Oxidative Stress Status Increase in Patients with Nonischemic Heart Failure

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Key Words
Heart failure · Oxidative stress index · Total oxidative stress · Uric acid

Abstract
Objective: It was the aim of this study to investigate the serum oxidative stress level in nonischemic patients with heart failure (HF). Subjects and Methods: The study included 37 patients who presented to the Department of Cardiology, Suleyman Demirel University, Isparta, Turkey, with a diagnosis of asymptomatic HF (New York Heart Association class I–II). The patients had a left ventricular (LV) ejection fraction (EF) of ≤40% and normal coronary arteries or nonsignificant stenosis (stenosis <40%). In addition, 30 age- and sex-matched normal patients were selected as the control group. Clinical and laboratory characteristics presumed to be associated with oxidative stress were evaluated. Results: Demographic characteristics were comparable. However, creatinine and potassium levels were higher in the HF than in the control group. Total oxidative status [2.42 μmol H₂O₂ Eq/l (range 0.74–5.86) vs. 1.81 μmol H₂O₂ Eq/l (range 0.42–3.45); p < 0.01], oxidative stress index [2.24 (range 0.63–5.33) vs. 1.53 (range 0.28–2.51); p < 0.01] and uric acid (6.1 ± 1.8 vs. 4.4 ± 1.1 mg/dl; p < 0.01) levels were significantly higher in the HF than in the control group. The total antioxidant capacity was similar in both groups [1.22 mmol Trolox Eq/l (range 0.61–1.99) vs. 1.18 mmol Trolox Eq/l (range 0.82–1.80); p = 0.77]. The γ-glutamyltransferase levels were also comparable in both groups [32 U/l (range 11–106) vs. 23 U/l (range 11–72); p = 0.10]. Conclusion: The oxidative stress levels were higher in HF patients, and hence, oxidative stress may play an important role in poor prognosis of HF. Therefore, antioxidant treatment might be reasonable.

Introduction

Heart failure (HF) has a poor prognosis despite new improvements in its management [1]. Activation of the sympathetic nervous system is one of the major pathophysiological abnormalities in HF patients [1, 2]. It leads to ventricular remodeling and progression of cardiac dysfunction through various mechanisms, including oxidative stress [2]. Moreover, HF is a state of chronic deterioration of oxidative mechanisms due to enhanced oxidative stress and consequent subcellular alterations [3].

Oxidative stress is defined as an excess production of reactive oxygen species (ROS) relative to the levels of an-
tioxidants, thereby creating an imbalance between pro-
and antioxidant factors in favor of pro-oxidants, and so
potentiating oxidative damage [4]. Over several decades,
clinical and experimental studies have provided substan-
tial evidence that increased oxidative stress could lead to
HF [5–8].

It is difficult to measure each antioxidant separately
because of the number of different antioxidants in plas-
ma, serum, urine or other biological samples. Thus, mea-
surements of the total antioxidant capacity (TAC) and
total oxidant status (TOS) have been used in assessing
oxidant and antioxidant systems in organisms [9, 10]. In
addition, the oxidative stress index (OSI), the ratio of
the total plasma TOS level to TAC, is an indicator of oxidative
stress, in patients with dilated cardiomyopathy in the
literature [15]. In the current study, OSI was calculated
because of the number of different antioxidants in plas-
ma.

Increased generation of ROS has been described in pa-
patients with congestive HF; ROS may play an important
role in the sudden death of these patients [5, 12–14]. To
the best of our knowledge, there is only one study on plas-
ma TOS, TAC levels and OSI, a method for global oxidative
stress, in patients with dilated cardiomyopathy in the
literature [15], and we have found that the oxidative sta-
tus in patients with nonischemic HF was not evaluated.
Hence, we aimed to evaluate the global oxidative status in
patients with nonischemic HF.

