ORIGINAL ARTICLE

The relationship between low thiol levels and major adverse cardiovascular events after primary percutaneous coronary intervention in patients with STEMI

STEMI hastalarında primer perkütan koroner girişim sonrası düşük tiyol seviyeleri ve kardiyovasküler olay gelişimi arasındaki ilişki

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ABSTRACT

Objective: The aim of this study was to investigate whether low thiol levels are associated with peri-procedural factors during primary percutaneous coronary intervention (pPCI) upon admission with ST-segment elevation myocardial infarction (STEMI), and the prognostic value at 6-month follow-up.

Methods: A total of 241 consecutive acute STEMI patients who underwent pPCI and a control group of 67 individuals with a normal coronary angiography were enrolled in the study.

Results: While age, contrast-induced nephropathy, N-terminal prohormone of brain natriuretic peptide (NT-proBNP), stent length, and creatinine were related to native thiol, NT-proBNP, contrast-induced nephropathy, and creatinine were related to total thiol. NT-proBNP was also related to the disulphide level. The left ventricular ejection fraction (LVEF) and the levels of native thiol, total thiol, low-density lipoprotein, and serum albumin were found to be independent predictors of major adverse cardiovascular events (MACEs) during 6 months of follow-up.

Conclusion: Initial lower native thiol, total thiol, LVEF, LDL, and serum albumin may be used to identify patients with an increased long-term risk of unfavorable cardiac events in case of STEMI.

ÖZET

Amaç: Bu çalışmanın amacı, primer perkütan koroner girişim yapılan ST-segment yükselmeli miyokart enfarktüsü (STEMI) hastalarında periprosedürel faktörlerle düşük tiyol seviyelerinin ilişkisi olup olmadığını ve 6 aylık takipte prognoza etkilerini belirlemektir.

Yöntemler: Primer perkütan koroner girişim yapılan 241 STEMI ve normal koroner anjiyografi saptanan 67 bireyden oluşan kontrol grubu dahil edildi.

Bulgular: Yaş, kontrast nefropatisi, N-terminal beyin natriüretik peptit (NT-proBNP), stent uzunluğu ve kreatinin native thiol için, NT-proBNP, kontrast nefropatisi ve kreatinin total tiyol ile ilişkili faktörler iken; NT-proBNP disülfit ile ilşkili faktör olarak saptandı. Sol ventrikül ejeksiyon fraksiyonu, nativ tiyol, total tiyol, düşük yoğunluklu lipoprotein (LDL) ve serum albümin altı aylık majör istenmeyen kardiyovasküler olaylar (MACE) gelişimine etki eden en etkili parametreler olarak bulundu.

Sonuç: ST-segment yükselmeli miyokart enfarktüs hastalarında sol ventrikül ejeksiyon fraksiyonu, nativ, total tiyol, LDL ve serum albümin uzun dönem morbititeye etki eden faktörler olarak değerlendirilebilir.

Oxidative stress occurs due to damage caused by reactive oxygen species (ROS) and a deficiency in the antioxidant defense system.[1] Oxidative stress can cause harmful cellular effects involving altered protein and DNA structures, resulting in inflammation and progressive atherosclerosis. Moreover, increased oxidative stress is correlated with the pathogenesis of hypertension and cardiovascular disease severity.[2–4]

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Thiols are components of the amino acid cysteine and the disulphide/thiol redox couple. They are organic composites in cytosol and mitochondria.[5] Thiols may transform into disulphide forms in proteins after oxidative reactions and bring out various products. Disulphide bonds can convert into thiols once again, and the reactions continue.[6] These reactions are reversible, and they are known as thiol-disulphide exchange.[5–7]

Abbreviations:

AMI	*Acute myocardial infarction*
CIN	*Contrast-induced nephropathy*
DBP	*Diastolic blood pressure*
DBT	*Door-balloon time*
ECG	*Electrocardiogram*
GRACE	*Global registry of acute coronary events*
hsCRP	*High-sensitivity C-reactive protein*
HT	*Hypertension*
IRA	*Infarct-related artery*
LDL	*Low-density lipoprotein*
LVEF	*Left ventricular ejection fraction*
MACE	*Major adverse cardiovascular event*
NaBH4	*Sodium borohydride*
NT-proBNP	*N-terminal prohormone of brain natriuretic peptide*
PPCI	*Primary percutaneous coronary intervention*
ROC	*Receiver operating characteristic*
ROS	*Reactive oxygen species*
SBP	*Systolic blood pressure*
STEMI	*ST-elevation myocardial infarction*
SYNTAX	*Synergy Between Percutaneous Coronary Intervention With Taxus and Cardiac Surgery*
TIMI	*Thrombolysis in Myocardial Infarction*

Thiol groups are highly sensitive to oxidative stress, which may precipitate the formation of thiol-disulphide over-accumulation, which is often associated with a variety of disorders, such as fibrogenesis, hypertension, and atherosclerosis.[2–8] These components of proteins provide a protective barrier against oxidative damage caused by a direct reaction with ROS and other detrimental free radicals.[9] Proteins and their oxidized disulphide formations have been found to be associated with cardiovascular disease progression because of the pro-inflammatory redox state, which triggers inflammation, apoptosis, and vascular proliferation.[10] Acute myocardial infarction (AMI) is the visible result of these unfavorable processes. Despite advancements in diagnosis and treatment, AMI is still an abrupt and subtle cause of mortality.[11] Thiol oxidation and its products, disulphide groups, have been shown to be markers in AMI.[12] The aim of this study was to investigate whether lower thiol levels are associated with adverse prognostic factors during primary percutaneous coronary intervention (pPCI) upon admission with ST-segment elevation myocardial infarction (STEMI) or not, and their prognostic value at 6-month follow-up.

