ORIGINAL RESEARCH

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Inducible Nitric Oxide Synthase (iNOS) is a Potential Marker of Myocardial Infarction with Non-obstructive Coronary Artery Disease (MINOCA)

İndüklenebilir Nitrik Oksit Sentaz (iNOS), Obstrüktif Olmayan Koroner Arter Hastalığı ile Birlikte Miyokard Enfarktüsü (MINOCA) için Potansiyel Bir Belirteçtir

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Abstract

Objective: The currently available cardiac biomarkers are not sufficient to differentiate between myocardial infarction with non-obstructive coronary arteries (MINOCA) and acute coronary syndrome (ACS) without the use of coronary angiography. In this context, this study aimed to evaluate inducible nitric oxide synthase (iNOS) activity as a potential marker for the differential diagnosis of MINOCA and ACS.

Method: The study population comprised 734 consecutive patients who presented to our hospital with chest pain between July 2022 and January 2023. Leftover plasma samples collected in EDTA vials were sent for troponin T estimation within 24 hours of the onset of chest pain. Patients' blood samples were collected into tubes for malondialdehyde (MDA), iNOS, and total sialic acid (TSA) measurements and centrifuged at 4000 g, 4 °C for 10 min, and the sera obtained as a result were kept at -25 °C until the analyses were performed.

Results: The mean age of the study population, which consisted of 648 patients [421 (65.6%) males, was 62±12] years. There were no significant difference between the MINOCA and ACS patients in MDA and homocysteine levels. Univariate logistic regression analysis revealed significant correlations between gender, age, diabetes mellitus (DM), glucose, urea, iNOS, smoking, hemoglobin, platelets, lymphocytes, monocytes, neutrophils, triglycerides, low-density lipoprotein cholesterol, high-density lipoprotein cholesterol, creatine, and TSA as significant predictors of MINOCA. Further analysis of these variables using

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Amaç: Mevcut kardiyak biyobelirteçler, koroner anjiyografi kullanılmadan, obstrüktif olmayan koroner arterlerin eşlik ettiği miyokard enfarktüsü (MINOCA) ile akut koroner sendrom (AKS) arasında ayrım yapmak için yeterli değildir. Bu bağlamda bu çalışma, MINOCA ve AKS ayırıcı tanısında potansiyel bir belirteç olarak indüklenebilir nitrik oksit sentaz (iNOS) aktivitesinin değerlendirilmesi amacıyla yapılmıştır.

Yöntem: Çalışma evrenini Temmuz 2022 ile Ocak 2023 tarihleri arasında hastanemize göğüs ağrısı şikayetiyle başvuran 734 hasta oluşturdu. EDTA şişelerinde toplanan kalan plazma örnekleri göğüs ağrısının başlamasından sonraki 24 saat içinde troponin T çalışılması için gönderildi. Hastalardan malondialdehit (MDA), iNOS ve total sialik asit (TSA) ölçümleri için kan örnekleri tüplere alınarak 4000 g, 4 °C'de 10 dakika santrifüj edildi ve elde edilen serumlar -25 °C'de analizler yapılana kadar saklandı.

Bulgular: Dört yüz yirmi biri (%65,6) erkek olmak üzere 648 hastadan oluşan çalışma örnekleminin yaş ortalaması 62±12 idi. MINOCA ve AKS hastaları arasında malondialdehit (MDA) ve homosistein düzeyleri arasında anlamlı fark yoktu. Tek değişkenli lojistik regresyon analizi cinsiyet, yaş, diabetes mellitus (DM), glikoz, üre, iNOS, sigara içme durumu, hemoglobin, trombosit, lenfosit, monosit, nötrofil, trigliserit, düşük yoğunluklu lipoprotein kolesterol, yüksek yoğunluklu lipoprotein kolesterol, kreatin ve toplam sialik asit (TSA) için MINOCA'nın önemli belirleyicileri olarak anlamlı ilişkiler ortaya çıkardı. Bu değişkenlerin çok

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Abstract

multivariate logistic regression revealed that sex, age, presence of DM, glucose and urea levels, and iNOS were independent predictors of MINOCA. The iNOS level was significantly higher in patients with MINOCA than in those with ACS. The optimal iNOS cut-off value of >1372.9 pg/mL predicted MINOCA with 99.4% sensitivity and 93.7% specificity (area under the curve: 0.99, 95% confidence interval: 0.979-0.996, p<0.001).

