAK ve TOS seviyelerine bakıldı. Koroner arter hastalığı şiddeti Gensini skor indeksi ile hesaplandı.

Bulgular: Plazma TAK ve TOS değerleri hastalarda kontrollere göre daha düşüktü (sırası ile p=0.008 ve p=0.004). Doku TAK seviyesi plazmaya göre anlamlı derecede düşüktü (0.11±0.03 mmol Trolox Equiv./L'ye karşın 0.65±0.25 mmol Trolox Equiv./L, p<0.001). Total oksidan seviye ise plazmada dokuya göre belirgin derecede yüksekti (0.80±0.46 μmol H_2O_2 equiv./L'ye karşın 10.9±3.7 μmol H_2O_2 equiv./L, p<0.001). Çoklu regresyon analizinde Gensini skor indeksi sadece plazma TAK seviyeleri (r=-0.898, p<0.001) ve yaş ile (r=0.258, p=0.023) bağımsız ilişki göstermekteydi.

Sonuç: Doku oksidatif stres ve antioksidan kapasiteden ziyade plazma TAK seviyeleri ateroskleroz ile ilişki göstermektedir.

Anahtar sözcükler: Aterosklerotik plak; koroner arter hastalığı; total antioksidan kapasite; total oxidan status.

Coronary artery disease (CAD) is believed to have a multifactorial etiology, composed of numerous biological, environmental, behavioral and sociocultural factors.[1] In addition to traditional risk factors, oxidative stress has been regarded as one of the most important contributors to the initiation and progression of atherosclerosis.[2] The mechanisms leading to the initiation and development of atherosclerosis have not yet been clearly identified. A number of reports in the literature implicate excessive plasma oxidative stress and/or inadequate antioxidant defenses as the cause of CAD.[2-6]

It is also known that in patients with CAD, the risk of cardiovascular events has been predicted by endothelial dysfunction-mediated oxidative stress.[7] According to

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the LDL oxidation theory of atherosclerosis, oxidation of LDL occurs in the disease process, leading to accumulation of foam cells and fatty streaks.[8,9] Oxidized molecules generally form new radicals leading to radical chain reactions while its effects are neutralized by antioxidants. Direct measurement of free radicals in humans is difficult because of their transient nature and the complexity of available techniques. Since the separate measurements of different oxidant and antioxidant molecules were not practical, measurement of the total oxidant status (TOS) and total antioxidant capacity (TAC) has been suggested.[10,11]

Unlike our knowledge of oxidant and antioxidant changes in plasma, little is known about the accompanying changes to atherosclerotic plaque oxidants and antioxidants.[12] Because the data currently available are usually restricted to animal studies of advanced vascular disease, our study involved measuring the TAC and TOS in plasma and atherosclerotic tissue plaques of patients with atherosclerosis.[12]

PATIENTS AND METHODS

Patients and controls

Thirty patients who had previously undergone coronary artery bypass surgery (CABS) and coronary endarterectomy were enrolled in the study group. The control group included 30 age- and sex-matched healthy individuals at the same period. All participants were assessed with a detailed medical history, current medications, complete physical examination and electrocardiographic evaluation before bypass surgery. Blood pressure measurements were performed with a mercury manometer. Height and weight were measured according to a standardized protocol. Body mass index (BMI) was calculated by dividing the weight in kilograms by the height in meters squared (kg/m^2).

Exclusion criteria were as follows: heart failure, valvular disease, diabetes mellitus, cerebrovascular disease or malignant tumor, receiving any antioxidant drugs, smoking, chronic respiratory insufficiency and renal disease. Patients with myocardial infarction within the previous three months, unstable angina and previously - performed CABS were also excluded.

The study protocol conforms to the principles outlined in Declaration of Helsinki and approved by ethic committee of our hospital. Informed consent for participation in the study was obtained from all individuals.