METHODS

Study population

A total of 241 consecutive acute STEMI patients who underwent pPCI and a control group of 67 individuals with a normal coronary angiography were enrolled in the study. Patients with an infectious disease, malignancy, pulmonary embolism, more than mild liver or kidney disease, patients previously using drugs with antioxidant effects (nebivolol, captopril, statins, etc.) before admission, and patients not taking dual antiplatelet medication after discharge were excluded from the study.

The Killip classification of the patients was recorded to demonstrate hemodynamic status.[13]

The local ethics committee approved the study, and written informed consent was obtained from all of the participants. The patients were followed up for 6 months for the occurrence of major adverse cardiovascular events (MACEs: acute coronary syndrome, stroke, death, target vessel revascularization).

Diagnosis of acute myocardial infarction

Diagnosis of AMI was based on symptoms, elevated cardiac markers, and electrocardiogram (ECG) changes. Patients with typical chest pain plus ECG changes (pathological Q waves, at least 1 mm ST-segment elevation in any 2 or more contiguous limb and chest leads, or new left bundle branch block) or a plasma level of abnormal cardiac troponin-I were diagnosed with AMI.

Primary percutaneous coronary intervention and SYNTAX score

Coronary angiography and primary pPCI were performed via the standard poses with Siemens Medical Systems (Siemens Healthineers, GmbH, Erlangen, Germany) or Toshiba Infinix CC-i (Toshiba Corp., Tokyo, Japan) monoplane cardiac angiography equipment using 6–7F diagnostic/guiding catheters. The standard Judkins technique was employed by experienced, blinded interventional cardiologists via the left/right femoral or radial approach. The Synergy Between Percutaneous Coronary Intervention with Taxus and Cardiac Surgery (SYNTAX) score was calculated from the angiographic analysis of coronary lesions with ≥50% stenosis in vessels ≥1.5 mm in diameter, according to the presence of total occlusion, bifurcation,

trifurcation, distal vessel bed, thrombus formation, and so on.[14] The score was calculated before wiring or pre-dilatation of the infarct-related artery (IRA). The grade of coronary collaterals was determined according to the Rentrop classification as follows: grade 0: no collaterals; grade 1: collaterals that did not reach the epicardial artery; grade 2: partial filling of the epicardial artery; and grade 3: total filling up to the occlusion.[15] Thrombolysis in myocardial infarction (TIMI) flow grades were assessed as previously described.[16]

Spontaneous reperfusion, no reflow, and thrombolysis in myocardial infarction flow grade

TIMI 3 flow in the IRA before pPCI was defined as spontaneous reperfusion.[17] Attenuation of coronary flow in the infarct vessel territories after appropriate balloon and/or stent placement is one of the most important decisive elements for the prognosis of STEMI; this is known as no reflow without coronary dissection, thrombus, or spasm.[18,19] A reduction of at least one TIMI flow grade after stent placement or a post-procedural TIMI flow of less than grade 3 represents the no-reflow phenomenon. In addition, diminished and delayed perfusion of the myocardium (low myocardial blush grade) is also defined as no reflow, even with TIMI 3 flow.

Blood sample collection

Blood samples were collected to measure biochemical parameters after 8 hours of overnight fasting. Thiol/disulphide homeostasis tests were obtained before medication and invasive approach. The samples were drawn from a cubital vein into blood tubes. Collected samples were immediately centrifuged at 3000 rpm for 10 minutes to separate the serum, and the serum was stored at -80°C until analysis. Biochemical analyses were performed using an automatic blood counter (Roche Diagnostics, Basel, Switzerland) with commercially available kits. The levels of N-terminal pro-brain natriuretic peptide (NT-proBNP) were assessed using immunoturbidimetry (Beckman assay 360; Beckman Coulter, Inc. Brea, CA, USA). Hematological parameters were measured from tripotassium ethylenediaminetetraacetic acid-based anticoagulated blood samples and assessed by a Sysmex K-1000 (Block Scientific, Inc., Bohemia, NY, USA) autoanalyzer within 30 minutes of sampling.

Serum disulphide/thiol hemostasis

Thiol and oxidized disulphide homeostasis tests were performed using a novel spectrophotometric method described in a previous work.[7] A Shimadzu UV-1800 spectrophotometer with a temperature controlled cuvette holder (Shimadzu Corp., Hadano, Japan) and a Cobas c501 automated analyzer (Roche Diagnostics, Basel, Switzerland) were used for this reduction analysis process. Briefly, dynamic disulphide bonds were reduced to free functional thiol groups by sodium borohydride (NaBH4). Formaldehyde was incinerated and unused reductant NaBH4 residue was removed after reaction with 5,5′-dithiobis-(2-nitrobenzoic acid). This prevented undesirable additional reductions of dynamic disulphide bonds. After these reactions were complete, measurements of the reduced and native thiol groups were obtained.