Conclusion: iNOS activity may help distinguish between ACS and MINOCA without the need for coronary angiography.

Keywords: Acute coronary syndrome (ACS), inducible nitric oxide synthase (iNOS), myocardial infarction with non-obstructive coronary arteries (MINOCA)

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değişkenli lojistik regresyon analizi ile; cinsiyet, yaş, DM varlığı, glikoz, üre seviyeleri ve iNOS'nin MINOCA için bağımsız belirleyiciler olduğunu ortaya çıkardı. MINOCA hastalarında iNOS düzeyi AKS hastalarına göre anlamlı derecede yüksekti. Optimum iNOS cut-off değeri >1372,9 pg/mL, MINOCA'yı %99,4 duyarlılık ve %93,7 özgüllükle öngördü (eğri altında kalan alan: 0,99, %95 güven aralığı: 0,979-0,996, p<0,001).

Sonuç: iNOS aktivitesinin ölçülmesinin koroner anjiyografiye gerek kalmadan AKS ile MINOCA ayrımında yardımcı olabileceğini düşünüyoruz.

Anahtar kelimeler: Akut koroner sendrom (AKS), indüklenebilir nitrik oksit sentaz (iNOS), obstrüktif olmayan koroner arterlerle birlikte miyokard enfarktüsü (MINOCA)

Introduction

Ischemic heart disease is the leading cause of death worldwide, accounting for 12.7% of all deaths. Acute coronary syndrome (ACS) encompasses a wide spectrum of clinical conditions, ranging from unstable angina to non-ST-segment elevation myocardial infarction (NSTEMI) and ST-segment elevation myocardial infarction (STEMI). Most patients with anginal symptoms have no obstructive coronary artery disease (CAD) (1). Myocardial infarction with non-obstructive coronary arteries, that is, ≤50% stenosis in a major epicardial artery, is termed MINOCA. MINOCA is characterized by a heterogeneous group of conditions, including causes of epicardial and microvascular myocardial ischemia, such as plaque disruption, epicardial coronary spasm, spontaneous coronary dissection, microvascular spasm, and coronary distal embolization (2,3). Takotsubo cardiomyopathy and myocarditis, which were initially included in MINOCA, were later excluded. The prevalence of MINOCA among all type 1 myocardial infarctions (MI) is approximately 10%. It is more common in female patients with NSTEMI (4). Up to 50-60% of patients undergoing elective coronary angiography for suspected CAD actually have non-obstructive CAD. Various biochemical parameters have been investigated in the differential diagnosis of ACS and MINOCA without the need for coronary angiography. Coronary angiography performed to rule out epicardial stenosis is an invasive procedure with the risk of various complications.

A family of enzymes known as NO synthases (NOSs) generates NO. NOSs have three distinct isoforms: neuronal NOS (nNOS, NOS1), inducible NOS (iNOS, NOS2), and endothelial NOS [endothelial NOS (eNOS), NOS3]. In humans, these isoforms have ~55% homology; they differ in their intracellular location, regulation, and enzymatic properties, including their activity and susceptibility to various inhibitors (5). In ACS, platelets, which are bound to the subendothelium, are activated following plaque rupture and further stimulate thrombus formation by producing thromboxane A2 (TxA2) and increasing thrombin formation. Endothelial products such as prostacyclins and nitric oxide (NO) play an important role in the regulation of platelet activation and aggregation (6). NO impairment is common in patients with MINOCA via microvascular dysfunction and in those with ACS (7-9). In ACS, excess nitric oxide is generated in excess during ischemic attack because of marked activation of the inducible nitric oxide synthase (iNOS) enzyme by different cytokines, resulting in serious adverse effects. However, there are no studies on iNOS activity in patients with MINOCA.

