ORIGINAL ARTICLE

Are oxidative stress markers helpful for diagnosing the disease and determining its complexity or extent in patients with stable coronary artery disease?

Oksidatif stres belirteçleri kararlı koroner arter hastalığının tanısında, yaygınlığını ve kompleksliğini değerlendirmede yardımcı mıdır?

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ABSTRACT

Objective: The aim of this study was to investigate the relationship between oxidative/antioxidative stress markers and the diagnosis and complexity of coronary artery disease (CAD) in patients with stable CAD.

Methods: A total of 145 patients were enrolled in the study. Based on coronary angiography results, the patients were categorized into 2 groups: those without CAD (Group 1) and those with CAD (Group 2). The patients in Group 2 were also categorized into low score and moderate/high score groups according to their SYNTAX score. The serum malondialdehyde (MDA) and total antioxidant capacity (TAOC) levels of Group 1 and Group 2 were compared. Finally, MDA and TAOC levels were compared between the moderate/high-risk and low-risk groups formed according to SYNTAX score.

Results: There was a significant difference with respect to both serum TAOC and MDA levels between Group 1 and Group 2 (p=0.036 and p=0.029, respectively). The groups with a SYNTAX score 1-22 and with a SYNTAX score >22 were not significantly different with respect to serum TAOC or MDA level (p=0.582 and p=0.85, respectively).

Conclusion: The serum MDA level was significantly higher and the TAOC level was significantly lower in patients with stable CAD compared to those without; however, these molecule levels failed to predict disease complexity in patients with stable CAD.

ÖZET

Amaç: Bu çalışmanın amacı kararlı koroner arter hastalığı (KAH) olan hastalarda, hastalığın tanısında, kompleksitesi ve yaygınlığının değerlendirilmesinde serum oksidatif ve antioksidatif stress belirteçlerinin yerinin olup olmadığının araştırılmasıdır.

Yöntemler: Çalışmaya toplam 145 hasta dahil edildi. Koroner anjiyografi (KAG) sonuçlarına göre hastalar KAH'ı olmayan (grup 1) ve olan (grup 2) olmak üzere 2 gruba ayrıldı. Grup 2'deki hastalar SYNTAX skorlarına göre düşük skorlu ve orta-yüksek skorlu olmak üzere tekrar 2 gruba ayrıldı. Serum malondialdehit (MDA) ve total antioksidan kapasitesi (TAK) seviyeleri hem grup 1 ve grup 2 arasında hem de düşük skorlu ve orta-yüksek skorlu gruplar arasında karşılaştırıldı.

Bulgular: Grup 1 ile grup 2'nin TAK ve MDA değerleri arasında istatistiksel olarak anlamlı fark saptandı (sırasıyla, p=0.036 ve p=0.029). SYNTAX skoru 1-22 olan grup ile SYNTAX skoru >22 olan grup arasında da TAK ve MDA seviyeleri yönünden istatistiksel olarak anlamlı fark bulunmadı (sırasıyla, p=0.582 ve p=0.85).

Sonuç: Kararlı KAH olan hastalarda, KAH olmayanlara göre serum MDA seviyesi yüksek, TAK seviyesi ise düşüktür, ancak bu parametreler hastalığın kompleksitesi göstermede yeterli değildir.

Atherosclerotic cardiovascular disease (ASCVD) is a major global cause of death. Today, it accounts for approximately 30% of deaths worldwide.[1] Dyslipidemias, diabetes mellitus, hypertension, and smoking are contributors to the occurrence and progression of ASCVD.[2] It is well known that oxidative/antioxidative parameters and inflammation play a critical role in pathophysiology of the atherosclerosis development that leads to ASCVD.[3,4] Furthermore lipid abnormalities play a major role in the development of

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ASCVD. Studies have demonstrated that malondialdehyde (MDA) is an important marker of lipid peroxidation and that progression of atherosclerosis is correlated with oxidative stress and can be followed up using MDA.[5] Total antioxidant capacity (TAOC) is a global indicator of all antioxidants. This is a very useful parameter to show true antioxidant capacity.[6] Although the relationship between oxidative stress and atherosclerosis is well known, the association between oxidative stress and stable coronary artery disease (CAD) complexity has not been clearly determined. Therefore, the aim of this study was to investigate the relationship between oxidative/antioxidative stress markers (MDA, TAOC) and the diagnosis and complexity of CAD in patients with stable CAD using Synergy between Percutaneous Coronary Intervention with TAXUS and Cardiac Surgery (SYNTAX) scores.