The total thiol measurement was performed using modified Ellman's reagent. Half the difference between the total thiol and native thiol yields the amount of dynamic disulphide bonds. After determining the levels of native (–SH) and total thiols (–S–S– + –SH), as well as disulphide (–S–S–), the disulphide/native thiol (–S–S–)/(–SH) ratio was calculated.

Echocardiographic examination

All echocardiographic examinations were performed using commercially available equipment (Vivid-7; GE Healthcare, Inc. Chicago, IL, USA) with a 2.5-3.5 MHz transducer. Simultaneous ECG recordings were also obtained. One echocardiographer who was blinded to the patients' clinical and laboratory data interpreted each echocardiographic examination independently. Echocardiographic techniques and calculations were performed according to recommendations; the left ventricular ejection fraction (LVEF) was calculated using a modified Simpson's rule technique.[20]

Statistical analysis

SPSS for Windows, Version 16.0 (SPSS, Inc., Chicago, IL, USA) software was used for the statistical analysis of the data. In order to determine normal distribution, first skewness and kurtosis measures were examined, and then the Kolmogorov-Smirnov normality test was applied. Continuous variables were expressed as mean±SD or median (interquartile range), otherwise the number of cases and percentages were used for categorical data. Comparisons between groups of patients were carried out using a chi-square test for categorical variables, an independent-samples t-test for normally distributed continuous variables, and

the Mann-Whitney U test when the assumption of normality was invalid. The correlation of both native thiol and disulphide levels with other study parameters was evaluated using the Pearson or Spearman correlation test. The Pearson correlation test was used for variable pairs that demonstrated normal distribution and a linear image in scatter plots; otherwise, the Spearman correlation test was applied. Stepwise multiple regression was conducted to evaluate whether the selected study parameters were necessary to predict disulphide, native thiol, and total thiol. Variables with a univariate analysis result of $p<0.10$ in terms of the relationship with native thiol (disease, age, hemoglobin [Hb], NT-proBNP, creatinine, SYNTAX score, systolic blood pressure [SBP], diastolic blood pressure [DBP], pain duration, stent length and high-sensitivity C-reactive protein [hsCRP]), total thiol (disease, age, Hb, NT-proBNP, creatinine, hsCRP, SBP, pain duration, stent length, and SYNTAX score), or with disulphide (disease, mean platelet volume, peak troponin I, NT-proBNP, hsCRP, and low-density lipoprotein [LDL]) were accepted as candidate factors and included in multivariate analysis. Thus, the most impact factors on the dependent variables (native thiol, total thiol, and disulphide) were determined using the stepwise method, and an ultimate model depicted those that provided a significant contribution to the models. Possible variables in predicting 6-month MACE rate: age, native thiol, total thiol, disulphide, disulphide/total thiol, LVEF, NT-ProBNP and SYNTAX score, were included in the univariate logistic regression analysis. In accordance with the

Table 1. Effect of several variables on the development of 6-month MACE in univariate logistic regression analysis

	Patients with MACE (n=36)	Patients with no MACE (n=205)	Odds Ratio (95%CI)	*p*
	Mean±SD	Mean±SD		
Gender (%)	57.1 m	58.3 m	1.053 (0.514–2.159)	0.888
Diabetes mellitus (%)	44.4	27.8	0.481 (0.233–0.994)	0.048
Age (years)	63.64±11.46	58.48±11.17	1.041 (1.008–1.074)	0.013
Systolic blood pressure (mm Hg)	118.56±23.54	128.46±16.92	0.972 (0.953–0.991)	0.004
body mass index (kg/m^2)	27.27±2.87	27.03±2.34	1.040 (0.902–1199)	0.593
Hypertension	0.56±0.50	0.52±0.50	1.145 (0.562–2.334)	0.710
Contrast induced nephropathy (%)	36.1	10.7	0.213 (0.095–0.479)	<0.001
hemoglobin (gr/dL)	12.85±2.18	13.80±1.88	0.777 (0.646–0.936)	0.008
Mean platelet volume (fL)	10.18±5.69	10.60±13.35	0.997 (0.963–1.031)	0.853
High-sensitivity C-reactive protein (mg/L)	4.91±7.07	1.85±3.46	1.125 (1.049–1.206)	0.001
Creatinine (mg/dL)	1.04±0.28	0.87±0.22	17.495 (4.116–74.364)	<0.001
Total cholesterol (mg/dL)	163.81±49.67	182.38±38.66	0.988 (0.979–0.997)	0.013
Low-density lipoprotein (mg/dL)	111.28±47.06	130.89±39.53	0.987 (0.978–0.997)	0.009
High-density lipoprotein (mg/dL)	37.56±11.41	38.08±11.28	0.996 (0.965–1.028)	0.795
Triglyceride (mg/dL)	135.31±86.87	161.32±101.08	0.997 (0.992–1.001)	0.152
Serum albumin (g/dL)	3.66±0.54	4.05±0.46	0.177 (0.079–0.397)	<0.001
Native thiol (µmol/L)	227.20±68.69	262.63±64.65	0.992 (0.987–0.997)	0.004
Total thiol (µmol/L)	295.14±70.70	317.94±73.06	0.996 (0.991–1.001)	0.085
Disulphide (µmol/L)	33.97±11.13	28.04±13.33	1.033 (1.006–1.060)	0.015
Disulphide/total thiol (%)	17.15±10.22	11.71±10.57	1.040 (0.999–1.082)	0.055
Left ventricular ejection fraction (%)	34.94±7.74	48.08±9.06	0.823 (0.772–0.877)	<0.001
Nt-ProBNP (pg/mL)	1648.91±2602.25	1026.75±2729.10	1.000 (1.000–1.000)	0.220
SYNTAX score	25.60±9.54	15.69±8.64	1.119 (1.072–1.168)	<0.001