The currently available cardiac biomarkers are not sufficient to differentiate between MINOCA and ACS without the use of coronary angiography. In this context, this study aimed to evaluate iNOS activity as a potential marker in the differential diagnosis of MINOCA and ACS.

Materials and Methods

Population and Sample

The study population consisted of 734 consecutive patients admitted to our hospital between July 2022 and January 2023 with chest pain, as indicated by changes in electrocardiogram or elevated cardiac troponin T levels (>0.03 ng/mL).

A total of 86 patients with 1) recent hospitalization for myocardial infarction, unstable angina, acutely decompensated heart failure (HF), deep vein thrombosis or pulmonary embolism or conduction of any cardiac re-vascularization procedure; 2) any history of surgery in the

preceding few months; 3) evidence of hepatic dysfunction; 4) presence of concomitant illness(es) including infectious and connective tissue diseases and neoplasm; and, 5) intake of immunosuppressant agents were excluded from the study. Therefore, 86 patients were excluded from the study. In conclusion, the study sample consisted of 648 patients. Leftover plasma samples collected in EDTA vials were sent for troponin T estimation within 24 hours of the onset of chest pain.

Patients' blood samples were collected into tubes for malondialdehyde (MDA), iNOS, and total sialic acid (TSA) measurements and centrifuged at 4000 g, 4 °C for 10 min, and the sera obtained as a result were kept at -25 °C until the analyses were carried out. MDA, an end-product of lipid peroxidation, was measured using the method described by Yoshioka et al. (10) based on the reaction between thiobarbituric acid and MDA. The optical density of the end products was measured at 535 nm. iNOS activity was evaluated using the method described by Miranda et al. (11). Accordingly, nitrate was reduced to nitrite by $VaCl_3$ and then reacted with sulfanilamide in an acidic environment to produce a colored diazonium compound, the optical density of which was measured at 540 nm. TSA was measured colorimetrically based on the method described by Sydow (12) using a spectrophotometer (UV-1201, Shimadzu, Japan). Accordingly, all bound sialic acid was separated using perchloric acid in serum, and the supernatants were boiled by Ehrlich's reagent. The optical density of the product was measured at 525 nm (12). Among the cardiac markers, human cardiac troponin-I (cTn-I) and iNOS were measured using commercial enzyme-linked immunoassay (ELISA) kits (Elabscience Biotechnology, Beijing, China), and homocysteine was measured using a spectrophotometer (Epoch, Biotech, USA) and another commercial ELISA kit (ELK Biotechnology, Wuhan, China).

Data Collection

Complete blood counts and biochemical test results were retrospectively obtained from intravenous blood samples before coronary angiography. Blood samples were collected from patients after 12 hours of fasting in the morning. Standard methods were used for routine biochemical tests, including lipid profile, glucose, urea, and creatinine measurements. Sialic acid was estimated spectrophotometrically using thiobarbituric acid, as described by Aminoff (13). Plasma samples of 10 µL were reacted with a strong acid, i.e., periodate reagent (25 mM periodate in 0.125 N H_2SO_4), to release sialic acid. Excess periodate reagent was reduced using sodium arsenite solution (2% sodium arsenite in 0.5 N HCl). 2 mL of thiobarturic acid solution (0.1 M thiobarturic acid in 1 N NaOH) was added to the resulting mixture, incubated for 8 min. in boiling water bath, and then cooled in ice. The resulting colored complex was extracted by acid butanol (butanol in 5% HCl), and its optical density was measured at 549 nm.

Angiographic Analysis

Coronary angiography was performed using the standard Judkins technique without nitroglycerin (Siemens Medical Solutions, Erlangen, Germany). Two experienced physicians who were blinded to the study evaluated the angiograms, and the visually smooth contours with no wall irregularities were considered normal.

