

## 8-Hydroxy-2'-Deoxyguanosine and Lipid Peroxidation in Patients With Heart Failure

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**Introduction and objectives.** Heart failure is associated with increased free radical production, which leads to a state of oxidative stress. Known markers of oxidative stress include 8-hydroxy-2'-deoxyguanosine, which reflects oxidative damage to DNA, and lipid peroxidation, which can be used to quantify damage to lipid-rich structures. The aims of this study were to compare 8-hydroxy-2'-deoxyguanosine and lipid peroxidation levels in heart failure patients and healthy subjects and to assess how these levels are influenced by heart failure etiology.

**Methods.** The study included 78 patients (57 male, age 64 [14] years) with heart failure and 12 control subjects. Patients completed a questionnaire and were graded according to the New York Heart Association classification. Doppler echocardiography was performed and blood samples were obtained. 8-hydroxy-2'-deoxyguanosine and lipid peroxidation levels were determined.

**Results.** Significant differences were observed between patients and control subjects in 8-hydroxy-2'-deoxyguanosine and lipid peroxidation levels, at 0.34 (0.54) ng/mL vs 0.04 (0.07) ng/mL ( $P < .05$ ), and 18 (10)  $\mu$ M vs 8 (3)  $\mu$ M ( $P < .01$ ), respectively. Subsequent analysis showed that heart failure etiology had a significant effect on the levels of the two markers ( $P < .05$ ), which were highest in patients with hypertensive cardiomyopathy.

**Conclusions.** Levels of 8-hydroxy-2'-deoxyguanosine and lipid peroxidation were higher in heart failure patients than in control subjects. The most significant increases were found in patients with hypertensive cardiomyopathy.

**Key words:** Oxidative stress. Heart failure. Free radicals.

### Valores de 8-hidroxi-2'-desoxiguanosina y de peroxidación lipídica en pacientes con insuficiencia cardiaca

**Introducción y objetivos.** La insuficiencia cardiaca está asociada con un incremento en la producción de radicales libres, llegándose al estado de estrés oxidativo. Se conocen diversos marcadores de estrés oxidativo, como la 8-hidroxi-2'-desoxiguanosina, marcador del daño oxidativo en el ADN, y la peroxidación lipídica que permite cuantificar el daño en las estructuras ricas en lípidos. El propósito de este estudio es comparar los valores de 8-hidroxi-2'-desoxiguanosina y de peroxidación lipídica en pacientes con insuficiencia cardiaca y sujetos sanos, y evaluar la influencia de la etiología.

**Métodos.** Estudiamos a 78 pacientes (57 varones, edad 64  $\pm$  14 años) diagnosticados de insuficiencia cardiaca y a 12 controles. Los pacientes completaron un cuestionario y fueron clasificados de acuerdo con la New York Heart Association. Se les realizó un estudio eco-Doppler y extracción de sangre. Medimos las concentraciones de 8-hidroxi-2'-desoxiguanosina y de peroxidación lipídica.

**Resultados.** Al comparar los valores de 8-hidroxi-2'-desoxiguanosina y peroxidación lipídica entre pacientes y controles obtuvimos diferencias significativas (0,34  $\pm$  0,54 frente a 0,04  $\pm$  0,07 ng/ml,  $p < 0,05$  y 18  $\pm$  10 frente a 8  $\pm$  3  $\mu$ mol/l,  $p < 0,01$ , respectivamente). Cuando comparamos las concentraciones de los 2 marcadores según la etiología de la insuficiencia cardiaca encontramos diferencias significativas en ambos ( $p < 0,05$ ), que fueron mayores en la miocardiopatía hipertensiva.

**Conclusiones.** Los valores de 8-hidroxi-2'-desoxiguanosina y peroxidación lipídica se encuentran aumentados en los pacientes con insuficiencia cardiaca al compararlos con los controles. El incremento más importante lo encontramos en pacientes con miocardiopatía hipertensiva.

**Palabras clave:** Estrés oxidativo. Insuficiencia cardiaca. Radicales libres.

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## ABBREVIATIONS

ROS: reactive oxygen species.  
 LPO: lipid peroxidation.  
 8-OHdG: 8-hydroxy-2'-deoxyguanosine.  
 ARBs: angiotensin II receptor blockers.  
 ACE inhibitors: angiotensin converting enzyme inhibitors.  
 LVEF: left ventricular ejection fraction.