Subjects and Methods

Patients and Study Drugs

Seventy-one consecutive patients presented to the Department
of Cardiology with a diagnosis of asymptomatic HF (New York
Heart Association class I–II) between June 2011 and August 2013.
They had undergone coronary angiography to define the etiology
of HF, with normal coronary arteries or nonsignificant stenosis
(stenosis <40%) and a left ventricular (LV) ejection fraction (EF)
of ≤40% in the previous 3 months. Forty-four patients were ex-
cluded from the study (detailed below). The remaining 37 patients
were included in the patient group. In addition, 30 age- and sex-
matched normal patients recruited from the population were in-
cluded as the control group.

Exclusion criteria were HF with significant coronary stenosis,
history of myocardial infarction, moderate or severe valvular heart
disease, severe hypertension (systolic blood pressure ≥180 mm Hg
and/or diastolic blood pressure ≥120 mm Hg), peripheral arterial
disease, diabetes mellitus, dyslipidemia (either a low-density lipo-
protein cholesterol level of ≥155 mg/dl, a triglyceride level of ≥200
mg/dl or the use of lipid-lowering drugs), hypo- or hyperthyroid-
ism, hepatic or renal failure (serum creatinine >2.0 mg/dl), hema-
tological disorders, history of malignancy, inflammatory or infec-
tious disease, asthma, chronic obstructive pulmonary disease, ob-
structive sleep apnea syndrome, Cheyne-Stokes respiration,
cigarette smoking, alcohol use, and any drug use that may affect
the oxidative stress status. All study and control patients gave in-
formed consent, and the study was approved by the institution’s
Ethics Committee.

Complete blood counts, biochemical analyses and echocardi-
ographic measurements were made of all study subjects. Heart rate,
blood pressure and body weight were measured. All medications
were administered according to the current chronic HF guidelines
[1]. All patients received β-blockers, angiotensin-converting en-
zyme inhibitors (lisinopril) and diuretics (spironolactone, thia-
zides, furosemide) in appropriate doses. Angiotensin receptor
blockers were given when intolerance to angiotensin-converting
enzyme inhibitor occurred.

Blood Sample Collection

Blood samples were drawn from an antecubital vein by careful
venipuncture using a 21-gauge needle without stasis between 08.00
and 10.00 a.m. after a resting time of 30 min and a fasting period
of 12 h. Routine biochemical parameters were determined by stan-
dard methods. Hematological indices were measured within 30
min of collecting the blood samples in tubes containing dipotas-
sium EDTA. Biochemical analyses were performed using an auto-
alyzer Olympus AU-640 (Olympus Diagnostica, Hamburg,
Germany). An automatic blood counter (Beckman-Coulter Com-
pany, Miami, Fla., USA) was used for whole blood counts.

For TAC/TOS measurements, a 5-ml blood sample was col-
lected into a plastic tube containing potassium EDTA. TAC and
TOS were measured in every patient and the control group. Blood
samples (4 ml) were obtained following overnight fasting. The se-
rum was separated from the cells by centrifugation for 10 min and
then stored at –80 °C until biochemical examination. TAC and
TOS levels were measured using commercially available kits (Rel
Assay, Gaziantep, Turkey). The level of TAC was measured using
an automated method [16], based on the bleaching of the charac-
teristic color of a more stable 2,2′-azino-bis(3-ethylbenzthiazio-
line-6-sulfonic acid) radical cation by antioxidants. The results
were expressed in mmol Trolox Eq/l. The level of TOS was mea-
sured by a method, in which oxidants present in the sample oxidize
the ferrous ion–o-dianisidine complex to ferric ion. The oxidation
reaction is enhanced by glycerol molecules abundantly present in
the reaction medium. The ferric ion produces a colored complex
with xylene orange in an acidic medium. The color intensity,
which was measured spectrophotometrically, was related to the
total amount of oxidant molecules present in the sample. The assay
was calibrated with hydrogen peroxide and the results were ex-
pressed in µmol H 2 O 2 Eq/l [16].

Determination of OSI

The ratio of TOS to TAC was accepted as the OSI. For calcula-
tion, the resulting unit of TAC was converted to mmol/l, and the
OSI value was calculated according to the following formula: OSI
(AU) = TOS (µmol H2O2 Eq/l)/TAC (mmol Trolox Eq/l) [11].