Abbreviations:

ASCVD	*Atherosclerotic cardiovascular disease*
CAD	*Coronary artery disease*
ESC	*European Society of Cardiology*
MDA	*Malondialdehyde*
ROS	*Reactive oxygen species*
SYNTAX	*Synergy between Percutaneous Coronary Intervention with TAXUS and Cardiac Surgery*
TAOC	*Total antioxidant capacity*

METHODS

Study population

This was a cross-sectional study. A total of 145 consecutive participants who underwent diagnostic coronary angiography in the Baskent University, Ankara Hospital, Department of Cardiology from January 2010 to July 2011 were evaluated. The indications for performing coronary angiography procedures were based on symptoms, risk factors, and results of appropriate noninvasive tests as per guidelines. The study was conducted in accordance with the guidelines proposed in the Declaration of Helsinki and was approved by the local ethics committee. Each patient provided a signed, informed consent form.

The following patients were excluded from the study: Patients who had cardiovascular disease (ASCVD, peripheral artery disease, cerebrovascular disease), renal disease (creatinine >1.5 mg/dL), patients with known malignancy, liver disease, active infection, allergy to contrast media, patients with unstable angina or who required immediate percutaneous coronary intervention, and hemodynamically unstable patients.

In order to evaluate each patient's serum MDA and TAOC levels, venous blood samples were taken 30 minutes before coronary angiography.

Study design

In all, 145 patients who underwent coronary angiography were enrolled in the study. Based on those results, the patients were categorized into 2 groups: those with no CAD (Group 1) and those with CAD (Group 2). Group 1 was similar to Group 2 in terms of clinical findings, demographics, and anthropometric measurements. SYNTAX scores were calculated for the patients in Group 2. Patients with CAD were further divided into 2 groups according to SYNTAX score (less complex: SYNTAX score 1–22 and moderate/high complexity: SYNTAX score >22) as described in the European Society of Cardiology (ESC) revascularization guideline.[7] First, serum MDA and TAOC levels were compared between Group 1 and Group 2. Then, TAOC and MDA levels were compared between the moderate/high and low-risk groups formed according to SYNTAX score.

Biochemical analysis

Serum TAOC measurement is generally based on the loss of the characteristic color of a stable 2,2'-azinobis (3-ethylbenz-thiazoline-6-sulfonic acid) radical cation through oxidation.[6] Serum MDA levels were determined using the spectrophotometric method at 532 nm after boiling the sample and condensing it with thiobarbituric acid.[8]

Coronary angiography imaging, intensity, and complexity of coronary artery disease

The indications for performing coronary angiography procedures were based on symptoms, risk factors, and the results of appropriate noninvasive tests according to guidelines. A coronary angiographic examination was performed after the administration of local anesthesia, using the modified Seldinger technique through the femoral artery. All coronary arteries were visualized at right and left anterior oblique projections, with caudal and cranial angulations and left lateral projection.

The complexity of CAD was defined according to SYNTAX score.[9] Each coronary lesion with a diameter stenosis of at least 50% and in vessels at least 1.5 mm was scored. The current online calculator (version 2.28; www.syntaxscore.com) was used to calculate the SYNTAX score.[10] An operator who

was blinded to other parameters calculated the scores based on angiographic findings.

Statistical analysis

Continuous variables were tested for normal distribution using the Kolmogorov-Smirnov test. Variables not normally distributed were expressed as medians (interquartile range). Normally distributed continuous variables were expressed as mean±SD. Categorical variables were summarized as frequency percentages and absolute numbers. The means for normally distributed continuous variables were compared with the independent samples t-test. Continuous variables with non-normal distribution were analyzed using the Mann-Whitney U test. Chi-square test or Fisher's exact test was used to compare categorical variables as appropriate. The degree of association between continuous variables was evaluated using the Pearson or Spearman correlation test, as appropriate. Independent determinants of variables were ascertained using standard multivariate logistic regression analysis. IBM SPSS Statistics for Windows, Version 21.0 (IBM Corp., Armonk, NY, USA) was used for all statistical calculations. P value <0.05 was considered statistically significant.