All patients underwent coronary angiography for ACS via the femoral artery within 90 minutes of hospital admission. Patients with STEMI were administered 300 mg of acetylsalicylic acid and 180 mg of oral loading ticagrelor at admission. Patients who could not be administered ticagrelor were given a 300-600 mg oral clopidogrel loading dose according to the myocardial revascularization guidelines of the European Society of Cardiology instead of ticagrelor (14,15). Standard intravenous bolus unfractionated heparin (70-100 U/kg) and additional doses were administered as needed to achieve an activating clotting time of >250 s before coronary intervention. Stenting of the infarct-related artery with drug-eluting stent was successfully completed immediately after coronary angiography in suitable patients.

The study protocol was approved by the Local Ethics Committee (Ethics Committee of the Deanery of the Faculty of Medicine of Kafkas University; approval no. 80576354-050-99/83, approval date: 08/06/2023).

Statistical Analysis

The SPSS 22.0 (Statistical Product and Service Solutions for Windows, Version 22.0, IBM Corp., Armonk, NY, U.S., 2013) software package was used for statistical analyses. The descriptive statistics obtained from the collected data were expressed as mean ± standard deviation for continuous variables determined to conform to the normal distribution, as median with 0.25 and 0.75 quantiles for continuous variables determined not to conform to the normal distribution, and as percentage values for categorical variables. The t-test or Mann-Whitney U test was used to compare continuous variables between the groups, whereas Fisher's exact test or the chi-square test was used to compare categorical variables between the

groups. Univariate Cox proportional hazards analyses were conducted for all clinically relevant variables that could potentially predict MINOCA. Multivariate Cox regression analysis of variables found to be significant in univariate analyses was performed with stepwise backward conditional elimination to determine independent predictors of MINOCA ($p<0.05$). The receiver operating characteristic curve analysis was used to determine the optimal iNOS cut-off value for predicting MINOCA.

Results

The baseline demographic and laboratory characteristics of patients are presented in Table 1. The study sample consisted of 648 patients, including 477 with ACS and 171 with MINOCA. The mean age of the study population, of which 421 (65.6%) were male, was 62±12 years. There were no significant differences between the ACS and MINOCA groups in terms of hypertension, lymphocyte and eosinophil counts, total cholesterol, C-reactive protein, homocysteine, and MDA levels. Platelet and lymphocyte counts were significantly higher in patients with MINOCA, whereas hemoglobin levels and neutrophil and monocyte counts were significantly higher in patients with ACS. High-density lipoprotein cholesterol (HDL-c) and triglyceride levels were significantly lower and low-density lipoprotein cholesterol (LDL-c) was significantly higher in patients with ACS than in those with MINOCA. Among the renal parameters investigated within the scope of the study, urea and creatine levels were significantly higher in patients with ACS than in those with MINOCA. Troponin levels were also significantly higher in patients with ACS than in those with MINOCA. On the other hand, iNOS activity and TSA levels were significantly higher in patients with MINOCA than in those with ACS.

Table 1. Distribution of patients' baseline characteristics and laboratory test results among the study groups

