The usability of MCP-1, fetuin-A, TAS, and TOS levels in the diagnosis of acute myocardial infarction

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Abstract. – OBJECTIVE: Our aims were to determine whether the levels of plasma monocyte chemotactic protein-1 (MCP-1), fetuin-A, serum total antioxidant status (TAS), and serum total oxidant status (TOS) are cardiac biomarkers and to clarify their relationship with each other in acute myocardial infarction (AMI).

PATIENTS AND METHODS: The study included 90 participants: 60 patients with AMI [30 with and 30 without ST-segment elevation myocardial infarction (STEMI)] and 30 cardiac patients without AMI. The diagnostic values of serum Hs-cTnT, MCP-1, fetuin-A, TAS, and TOS levels in predicting AMI were evaluated statistically.

RESULTS: Median levels of MCP-1 [120.10 ng/L (interquartile range: 76.94-230.54 ng/L)] and TOS [2.89 U/MI (IQR: 2.31-3.94 U/MI)] were statistically higher, and median levels of fetuin-A [433.52 mg/L (IQR: 387.89-584.49 mg/L)] and TAS (3.10 ± 0.86 U/mL) were lower in patients with AMI than in controls. The parameter with the area under the curve (0.815), sensitivity (73.3%), and specificity (66.7%) closest to those of Hs-cTnT was fetuin-A, followed by MCP-1, TOS, and TAS, respectively. A one-unit increase in MCP-1 levels increased the probability of AMI by 1.023 times ($p = 0.002$). A one-unit increase in fetuin-A levels decreased the probability of AMI by 0.995 times ($p = 0.003$). A one-unit increase in serum TOS levels was 1.29 times more characteristic of STEMI than of NSTEMI ($p = 0.044$).

CONCLUSIONS: MCP-1, oxidative stress parameters, and fetuin-A might support Hs-cTnT levels in the early diagnosis of AMI. Fetuin-A and MCP-1 levels may be independent risk factors for AMI, whereas TOS could be used to distinguish STEMI from NSTEMI.

Introduction

Cardiovascular diseases are the leading causes of death worldwide[1]. The World Health Organization reported[2] that approximately 17.9 million people died of cardiovascular diseases in 2019, which represented 32% of all global deaths, and that 85% of these cardiovascular disease-related deaths were caused by acute myocardial infarction (AMI) and stroke. Established risk factors in the development of cardiovascular diseases include tobacco use, unhealthy nutrition, obesity, physical inactivity, alcohol consumption, hypertension, diabetes, and hyperlipidemia[3].

Acute coronary syndrome (ACS) is the presence of conditions compatible with acute myocardial ischemia that are caused by a sudden decrease in coronary blood flow. In the diagnosis of ACS, of importance is the appearance of ST-segment elevation or a new left bundle branch block on an electrocardiogram (ECG). Such conditions indicate the occurrence of myocardial infarction with ST-elevation (STEMI), and reperfusion therapy is necessary to open completely occluded coronary arteries. Noncontinuous ST-elevation suggests non-ST-elevation myocardial infarction (NSTEMI) or unstable angina pectoris. If levels

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of cardiac biomarkers are elevated and clinical manifestations are present, NSTEMI is diagnosed; otherwise, unstable angina pectoris is diagnosed[4]. Biochemical parameters should also be evaluated to determine the subtype of ACS (STEMI or NSTEMI)[5].

Cardiac troponins T and I are the most sensitive and specific markers of myocardial necrosis[6]. However, the levels of troponins T and I may also be the indicators of myocardial damage arising from conditions other than ACS, such as chronic renal failure, acute pulmonary embolism, or heart failure. High-sensitivity cardiac troponins T (Hs-cTnT) and I are more sensitive for detecting ischemia, but a worrisome number of results are falsely positive. Therefore, troponin values cannot be used alone in the diagnosis of AMI[5]. Nevertheless, serial troponin tests are still important in the diagnosis of myocardial infarction[7].

In the diagnosis of ACS, inflammation caused by atherosclerosis and many chemokines involved in these pathways have long been known to be associated with vascular diseases. Monocyte chemotactic protein-1 (MCP-1), one of these chemokines, is released by various cells, such as smooth muscle cells, monocytes, and endothelial cells[8]. MCP-1 mediates the collection of mononuclear cells, modulates monocyte and lymphocyte phenotypes, and regulates fibrous tissue accumulation and angiogenesis. Its expression increases after proinflammatory stimulation and tissue damage[9]. Hence, continuous overexpression of MCP-1 contributes to AMI caused by atherosclerosis[10].

Another protein associated with inflammation caused by atherosclerosis is fetuin-A (a_2-Heremans-Schmid glycoprotein). Fetuin-A is a glycoprotein released from hepatocytes into the circulation as an acute-phase protein and has anti-inflammatory action that inhibits the production of proinflammatory cytokines such as tumor necrosis factors. Fetuin-A can increase the phagocytosis of apoptotic cells in macrophages and prevent the accumulation of calcium in the vascular wall by combining with circulating calcium. Although a high level of serum fetuin-A helps prevent inflammation in the vascular wall and inhibits the development of coronary artery disease, the relationship between serum fetuin-A level and AMI remains uncertain[11].