RESULTS

A total of 145 patients were included in the study. The baseline characteristics of the study population are shown in Table 1. According to the coronary angiography results, CAD was absent in 43 (Group 1) of 145 patients and present in 102 patients (Group 2). A comparison of the serum TAOC and MDA levels in Group 1 and Group 2 is illustrated in Figure 1. The serum TAOC level was 8.61±2.42 mM in Group 1 and 7.47±3.16 mM in Group 2. There was a statistically significant difference between Group 1 and Group 2 in terms of TAOC (p=0.036). Similarly, the serum MDA level was determined to be 6.65±1.30 nmol/L in Group 1 and 7.13±1.15 nmol/L in Group 2. There was

Table 1. Baseline characteristics of the study population

	Non CAD group (n=43)	CAD group (n=102)	*p*
Age, years, Mean±SD	61.49±10.66	64.35±9.86	0.121
Male gender, n (%)	24 (55.8)	67 (65.7)	0.261
Body mass index (kg/m^2), Mean±SD	29.21±4.41	28.73±4.12	0.143
Ejection fraction (%), Mean±SD	56.6±9.55	55.43±8.63	0.47
Hypertension, n (%)	42 (97.67)	100 (98.03)	1
Diabetes mellitus, n (%)	13 (30.23)	41 (40.19)	0.257
Hyperlipidemia, n (%)	19 (44.18)	55 (53.92)	0.284
Smokers, n (%)	11 (25.58)	38 (37.25)	0.175
Beta-blocker use, n (%)	43 (100)	102 (100)	–
ACEI/ARB use, n (%)	22 (51.16)	49 (48.03)	0.731
Statin use, n (%)	18 (41.86)	56 (54.9)	0.151
Oral anti diabetic, n (%)	12 (27.96)	39 (38.23)	0.159
Insulin, n (%)	6 (13.95)	29 (28.43)	0.17
Creatinine (mg/dL), Mean±SD	0.79±0.23	0.86±0.21	0.131
Total cholesterol (mg/dL), Mean±SD	212.53±36.13	213.49±33.26	0.877
Low-density lipoprotein (mg/dL), Mean±SD	138.23±30.55	139.15±26.75	0.857
High-density lipoprotein (mg/dL), Mean±SD	47.16±12.58	47.19±11.08	0.991
Triglyceride, (mg/dL)	105 (70–140)	116 (87–146)	0.881
Hemoglobin (gr/dL), Mean±SD	14.09±1.4	13.72±1.42	0.155
White blood cell (/mm^3), Mean±SD	8143±1369	8114±1470	0.913
Platelets (100/m^3)	280 (230–340)	278 (256–296)	0.650

CAD: Coronary artery disease; ACEI: Angiotensin-converting enzyme inhibitor; ARB: Angiotensin receptor blocker; SD: Standard deviation.