## INTRODUCTION

Heart failure is a complex clinical syndrome characterized by the activation of different neurohormonal systems and proinflammatory mediators.<sup>1,2</sup>

A number of studies have reported that oxidative stress plays an important part in the pathophysiology and development of heart failure via the production of free radicals.<sup>3-5</sup> Reactive oxygen species (ROS) have a key role in the onset and progression of coronary heart disease, tissue necrosis (within the framework of myocardial infarction), and contractile dysfunction.<sup>6,7</sup> An increase in the presence of ROS is also associated with damage to the mitochondrial<sup>8,9</sup> and nuclear<sup>10</sup> DNA.

Reactive oxygen species, including the superoxide anion ( $O_2^-$ ), the hydroxyl radical ( $-OH$ ), and hydrogen peroxide, are products of metabolism. Under normal circumstances, antioxidant enzyme systems, such as that represented by superoxide dismutase, as well as non-enzymatic systems, such as those represented by uric acid or ceruloplasmin, maintain ROS in low concentrations. Oxidative stress occurs when there is a shift in the equilibrium in favor of free radical formation.<sup>11</sup>

Several plasma biomarkers of oxidative damage have been proposed, including lipid peroxidation (LPO) and 8-hydroxy-2'-desoxyguanosine (8-OHdG) levels.<sup>12-14</sup> Lipid peroxidation is a marker of the oxidation of fatty acids, and has been studied by other authors in connection with heart failure.<sup>4</sup> 8-hydroxy-2'-desoxyguanosine, however, is a new marker of oxidative damage to genetic material; indeed, it has been proposed as a very sensitive and specific marker of DNA damage.<sup>15,16</sup> However, no studies have been performed that compare the capacity of these biomarkers in heart failure. Differences have been reported, however, in endothelial function and immune activation parameters depending on the cause of heart failure.<sup>17</sup> The present work tests the hypothesis that, in a cohort of patients with heart failure, the level of oxidative stress may be related to the etiology of this disease. The lipid peroxidation and 8-OHdG levels of these patients and of healthy controls were therefore analyzed and compared, and their influence on heart failure etiology determined.

## METHODS

### Patients

The study subjects were 78 consecutive patients diagnosed with heart failure<sup>18</sup> (57 men, age 64 [14] years) and 12 controls of similar age and sex ratio. The control patients were referred to the cardiology departments of the participating hospitals from their internal medicine units. In order to confirm their validity as controls, their medical histories were studied and they underwent electrocardiographic, echocardiographic, and biochemical analyses similar to those to which the heart failure patients were subjected. All subjects underwent a physical examination, electrocardiographic, and Doppler echocardiographic studies, and a chest x-ray. Blood was extracted for biochemical and hematological analyses. The cardiological diagnoses reached were: ischemic heart disease (39%), dilated cardiomyopathy (42%), and hypertensive cardiomyopathy (19%).

The patients were classified according to the criteria of the New York Heart Association<sup>19</sup> and received medical treatment according to those of the European Society of Cardiology<sup>18</sup>: 74% received diuretics, 73% received angiotensin converting enzyme inhibitors (ACE inhibitors), 51% were treated with beta-blockers, 42% with anti-aldosterone agents, 26% with digoxin, 10% with calcium antagonists, and 16% with angiotensin II receptor blockers (ARBs). No patient regularly took vitamins A, E, or C. All had received stable medical treatment for at least one month before their inclusion in the study; this helped avoid any effects due to changes in medication.

The distribution of the treatments for the three etiologies was as follows. Among the patients with ischemic etiology, 11% received ARBs, 83% received ACE inhibitors, 57% received beta-blockers, and 76% received diuretics. Among those with dilated cardiomyopathy, 21% received ARBs, 78% received ACE inhibitors, 46% received beta-blockers, and 82% received diuretics. Among those with hypertensive cardiomyopathy, 14% received ARBs, 57% received ACE inhibitors, 50% received beta-blockers, and 64% received diuretics.

Patients with atrial fibrillation, acute coronary syndromes, chronic or acute liver disease, chronic infections, kidney disease, or chronic obstructive pulmonary disease were excluded.

The study was performed in accordance with good clinical practice guidelines and following the ethical guidelines for human experimentation established by the Declaration of Helsinki. All subjects gave their written consent to be included.