In recent studies[12], investigators predicted that oxidative stress in cells that results from ischemia caused by atherosclerosis in the vascular wall may be useful in the diagnosis of AMI. Total oxidant status (TOS) and total antioxidant status (TAS) are among the oxidative stress parameters. Elevated levels of oxidant parameters in atherosclerosis and resulting AMI pathogenesis may indicate vascular damage[13]. Deteriorations in all these oxidant/antioxidant balances suggest that their measurements may serve as an alternative to long-term troponin follow-up in the diagnosis of ACS (especially NSTEMI)[12].

This aim of this study was to determine whether the levels of plasma MCP-1, plasma fetuin-A, serum TAS, and serum TOS are cardiac biomarkers and to clarify their relationship with each other in AMI. Accordingly, we investigated the usefulness of these parameters in the early diagnosis of STEMI *vs*. NSTEMI.

Patients and Methods

Our study was conducted in a prospective cross-sectional manner with the approval of the local Ethics Committee of Kafkas University Faculty of Medicine Deanship, Kars, Turkey (number 80576354-050-99/130; May 29, 2019).

Patients

Patients who presented to the Emergency Department of Kafkas University Health Research and Application Center Hospital between June 2019 and June 2020 within 6 hours of the onset of cardiac symptoms were included in the study. The American Heart Association's case definitions were used in cardiac symptoms (acute chest, epigastric, neck, jaw, or arm pain, discomfort or pressure sensation without a visually noncardiac source)[14]. Our study population included patients in whom AMI with cardiac symptoms was diagnosed (STEMI and NSTEMI, Thrombolysis in Myocardial Infarction score of ≥ 3) and who were referred to the coronary intensive care unit. These patients were divided into two groups: (1) 30 who had at least a 1-mm ST-segment elevation in at least two adjacent ECG derivations, regardless of whether levels of serum creatine kinase and cardiac Hs-cTnT were elevated, and were considered to have STEMI (the STEMI subgroup), and (2) 30 who had elevated levels of serum creatine kinase and cardiac Hs-cTnT but no ST-segment elevation evident on ECGs and who were considered to have NSTEMI (the NSTEMI subgroup). The control group comprised 30 patients who presented to the Emergency Department within 6 hours of the onset of cardiac symptoms, did not have any chronic diseases, and were discharged from the

Emergency Department without a diagnosis of AMI according to the examination and test results. Written informed consent to participate in this study was obtained from the patients before the study began. Individuals with any infection, malignancy, autoimmune disease, or hyperthyroidism and those undergoing treatment with anticoagulants or immunosuppressive agents were excluded from the study. In the data collection form for each participant, we recorded age, gender, arterial systolic blood pressure, arterial diastolic blood pressure, and pulse (beat/min).

Collection of Blood Samples

For biochemical examinations, 5 mL of whole blood and 5 mL of blood collected into biochemistry tubes with gel were obtained from individuals with brachial vascular access who signed the informed consent form. VACUETTE® blood collection tubes (Greiner Bio-One GmbH, Kremsmünster, Austria) were used for serum analysis, and K2EDTA tubes (BD, Wokingham, UK) were used for plasma analysis. Serum and plasma samples were collected before angiography and heparin administration. Blood samples were immediately centrifuged at +4°C for 15 minutes at 1,600 g in the Biochemistry Laboratory of Kafkas University Health Research and Application Center Hospital. This process was continued until the plasma and serum samples became clear. Routine biochemical and cardiac markers in the serum samples were then studied (detailed in the following section). After the routine parameters were analyzed, the remaining plasma and serum samples were divided into portions in Eppendorf tubes and stored at -80°C until enzyme-linked immunosorbent assay (ELISA) analyses.

Analyses of Laboratory Parameters
Routine analyses

To analyze levels of serum glucose, lactate dehydrogenase (LDH), triglycerides, total cholesterol, low-density lipoprotein (LDL), high-density lipoprotein (HDL), creatine kinase, and creatine kinase-MB (CK-MB), we used photometric methods with the cobas c 501 system (Roche Diagnostics, Mannheim, Germany). Serum Hs-cTnT was assessed with the electrochemiluminescence method in a hormone analyzer (cobas c 601, Roche Diagnostic, Mannheim, Germany). The analytical methods in the study were performed according to the procedures specified in the commercial kits suitable for each device. The reference test ranges of the analytical methods were 70-100 mg/dL for glucose, 135-225 U/L for LDH, 0-150 mg/dL for triglycerides, 0-200 mg/dL for total cholesterol, 0-130 mg/dL for LDL, 35-55 mg/dL for HDL, 0-190 IU/L for creatine kinase, 0-25 U/L for CK-MB, and 0-14 pg/mL for Hs-cTnT.

