DNA damage and plasma total antioxidant capacity in patients with slow coronary artery flow

Koroner yavaş akımli hastalarda DNA hasarı ve total antioksidan kapasite

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Objectives: Although clinical and pathophysiologic features of slow coronary artery flow (SCAF) have been previously described, the underlying pathophysiology has not been fully elucidated. The aim of this study was to investigate the association between DNA damage and SCAF.

Study design: The study was comprised of 23 patients with SCAF and 23 healthy volunteers. DNA damage was assessed by the alkaline comet assay in peripheral lymphocytes and plasma total antioxidant capacity (TAC) was determined by a novel, automated method.

Results: Although DNA damage was higher in the SCAF group than in controls, this did not reach significance (106.6±38.2 AU vs 80.5±51.7 AU; p=0.055). Similarly, TAC levels did not differ significantly between the two groups (1.32±0.32 mmol Trolox equiv./L vs 1.35±0.26 mmol Trolox equiv./L, p=0.667). In the patient group, DNA damage showed a positive correlation with age (r=0.775, p<0.001) and a negative correlation with TAC (r=−0.791, p<0.001) and HDL cholesterol levels (r=−0.456, p=0.029). In multiple linear regression analysis, TAC and age were found to be independent predictors of DNA damage in patients with SCAF.

Conclusion: These findings indicate that SCAF is not associated with increased DNA damage and decreased TAC, suggesting that DNA damage may not be useful in the differential diagnosis of SCAF.

Key words: Antioxidants; coronary circulation; coronary atherosclerosis/blood; DNA damage.

Amaç: Koroner yavaş akımının klinik ve patofizyolojik özellikleri tarif edilmesine karşın, alıta yatan esas mekanizma tam olarak aydınlatılamamıştır. Bu çalışmada koroner yavaş akım ile DNA hasarı arasındaki ilişkini değerlendirildi.

Çalışma planı: Yavaş akımı 23 hasta ve 23 sağlıklı göğüslen çalıﬂma gruplarını oluﬂturdu. DNA hasarı periferik kanda lenfositte alkalen comet yöntemiyle, plazma total antioksidan kapasitesi ise yeni geliştirilen otomatik yöntemle ölçüldü.

Bulgular: Koroner yavaş akımı olgularda DNA hasarı kontrol grubundan daha fazla bulunmasına karşın aradaki fark anlamlı değildi (sirasıyla 106.6±38.2 AU ve 80.5±51.7 AU; p=0.055). İki grup arasında total antioksidan kapasite değerleri açısından da anlamlı farklılık bulunmadı (1.32±0.32 mmol Trolox equiv./L’e karşın 1.35±0.26 mmol Trolox equiv./L, p=0.667). Yavaş akımı olgularda DNA hasarı yaşla pozitif (r=0.775, p<0.001), total antioksidan kapasite (-0.791, p<0.001) ve HDL-kolesterol (-0.456, p=0.029) ile negatif ilişki gösterdi. Çoklu regresyon analizinde, yaş ve total antioksidan kapasitenin yavaş akımı olgularında DNA hasarıyla baﬂımsız ilişkiye olduğu bulundu.

Sonuç: Bulgularımız, koroner yavaş akımı olgularında DNA hasarının artmadığı ve total antioksidan kapasitenin azalmasının göstermektedir. Bu durumda, DNA hasarı koroner yavaş akımın ayrıntıları tanısında yararlı olmayabilir.

Anahtar sözcükler: Antioksidan; koroner dolaﬂım; koroner ateroskleroz/kan; DNA hasarı.
A reduction in velocity in coronary artery contrast filling during selective coronary angiography is known as slow coronary artery flow (SCAF). It was first described as slow coronary flow by Tambe et al.\[1\] in 1972. Although the clinical and pathological features of this disorder have been described, its pathophysiology is poorly understood. Microvascular dysfunction and occlusive disease of small coronary arteries have been suggested as the predominant etiology.\[1-3\] Increased plasma endothelin-1 levels and the presence of endothelial dysfunction were reported in patients with SCAF.\[4\]

It is known that there is a decrease in plasma antioxidant capacity and an increase in lymphocyte DNA damage in patients with coronary artery disease.\[5,6\] Decreased plasma antioxidant capacity alters normal endothelial function, resulting in proinflammatory, prothrombotic, proliferative, and vasoconstrictor effects that contribute to the atherogenic process.\[7\] It is also speculated that reduced antioxidant capacity might play a major role in the initiation of DNA damage.\[8-10\]

Plasma concentrations of antioxidants can be measured separately in the laboratory, but these measurements are time-consuming, labor-intensive and costly. Total antioxidant capacity (TAC) reflects total potential of the antioxidant system.\[11,12\]

It is not known whether there is a relationship between plasma TAC and DNA damage and SCAF. This study was designed to evaluate DNA damage in peripheral blood lymphocytes and plasma TAC in patients with SCAF.