NT-proBNP: N-terminal proB-type natriuretic peptide. SYNTAX: Synergy Between Percutaneous Coronary Intervention with Taxus and Cardiac Surgery.

criteria of Hosmer, Lemeshow, and Sturdivant , variables with p <0.25 in the univariate analysis (Table 1) were used in a multivariate logistic regression model with the backward stepwise method to determine the independent prognostic factors of early mortality. Variables were evaluated as continuous variables in the multivariate regression analysis. A p value <0.05 was accepted as statistically significant. Two receiver operating characteristic (ROC) curve analyses were performed to determine the ability of native thiol and disulphide measurements to distinguish between patients with and without MACE. The optimal cut-off value was calculated by determining the value for native thiol and disulphide that provided a balance between sensitivity and specificity.

RESULTS

Comparison of clinical and laboratory findings

Gender, age, and body mass index were similar between groups (p>0.05 for all). Table 2 shows the demographic and clinical characteristics of the study population. Contrast-induced nephropathy (CIN), hsCRP, NT-proBNP, creatinine, and LDL were significantly higher in the STEMI patients (p<0.05 for all). The HDL, native thiol, total thiol, and disulphide values were significantly lower in the STEMI patients (p<0.05 for all). The disulphide/native thiol ratio was similar between the two groups (p>0.05).

Relationships between native thiol level, total thiol level, disulphide level, and disulphide/native thiol ratio and clinical and laboratory parameters

Both native thiol and total thiol levels were positively correlated with Hb (r=0.138, p=0.016; r=0.149, p<0.001, respectively) and negatively correlated with age, NT-proBNP, creatinine, hsCRP, pain duration, and the SYNTAX score (r=–0.242, p<0.001; r=–0.283, p<0.01; r=–0.247, p<0.001, r=–0.165, p<0.001; r=–0.199, p<0.001; r=–0.142, p=0.028 for native thiol and r=–0.294, p<0.001; r=–0.329, p<0.001; r=–0.234, p<0.001; r=–0.207, p<0.001, r=–0.146, p=0.024; r=–0.142, p=0.027 for total thiol, respectively). In addition, the native thiol level was

Table 2. Demographic and clinical characteristics of study population

Variables	Controls	Patients	p
Gender, female/male (%)	29 (43%)/38 (57%)	103 (43%)/138 (57%)	0.936
Age (years)	56.4±10.48	59.2±11.34	0.072
Body mass index (kg/m^2)	28.4 [24.5; 31.1]	26.9 [25.8; 28.3]	0.078
Hypertension	28 (41.2%)	127 (52.7%)	0.093
Diabetes mellitus	18 (26.5%)	73 (30.3%)	0.542
Contrast induced nephropathy	0 (0%)	35 (14.5%)	<0.001
Hemoglobin (gr/dL)	13.4±1.70	13.7±1.95	0.391
Mean platelet volume (fL)	10.4 [10.1; 11.4]	9.0 [7.7; 10.8]	<0.001
High-sensitivity C-reactive protein (mg/L)	0.2 [0.1; 0.3]	0.6 [0.3; 2.0]	<0.001
N-terminal proB-type natriuretic peptide (pg/mL)	31.0 [19.0; 51.0]	221.0 [87; 708.5]	<0.001
Creatinine (mg/dL)	0.6 [0.6; 0.8]	0.9 [0.7; 1.0]	<0.001
Total cholesterol (mg/dL)	180.2±36.30	176.9±40.92	0.911
Low-density lipoprotein (mg/dL)	115.3± 28.85	127.9±41.24	0.019
High-density lipoprotein (mg/dL)	40.0 [32.0; 49.0]	37.0 [30.5; 44.0]	0.027
Triglyceride (mg/dL)	141.0 [99.0; 209.0]	130.0 [92.5; 181.5]	0.302
Serum albumin (g/dL)	4.0 [3.9; 4.3]	4.0 [3.7; 4.3]	0.251
Native thiol (µmol/L)	286.9±69.99	257.3±66.34	0.002
Total thiol (µmol/L)	357.0±77.64	314.5±73.03	<0.001
Disulphide (µmol/L)	35.1±11.70	28.9±13.17	<0.001
Disulphide/native thiol	12.8 [9.2; 15.5]	11.5 [7.9; 15.0]	0.612

Values of p<0.05 were considered statistically significant and are indicated in bold.

positively correlated with SBP (r=0.128, p=0.048) and negatively correlated with door-balloon time (DBT) (r=–0.135, p=0.036) and stent length (r=–0.158, p=0.014). Disulphide levels were positively associated with peak troponin I and negatively associated with NT-proBNP and hsCRP (r=0.165, p=0.010; r=–0.234, p<0.001, r=–0.119, p=0.038, respectively). The disulphide/native thiol ratio was positively associated with DBT (r=0.150, p=0.020). Table 3 shows the correlations between the native thiol level, total thiol level, disulphide level, disulphide/native thiol ratio, and other variables.