ELISA Method

We used human ELISA kits (BT Lab/Bioassay Technology Laboratory, Zhejiang, China) to measure TOS and TAS levels in the serum samples (catalog No. E1599Hu and E4350Hu, respectively) and MCP-1 and fetuin-A levels in the plasma samples (catalog No. E0124Hu and E1386Hu, respectively; lot No. 202011017 for each), as specified in the kit procedures. The absorbances were spectrophotometrically read at 450 nm with the BioTek ELx800 ELISA reader (BioTek Instruments, Winooski, VT, USA). In plate washing, BioTek ELx50 (BioTek Instruments, Winooski, VT, USA) was used as an automatic washer. The measurement ranges of the kits were 0.05-30 U/mL for serum TOS level, 0.05-30 U/mL for serum TAS level, 5-1,500 ng/L for plasma MCP-1 level, and 20-4,000 mg/L for fetuin-A levels. The minimum measurable levels of the kits were 0.023 U/mL for both serum TOS and serum TAS, 2.43 ng/L for plasma MCP-1, and 9.52 mg/L for plasma fetuin-A. The intra-assay and interassay coefficients of variation for all kits were < 8% and < 10%, respectively. All ELISA analyses in this study were performed in the Medical Biochemistry R&D Laboratory of Kafkas University Faculty of Medicine.

Statistical Analysis

The data obtained from the research were analyzed with SPSS 24.0 packaged software (IBM Corp., Armonk, NY, USA). In descriptive analyses, frequency data were calculated as numbers and percentages, and numerical data were calculated as means ± standard deviations or as medians [with interquartile ranges (IQRs)]. The Chi-square test and Fisher's exact test were used to compare categorical data. To examine the conformity of numerical data to a normal distribution, we used the Kolmogorov-Smirnov and Shapiro-Wilk tests. The numerical data distributed normally in two independent groups were assessed with the independent-samples *t*-test, and those distributed normally in more than two independent groups were assessed with one-way analysis of variance. Tukey's test was used for the post hoc analysis of the data whose results in the analysis of variance

were significant. For numerical variables without a normal distribution, the Mann-Whitney U test was used to compare two groups, and the Kruskal-Wallis test was used to compare more than two groups. In the groups with significant differences according to the Kruskal-Wallis test, the group or groups that differed were identified in a post hoc test, and the Dunn-Bonferroni correction was applied. To examine the correlation between two non-normally distributed numerical variables, we used Spearman's correlation analysis. We analyzed the receiver operating characteristic (ROC) curves to discern the levels of Hs-cTnT and cardiac markers in the blood, measured with the ELISA, that were involved in diagnostic decision making and to predict the presence of disease; binary logistic regression analysis was conducted to predict whether they were independent risk factors in the diagnosis of AMI; and multinomial logistic regression analysis was performed to distinguish patients by AMI type and the control group. A p level of < 0.05 denoted statistical significance in all tests.

Results

Data from 60 patients with AMI (30 with STEMI and 30 with NSTEMI) and 30 controls were evaluated in this study. Of the 60 patients with AMI, 33 (55%) had a comorbid disease, 19 (31.7%) had coronary artery disease, 9 (15%) had diabetes mellitus, and 6 (10%) had chronic obstructive pulmonary disease.

The demographic characteristics and laboratory values of the patient and control groups are listed in Table I. According to gender, the female and male patients were significantly older on average than the female and male controls ($p < 0.001$, and $p = 0.002$, respectively). Male patients outnumbered female patients ($p = 0.001$). The mean or median serum levels of Hs-cTnT, creatine kinase, CK-MB, triglycerides, glucose, and LDH, which are among the routine blood parameters measured in patients, were significantly higher in the patients than in the control group ($p < 0.05$).

In this study, the cardiac markers measured by ELISA in the patient and control groups are listed in Table II. In comparison with the control group, the patients had significantly higher median serum TOS and median plasma MCP-1 levels and significantly lower mean serum TAS and median plasma fetuin-A levels ($p < 0.05$).

The values of routine blood parameters for the NSTEMI, STEMI, and control groups are listed in Table III. The median values of serum Hs-cTnT, creatine kinase, CK-MB, triglycerides,

Table I. Demographic characteristics and laboratory values of the patients and controls.

Variable	Patients (n = 60)	Controls (n = 30)	*p*-value
Age (years)[†]	67.03 ± 12.81	52.73 ± 13.92	< 0.001***
Age, women (years)[†]	74.21 ± 17.15	55.56 ± 15.43	< 0.001***
Age, men (years)[†]	64.85 ± 13.39	49.80 ± 11.02	0.002**
Sex (n)[‡]			0.001***
Female	14 (23.30%)	18 (60.00%)	
Male	46 (76.70%)	12 (40.00%)	
SBP (mm Hg)[§]	140.00 (120.00-160.00)	130.00 (120.00-140.00)	0.078
DBP (mm Hg)[§]	80.00 (70.00-93.75)	80.00 (70.00-80.00)	0.098
Heart rate (beat/min)[§]	84.00 (76.00-97.50)	88.00 (78.50-89.50)	0.681
Hs-cTnT (pg/mL)[§]	481.05 (129.92-1,430.70)	7.00 (4.75-13.50)	< 0.001***
Creatine kinase (IU/L)[§]	205.00 (133.50-374.00)	72.00 (48.75-110.75)	< 0.001***
CK-MB (U/L)[§]	36.00 (30.00-72.00)	23.50 (19.75-32.00)	< 0.001***
Triglycerides (mg/dL)[§]	99.00 (65.75-190.50)	57.50 (54.00-95.50)	0.029*
Total cholesterol (mg/dL)[†]	179.60 ± 58.42	160.33 ± 17.00	0.074
HDL (mg/dL)[§]	41.00 (32.00-44.00)	51.00 (37.75-52.25)	0.065
LDL (mg/dL)[§]	112.60 (77.10-137.00)	104.00 (95.55-104.25)	0.277
Glucose (mg/dL)[§]	137.50 (117.00-181.00)	110.00 (98.25-113.50)	< 0.001***
LDH (U/L)[§]	244.00 (214.00-281.00)	190.50 (175.00-213.00)	< 0.001***

