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

Koroner yavaş akımlı 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 arteriosclerosis/blood; DNA damage.

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

Çalışma planı: Yavaş akımlı 23 hasta ve 23 sağlıklı gönüllü çalışma gruplarını oluşturdu. DNA hasarı periferik kanda lenfositte alkalen *comet* yöntemiyle, plazma total antioksidan kapasitesi ise yeni geliştirilen otomatik yöntemle ölçüldü.

Bulgular: Koroner yavaş akımlı olgularda DNA hasarı kontrol grubundan daha fazla bulunmasına karşın aradaki fark anlamlı değildi (sırasıyla 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 akımlı olgularda DNA hasarı yaşla pozitif (r=0.775, p<0.001), total antioksidan kapasite (r=-0.791, p<0.001) ve HDL-kolesterol (r=-0.456, p=0.029) ile negatif ilişki gösterdi. Çoklu regresyon analizinde, yaş ve total antioksidan kapasitenin yavaş akımlı olgularda DNA hasarıyla bağımsız ilişkide olduğu bulundu.

Sonuç: Bulgularımız, koroner yavaş akımlı olgularda DNA hasarının artmadığını ve total antioksidan kapasitenin azalmadığını göstermektedir. Bu durumda, DNA hasarı koroner yavaş akımın ayırıcı tanısında yararlı olmayabilir.

Anahtar sözcükler: Antioksidan; koroner dolaşım; koroner ateroskleroz/kan; DNA hasarı.

Received: May 20, 2005 *Accepted:* December 22, 2005

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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 and increase in lymphocyte DNA damage in patients with coronary artery disease.[5-9] Decreased plasma antioxidant capacity alters normal endothelial function, resulting in proinflammatory, prothrombotic, proliferative, and vasoconstrictor effects that contribute to the atherogenic process.[10] It is also speculated that reduced antioxidant capacity might play a major role in the initiation of DNA damage.[11-13]

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.[14,15]

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 quasi-typical 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/m^2). 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

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

	SCAF group (n=23)	Control group (n=23)	*p*
Age (year)	58±13	51±13	0.086
Men (n, %)	15 (65.2)	18 (78.3)	0.257
Body mass index (kg/m^2)	24.4±2.8	24.3±2.9	0.787
Waist circumference (cm)	91.7±13.5	90.2±9.5	0.653
Systolic blood pressure (mmHg)	122.2±11.2	121.7±12.8	0.875
Diastolic blood pressure (mmHg)	78.3±12.0	82.2±10.6	0.249
Triglyceride (mg/dl)	186±85	160±82	0.299
Total cholesterol (mg/dl)	198±43	171±49	0.055
HDL cholesterol (mg/dl)	36±11	38±9	0.299
LDL cholesterol (mg/dl)	128±19	103±42	0.094
Mean TIMI frame count (frame)	40.2±2.33	27.4±1.2	<0.001

Values are mean ± SD or percent. SCAF: Slow coronary artery flow; TIMI: Thrombolysis in Myocardial Infarction.

was performed as proposed by Singh et al.,[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

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

	Bivariate analysis		Multivariate analysis	
	r	*p*	β	*p*
Age	0.775	<0.001	0.445	0.005
Body mass index	0.029	0.896	–	–
Waist circumference	0.044	0.843	–	–
Systolic blood pressure	0.189	0.388	–	–
Diastolic blood pressure	0.379	0.074	–	–
Total cholesterol	0.013	0.952	–	–
Triglyceride	0.117	0.632	–	–
HDL cholesterol	-0.456	0.029	0.057	0.666
LDL cholesterol	0.173	0.596	–	–
Total antioxidant capacity	-0.791	<0.001	-0.485	0.008
Mean TIMI frame count	-0.243	0.264	–	–

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,[19,20] activation of the complement system,[21] adherence of neutrophils to the coronary endothelium,[22] lymphocyte-mediated injury to myocardial cells, and production of cytokines and free radicals.[23,24]

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.[25] 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.[26,27] Honda et al.[28] 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.[29,30] Previous reports showed that risk factors for coronary artery disease, including aging, hypertension, hypercholesterolemia, diabetes, and smoking resulted in elevated oxidative stress.[29,31] Oxidative stress is the major causative mechanism for DNA damage in coronary artery disease[9,12,32] and increased levels of DNA damage have been reported in these patients.[7,13,32] 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