### Doppler Echocardiography

Doppler echocardiography was performed using standard equipment with 2.5 MHz transducers. Apical

and parasternal views were obtained. The echocardiographic and Doppler studies were recorded on videotape for later, centralized analysis using Eco-Dat software (Eco-Dat, Software de Medicina S.A.); this was performed blind to other results. The area-length method was used to determine the left ventricular ejection fraction (LVEF).<sup>20</sup> Pulsed Doppler analysis was used to determine the early maximum diastolic velocity of the transmitral flow (E wave), and the deceleration time was calculated.

### Determination of Oxidative Stress Markers

Blood samples were taken by venipuncture and centrifuged at 3000 rpm for 10 min. Serum and plasma was divided into aliquots at room temperature and 4°C respectively, and then stored at -80 °C.

The serum 8-OHdG concentration was determined by ELISA immunoenzyme assay using the Bioxytech® 8-OHdG-EIA™ kit; the results are expressed in ng/mL. The determination of LPO in the plasma was based on a colorimetric technique using the Bioxytech® LPO-560™ kit; the results are expressed in μM/L.

### Statistical Analysis

Quantitative variables were expressed as means±standard deviation (SD). The normality of the distribution of the variables was analyzed using the Kolmogorov-Smirnov test. The Mann-Whitney U test was used to compare the 8-OHdG and LPO values of patients and controls. Kruskal-Wallis analysis of variance was used to compare the values of the oxidative stress markers according to disease etiology. Significance was set at *P*<.05. SPSS 11.5 software (SPSS Inc., Chicago, Illinois) was used for all calculations.

## RESULTS

Table 1 shows the functional and clinical characteristics of the patients. For the patients as a whole, the mean 8-OHdG value was 0.34 (0.54) ng/mL; the mean LPO value was 18 (10) μM/L.

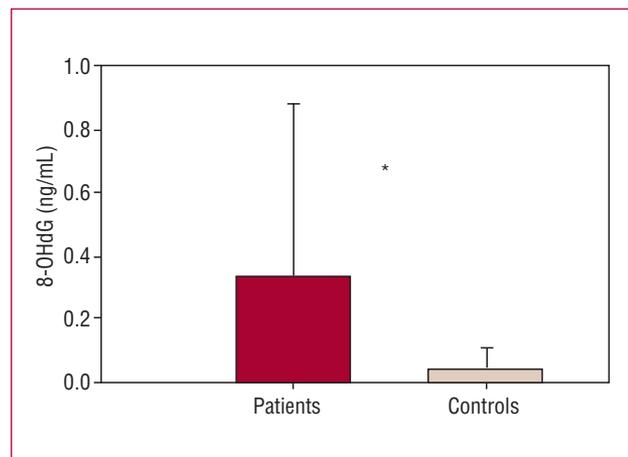
The mean 8-OHdG value of the patients was significantly higher than that of the controls (0.34 [0.54] compared to 0.04 [0.07] ng/mL; *P*<.05) (Figure 1); the same was seen with respect to the LPO values (18 [10] compared to 8 [3] μM/L; *P*<.01) (Figure 2).

With respect to disease etiology, the 8-OHdG levels were 0.22 [0.38] ng/mL in the dilated cardiomyopathy group, 0.25 [0.35] ng/mL in the ischemic etiology group, and 0.78 [0.87] ng/mL in the hypertensive cardiomyopathy group (Figure 3) (*P*<.05, largely due to the high values of the patients with hypertensive cardiomyopathy). Finally, the LPO levels for the three etiology groups were: 15.8 [9.4] μM/L in the dilated cardiomyopathy group, 19.5 [8.8] μM/L in the ischemic etiology group, and 21.6

**TABLE 1. Clinical Characteristics of the Patients**

Variables	Patients (n=78)
Male sex (%)	73
Age (years)	64 (14)
SBP (mm Hg)	127 (20)
HR (beats/min)	78 (13)
HDL (mg/dL)	45(13)
Total cholesterol (mg/dL)	192 (44)
Hematocrit (%)	42 (6)
Smokers (%)	10
Hypertension (%)	51
Diabetes (%)	37
NYHA (%)	
I	9
II	70
III	21
LVEF (%)	35 (12)
Deceleration time (ms)	197 (79)
Markers of oxidative stress	
8-OHdG (ng/mL)	0.34 (0.54)
LPO (μM/L)	18 (10)

HR: heart rate; LVEF: left ventricular ejection fraction; HDL: high density lipoproteins; LPO: lipid peroxidation; NYHA: New York Heart Association; SBP: systolic blood pressure; 8-OHdG: 8-hydroxy-2'-deoxyguanosine.