[†]Independent-samples *t*-test; values are expressed as means ± standard deviations. [‡]Pearson's Chi-square test. [§]Mann-Whitney U test; values are expressed as medians and interquartile intervals. *$p \leq 0.05$, **$p \leq 0.01$, ***$p \leq 0.001$; values are expressed as statistically significant for each test. SBP: arterial systolic blood pressure; DPB: arterial diastolic blood pressure; Hs-cTnT, high-sensitivity cardiac troponin T; CK-MB: creatine kinase-MB; HDL: high-density lipoprotein; LDL: low-density lipoprotein; LDH: lactate dehydrogenase.

Table II. ELISA parameters in the patient and control groups.

Variable	Patients (n = 60)	Controls (n = 30)	*p*-value
TAS (U/mL)†	3.10 ± 0.86	3.55 ± 0.68	0.014**
TOS (U/mL)‡	2.89 (2.31-3.94)	2.62 (1.93-3.00)	0.015*
MCP-1 (ng/L)‡	120.10 (76.94-230.54)	79.61 (45.02-104.95)	< 0.001***
Fetuin-A (mg/L)‡	433.52 (387.89-584.49)	565.71 (472.81-1148.25)	< 0.001***

†Independent-samples t-test; values are expressed as means ± standard deviations. ‡Mann-Whitney U test; values are expressed as medians and interquartile ranges. *$p \leq 0.05$, **$p \leq 0.01$, ***$p \leq 0.001$; values are expressed as statistically significant for each test. ELISA: enzyme-linked immunosorbent assay; TAS: total antioxidant status; TOS: total oxidant status; MCP-1: monocyte chemotactic protein-1.

glucose, and LDH of the control group were significantly lower than those of the NSTEMI and STEMI subgroups ($p < 0.05$). The mean serum total cholesterol level of the NSTEMI subgroup was significantly higher than that of the STEMI subgroup ($p < 0.05$).

The values of cardiac parameters measured with ELISA in the NSTEMI, STEMI, and control groups are listed in Table IV. The median levels of plasma MCP-1 and fetuin-A of the three groups differed significantly ($p < 0.05$): the median MCP-1 level in the plasma was lower and the median fetuin-A level was higher in the control group than in both the NSTEMI and STEMI subgroups ($p < 0.05$). The median serum TAS level of the control group was significantly higher than that of the STEMI subgroup ($p < 0.05$). Moreover, the median serum TOS level was significantly lower in the control group than in the NSTEMI subgroup ($p < 0.05$).

The correlations of Hs-cTnT levels with those of other blood parameters measured with ELISA are listed in Table V. The correlation between Hs-cTnT and TOS levels was low, positive, and significant ($r = 0.213$, $p = 0.044$); that between Hs-cTnT and MCP-1 levels was positive and significant ($r = 0.365$, $p < 0.00$); and that between Hs-cTnT and fetuin-A levels was low to moderate, negative, and significant ($r = -0.348$, $p = 0.001$).

We performed ROC analysis to discern the characteristics of Hs-cTnT, TOS, MCP-1, TAS, and fetuin-A levels that influenced diagnostic decision making and signified the presence of AMI. For predicting the diagnosis of ACS, the optimum cutoff value of serum Hs-cTnT was 19.5 pg/mL (93.3% sensitivity, 93.3% specificity, and AUC of 0.970; $p < 0.001$; 95% CI: 0.935-1.000); the optimum cutoff value of plasma fetuin-A was 498.71 mg/L (73.3% sensitivity, 66.7% specificity, and AUC of 0.815; $p < 0.001$; 95% CI:

0.727-0.903); the optimum cutoff value of plasma MCP-1 was 93.62 ng/L (65.0% sensitivity, 70.0% specificity, and AUC of 0.736; $p < 0.001$; 95% CI: 0.635-0.838); the optimum cutoff value of serum TAS was 3.21 U/mL (60.0% sensitivity, 60.0% specificity, and AUC of 0.667; $p = 0.010$; 95% CI: 0.557-0.778); and the optimum cutoff value of serum TOS was 2.69 U/mL (56.7% sensitivity, 56.7% specificity, and AUC of 0.658; $p = 0.015$; 95% CI: 0.540-0.777; Figure 1).

A multinomial logistic regression model was established to determine whether serum Hs-cTnT, TAS, TOS, plasma MCP-1, and fetuin-A levels could signify STEMI or NSTEMI. The model fit was good (Nagelkerke $R^2 = 0.833$). According to this analysis, only a one-unit increase in serum TOS levels was 1.29 times more characteristic of STEMI than of NSTEMI, and this increase was statistically significant ($p = 0.044$; 95% CI: 1.007-1.676; Table VI).