Tissue and blood samples

Blood sample collection: Blood samples were obtained prior to valve surgery, following an overnight fasting state. Samples were withdrawn from a cubital vein into blood heparinized tubes. The plasma was separated from the cells by centrifugation at 3000 rpm for 10 min and stored on at –80°C.

Tissue sampling and homogenization: Specimens of the coronary endarterectomy in patients were obtained during cardiac surgery, and stored at –80°C until use. Before biochemical assays, all tissues were weighed and then placed in empty glass tubes. 10 mL of 140 mM KCl solution per 1 gram of tissue were added to each tube containing tissue samples and then all tissues were homogenized in a motor-driven homogenizer. The homogenate was centrifuged at 2800 g for 10 min at 4 °C. The obtained supernatant was used for the levels of TAC and TOS.

Measurement of the total oxidant and anti-oxidant status

Total antioxidant capacity and TOS levels were measured by the Erel methods.[10,11] These methods are automatic and colorimetric. The total antioxidant capacity (TAC) measurement method is based on the bleaching of the characteristic color of a more stable 2.2′-azino-bis (3-ethylbenzthiazoline-6-sulfonic acid) (ABTS) radical cation by antioxidants. The total oxidant status (TOS) method is based on the oxidation of ferrous ion to ferric ion in the presence of various oxidant species in an acidic medium and the measurement of the ferric ion by xylenol orange. The TAC and TOS results were expressed in mmol Trolox equivalent/L, μmol H_2O_2 /L and mg/dL, respectively, and the precision error of this assay is lower than 3%.

Severity of CAD

The severity of coronary atherosclerosis in patients was assessed by using the Gensini score,[13] which grades the narrowing of the lumen of the coronary arteries as 1 for 1-25% narrowing, 2 for 26-50% narrowing, 4 for 51-75% narrowing, 8 for 76-90% narrowing, 16 for 91-99% narrowing and 32 for total occlusion. This score is then multiplied by a factor that takes into account the importance of the lesion's position in the coronary arterial tree, for example: 5 for the left main coronary artery, 2.5 for the proximal left anterior descending (LAD) coronary artery or proximal left circumflex (LCX) coronary artery, 1.5 for the mid-region of the LAD, and 1 for the distal LAD or mid-distal region of the LCX. The Gensini score was expressed as the total of the scores for the all coronary arteries.

Statistical analysis

Results are presented as mean±SD or frequency values expressed as a percentage. Comparison of the parameters was performed using the paired T-test. The correlations between Gensini score index and clinical and laboratory parameters were assessed by the Pearson correlation test. Independent predictors of Gensini score index were assessed by multiple linear regression

Table 1. Demographic and clinical characteristics of the patient and controls

	Group 1 (n=30)	Group 2 (n=30)	*p*
Age (years)	56±8	54±14	0.733
Male/female (n)	18/12	16/14	0.219
Body mass index (kg/m^2)	29±3	27±5	0.794
Systolic blood pressure (mmHg)	116±12	118±21	0.581
Diastolic blood pressure (mmHg)	77±10	72±9	0.032
Statin use, (%)	56	–	
Acetylsalicylic acid use, (%)	64	–	
ACEI / ARB use, (%)	54	–	
Beta blocker use, (%)	42	–	
Heart rate (minute)	74±7	70±12	0.232
Plasma TAC	0.65±0.25	0.83±0.25	0.008
Plasma TOS	10.94±3.75	14.79±4.87	0.004
Gensini score index	52±24	–	
Tissue TAC	0.11±0.03	–	
Tissue TOS	0.80±0.46	–	

ACE: Angiotensin-converting enzyme inhibitors; ARB: Angiotensin II receptor blockers; TAC: Total antioxidant capacity (mmol Trolox Equiv./L); TOS: Total oxidant status (mmol Trolox Equiv./L); Values are mean±SD or %.

analysis. For multiple regressions, the factors showing a significant relationship in bivariate Pearson's correlation test were selected. A $p<0.05$ was considered statistically significant. Data were analyzed by using SPSS for Windows 11.5 version (SPSS Inc., Chicago, Illinois, USA).