	ACS group (n=477)		MINOCA group (n=171)		Overall study group (n=648)		p-value
Age (years)	63	±12	60	±11	62	±12	**0.017**
Sex, n (%) (male) (mean)	352	74.7	69	40.4	421	65.6	**<0.001**
Smoking status, n (%), (mean)	213	46.6	51	29.8	264	42	**<0.001**
Presence of DM, n (%) (mean)	176	38	40	23.4	216	34.1	**0.001**
Presence of HT, n (%) (mean)	285	61.4	90	53.3	375	59.2	0.065
EF (%) (mean)	56	±10	61	±7	58	±9	**<0.001**
Hemoglobin level (g/dL) (mean)	14.11	±1.94	13.3	±2.1	13.91	±1.98	**<0.001**
Platelet count (10^3/mL) (mean)	229	±62	243	±67	233	±64	**0.007**
Neutrophil count (10^3/mL) (median)	6.7	(5.02-8.89)	4.60	(3.6-6)	6.1	(4.4-8.3)	**<0.001**
Lymphocyte count (10^3/mL) (median)	1.87	(1.3-2.59)	2.17	(1.5-2.9)	1.91	(1.4-2.7)	0.005
Eosinophil count (10^3/mL) (median)	0.12	(0.1-0.2)	0.14	(0.1-0.2)	0.12	(0.1-0.2)	0.134
Monocyte count (10^3/mL) (median)	0.56	(0.4-0.7)	0.48	(0.37-0.63)	0.5	(0.4-0.7)	**0.001**
Glucose level (mg/dL) (median)	132	(107-169)	103	(95-128)	121.5	(101.5-158)	**<0.001**
Total cholesterol level (mg/dL) (mean)	178.02	±47.68	179.39	±50.98	178.38	±48.54	0.407
LDLC level (mg/dL) (mean)	115.7	±40.5	107.5	±38.3	113.5	±40	**0.033**
HDLC level (mg/dL) (mean)	42	±10	49	±12	43	±11	**<0.001**
Triglyceride level (mg/dL) (median)	92	(59-139)	107	(75-163)	95	(63-147)	**0.002**
Troponin level (ng/dL) (median)	712.9	(109.4-3657.8)	90.1	(26.4-492.9)	401	(58.7-2345)	**<0.001**
Urea level (mg/dL) (median)	37	(39-40)	34	(28-44)	36	(29-47)	**0.008**
Creatine level (mg/dL) (median)	0.94	(0.82-1.12)	0.85	(0-73-1.07)	0.93	(0.78-1.12)	**<0.001**
CRP level (mg/dL) (median)	5.5	(2.71-12.3)	5.51	(2.8-13.3)	5.51	(2.72-12.17)	0.661
Homocysteine level (µmol/L) (mean)	13.65	±2.47	13.37	±6	13.58	±3.73	0.329
iNOS level (pg/mL) (median)	792.6	(692.7-928.6)	2036.8	(1756.8-2394.3)	893.4	(723.9-1637.05)	**<0.001**
TSA level (mg/dL) (median)	84.2	(76.2-96.4)	93.7	(81.9-136.4)	86.7	(77.2-98.95)	**<0.001**
MDA level (µmol/L) (mean)	26.8	5.9	27.5	5.9	27	5.9	0.191

ACS: Acute coronary syndrome, MINOCA: Myocardial infarction with non-obstructive coronary artery disease, DM: Diabetes mellitus, HT: Hypertension, EF: Ejection fraction, LDLC: Low-density lipoprotein-cholesterol, HDLC: High-density lipoprotein-cholesterol, CRP: C-reactive protein, iNOS: Inducible nitric oxide synthase, TSA: Total sialic acid, MDA: Malondialdehyde

Univariate logistic regression analysis revealed significant correlations between sex, age, diabetes mellitus (DM), glucose, urea, iNOS, smoking, hemoglobin, platelets, lymphocytes, monocytes, neutrophils, triglycerides, LDL-c, HDL-c, creatine, and total sialic acid (TSA) as significant predictors of MINOCA (Table 2). Further analysis of these variables with the multivariate logistic regression analysis revealed gender [odds ratio (OR): 0.107, 95% confidence interval (CI): 0.030-0.388; p=0.001], age (OR: 0.940, 95% CI: 0.893-0.990; p=0.002), presence of DM (OR: 0.183, 95% CI: 0.049-0.681; p=0.011), glucose (OR: 0.988, 95% CI: 0.977-1.000; p=0.045), urea (OR: 1.028, 95% CI: 1.000-1.057; p=0.049) levels, and iNOS (OR: 1.010, 95% CI: 1.007-1.013; p<0.001) as independent predictors of MINOCA (Table 2).

The optimal iNOS cut-off value of >1372.9 pg/mL predicted MINOCA with 99.4% sensitivity and 93.7% specificity [AUC: 0.99 (95% CI: 0.979-0.996, p<0.001)] (Figure 1).