Moreover, a binary logistic regression model was established to discern whether TAS, TOS, MCP-1, and fetuin-A levels could signify AMI. The model fit was good (Nagelkerke $R^2 = 0.595$). According to this analysis, a one-unit increase in MCP-1 levels increased the probability of AMI by 1.023 times ($p = 0.002$; 95% CI: 1.008-1.037), and a one-unit increase in fetuin-A levels reduced the probability of AMI by 0.995 times ($p = 0.002$; 95% CI: 1.008-1.037; Table VII).

Discussion

Coronary artery disease and ACS in particular are major causes of mortality and morbidity in women and men in the western hemisphere. Therefore, it is aimed to reduce the risk of acute event and death due to ACS and AMI as much as possible[15,16]. In investigating gender differences in ACS, Radovanovic et al[15] found that 28% of

Table III. Laboratory measurements according to the acute coronary syndrome type and control group.

Variable	NSTEMI (n = 30)	STEMI (n = 30)	Control (n = 30)	p-value	Post-hoc test: group differences
Hs-cTnT (pg/mL)[†]	374.00 (111.50-1,218.45)	665.00 (232.07-1,605.50)	7.00 (4.75-13.50)	< 0.001***	NSTEMI *vs.* control STEMI *vs.* control
Creatine kinase (IU/L)[†]	205.00 (108.50-410.00)	204.50 (172.00-326.75)	72.00 (48.75-110.75)	< 0.001***	NSTEMI *vs.* control STEMI *vs.* control
CK-MB (U/L)[†]	37.00 (30.00-77.00)	36.00 (29.75-52.00)	23.50 (19.75-32.00)	< 0.001***	NSTEMI *vs.* control STEMI *vs.* control
Triglycerides (mg/dL)[†]	163.00 (99.50-222.00)	77.00 (57.25-98.50)	57.50 (54.00-95.50)	< 0.001***	NSTEMI *vs.* STEMI NSTEMI *vs.* control
Total cholesterol (mg/dL)[‡]	197.33 ± 68.89	161.86 ± 39.34	160.33 ± 17.00	0.034*	NSTEMI *vs.* STEMI
HDL (mg/dL)[†]	40.50 (29.00-154.25)	42.00 (34.25-44.50)	51.00 (37.75-52.25)	0.137	
LDL (mg/dL)[†]	121.00 (75.20-154.25)	108.80 (83.50-122.80)	104.00 (95.55-104.25)	0.404	
Glucose (mg/dL)[†]	140.50 (114.50-181.75)	136.50 (116.75-184.75)	110.00 (98.25-113.50)	< 0.001***	NSTEMI *vs.* control STEMI *vs.* control
LDH (U/L)[†]	236.50 (197.50-285.00)	250.00 (218.50-299.00)	190.50 (175.00-213.00)	< 0.001***	NSTEMI *vs.* control STEMI *vs.* control

[†]Kruskal-Wallis test; values are expressed as medians and interquartile ranges. [‡]One-way analysis of variance; values are expressed as means ± standard deviations. *$p \leq 0.05$, **$p \leq 0.01$, ***$p \leq 0.001$; values are expressed as statistically significant for each test. NSTEMI: non-ST-elevation myocardial infarction; STEMI: ST-elevation myocardial infarction; Hs-cTnT: high-sensitivity cardiac troponin T; LDL: low-density lipoprotein; HDL: high-density lipoprotein; CK-MB: creatine kinase-MB; LDH: lactate dehydrogenase.

Table IV. Cardiac parameters measured with ELISA according to acute coronary syndrome type and control group.

Variable	NSTEMI (n = 30)	STEMI (n = 30)	Control (n = 30)	p-value	Post-hoc test: group differences
TAS (U/mL)[†]	2.98 (2.44-3.81)	2.92 (2.42-3.65)	3.44 (3.05-4.08)	0.032*	STEMI *vs.* control
TOS (U/mL)[†]	3.15 (2.55-7.62)	2.67 (2.20-3.84)	2.62 (1.93-3.00)	0.022*	NSTEMI *vs.* control
MCP-1 (ng/L)[†]	118.59 (78.80-229.55)	131.94 (73.13-234.74)	79.61 (45.02-104.95)	0.001***	NSTEMI *vs.* control STEMI *vs.* control
Fetuin-A (mg/L)[†]	419.83 (375.73-490.76)	447.13 (402.08-638.87)	565.71 (472.81-1,148.25)	< 0.001***	NSTEMI *vs.* control STEMI *vs.* control

[†]Kruskal-Wallis test; values are expressed as medians and interquartile ranges. *$p \leq 0.05$, **$p \leq 0.01$, ***$p \leq 0.001$; values are expressed as statistically significant for each test. ELISA: enzyme-linked immunosorbent assay; NSTEMI: non-ST-elevation AMI; STEMI: ST-elevation myocardial infarction; TAS: total antioxidant status; TOS: total oxidant status; MCP-1: monocyte chemotactic protein-1.