Multiple regression analysis illustrated that the initial native thiol level was independently associated with creatinine, age, stent length, NT-proBNP, CIN, and HT (p=0.038, p=0.08, p=0.029, p=0.010, p=0.013, and p=0.036, respectively). The initial total thiol level was independently associated with NT-proBNP, stent length, CIN, age, and HT (p<0.001, p<0.001, p=0.016, p=0.015, and p=0.041, respectively). The disulphide level was independently associated with disease and NT-proBNP (p=0.003 and p=0.004. respectively). The multiple stepwise linear regression analysis is shown in Table 4.

According to ROC analysis, the optimal cut-off value to predict the occurrence of MACE was 241.8 for native thiol. With this cut-off value, native thiol had 52.8% sensitivity, 62.4% specificity, an area un-

Table 3. Relationship between native thiol, total thiol, disulphide, disulphide/native thiol and other variables

	Native thiol (µmol/L)		Total thiol (µmol/L)		Disulphide (µmol/L)		Disulphide/ native thiol (%)	
	r	*p*	r	*p*	r	*p*	r	*p*
Age (years)	-0.242	<0.001	-0.229	<0.001	-0.036	0.530	0.077	0.181
Body mass index (kg/m^2)	-0.032	0.594	-0.054	0.361	0.050	0.401	0.031	0.608
Hemoglobin (gr/dL)	0.138	.016	0.149	<0.001	0.057	0.321	-0.008	0.892
Mean platelet volume (fL)	-0.074	0.197	-0.088	0.122	-0.101	0.075	-0.028	0.624
Peak troponin I (mg/dL)	0.046	0.480	0.097	0.134	0.165	0.010	0.109	0.090
NT-proBNP (pg/mL)	-0.283	<0.001	-0.329	<0.001	-0.234	<0.001	-0.077	0.176
Creatinine (mg/dL)	-0.247	<0.001	-0.234	<0.001	-0.037	0.518	0.081	0.157
hsCRP (mg/L)	-0.165	<0.001	-0.207	<0.001	-0.119	0.038	-0.030	0.594
Total cholesterol (mg/dL)	0.015	0.795	0.011	0.853	-0.040	0.482	-0.043	0.454
Low-density lipoprotein (mg/dL)	0.013	0.825	0.035	0.537	-0.099	0.084	-0.079	0.164
High-density lipoprotein (mg/dL)	0.025	0.667	0.029	0.618	-0.007	0.908	0.008	0.887
Triglyceride (mg/dL)	0.092	0.108	0.109	0.55	0.030	0.600	0.002	0.967
Glucose (mg/dL)	-0.077	0.176	-0.082	0.153	0.070	0.224	0.015	0.791
Systolic blood pressure (mmHg)	0.128	0.048	0.115	0.076	0.002	0.980	-0.055	0.397
Diastolic blood pressure (mmHg)	0.068	0.291	0.064	0.325	0.010	0.880	-0.005	0.941
Door-balloon time (min)	-0.135	0.036	-0.097	0.134	0.098	0.131	0.150	0.020
Pain duration (mm)	-0.199	<0.001	-0.146	0.024	0.027	0.677	0.125	0.054
Stent length (mm)	-0.158	0.014	-0.126	0.051	0.015	0.820	0.104	0.107
Stent diameter (mm)	0.005	0.942	-0.002	0.971	-0.029	0.656	-0.065	0.316
Latest vessel diameter (mm)	-0.007	0.916	-0.038	0.558	-0.104	0.107	-0.116	0.072
SYNTAX score	-0.142	0.028	-0.142	0.027	-0.008	0.899	0.068	0.291
Left ventricular ejection fraction (%)	0.041	0.526	0.017	0.798	-0.068	0.295	0.066	0.306
Contrast induced nephropathy	3.904*	<0.001	3.346*	0.001	-0.251*	0.802	-1.791*	0.074

hsCRP: High-sensitivity C-reactive protein; NT-proBNP: N-terminal proB-type natriuretic peptide; SYNTAX: Synergy Between Percutaneous Coronary Intervention with Taxus and Cardiac Surgery.
• Because contrast induced nephropathy is a categorical variable, T-score was given instead of correlation coefficient.