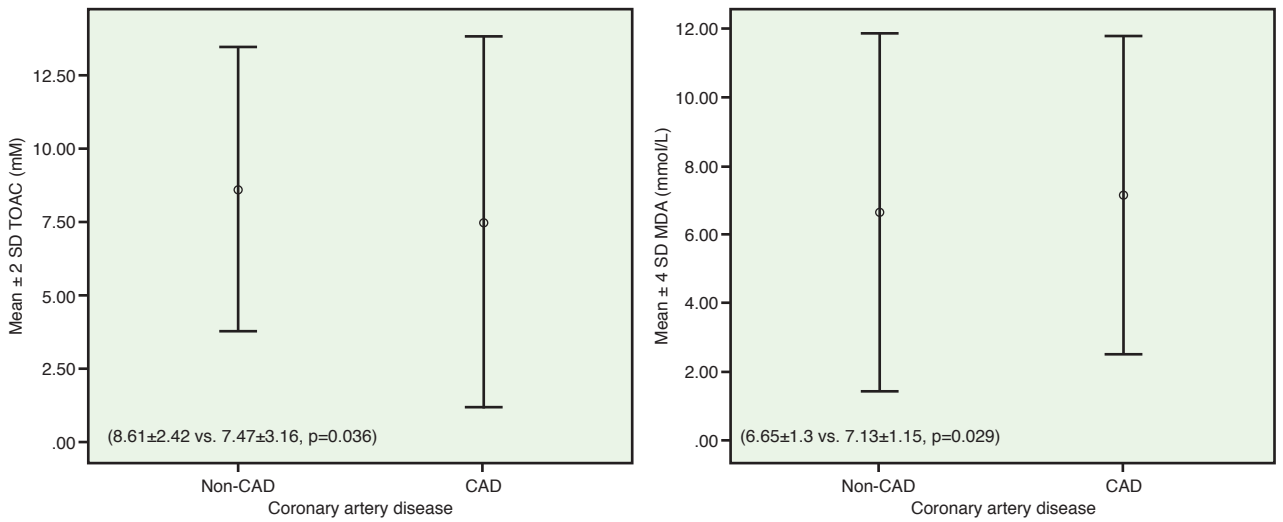

Figure 1. Comparison of serum TAOC and MDA levels between with and without CAD groups.
CAD: Coronary artery disease; MDA: Malondialdehyde; TAOC: Total antioxidant capacity.

a statistically significant difference between Group 1 and Group 2 in terms of MDA (p=0.029).

A SYNTAX score was calculated for patients with CAD to determine CAD complexity. Patients with CAD were divided into less complex and moderate-high complexity groups based on the SYNTAX score, according to the ESC revascularization guideline. Of 102 patients with CAD, 80 (80.3%) were assessed to be in the less complex group (SYNTAX score 1–22) and 20 (19.6%) in the moderate/high group (SYNTAX score >22). Eighteen patients had a moderate SYNTAX score of 23-32 and 2 had high complexity SYNTAX score >32. There was no significant difference between the less complex and moderate/high groups in terms of serum TAOC or MDA levels. The less complex group had TAOC level of 7.55±3.11 mM and the moderate/high score group had a TAOC level of 7.12±3.4 mM (p=0.582). Similarly, the serum MDA level was found to be 7.12±1.14 nmol/L in the less complex group and 7.17±1.24 nmol/L in the moderate/high SYNTAX score group (p=0.85).

Correlation analyses of clinical parameters (age, body mass index, creatinine, light-density lipoprotein, high-density lipoprotein, systolic blood pressure, fast-

Table 2. Correlation analyses between some clinical parameters and serum total antioxidant capacity and malondialdehyde levels

	Total antioxidant capacity		Malondialdehyde	
Clinical variables	r	*p*	r	*p*
Age (years)	-0.031	0.712	0.098	0.24
Body mass index (kg/m^2)	0.022	0.792	0.1	0.232
Creatinine (mg/dL)	0.04	0.631	0.095	0.255
Low-density lipoprotein (mg/dL)	0.048	0.569	-0.086	0.306
High-density lipoprotein (mg/dL)	0.079	0.347	-0.027	0.745
Systolic blood pressure (mmHg)	0.126	0.131	0.053	0.528
Fasting plasma glucose (mg/dL)	0.12	0.151	-0.102	0.222
SYNTAX score	-0.117	0.241	-0.03	0.765

SYNTAX: Synergy between Percutaneous Coronary Intervention with TAXUS and Cardiac Surgery trial.

Table 3. Independent predictors of SYNTAX score in multivariate logistic regression analysis

	β	*p*
Total antioxidant capacity	-0.168	0.033
Malondialdehyde	0.113	0.153
Age	0.041	0.608
Sex	0.155	0.047
Creatinine	0.139	0.079
Hypertension	0.078	0.326
Diabetes mellitus	0.004	0.963
Hyperlipidemia	0.337	<0.001

SYNTAX: Synergy between Percutaneous Coronary. Intervention with TAXUS and Cardiac Surgery trial.

ing plasma glucose, SYNTAX score) and TAOC, and MDA level revealed no statistical correlation between the parameters ($p>0.05$), as shown in Table 2.

Multivariate logistic regression analyses indicated that TAOC, male sex gender and hyperlipidemia were independent predictors of high SYNTAX score, as can be seen in Table 3.

DISCUSSION

In the present study, we explored whether serum oxidative stress and antioxidative stress markers (MDA and TAOC) were helpful in making a diagnosis of CAD and whether they were predictors of the extent and complexity of CAD. The results revealed that although serum MDA and TAOC levels were helpful in making a diagnosis of stable CAD, they were not beneficial in assessing the extent and complexity of CAD. The low overall SYNTAX score of the study participants may have affected the results.