Table V. Correlations of Hs-cTnT levels with other parameter levels measured by ELISA.

Hs-cTnT (pg/mL)	r†	*p*-value
TAS (U/mL)	-0.122	0.252
TOS (U/mL)	0.213	0.044*
MCP-1 (ng/L)	0.365	< 0.001***
Fetuin-A (mg/L)	-0.348	0.001***

†Spearman's correlation analysis, r† correlation coefficient, *p*-value < 0.05 statistically significant; *$p \leq 0.05$, **$p \leq 0.01$, ***$p \leq 0.001$; values are expressed as statistically significant for each test; Hs-cTnT: high-sensitivity cardiac troponin T; ELISA: enzyme-linked immunosorbent assay; TAS: total antioxidant status; TOS: total oxidant status; MCP-1: monocyte chemotactic protein-1.

patients who presented to the hospital with ACS were women, and their mean age was 70.9 years. They also found that female patients had more comorbid diseases and were admitted to the hospital sooner after the onset of symptoms than male patients. In our study, 23.3% of the patients with a diagnosis of AMI were women, and they were older on average than men (mean age: 74.21 ± 17.15 years).

Chest pain and cardiac symptoms are common among patients who visit emergency departments and account for 10% of all emergency department consultations. Therefore, diagnosing life-threatening diseases such as AMI on the
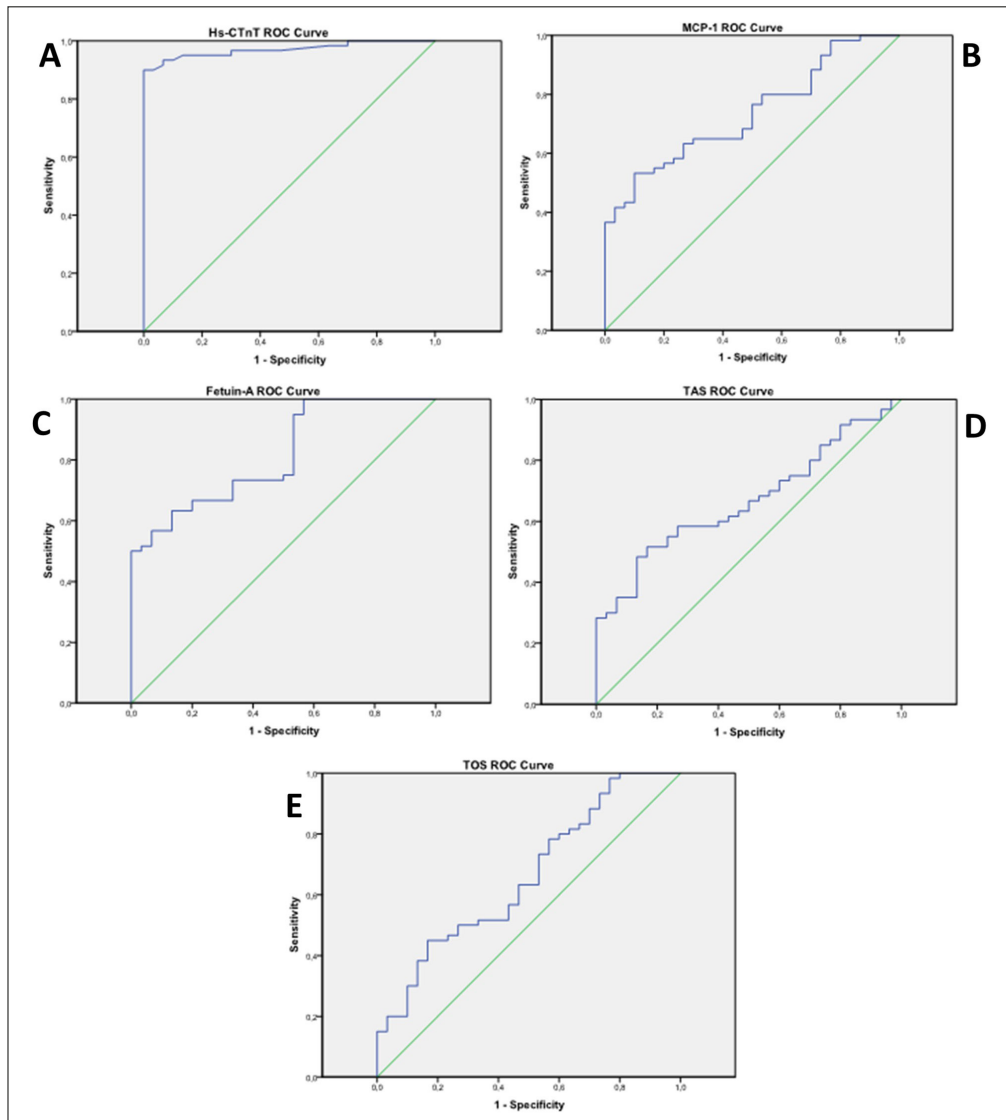

Figure 1. Receiver operating characteristic curves for high-sensitivity cardiac troponin T (Hs-cTnT) (**A**), monocyte chemotactic protein-1 (MCP-1) (**B**), fetuin-A (**C**), total antioxidant status (TAS) (**D**), and total oxidant status (TOS) (**E**).