Table 4. Linear regression analysis for native thiol, total thiol and disulphide

	B	95% Confidence interval	t	*p*
Native thiol related determinants				
Creatinine	-0.123	-68.098 -2.024	-2.088	0.038
Age (years)	-0.150	-1.583 to -0.235	-2.654	0.008
Stent length (mm)	-0.123	-1.385 to -0.074	-2.191	0.029
N-terminal proB-type natriuretic peptide (pg/mL)	-0.148	-0.008 to -0.001	-2.594	0.010
Contrast induced nephropathy (absent=0. present=1)	-0.141	-53.629 to -6.444	-2.505	0.013
Hypertension	0.113	1.040 to 29.634	2.111	0.036
Stepwise multiple regression analysis; n=241				
Model R^2=0.162; F=9.059				
Model p<0.001				
β indicates regression coefficient				
Total thiol related determinants				
N-terminal proB-type natriuretic peptide (pg/mL)	-0.217	-0.010 to -0.003	-3.951	<0.001
Stent length (mm)	-0.192	-1.978 to -0.561	-3.526	<0.001
Contrast induced nephropathy (absent/present)	-0.132	-57.108 to -5.922	-2.423	0.016
Age (years)	-0.138	-1.676 to -0.185	-2.455	0.015
Hypertension	0.109	0.662 to 32.375	2.050	0.041
Stepwise multiple regression analysis; n=241				
Model R^2=0.132; F=11.995				
Model p<0.001				
β indicates regression coefficient				
Disulphide related determinants				
Disease	-0.169	-8.861 to -1.858	-3.012	0.003
N-terminal proB-type natriuretic peptide (pg/mL)	-0.163	-0.001 to 0.000	-2.905	0.004
Stepwise multiple regression analysis; n=241				
Model R^2=0.036; F=8.818				
Model p=0.003				
β indicates regression coefficient				

der the ROC curve of 0.629 (95% confidence interval [CI]: 0.529–0.729; p=0.014), a positive predictive value of 19.8%, and a negative predictive value of 88.3%. Applying this cut-off value to the study population, 115 patients (37.2%) had a native thiol value <241.75. Multiple regression analysis was performed using variables triggering MACE to predict the diagnostic accuracy of cut-off values.

The optimal cut-off value, according to ROC analysis, to predict the occurrence of MACE was 27.3 for disulphide. With this cut-off value, disulphide had 50.2% sensitivity, 63.9% specificity, an area under the ROC curve of 0.646 (95% CI: 0.555–0.737; p=0.005), a positive predictive value of 91.15% and a negative predictive value of 18.4%. Applying this cut-off value to the study population, there were 132 patients (42.72%) with a disulphide value <27.3.

Relationship between prognostic indicators and thiol-disulphide values in the acute phase of STEMI

In this study, the mean DBT was quite short. It was 30.6 minutes with a SD of 14.8. Perioperative coronary circulation parameters like spontaneous reperfusion, Rentrop grades of coronary collaterals, SYNTAX score and TIMI flow grades were recorded in patients with STEMI. In all, 149 (61.8%) patients had a Rentrop grade of 0.74, xx (30.7%) patients were classified as grade 1, 16 (6.6%) patients were catego-

Table 5. Effect of several variables on the development of 6-month MACE in multivariate logistic regression analysis

	Odds Ratio (95%CI)	p
Native thiol (µmol/L)	0.971 (0.951–0.991)	0.005
Total thiol (µmol/L)	1.019 (1.001–1.038)	0.041
LVEF (%)	0.834 (0.768–0.907)	<0.001
LDL (mg/dL)	0.981 (0.967–0.995)	0.007
Serum albumin (g/dL)	0.310 (0.097–0.993)	0.049

MACE: Major adverse cardiac event; CI: Confidence interval; LVEF: Left ventricular ejection fraction; LDL: Low-density lipoprotein.

rized as grade 2, and 2 (0.8%) patients had grade 3 collaterals. No reflow (TIMI 0–2) was observed in 43 patients (17.8%).

There was no significant relationship between those perioperative coronary circulation parameters or in-hospital mortality rates with native thiol, total thiol, disulphide level, or disulphide/native thiol ratio in the acute phase of MI (p>0.05 for all).

Relationships between prognostic indicators and thiol-disulphide values in the chronic phase of STEMI

MACE occurred in 36 patients during the 6-month study period. Two patients experienced stroke. In-hospital mortality occurred in 8 (3.3%) patients. Mortality after discharge occurred in 8 (3.3%) patients. Thirteen (5.3%) patients had target stent thrombosis and target vessel revascularization. The remaining 5 patients were admitted due to acute coronary syndromes of non-target vessel thrombosis. The distribution of native thiol, total thiol, disulphide and disulphide/native thiol levels according to MACE status is illustrated in Figure 1. In multivariate logistic regression analysis;