RESULTS

Demographic and clinical characteristics of the patients and controls are shown in the Table 1. There were no significant differences in age, sex and body mass index. Among the two groups, the diastolic blood pressure and BMI were higher in patients than in the control group ($p<0.05$, for both variables).

The plasma TAC and TOS were lower in patients than the controls (both with $p<0.05$). Total antioxidant capacity and TOS levels in the tissues were significantly lower than plasma levels in patients.

In the patients' group, the presence of correlations between the Gensini score index and other factors is demonstrated in the Table 2. The Gensini score was correlated with age ($r=0.371$, $p=0.043$), plasma TAC ($r=-0.785$, $p<0.001$) and tissue TOS ($r=0.394$, $p<0.031$). There was no correlation between medicine taken and other parameters (all of $p>0.05$). Plasma TAC level ($\beta=-0.898$, $p<0.001$) and age ($\beta=0.258$, $p=0.023$) were independently related with the Gensini score index.

DISCUSSION

In our study, we used the TAC and TOS to measure the level of plasma and tissue oxidative status in patients with CABS. We found that these parameters in plasma levels were significantly higher than in tissue levels. The severity of CAD was negatively correlated with TAC and positively correlated with TOS values. There was also a close relationship between the severity of CAD, and plasma TAC and age.

Table 2. Bivariate and multivariate relationships of the Gensini score index to clinical, demographic, and oxidative variables in patient groups

	Pearson correlation coefficient	*p*	Standardized ß regression coefficients[a]	*p*
Age	0.371	0.043	0.258	0.023
Body mass index	0.088	0.645		
Systolic blood pressure	0.056	0.768		
Diastolic blood pressure	0.047	0.804		
Heart rate	0.030	0.875		
Plasma total antioxidant capacity	-0.785	<0.001	-0.898	<0.001
Tissue total antioxidant capacity	-0.095	0.619		
Plasma total oxidant status	0.159	0.401		
Tissue total oxidant status	0.394	0.031	0.227	0.120

[a]: From multiple linear regression.

Reactive oxygen species (ROS) are produced in all biological systems and play an important role in the pathophysiology of CAD.[2] The antioxidant system reacts with ROS and inactivates it. Oxidative stress occurs as a consequence of a general increase in ROS generation or a depression of the antioxidant systems.[12,14] It has been well-established that age is one of the most common CAD risk factors and oxidative stress.[15,16] The outcome of this study complies with well known age factors in CAD and oxidative stress.

Previous studies have reported that atherosclerotic plaques contain large amount of oxysterols compared to normal arteries. The presence of oxysterols in plaques is interpreted as a consequence of LDL oxidation.[17,18] In another study, the activity of the antioxidant superoxide dismutase enzyme in the arterial wall was decreased in advanced atherosclerotic lesions.[19] Another study showed that the glutathione peroxidase and glutathione reductase activities are decreased in human carotid atherosclerotic plaques than normal internal mammary arteries.[20] We have demonstrated that TAC levels in plaques were lower than in plasma. Our data suggests that TAC and TOS levels in atherosclerotic plaques are reduced in patients with advanced atherosclerosis.

A previous study implied that antioxidant levels are reduced in plasma and atherosclerotic plaques in patients with advanced atherosclerosis. The oxidant capacity was not evaluated in this study. In addition, it demonstrated that Vitamin E supplementation in patients with advanced atherosclerosis improved an imbalance between oxidative stress and antioxidant status in plasma, but not in plaques.[21] In our study, we measured both the antioxidant capacity and oxidant status in plasma and in human atherosclerotic plaques. We shows that TAC and TOS levels in plasma were significantly elevated compared to the atherosclerotic plaques.

In conclusion, we demonstrated that the severity of the atherosclerosis is significantly related to plasma antioxidant levels than tissue levels. Facilitation of plasma TAC may represent an important target for the treatment of atherosclerosis disease.

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