Table VI. Multinomial logistic regression analysis; comparison of STEMI and NSTEMI subgroups.

Model (according to STEMI subgroup)		Odds ratio	Sig.	5% confidence interval for odds ratio	
				Lower limit	Upper limit
NSTEMI	Hs-cTnT (pg/mL)	1.000	0.684	0.999	1.000
	TAS (U/mL)	1.493	0.252	0.752	2.965
	TOS (U/mL)	1.299	0.044*	1.007	1.676
	MCP-1 (ng/L)	1.000	0.953	0.995	1.006
	Fetuin-A (mg/L)	0.998	0.277	0.995	1.002

*$p \leq 0.05$, **$p \leq 0.01$, ***$p \leq 0.001$; values are expressed as statistically significant for each test. STEMI: ST-elevation myocardial infarction; NSTEMI: non-ST-elevation myocardial infarction; TAS: total antioxidant status; TOS: total oxidant status; MCP-1: monocyte chemotactic protein-1.

basis of these symptoms is difficult[17,18]. To diagnose ACS, ECGs, and cardiac troponin levels are evaluated together with cardiac symptoms. However, it takes time for cardiac troponin to reach a measurable level in blood, and serial blood sampling over 6-12 hours may result in a delay in the diagnosis of AMI or in the exclusion of this diagnosis. Lower troponin concentrations can be measured with new-generation high-precision cardiac troponin tests; however, the rate of positive results of these tests that are attributable to acute and chronic heart diseases other than AMI has increased significantly[17]. In their meta-analysis, Arslan et al[19] found that serial measurement of Hs-cTnT levels took 2.5 hours, two Hs-cTnT measurements had high sensitivity (98.9%) for ruling out AMI, and a single Hs-cTnT value of > 50 ng/L had a baseline specificity of 94.6% for the diagnosis of AMI. Our study revealed that serum Hs-cTnT levels of 19.50 pg/mL could signify ACS with 93.3% sensitivity and 93.3% specificity in comparison to the control group. These sensitivity and specificity values are similar to those in Arslan et al's study[19], but the small differences in the results may be attributable to the differences in the sample sizes, in arrival times (0-6 hours after onset of symptoms) of patients, and in our cutoff levels.

Atherosclerosis is an inflammatory disease that results in the differentiation of circulating oxidized lipids into macrophages (foam cells), monocytes that exhibit adhesion, and endothelial cells and then the formation of lesions in the arterial wall[20]. For this reason, the balance between reactive oxygen species and the antioxidant defense system deteriorates, which, along with the worsening lipid profile, causes an increase in oxidative stress and the development of atherosclerotic heart diseases[21,22]. In some studies[23,24], the lipid profile was within or above normal limits in patients with AMI. For example, Aksoy et al[23] examined oxidant and antioxidant parameters in young male patients with AMI who smoked; the lipid profiles of the patients and controls were similar, but patients' TOS levels were higher and their TAS levels lower than those of the control group. In evaluating oxidative stress parameters in AMI, Surekha et al[24] found that lipid values were significantly higher in patients with AMI than in controls (healthy individuals). They stated

Table VII. Binary logistic regression analysis of TAS, TOS, MCP-1, and fetuin-A levels: comparison of NSTEMI and STEMI subgroups with control group.

Model (according to control group)	Odds ratio	Sig.	5% confidence interval for odds ratio	
			Lower limit	Upper limit
TAS (U/mL)	0.627	0.229	0.293	1.341
TOS (U/mL)	1.614	0.115	0.891	2.924
MCP-1 (ng/L)	1.023	0.002**	1.008	1.037
Fetuin-A (mg/L)	0.995	0.003**	0.991	0.998

*$p \leq 0.05$, **$p \leq 0.01$, ***$p \leq 0.001$; values are expressed as statistically significant for each test. TAS: total antioxidant status; TOS: total oxidant status; MCP-1: monocyte chemotactic protein-1.

that a decrease in HDL, along with increases in total cholesterol and LDL, was directly correlated with the incidence of coronary heart disease. The researchers reported[24] that mean serum TAS levels were significantly lower in patients with AMI than in the controls.

In another study, El-Mahdy et al[25] observed no significant difference between serum creatine kinase, CK-MB, troponin, HDL, LDL, total cholesterol, and triglyceride values in patients with ACS (STEMI, NSTEMI) and in control groups (healthy controls), but mean serum TAS levels were lower in patients with ACS than in the controls. Bhat and Ghandi[26] reported that serum lipid and TOS levels were higher in patients with ACS than in the controls, but the patients had lower TAS levels. We found no significant correlation between the lipid profile and the presence of AMI, but the TAS level was lower, whereas the TOS level was higher, in patients with AMI than in the control group. Advanced analyses showed that high sensitivity and specificity at optimum cutoff values of 3.21 U/mL for serum TAS and 2.69 U/mL for serum TOS indicate that TAS and TOS can be useful markers along with Hs-cTnT in patients with chest pain. Furthermore, the fact that a one-unit increase in serum TOS levels increases the probability of STEMI instead of NSTEMI by 1.29 times suggests that the combination of TOS and Hs-cTnT will aid in the diagnosis of AMI.