**PATIENTS AND METHODS**

**Study population.** The study consisted of 23 patients with SCAF, who underwent coronary angiography between January 2003 and January 2005 to determine whether or not obstructive coronary artery disease existed. All the patients had typical and atypical symptoms of angina and exhibited electrocardiography changes. The presence of SCAF was defined according to the Thrombolysis in Myocardial Infarction (TIMI) frame count (TFC) method.\[16\] Subjects with a TFC greater than two standard deviations (SD) from the normal range for a particular vessel were accepted as having SCAF. The mean TFC was calculated as the mean of three coronary artery TFCs. The voluntary patients with SCAF did not have coronary artery luminal irregularities. As a control group we studied 23 age- and gender-matched voluntary subjects who had normal coronary arteries without SCAF. Subjects with valvular heart disease, a history of myocardial infarction, cardiomyopathy, hypertension, smoking, diabetes mellitus, or any other systemic disease were excluded from the study. Patients with SCAF did not have coronary artery luminal irregularities. All the patients were in sinus rhythm and they did not receive any antioxidant medications or vitamin treatment. Informed consent was obtained from all the patients and controls prior to the study.

Body mass index was calculated as weight in kilograms divided by the square of the height in meters (kg/m²). Waist circumference was measured by a measuring tape in centimeters at the level of the umbilicus.

Fasting peripheral venous blood samples were collected from the patients and controls and were taken into heparinized tubes. To measure DNA damage, one milliliter of blood was pipetted into another tube immediately after collection. The remaining blood was centrifuged at 3000 rpm for 10 minutes for plasma separation. Plasma samples were stored at -80 °C until analysis for TAC. Plasma triglyceride, total cholesterol, low-density lipoprotein (LDL) cholesterol and high-density lipoprotein (HDL) cholesterol concentrations were measured by an automated chemistry analyzer (Aeroset, Abbott) using commercial kits (Abbott, USA).

**Measurement of total antioxidant capacity.** Serum TAC was determined using a novel automated measurement method developed by Erel.\[7,14,15,17\] In this method, hydroxyl radical, which is the most potent biological radical, is produced, and antioxidative effect of the sample against potent free radical reactions, which is initiated by the produced hydroxyl radical, is measured. The assay has excellent precision values, which are lower than 3%. The results are expressed as mmol Trolox equivalent/L.

**Determination of lymphocyte DNA damage by the alkaline comet assay.** All the samples were processed within 2 hours. Lymphocyte isolation for the comet assay was performed using the Lymphoprep (Axis-Shield Poc, Oslo, Norway). One milliliter of heparinized blood was carefully layered over 1 ml Lymphoprep and centrifuged for 35 min at 500 x g and 25 °C. The interface band containing lymphocytes was washed with phosphate buffered saline (PBS) and then collected by 15-min centrifugation at 400 x g. The resulting pellets were resuspended in PBS to obtain 20,000 cells in 10 µl. Membrane integrity was assessed by means of trypan blue exclusion. The comet assay
was performed as proposed by Singh et al.,\cite{18} with modifications. Each image was classified according to the intensity of fluorescence in the comet tail and was rated from 0 to 4 (from undamaged class 0 to maximally damaged class 4) so that the total score of the slide could be between 0 and 400 arbitrary units (AU).

**Statistical analysis.** The results were expressed as mean ± SD or median (range) values for all continuous variables. Differences between the two groups in baseline continuous clinical variables were compared by Student’s t-test, whereas categoric variables were compared by the chi-square test. Correlations were sought by the Pearson correlation test. A multivariate linear regression analysis was performed to identify discriminate predictive parameters. A \( p \) value of less than 0.05 was considered significant. Data were analyzed using SPSS (ver. 11.0) for Windows.

**RESULTS**

Clinical and laboratory characteristics of the two groups are given in Table 1. There were no differences between the two groups with respect to age, sex, body mass index, waist circumference, systolic and diastolic blood pressures, and serum levels of total cholesterol, HDL and LDL cholesterol. The mean TFC was significantly higher in patients with SCAF than controls (\( p<0.001 \)).

Lymphocyte DNA damage (106.6±38.2 AU vs 80.1±51.7 AU, \( p=0.055 \)) and TAC levels (1.32±0.32 mm Trolox equiv./L vs. 1.35±0.26 mm Trolox equiv./L, \( p=0.667 \)) did not differ significantly between the patient and control groups.

In bivariate analysis, DNA damage showed significant correlations with age, TAC, and HDL cholesterol (Table 2). Multiple linear regression analysis showed that age and TAC were independent predictors of DNA damage (Table 2). No correlations were found between TFC and DNA damage (\( r=0.243, p=0.264 \)) and TAC levels (\( r=0.331, p=0.123 \)).