Discussion

Several studies have investigated the role of nitric oxide in ACS, with a particular focus on its impact on vascular function, plaque stability, and thrombosis. This study examined the use of iNOS activity in the differential diagnosis of ACS and MINOCA for the first time in the literature. Accordingly, the primary outcome of this study was that iNOS levels were significantly higher in patients with MINOCA than in those with ACS, whereas the secondary outcome was that an optimal iNOS cut-off value of >1372.9 predicted MINOCA with 99.4% sensitivity and 93.7% specificity.

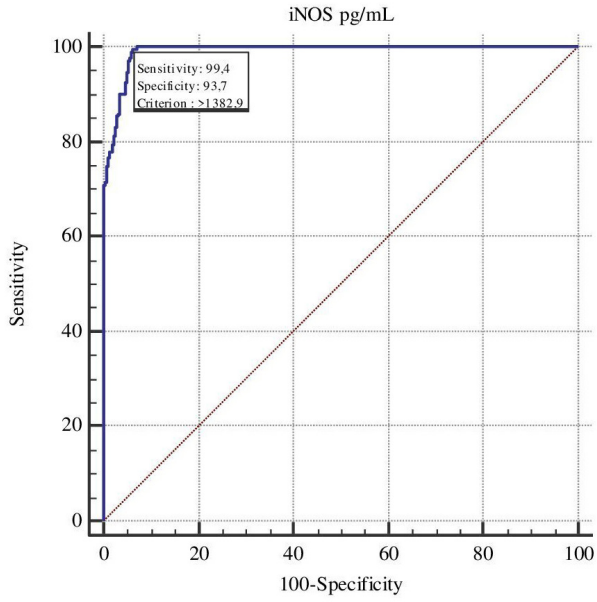

Figure 1. Receiver operating characteristic curve analysis of the efficacy of using iNOS activity to predict MINOCA

iNOS: Inducible nitric oxide synthase, MINOCA: Myocardial infarction with non-obstructive coronary arteries

Table 2. Results of univariate and multivariate models for predicting MINOCA in patients with ACS

	Univariate			Multivariate		
	Univariate OR, 95% CI		p	Multivariate OR, 95% CI		p
Gender	0.224	(0.155-0.324)	**<0.001**	0.107	(0.030-0.388)	**0.001**
Age	0.981	(0.966-0.996)	**0.014**	0.940	(0.893-0.990)	**0.020**
Diabetes mellitus	0.498	(0.334-0.743)	**0.001**	0.183	(0.049-0.681)	**0.011**
Glucose	0.987	(0.982-0.992)	**<0.001**	0.988	(0.977-1.000)	**0.045**
Urea	0.993	(0.984-1.003)	0.166	1.028	(1.000-1.057)	**0.049**
iNOS	1.008	(1.006-1.010)	**<0.001**	1.010	(1.007-1.013)	**<0.001**
Smoking	0.558	(0.152-0.046)	0.378	-	-	-
Hemoglobin	1.000	(0.995-1.004)	0.959	-	-	-
Platelet	0.994	(0.984-1.004)	0.230	-	-	-
Lymphocyte	0.940	(0.534-1.652)	0.829	-	-	-
Monocyte	1.440	(0.263-7.897)	0.674	-	-	-
Neutrophil	0.972	(0.825-1.144)	0.732	-	-	-
Triglyceride	1.003	(0.995-1.011)	0.472	-	-	-
LDL-c	1.000	(1.000-1.000)	0.851	-	-	-
HDL-c	1.018	(0.963-1.075)	0.530	-	-	-
Creatine	1.447	(0.565-3.707)	0.441	-	-	-
TSA	0.996	(0.991-1.002)	0.184	-	-	-