The *MCP-1* gene is located on chromosome 17, and the protein contains 76 amino acids. It is known to be produced in many cells, such as monocytes, macrophages, fibroblasts, microglial cells, and smooth muscle cells. Atherosclerotic lesions are rich in macrophages, and MCP-1 expression in these macrophages is increased. Moreover, MCP-1 is involved in monocyte diapedesis to the subendothelial space and in the accumulation of lipid-loaded foam cells in the arterial wall. MCP-1 may therefore be a target of anti-atherosclerotic drugs to prevent the development of atherosclerosis[27]. In one study[9], the expression of MCP-1 was observed to have two phases: first, levels were high very soon (0-4 hours) after chest pain started, and second, its levels decreased 6-8 hours later. In STEMI, fibrocytes accumulate in the area affected by AMI and bind to MCP-1 with CC chemokine receptor type 2 on the surface in this region. They then stimulate fibrocyte proliferation and migration and collagen accumulation. Circulating MCP-1 levels are higher in patients with

STEMI, NSTEMI, and unstable angina pectoris than in healthy controls[8]. In our study, plasma MCP-1 levels were higher in patients with AMI than in the control group; the optimum cutoff value of 93.62 ng/L had 65.0% sensitivity and 70.0% specificity. Therefore, MCP-1 may be a supportive marker with other parameters in the diagnosis of AMI, especially soon (0-6 hours) after the onset of symptoms.

In inflammation at every stage of ACS development, levels of proinflammatory mediators increase, whereas those of anti-inflammatory mediators decrease. Fetuin-A has an anti-inflammatory effect and is a cofactor in the opsonization of macrophage-deactivating molecules. During inflammation, proinflammatory cytokines decrease fetuin-A synthesis in the liver; thus, the activity of many anti-inflammatory mediators is limited, but the activity of proinflammatory mediators increases[28]. In one study[29], the fetuin-A level was shown to decrease in the first 3 days after AMI as a result of the inflammatory process, and 4 months after AMI, plasma fetuin-A levels were still significantly lower than the baseline level. Schernthaner et al[30] investigated the role of new biomarkers in patients with AMI and reported that mean plasma fetuin-A levels were higher in the controls than in the patients with AMI (STEMI and NSTEMI). According to their ROC analysis, 70 μg/mL and lower values for fetuin-A could be helpful in predicting AMI with 46% sensitivity and 89% specificity. In our study, the median plasma fetuin-A level was similarly lower in the patients with AMI than in the control group. However, the values of plasma fetuin-A levels for predicting the occurrence of AMI differed for STEMI and NSTEMI. These differences may be supported by results of future studies. Our study showed that fetuin-A values of 498.71 mg/L could predict AMI with 73.3% sensitivity and 66.7% specificity. Moreover, the relatively lower plasma fetuin-A levels in the AMI group in our study can be interpreted as an indicator of impaired proinflammatory/anti-inflammatory balance and insufficiency of anti-inflammatory activity in AMI.

Furthermore, to determine whether TAS, TOS, MCP-1, and fetuin-A levels were independent risk factors for the diagnosis of AMI, we used a binary logistic regression model, which showed that a one-unit increase in MCP-1 levels increased the probability of AMI by 1.023 times and that a one-unit increase in fetuin-A levels reduced the probability of AMI by 0.995 times.

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Limitations

Our study had several limitations. Because it was conducted at a single center and the sample size was small, the generalizability of the results is limited. The patients included in the study were patients who underwent coronary angiography after being diagnosed with NSTEMI and STEMI in the Emergency Department. The results reflect the patients' status before coronary angiography, and therefore the results of angiography could not be obtained. With larger samples, these markers should be measured in blood samples taken both at the time of admission to the Emergency Department and after AMI treatment.

Conclusions

In patients with AMI, TOS, and MCP-1 levels increased along with Hs-cTnT levels, whereas TAS and fetuin-A levels decreased. According to the regression models, fetuin-A and MCP-1 levels may be independent risk factors for the diagnosis of AMI, and TOS helped distinguish NSTEMI from STEMI. In patients presenting to the Emergency Department with chest pain, levels of MCP-1, fetuin-A, and oxidative stress parameters may provide evidence supportive of Hs-cTnT levels in the differential diagnosis of AMI 0-6 hours after the onset of symptoms.

Conflict of Interest

The authors declared no potential conflicts of interest with respect to the research, authorship, and publication of this article.

Ethics Approval

This study was conducted in a prospective cross-sectional pattern with the approval of the local Ethics Committee of Kafkas University Faculty of Medicine Deanship dated 29.05.2019 and numbered 80576354-050-99/130.

Authors' Contribution

Handan Ciftci: conceptualization, writing - original draft, project administration, investigation. Huseyin Fatih Gul: conceptualization, project administration, investigation. Ömer Çanacik: data curation, methodology, investigation. Turgut Dolanbay: data curation, methodology, investigation. Emre Karsli: data curation, resources. Doğan Erçin: data curation, resources. Levent Şahin: data curation, validation. Mahmut Karapehlivan: writing - review and editing.

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Informed Consent

All patients provided written informed consent.

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