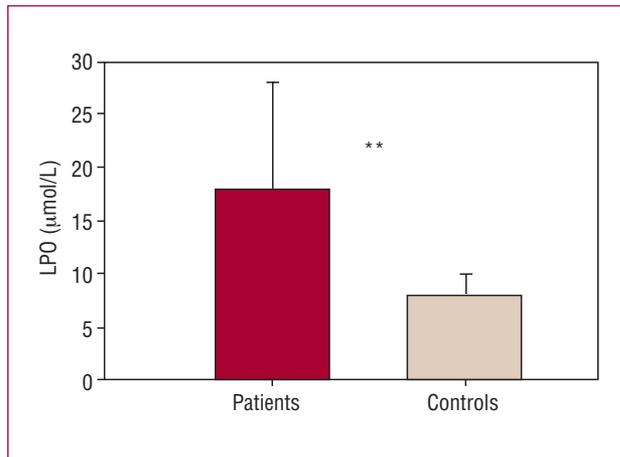


**Figure 1.** 8-hydroxy-2'-deoxyguanosine levels in patients with heart failure and controls. 8-OHdG: 8-hydroxy-2'-deoxyguanosine \**P*<.05. Data are means±SD.

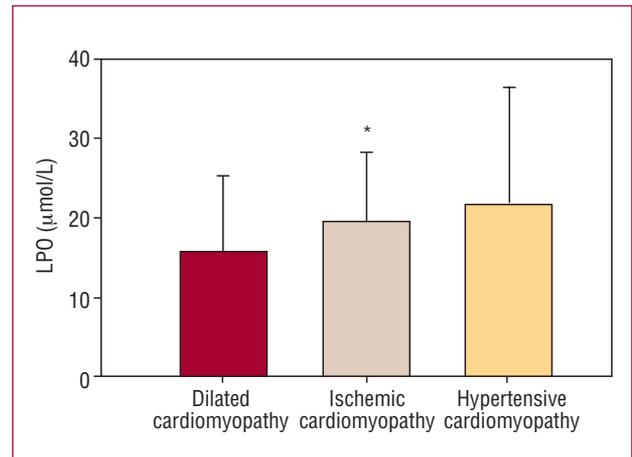
[14.6] μM/L in the hypertensive cardiomyopathy group (*P*<.05) (Figure 4).

## DISCUSSION

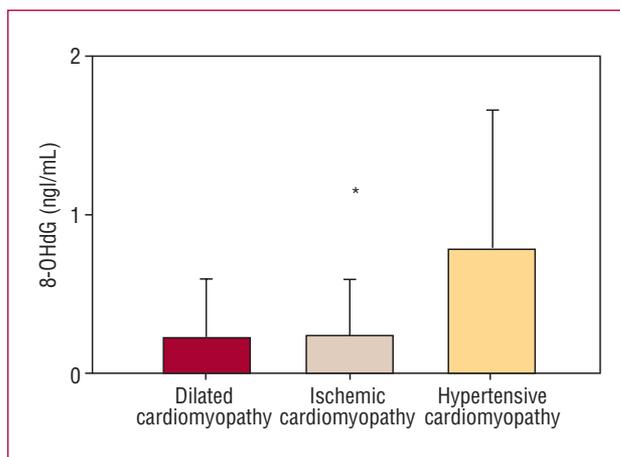
Free radicals are molecules whose atomic structures have an unpaired electron in their outer orbit. This renders them a spatial structure that generates instability. They are extremely reactive and have very short half-lives. Free radicals are made continuously as by-products of



**Figure 2.** Lipid peroxidation levels in patients with heart failure and controls.  
LPO: lipid peroxidation.  
\*\* $P < .01$ .  
Data are means $\pm$ SD.



**Figure 4.** Lipid peroxidation levels in the patients of the three etiological groups: dilated, ischemic and hypertensive cardiomyopathy.  
\* $P < .05$ .  
Data are means $\pm$ SD.