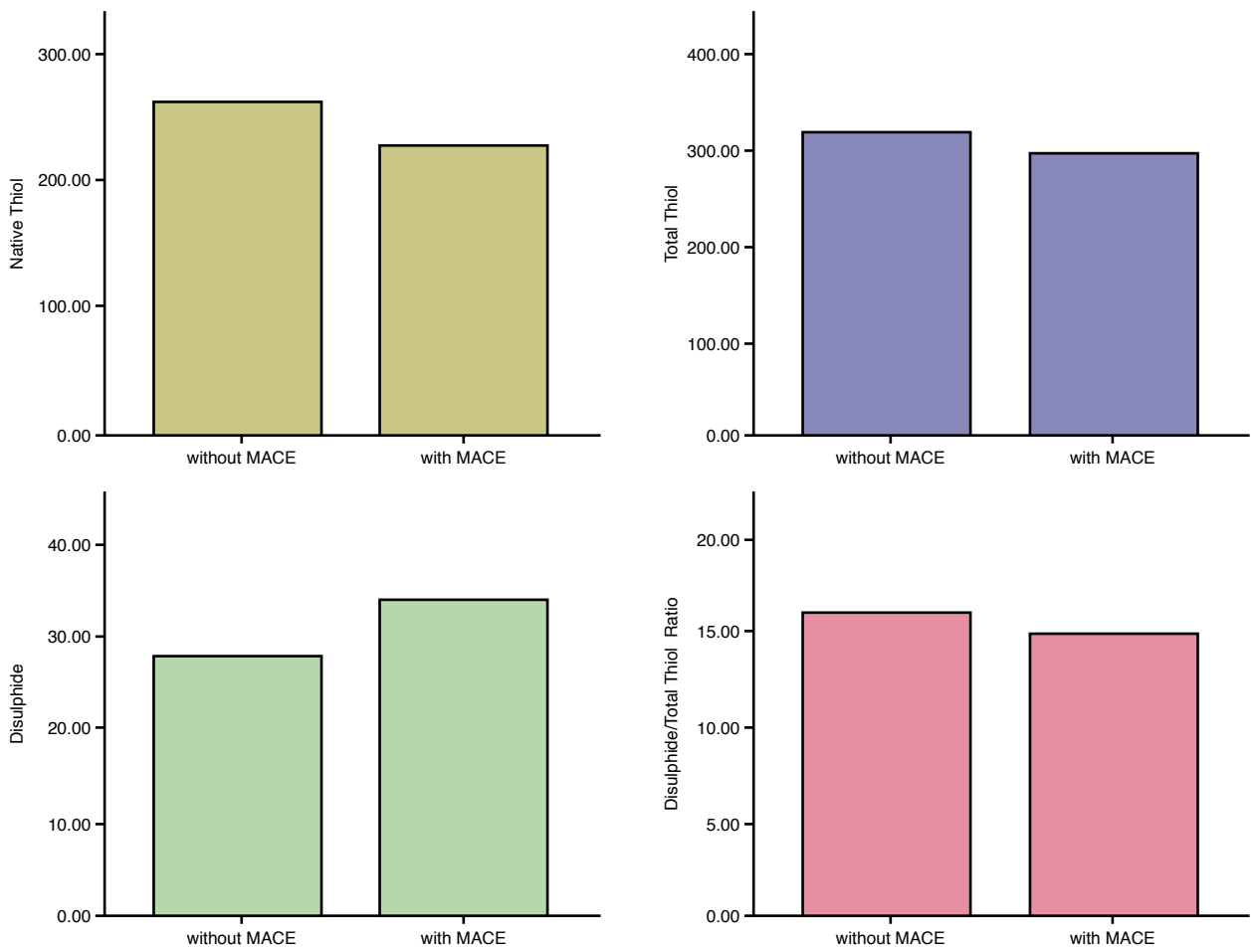

Figure 1. Distribution of native thiol, total thiol, disulphide, and disulphide/native thiol levels in major adverse cardiovascular events (MACE: Major adverse cardiac event).

LVEF, native thiol level, total thiol level, LDL, and serum albumin were most related to MACE during the 6 months of follow up (95% CI: 0.768–0.907. p<0.001; 95% CI: 0.951–0.991, p=0.005; 95% CI: 1.001–1.038, p=0.041; 95% CI: 0.967–0.995, p=0.007; 95% CI: 0.097–0.993, p=0.049, respectively) (Table 5).

DISCUSSION

Our study was designed to demonstrate possible relationships between the thiol/disulphide redox state and its influence on patients with acute MI over 6 months of follow-up. The main findings of the present study were as follows:

1) While native thiol was independently associated with NT-proBNP, CIN, age, stent length, and total thiol were independently associated with NT-proBNP, CIN, and creatinine in linear regression analysis;

2) The initial lower LVEF, native, total thiol level, LDL, and serum albumin were significantly associated with unfavorable events, such as acute coronary syndrome, target vessel revascularization, stroke, and death, occurring in the long term.

Disulphide/thiol reactions in the acute phase of myocardial infarction

Kundi et al.[12] investigated thiol disulphide change in patients with AMI and healthy volunteers. They found significantly lower native thiol, total thiol, and disulphide levels, and significantly higher disulphide/thiol values in the AMI group. In another study, the severity of coronary artery disease accompanied by oxidative stress was associated with a lower disulphide/thiol ratio.[8] In our research, we found significantly lower native thiol, total thiol, and disulphide levels in patients, representing similar results research to those of Kundi et al.[12] However, there was no significant difference in the disulphide/thiol ratio between the STEMI and the normal coronary angiography group. This may be explained by the higher levels of disulphide in our control group than in the Kundi et al. control group. Their control group was selected from individuals without any chronic disease. Our control population consisted of individuals with a normal coronary angiography, despite traditional risk factors and experiencing angina or a positive stress test, which are indications for coronary angiography. Some of these patients had microvascular angina. Thus, these findings might be the result of a similar disulphide/native thiol ratio between our groups. Similarly, it might be the result of a higher disulphide/native thiol ratio than that of Kundi et al. The larger number of patients may increase this ratio toward the STEMI group.