Echocardiographic Evaluation

Echocardiographic examinations were performed by the same
investigators M.K. and S.T. who were blinded to the data of pa-
patients and controls. Measurements were acquired at the end of ex-
piration during normal breathing in the left lateral decubitus posi-
tion. Two-dimensional, M-mode echocardiography was obtained

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Echocardiography with the System 5 Echocardiography Device (GE Vingmed Ultrasound) with a 2.5-MHz FPA transducer [17]. Echocardiographic results were calculated as the mean of 3 cardiac cycles during electrocardiographic monitoring. Left atrial size, LV diameters and wall thickness were measured using M-mode echocardiography. LVEF was calculated by Simpson’s rule [18].

**Statistical Analysis**

Data were analyzed with the SPSS software version 20.0 for Windows. Continuous variables from the study groups were reported as means ± standard deviations and categorical variables as percentages. To compare continuous variables, the Student t test or Mann-Whitney U test was used, as appropriate. Categorical variables were compared using the χ² test. Pearson’s correlation coefficients were calculated to evaluate the relationships between variables. A 2-tailed p < 0.05 was considered significant.

**Results**

Demographic, clinical and echocardiographic characteristics of the patients are given in table 1. The demographic and clinical characteristics are comparable between the HF patients and the control group. EF [30.2 ± 4.5 (patients group) vs. 64.2 ± 4.2 (control group); p < 0.01], LV end-diastolic volume, LV end-systolic volume (LVESV) and left atrium diameter were higher in the HF group (table 1). Creatinine [1.06 ± 0.29 mg/dl (patient group) vs. 0.89 ± 0.19 mg/dl (control group); p < 0.01], potassium (4.7 ± 0.5 vs. 4.7 ± 0.5 mg/dl; p < 0.01), red blood cell distribution width (RDW; 15.5 ± 1.5 vs. 14.5 ± 1.5%; p < 0.01) and neutrophil-lymphocyte ratio (3.01 ± 1.69 vs. 1.75 ± 0.53; p < 0.01) were significantly higher in the HF than in the control group. However, the hemoglobin level was higher in the control compared to the patient group (13.1 ± 2.1 vs. 14.2 ± 1.8 g/dl, respectively; p = 0.02). Other laboratory values were similar in the two groups (table 2).

Oxidative stress parameters in the patients with non-ischemic HF and the control group are shown in table 3. TOS levels were significantly higher in the patient compared to the control group [2.46 μmol H₂O₂ Eq/l (range 0.74–5.86) vs. 1.81 μmol H₂O₂ Eq/l (range 0.42–3.45), respectively; p < 0.01]. TAC levels were comparable in both groups [1.20 mmol Trolox Eq/l (range 0.61–1.99) vs. 1.7 mmol Trolox Eq/l (range 0.82–1.80), respectively; p = 0.77]. The OSI ratio was significantly higher in the patient than in the control group [2.24 (range 0.63–5.33) vs. 1.53 (range 0.28–2.51), respectively; p < 0.01; fig. 1]. γ-Glutamyltransferase [32 U/l (range 11–106) vs. 23 U/l (range 11–72); p = 0.10] and alkaline phosphatase (77 ± 36 vs. 70 ± 18 U/l; p = 0.58) levels were similar in both

| Table 1. Demographic, clinical and echocardiographic characteristics of the HF and the control group |
|---------------------------------|-----------------|-----------------|---|
| **HF group** (n = 37) | **Control group** (n = 30) | p |
| Mean age, years | 55±10 | 58±12 | 0.25 |
| Male/female | 23/14 | 15/15 | 0.33 |
| BMI | 27.3±5.6 | 25.8±3.1 | 0.09 |
| Waist circumference, cm | 92±13 | 91±10 | 0.60 |
| Systolic BP, mm Hg | 125±13 | 116±28 | 0.08 |
| Diastolic BP, mm Hg | 82±13 | 78±13 | 0.27 |
| Heart rate, bpm | 72±10 | 74±9 | 0.32 |
| EF | 30.2±4.5 | 64.2±4.2 | <0.01 |
| LVEDV, cm³ | 214±31 | 102±12 | <0.01 |
| LVESV, cm³ | 149±28 | 38±6 | <0.01 |
| Interventricular septum, mm | 12.2±1.0 | 11.5±0.9 | 0.02 |
| Posterior wall, mm | 11.4±0.7 | 10.7±1.1 | 0.01 |
| Left atrium, mm | 43±10 | 37±4 | 0.02 |