ACS: Acute coronary syndrome, MINOCA: Myocardial infarction with non-obstructive coronary artery disease, iNOS: Inducible nitric oxide synthase, OR: Odds ratio, CI: Confidence interval, LDL-c: Low-density lipoprotein cholesterol, HDL-c: High-density lipoprotein-cholesterol, TSA: Total sialic acid

NO levels can be relevant in ACS as nitric oxide plays a crucial role in regulating blood vessel function and blood flow. NO helps dilate blood vessels, thereby improving blood flow and reducing heart workload. However, the production and availability of NO may be impaired in ACS (16). Additionally, oxidative stress and inflammation, which are prevalent in ACS, may also negatively affect NO levels. Oxidative stress can decrease the availability of NO by promoting its reaction with reactive oxygen species, leading to the formation of peroxynitrite, a potent oxidant that further damages the endothelium (17). Decreases in NO levels during ACS may contribute to vasoconstriction, platelet aggregation, and inflammation, all of which are factors associated with disease progression. Given the complex nature of the relationship between NO and ACS, variations may occur in individual cases. Consultation with medical professionals is essential for the accurate diagnosis, treatment, and management of ACS (16-19).

In an animal model, it was demonstrated that the cytokine-mediated overexpression of iNOS was followed by massive NO production, which caused toxic effects on cardiomyocytes, including necrosis and reduced adenosine triphosphate (ATP) content (20). This can be achieved by direct action on the myocardium during ischemia-reperfusion (21). Yang et al. (22) demonstrated another effect of iNOS expression in patients with myocardial infarction. iNOS is not expressed in the healthy heart, and its formation is induced by pro-inflammatory factors (23). Expression of constitutive isoform eNOS decreased in ACS patients' neutrophils compared to healthy control subjects. In contrast, iNOS enzyme expression was markedly induced in acute myocardial infarction (24). This finding can be attributed to the possible action of cytokines released during ACS, stimulating iNOS expression and downregulating eNOS (19). El-Baheie et al. (25) reported that iNOS mRNA concentrations were significantly higher in patients with ACS than in healthy controls, and iNOS mRNA concentrations predicted ACS with 100% sensitivity and specificity. In parallel, this study emphasizes the estimation of iNOS mRNA expression as a highly sensitive and specific assay for ACS diagnosis compared with cardiac troponin. High iNOS expression in peripheral blood leukocytes of children affected by acute Kawasaki disease has been reported in the cardiovascular system (26), especially when associated with progressive coronary artery lesions (27). Comparable results have been reported in cases of dilated cardiomyopathy, ischemic and valvular disease, and ischemic disease-related HF (28).

Coronary microvascular dysfunction, which is expressed by coronary microvascular spasm, accounts for approximately 20% of MINOCA patients (29). Oxidative stress, endothelial dysfunction, and low-grade chronic inflammation contribute to the pathogenesis of coronary artery spasm. Yamada et al. suggested that thiol oxidation-induced oxidative stress causes coronary artery spasm, resulting in impaired endothelium-dependent vasodilation. The underlying mechanism of coronary vasomotor dysfunction may be endothelium-dependent or endothelium-independent. Endothelium-dependent dysfunction originates from an imbalance between endothelium-derived relaxing factors, such as NO, and endothelium-derived constrictors, such as endothelin. Endothelium-independent function is based on myocyte tone. Oxidative stress can cause vasoconstriction and endothelial damage, resulting in coronary microvascular dysfunction and vasospasm, leading to the pathogenesis of MINOCA. Although animal and human studies have provided evidence of the role of oxidative stress in cardiovascular disorders, antioxidant applications are ineffective in preventing cardiovascular mortality.