1. Tambe AA, Demany MA, Zimmerman HA, Mascarenhas E. Angina pectoris and slow flow velocity of dye in coronary arteries-a new angiographic finding. Am Heart J 1972;84:66-71.
2. Kaski JC, Tousoulis D, Galassi AR, McFadden E, Pereira WI, Crea F, et al. Epicardial coronary artery tone and reactivity in patients with normal coronary arteriograms and reduced coronary flow reserve (syndrome X). J Am Coll Cardiol 1991;18:50-4.
3. Mangieri E, Macchiarelli G, Ciavolella M, Barilla F, Avella A, Martinotti A, et al. Slow coronary flow: clinical and histopathological features in patients with otherwise normal epicardial coronary arteries. Cathet Cardiovasc Diagn 1996;37:375-81.
4. Pekdemir H, Polat G, Cin VG, Camsari A, Cicek D, Akkus MN, et al. Elevated plasma endothelin-1 levels in coronary sinus during rapid right atrial pacing in patients with slow coronary flow. Int J Cardiol 2004; 97:35-41.
5. Nojiri S, Daida H, Mokuno H, Iwama Y, Mae K, Ushio F, et al. Association of serum antioxidant capacity with coronary artery disease in middle-aged men. Jpn Heart J 2001;42:677-90.
6. Young IS, Woodside JV. Antioxidants in health and disease. J Clin Pathol 2001;54:176-86.
7. Demirbag R, Yilmaz R, Kocyigit A. Relationship between DNA damage, total antioxidant capacity and coronary artery disease. Mutat Res 2005;570:197-203.
8. Botto N, Rizza A, Colombo MG, Mazzone AM, Manfredi S, Masetti S, et al. Evidence for DNA damage in patients with coronary artery disease. Mutat Res 2001;493:23-30.
9. Botto N, Masetti S, Petrozzi L, Vassalle C, Manfredi S, Biagini A, et al. Elevated levels of oxidative DNA damage in patients with coronary artery disease. Coron Artery Dis 2002;13:269-74.
10. Nedeljkovic ZS, Gokce N, Loscalzo J. Mechanisms of oxidative stress and vascular dysfunction. Postgrad Med J 2003;79:195-9.
11. Holvoet P, Vanhaecke J, Janssens S, Van de Werf F, Collen D. Oxidized LDL and malondialdehyde-modified LDL in patients with acute coronary syndromes and stable coronary artery disease. Circulation 1998; 98:1487-94.
12. Wang G, Mao JM, Wang X, Zhang FC. Effect of homocysteine on plaque formation and oxidative stress in patients with acute coronary syndromes. Chin Med J 2004;117:1650-4.
13. Holvoet P, Perez G, Zhao Z, Brouwers E, Bernar H, Collen D. Malondialdehyde-modified low density lipoproteins in patients with atherosclerotic disease. J Clin Invest 1995;95:2611-9.
14. Erel O. A novel automated method to measure total antioxidant response against potent free radical reactions. Clin Biochem 2004;37:112-9.
15. Demirbag R, Yilmaz R, Erel O, Gultekin U, Asci D, Elbasan Z. The relationship between potency of oxidative stress and severity of dilated cardiomyopathy. Can J Cardiol 2005;21:851-5.
16. Gibson CM, Cannon CP, Daley WL, Dodge JT Jr, Alexander B Jr, Marble SJ, et al. TIMI frame count: a quantitative method of assessing coronary artery flow. Circulation 1996;93:879-88.

17. Yeni E, Gulum M, Selek S, Erel O, Unal D, Verit A, Savas M. Comparison of oxidative/antioxidative status of penile corpus cavernosum blood and peripheral venous blood. Int J Impot Res 2005;17:19-22.
18. Singh NP, McCoy MT, Tice RR, Schneider EL. A simple technique for quantitation of low levels of DNA damage in individual cells. Exp Cell Res 1988;175:184-91.
19. Engler RL. Free radical and granulocyte-mediated injury during myocardial ischemia and reperfusion. Am J Cardiol 1989;63:19E-23E.
20. Andreassi MG, Botto N, Rizza A, Colombo MG, Palmieri C, Berti S, et al. Deoxyribonucleic acid damage in human lymphocytes after percutaneous transluminal coronary angioplasty. J Am Coll Cardiol 2002;40:862-8.
21. Dreyer WJ, Michael LH, Nguyen T, Smith CW, Anderson DC, Entman ML, et al. Kinetics of C5a release in cardiac lymph of dogs experiencing coronary artery ischemia-reperfusion injury. Circ Res 1992;71:1518-24.
22. Kukielka GL, Hawkins HK, Michael L, Manning AM, Youker K, Lane C, et al. Regulation of intercellular adhesion molecule-1 (ICAM-1) in ischemic and reperfused canine myocardium. J Clin Invest 1993;92:1504-16.
23. Kukielka GL, Youker KA, Hawkins HK, Perrard JL, Michael LH, Ballantyne CM, et al. Regulation of ICAM-1 and IL-6 in myocardial ischemia: effect of reperfusion. Ann N Y Acad Sci 1994;723:258-70.
24. Hansen PR. Role of neutrophils in myocardial ischemia and reperfusion. Circulation 1995;91:1872-85.
25. Gutteridge JM. Lipid peroxidation and antioxidants as biomarkers of tissue damage. Clin Chem 1995;41(12 Pt 2):1819-28.
26. Vita JA, Keaney JF Jr, Raby KE, Morrow JD, Freedman JE, Lynch S, et al. Low plasma ascorbic acid independently predicts the presence of an unstable coronary syndrome. J Am Coll Cardiol 1998;31:980-6.
27. Nyyssonen K, Porkkala-Sarataho E, Kaikkonen J, Salonen JT. Ascorbate and urate are the strongest determinants of plasma antioxidative capacity and serum lipid resistance to oxidation in Finnish men. Atherosclerosis 1997;130:223-33.
28. Honda M, Yamada Y, Tomonaga M, Ichinose H, Kamihira S. Correlation of urinary 8-hydroxy-2'-deoxyguanosine (8-OHdG), a biomarker of oxidative DNA damage, and clinical features of hematological disorders: a pilot study. Leuk Res 2000;24:461-8.
29. Maytin M, Leopold J, Loscalzo J. Oxidant stress in the vasculature. Curr Atheroscler Rep 1999;1:156-64.
30. Ji LL. Antioxidants and oxidative stress in exercise. Proc Soc Exp Biol Med 1999;222:283-92.
31. Fenster BE, Tsao PS, Rockson SG. Endothelial dysfunction: clinical strategies for treating oxidant stress. Am Heart J 2003;146:218-26.
32. Ross R. Atherosclerosis-an inflammatory disease. N Engl J Med 1999;340:115-26.