TIMI flow is a valuable acute and chronic survival determinant of AMI (spontaneous reperfusion, no-reflow phenomenon, and TIMI flow grade).[17,21] In patients with AMI, several determinants (increased total oxidant status, oxidative stress index, lipid hydroperoxide), as well as oxidative stress, were shown to be predictors of diminished TIMI flow.[22] This is the first study to investigate the association between TIMI flow and thiol-disulphide values.

Distinct results have been obtained using different biomarkers. A study was performed to determine the effects of several biomarkers, including oxidative stress biomarkers (malondialdehyde and homocysteine), on slow coronary flow. However, there was no significant difference between groups; hence, endothelial and vascular dysfunction could not be explained by oxidative stress.[23] In our study, we did not see a significant relationship between disulphide-thiol values and the status of spontaneous reperfusion, no-reflow, or TIMI flow at STEMI admission. This may show there is not a continually thiol-disulphide switching process and that the oxidation may reach the upper limit until the generation of myocardial infarction. During 6 months, the occurrence of MACEs may be the result of disulphide/thiol-related factors, which are illustrated in Table 3. These thiol-disulphide-related factors: age, CIN, stent length, creatinine, and NT-proBNP, have also been demonstrated to be predictors of MACE in the follow-up period in previous studies,[24–26] and may explain our results.

Native thiol, total thiol and disulphide-related factors

A lower thiol level has been identified as an important marker for detecting chronic inflammation, atherosclerosis, and cardiovascular risk factors.[27] This not only served as a predictor in cardiovascular disease, but it was also predictive for several chronic diseases and cancer, as well as the occurrence of disulphide change. Thiol and disulphide were both lower in patients than in the control group in the presence of oxidative stress, as in our study, even when the study population had no heart disease.[28]

Age, CIN, stent length, and NT-proBNP have been identified as predictors of increased mortality, the development of heart failure, and 6-month MACE. Stent length is an important signifier of the length of significant coronary lesions.[24–26] According to our knowledge, culprit lesion plus previous and following culprit plaques must be covered during stent application. This method can prevent acute stent thrombosis. Therefore, longer coronary lesions may require longer stent applications, and vice versa. The more oxidative corruption and atherosclerosis, the more increase in coronary disease severity, and consequently the stent length will increase.[8–24]

In light of the collected data, our study – in which native thiol and total thiol values were found to be significantly related to age, CIN, NT-proBNP, creatinine, and stent length – helps to explain the coexistence of lower thiols and MACE occurrence.

Prognostic value of thiol and disulphide

In the setting of acute STEMI, in-hospital and 6-month mortality rates are approximately 5% to 7% and 12% to 13%, respectively.[29] Moreover, thiol-disulphide change and increased oxidative stress are independently associated with atherosclerosis before the development of significant coronary heart disease.[4]

Patel et al.[30] demonstrated that oxidative proteins, including thiols, are identifying parameters of coronary events and mortality in the long-term period in coronary heart disease. Furthermore, there was no significant difference between the in-hospital mortality rate, native thiol, total thiol, disulphide, or disulphide/native thiol values between patients. The 6-month MACE rate revealed the highest correlation with LVEF, and native and total thiol, but it was not related to the disulphide level or disulphide/thiol ratio.

In our study, AMI caused in-hospital mortality in 8 patients. This is exactly equal to one-half of the 6-month-mortality rate of our patients. The mean TIMI score of our patients with in-hospital mortality was 9.1, with a SD of 2.4. The mean TIMI score of the patients without in-hospital mortality was 3.3, with a SD of 2.4. Our 6-month mortality rate was 6.6%. Our total MACE rate was estimated to be about 15% in all MI patients. In our study, the mean Global Registry of Acute Coronary Events (GRACE) score of the patients with mortality in 6 months was 149.2, with a SD of 29.1. The mean GRACE score of the patients without mortality in 6 months was 113.0, with a SD of 30.1. Our mortality rate was lower than that of the GRACE study at 6 months (6.6% vs 8.1%).[31] Logically, this may have been the result of novel treatment strategies, new generation stents, and medications (especially novel antiplatelet drugs).

There were some limitations in our study. As it was a single-center study, our patient cohort may be different from that of other centers. The sample size was relatively small, and our results need to be confirmed in large, multicenter prospective trials in the future. Furthermore, because of the observational nature of our study, we did not seek to optimize the patients' cardiac drugs, and in the STEMI group we cannot be sure whether thiol and disulphide values were influenced by hunger or satiety. Our findings were not compared with other oxidative stress markers. Another limitation was that the follow-up time was relatively short in this study; however, prognostic follow up is ongoing. Beyond being a suspicious precipitating factor of STEMI, low thiol levels may be a by-product of STEMI.

In conclusion, initial lower native thiol, total thiol, LVEF, LDL, and serum albumin may be used to identify patients with an increased long-term risk of unfavorable cardiac events in STEMI cases. Future studies may clarify the effects of other oxidative pathways.

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