Many studies to date have examined the role of the serum level of oxidant and antioxidant molecules in making the diagnosis of unstable CAD and assessing its extent and complexity; however, as discussed below, their results varied greatly. In contrast to unstable CAD, no study yet has examined the level of these molecules in stable CAD. Therefore, our study is the first to contribute to the existing literature in this area.

In biological organisms, some free oxygen radicals are derived from reactive oxygen species (ROS). In normal tissue, ROS are produced to a certain limit, and there is a delicate balance between oxidant and antioxidant molecules. When free radicals are produced in excessive amounts or the production of antioxidant molecules is inadequate, this balance is disrupted. This imbalance is called oxidative stress and plays an important role in the pathophysiology of atherosclerosis.[11] Atherosclerosis is a complex process of multifactorial origin. In addition to classical risk factors, oxidized LDL has an important role in the development and progression of atherosclerosis.[12] ROS can stimulate LDL oxidation. Oxidized LDL particles are modified lipoproteins internalized by macrophages. These cells are converted into fatty foam cells, the precursors of atherosclerosis, and the atherosclerotic process is begun.[13] Sigala et al. found a significantly higher tissue MDA level in atherosclerotic carotid lesions compared with normal tissue, but could not relate this elevation to clinical presentation. They concluded that MDA is probably an important parameter in atherosclerosis development although it has no role in advanced stages of atherosclerosis and plaque rupture. In addition, the study found a significantly higher oxidized LDL level in unstable atherosclerotic carotid plaques compared with normal tissue.[14] Uno et al., in another study, found higher serum and tissue oxidized LDL levels in unstable carotid plaques and showed that this elevation was associated with clinical findings.[15] MDA is an important enzyme for LDL oxidation. In another study by Turan et al., serum oxidative stress marker levels were correlated to Gensini score, but not to SYNTAX score in unstable CAD. The authors concluded that serum oxidative stress marker levels were predictive of disease extent, but not complexity, in patients with acute coronary syndrome.[16] Segev et al. found no correlation between serum oxidized LDL level and angiographic complexity in stable CAD. In addition, they concluded that the serum oxidized LDL level was not predictive of stent restenosis in patients with stable CAD.[17] Meuwissen et al. found levels of inflammatory markers and amounts of oxidative substances to be significantly higher in atherectomy samples of patients with unstable CAD compared with those of stable CAD patients.[18] They concluded that inflammation and oxidation may be responsible for plaque rupture and thrombosis in atherosclerosis.[18] In another study, Sotoudeh et al. demonstrated that the level of TAOC was not found to be an independent predictor for the presence of CAD.[19] In addition to these studies, others have investigated the relationship between

oxidative stress and CAD diagnosed by coronary computed tomography. Cho et al. reported that low total bilirubin and high gamma-glutamyltransferase levels were concomitantly associated with coronary atherosclerosis assessed by multidetector computed tomography.[20] In another study, it was shown that increased phagocytic nicotinamide adenine dinucleotide oxidase activity was associated with coronary artery calcification in asymptomatic men.[21] Their results were similar to and support our findings. According to the literature data, serum oxidative and antioxidative substance markers may be responsible for instability and rupture of atherosclerotic plaques; they were, however, not helpful in determining the extent and complexity of stable atherosclerotic plaques.

Study limitations

Our study involved only patients with stable CAD, excluding patients with unstable CAD. Patients were using statins, which are known to exert antioxidant properties; thus, it is unknown to what degree statin use affected study results. Another limitation of the study is that the patients included had relatively low SYNTAX scores.

Conclusion

Serum MDA levels were significantly higher and TAOC levels were significantly lower in patients with stable CAD compared to those without; however, the level of these molecules failed to predict disease extent and complexity in patients with stable CAD.

Conflict-of-interest: The authors have indicated they have no financial relationships to disclose that are relevant to this article.

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Keywords: Coronary artery disease; malondialdehyde; oxidative stress; total antioxidant capacity.

Anahtar sözcükler: Koroner arter hastalığı; malondialdehit; oksidatif stres; total antioksidan kapasite.