**DISCUSSION**

The results of this study demonstrated that, compared with controls, the extent of DNA damage and the level of TAC were not statistically different in

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**Table 1. Clinical characteristics of the patients with SCAF and controls**

<table>
<thead>
<tr>
<th></th>
<th>SCAF group (n=23)</th>
<th>Control group (n=23)</th>
<th>( p )</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (year)</td>
<td>58±13</td>
<td>51±13</td>
<td>0.086</td>
</tr>
<tr>
<td>Men (n, %)</td>
<td>15 (65.2)</td>
<td>18 (78.3)</td>
<td>0.257</td>
</tr>
<tr>
<td>Body mass index (kg/m²)</td>
<td>24.4±2.8</td>
<td>24.3±2.9</td>
<td>0.787</td>
</tr>
<tr>
<td>Waist circumference (cm)</td>
<td>91.7±13.5</td>
<td>90.2±9.5</td>
<td>0.653</td>
</tr>
<tr>
<td>Systolic blood pressure (mmHg)</td>
<td>122.2±11.2</td>
<td>121.7±12.8</td>
<td>0.875</td>
</tr>
<tr>
<td>Diastolic blood pressure (mmHg)</td>
<td>78.3±12.0</td>
<td>82.2±10.6</td>
<td>0.249</td>
</tr>
<tr>
<td>Triglyceride (mg/dl)</td>
<td>186±85</td>
<td>160±82</td>
<td>0.299</td>
</tr>
<tr>
<td>Total cholesterol (mg/dl)</td>
<td>198±43</td>
<td>171±49</td>
<td>0.055</td>
</tr>
<tr>
<td>HDL cholesterol (mg/dl)</td>
<td>36±11</td>
<td>38±9</td>
<td>0.299</td>
</tr>
<tr>
<td>LDL cholesterol (mg/dl)</td>
<td>128±19</td>
<td>103±42</td>
<td>0.094</td>
</tr>
<tr>
<td>Mean TIMI frame count (frame)</td>
<td>40.2±2.33</td>
<td>27.4±1.2</td>
<td>&lt;0.001</td>
</tr>
</tbody>
</table>

\( \text{Values are mean ± SD or percent. SCAF: Slow coronary artery flow; TIMI: Thrombolysis in Myocardial Infarction.} \)

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**Table 2. Bivariate and multivariate regression analyses for predicting DNA damage in patients with slow coronary artery flow**

<table>
<thead>
<tr>
<th></th>
<th>Bivariate analysis</th>
<th>Multivariate analysis</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>( r )</td>
<td>( p )</td>
</tr>
<tr>
<td>Age</td>
<td>0.775</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Body mass index</td>
<td>0.029</td>
<td>0.896</td>
</tr>
<tr>
<td>Waist circumference</td>
<td>0.044</td>
<td>0.843</td>
</tr>
<tr>
<td>Systolic blood pressure</td>
<td>0.189</td>
<td>0.388</td>
</tr>
<tr>
<td>Diastolic blood pressure</td>
<td>0.379</td>
<td>0.074</td>
</tr>
<tr>
<td>Total cholesterol</td>
<td>0.013</td>
<td>0.952</td>
</tr>
<tr>
<td>Triglyceride</td>
<td>0.117</td>
<td>0.632</td>
</tr>
<tr>
<td>HDL cholesterol</td>
<td>-0.456</td>
<td>0.029</td>
</tr>
<tr>
<td>LDL cholesterol</td>
<td>0.173</td>
<td>0.596</td>
</tr>
<tr>
<td>Total antioxidant capacity</td>
<td>-0.791</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Mean TIMI frame count</td>
<td>-0.243</td>
<td>0.264</td>
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</table>
patients with SCAF and that the mean TFC was not correlated with DNA damage and TAC.

The exact mechanism of lymphocyte DNA damage is not known. Several studies have demonstrated that ischemia leads to production of oxygen-derived free radicals, activation of the complement system, adherence of neutrophils to the coronary endothelium, lymphocyte-mediated injury to myocardial cells, and production of cytokines and free radicals.

The biological oxidative effects of free radicals on lipids, DNA, and proteins are controlled by a spectrum of exogenous dietary antioxidants and by endogenous antioxidants. Oxidative stress occurs when there is an imbalance between free radical production and antioxidant capacity. This may be due to increased free radical generation and/or loss in normal antioxidant defense. Several studies emphasized the importance of antioxidant status in the clinical activity of coronary disease. Honda et al. reported that reduced activities of antioxidant enzymes were associated with increased levels of oxidative DNA damage.

Balance between prooxidants and antioxidants may vary in various pathological conditions, depending on the extent of oxidative stress induced and the status of the antioxidant defense system. Previous reports showed that risk factors for coronary artery disease, including aging, hypertension, hypercholesterolemia, diabetes, and smoking resulted in elevated oxidative stress. Elevated levels of oxidative DNA damage have been reported in these patients. The results of the present study suggest that, compared with normal subjects, DNA damage and TAC levels do not differ significantly in patients with SCAF. Therefore, DNA damage does not seem to be a useful laboratory parameter for determining SCAF. It was also observed that the extent of lymphocyte DNA damage was associated with age and TAC in this patient group.

REFERENCES