**Figure 3.** 8-hydroxy-2'-deoxyguanosine levels in the patients of the three etiological groups: dilated, ischemic and hypertensive cardiomyopathy.  
\* $P < .05$ .  
Data are means $\pm$ SD.

the metabolism of normal cells, and are inactivated by a number of mechanisms whose job it is to maintain these radicals and antioxidants in equilibrium, thus minimizing and delaying the appearance of damage. The best known ROS are the superoxide anion ( $O_2^-$ ), hydrogen peroxide ( $H_2O_2$ ), and the hydroxyl radical ( $-OH$ ).<sup>21</sup>

An increase or reduction in the physiological concentrations of ROS may give rise to important functional alterations. The mitochondria are the most important source of free radicals,<sup>22</sup> but they are normally produced in quantities that are controlled by the cellular defense mechanisms. In pathological situations, such as in cancer,<sup>23</sup> diabetes,<sup>24</sup> degenerative diseases,<sup>25,26</sup> or

cardiovascular disease,<sup>27,28</sup> the production of free radicals is increased substantially and/or the antioxidant mechanisms are in deficit; this can lead to oxidative stress and cell damage. The abnormal diffusion of oxygen in a hypertrophic myocardium generates a progressive energy deficit associated with the molecular mechanisms of oxidative stress in conditions of ischemia/reperfusion.<sup>29,30</sup> There is evidence suggesting the participation of oxidative stress in the pathophysiology of high blood pressure and its complications.<sup>31</sup>

When free radicals collide with biomolecules, their high atomic instability causes the latter to release an  $H^+$  ion and thus become oxidized. This leads to a chain reaction causing certain molecules in the cell to no longer be able to perform their functions. When fatty acid are oxidized in the cell membrane they become fatty acid radicals capable of oxidizing other molecules. This process is known as LPO, and its measurement allows the concentration of hydroperoxidised lipids — the products of this peroxidation — to be determined.<sup>21</sup>

DNA can also be damaged by free radicals. Damage to nucleic acids is reflected as modified bases, which could cause mutations and functional damage. DNA can be repaired in response to this aggression; this produces 8-OHdG which can also be used as a marker of oxidative stress.<sup>32</sup>

In the present study, the patients with heart failure showed concentrations of 8-OHdG and LPO levels significantly higher than those seen in the control subjects — values in agreement with those reported in other studies.<sup>4,33</sup> Differences in these two markers were also seen between the three etiological groups, ie, patients with dilated cardiomyopathy, ischemic etiology, and hypertensive cardiomyopathy; the values for both markers were highest in the last of these groups.

Together these results show that patients with heart failure, and particularly those in whom this disease is of hypertensive etiology, have higher LPO levels and suffer increased DNA damage. The administration of antioxidants could be of great benefit in such patients,<sup>34-37</sup> although the use of certain vitamins remains controversial.<sup>38,39</sup>

This study may have a limitation in that the most deteriorated functional classes are not very well represented – the majority of the present patients belonged to NYHA functional class II. However, a high percentage (21%) did belong to functional class III, which might, therefore, be considered sufficiently represented. In addition, only 19% of the patients had a hypertensive etiology, although the three etiology groups did show significant differences in terms of oxidative stress levels when compared.

The strict exclusion criteria favored the attainment of the goals set. Given that some of the diseases excluded are commonly associated with heart failure, it could be claimed that the results obtained are not fully representative of what is seen in daily practice. Even though this may be argued, this work still provides important knowledge and is of notable pathophysiological value.

The fact that the members of the three etiological groups were not treated in exactly the same way may have some influence on the results. However, no significant differences were seen in biomarker levels between patients treated with ARBs, ACE inhibitors, or beta-blockers.

It should be remembered that the most precise measurements of left ventricular function are achieved with magnetic resonance imaging.<sup>40</sup> However, the Doppler echocardiographic results of the present study were all examined by a cardiologist experienced in this area; this, and the variability seen in the present results, suggests they are reliable.

## CONCLUSIONS

The concentration of 8-OHdG and the level of LPO are increased in patients with heart failure compared to control subjects. Among these patients, the highest oxidative stress levels were seen in those with hypertensive cardiomyopathy. Given the information they provide with respect to therapy, the measurement of these markers should be included in the analysis of patients with heart failure.

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