Studies have shown that serum total sialic acid levels are elevated in cardiovascular diseases due to an increase in acute-phase reactants in these patients. Elevated sialic acid levels may be attributed to scattering or secretion from damaged cells following acute myocardial infarction. Although the role of sialic acid in the pathogenesis of atherosclerosis has been investigated in many studies, its role as a prognostic marker has not been investigated yet (30). A large-scale prospective study reported that high serum sialic acid content was a strong predictor of cardiovascular mortality (31). Serum total sialic acid levels were significantly higher in patients with ACS than in healthy controls. Serdar et al. (32) found that total sialic acid levels progressively increased significantly in patients with unstable angina, NSTEMI, and STEMI. Interestingly, in our study, TSA levels were significantly higher in patients with MINOCA than in those with ACS, as in iNOS. The fact that TSA and iNOS levels have not been compared to date in patients with ACS and MINOCA in the literature makes this finding even more valuable. However, further studies with larger populations are needed to confirm the findings of this study.

In line with the literature data (33), the ACS patients included in this study mostly consisted of female and older patients. Factors such as smoking status, presence of DM, and dyslipidemia, which were reported to increase the risk

of ACS in the literature (33), were also more common in the ACS patients included in this study.

The criteria for an ideal biomarker include its role in diagnosis and screening, as well as its ability to influence therapy and improve patient outcomes. In clinical practice, physicians rely on available biomarkers to support the diagnosis of ACS, including creatine phosphokinase and creatine kinase myoglobin binding tests. However, the positivity of both MINOCA and ACS patients for these markers renders differential diagnosis impossible without coronary angiography. To the best of our knowledge, no previous study has evaluated the efficacy of iNOS activity in the differential diagnosis of MINOCA and ACS. The findings of this study indicate that iNOS activity can be effectively used as a biomarker to differentiate between patients with ACS and those with MINOCA indistinguishable from cardiac biomarkers. Evaluation of iNOS activity can be routinely performed in daily clinical practice because it is feasible, cost-effective, and easy to perform. The results of the iNOS activity evaluation can be made available within 3-4 hours after the collection of blood samples. Moreover, the clinical efficacy of iNOS as a cardiac marker may be further enhanced if it is associated with a therapeutic target, as it can help tailor the therapeutic strategy, allowing the treatment to be personalized.

Study Limitations

There are several limitations to this study. First, this was a single-center, retrospective study with a relatively small sample size. Second, the fact that both ACS and MINOCA feature dynamic and rapidly evolving clinical situations may potentially hinder the generalizability of our findings. As a reason, this study was designed to evaluate iNOS activity as a biomarker in the differential diagnosis of ACS and MINOCA; however, iNOS activity is likely to change during the course of both ACS and MINOCA and due to therapeutic interventions, and the collected research data feature measurements performed in each individual at a single time point. Therefore, although it was possible to examine the correlations that exist between these parameters in this study, our findings did not provide much evidence on the dynamics of the system and how ACS, MINOCA, and therapeutic interventions might have affected these correlations.

Conclusion

In conclusion, iNOS activity may help distinguish between ACS and MINOCA without the need for coronary angiography. However, the mean iNOS levels of patients with ACS may vary depending on the study and patient populations investigated in these studies as well as the measurement techniques used. Therefore, further large-scale studies are needed to corroborate the findings of this study in general and to elucidate the role of oxidative stress in ACS and MINOCA.

Ethics

Ethics Committee Approval: The study protocol was approved by the Local Ethics Committee (Ethics Committee of the Deanery of the Faculty of Medicine of Kafkas University; approval no. 80576354-050-99/83, approval date: 08/06/2023).

Informed Consent: Informed consent was obtained from all patients.

Authorship Contributions

Concept: M.K., M.Ö., İ.R., M.A., Design: M.K., M.Ö., İ.R., M.A., Data Collection or Processing: İ.A., T.O., A.A., Z.Ç., Analysis or Interpretation: Y.K., D.İ., Drafting Manuscript: M.Ö., İ.A., T.O., A.A., Z.Ç., M.K., Critical Revision of Manuscript: İ.R., M.K., D.İ., M.A., Y.K., Final Approval and Accountability: M.K., M.Ö., İ.A., T.O., D.İ., A.A., Z.Ç., M.A., Y.K., İ.R., Writing: M.K., M.Ö., İ.A., T.O., D.İ., A.A., Z.Ç., M.A., Y.K., İ.R.

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