BMI = Body mass index; BP = blood pressure; LVEDV = LV end-diastolic volume. p values indicate the comparison between the control and the study population. Figures in bold are significant (p < 0.05).

| Table 2. Laboratory characteristics of the HF and the control group |
|-----------------|-----------------|-----------------|---|
| **HF group** (n = 37) | **Control group** (n = 30) | p |
| Glucose, mg/dl | 102±18 | 90±28 | 0.05 |
| Creatinine, mg/dl | 1.06±0.29 | 0.89±0.19 | <0.01 |
| Sodium, mg/l | 138±3 | 138±5 | 0.92 |
| Potassium, mg/l | 4.7±0.5 | 4.2±0.5 | <0.01 |
| AST, U/l | 22±7 | 19±5 | 0.13 |
| ALT, U/l | 22±14 | 18±5 | 0.23 |
| TSH | 1.71±1.23 | 1.58±1.12 | 0.69 |
| Hemoglobin, g/dl | 13.1±2.1 | 14.2±1.8 | 0.02 |
| Platelets, ×10⁹/mm³ | 237±57 | 260±65 | 0.12 |
| WBC, ×10³/ml | 7.0±2.0 | 6.6±1.6 | 0.36 |
| RDW, % | 15.5±1.5 | 14.5±1.5 | <0.01 |
| Neutrophils, ×10⁹/ml | 4.46±1.72 | 3.73±1.26 | 0.08 |
| Lymphocytes, ×10⁹/ml | 1.78±0.85 | 2.20±0.61 | 0.05 |
| NLR | 3.01±1.69 | 1.75±0.53 | <0.01 |

AST = Aspartate transaminase; ALT = alanine transaminase; TSH = thyroid-stimulating hormone; WBC = white blood cells; NLR = neutrophil-lymphocyte ratio. p values indicate the comparison between the control and the study population. Figures in bold are significant (p < 0.05).
groups. However, uric acid (6.1 ± 1.8 vs. 4.4 ± 1.1 mg/dl; p < 0.01) and high-sensitive C-reactive protein (7.6 ± 6.2 vs. 4.1 ± 2.2 mg/l; p < 0.01) levels were higher in the HF group compared to the control group (table 3).

In the correlation analysis, EF (p = 0.001, r = −0.43) and the TAC level (p < 0.001, r = −0.47) were negatively correlated with OSI. TOS (p < 0.001, r = 0.84), uric acid (p = 0.001, r = 0.42; fig. 2); the RDW level (p = 0.001, r = 0.43) and LVESV (p = 0.005, r = 0.37) were positively correlated with OSI.

Table 3. Comparison of parameters associated with oxidative stress in the HF and the control group

| Parameter          | HF group (n = 37) | Control group (n = 30) | p   |
|--------------------|-------------------|------------------------|-----|
| TOS, μmol H₂O₂ Eq/l| 2.42 (0.74–5.86)  | 1.81 (0.42–3.45)       | <0.01|
| TAC, mmol Trolox Eq/l| 1.22 (0.61–1.99)  | 1.17 (0.82–1.80)       | 0.77 |
| OSI, AU            | 2.24 (0.63–5.33)  | 1.53 (0.28–2.51)       | <0.01|
| ALP, U/l           | 77±36             | 70±18                  | 0.58 |
| GGT, U/l           | 32 (11–106)       | 23 (11–72)             | 0.10 |
| Uric acid, mg/dl   | 6.1±1.8           | 4.4±1.1                | <0.01|
| Hs CRP, mg/l       | 7.6±6.2           | 4.1±2.2                | <0.01|

ALP = Alkaline phosphatase; GGT = γ-glutamyltransferase; Hs CRP = high-sensitivity C-reactive protein. p values indicate the comparison between the control and the study population. Figures in bold are significant (p < 0.05).

Discussion

In the present study, OSI levels, a marker of oxidative status, were significantly higher in the patients with nonischemic HF compared to the control subjects; uric acid and the TOS level were also higher in nonischemic HF patients compared to the control group. More importantly, OSI was positively correlated with RDW, LVESV, uric acid and the TOS level and negatively correlated with the TAC level and EF.

Oxidative stress results from an imbalance between ROS generation and antioxidant defensive mechanisms. ROS play a key role in the pathogenesis of a variety of cardiovascular diseases; the generation of increased levels of ROS has been shown to contribute directly to the progression of atherosclerosis, hypertension, reperfusion injury due to acute myocardial infarction and HF [18, 19]. A number of experimental and clinical studies have demonstrated an increased generation of ROS in HF patients [4–7]; hence, the activity of antioxidants may be reduced [20]. The importance of oxidative stress in HF patients may be associated with the process underlying LV hypertrophy, adverse LV remodeling and HF [21–23]. The most widely recognized effects of increased oxidative stress in the heart that cause cellular dysfunction, protein and lipid peroxidation, and DNA damage, leading to irreversible cell damage and death, have been implicated in a wide range of pathological cardiovascular conditions [3,
The role of oxidative stress is increasingly emerging with respect to a pathophysiological mechanism of cardiac remodeling responsible for the development and progression of HF [3, 24]. Plasma lipid peroxidation, an indicator of oxidative stress, is increased in patients with dilated cardiomyopathy and positively correlates with the severity of symptoms [25, 26]. Also, similar to our study, there is an inverse correlation between lipid peroxidation parameters and cardiac performance (EF, exercise capacity) [25]. These results may be associated with the effect of increased oxidative stress on LV remodeling and disease progression.

As in our study, Demirbag et al. [15] reported that OSI and uric acid levels increased in patients with idiopathic dilated cardiomyopathy. However, the TAC levels of plasma were significantly lower in the control subjects. Previous studies by Hill and Singal [6] demonstrated that HF subsequent to myocardial infarction was associated with an antioxidant capacity deficit as well as with increased oxidative stress. However, unlike these studies, we excluded HF due to ischemic etiology. Therefore, in our study, the TAC level was comparable in both groups but the TOS levels were significantly higher in the HF group. In addition, the uric acid level was moderately positively correlated with OSI (fig. 2). Similarly to our study, Demirbag et al. [15] found a negative correlation between EF and OSI. In an animal experiment, it was also reported that there was a significant negative correlation between the plasma lipid peroxidation product, malondialdehyde and LVEF ($r = 0.35$) [5]. Diraman et al. [27] found that antioxidant enzyme levels for patients with an EF $<25\%$ were significantly lower than for patients with an EF $\geq 25\%$. A previous study has shown that in children with idiopathic dilated cardiomyopathy undergoing standard treatment, abnormal antioxidant enzyme activity was found [28]. Sezgin et al. [29] have found similar results in patients with less severe HF when an EF limit of 35% was used. In addition, Erdogan et al. [30] showed that a 3-month treatment with allopurinol, an antioxidant agent, was significantly associated with reduced uric acid levels and improvement in coronary flow reserve and LV functions in patients with idiopathic dilated cardiomyopathy and hyperuricemia. Based on the aforementioned data, we suggest that antioxidant treatment could be used widely in HF patients.

The limitations of this study include the small number of patients with nonischemic HF. In addition, the analysis was based on a simple baseline determination at a single time point, which might not reflect patient status over long periods.

**Conclusion**

The present study showed that OSI levels were significantly increased in patients with nonischemic HF compared to control participants. The change in TOS, OSI and uric acid levels in the study groups might indicate increased oxidative status in patients with HF. This may be associated with increased cardiovascular risk. Our findings also suggest that antioxidant treatment might be helpful for these patients. However, further prospective studies are needed to establish the pathophysiological and clinical significance of increased oxidative status in patients with HF.

**Disclosure Statement**

The authors have no conflicts of interest to declare.
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