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# Differences in oxidative stress markers based on the aetiology of heart failure: Comparison of oxidative stress in patients with and without coronary artery disease

YASUHIRO NAGAYOSHI[1], HIROAKI KAWANO[2], JUN HOKAMAKI[2], TAKASHI UEMURA[2], HIROFUMI SOEJIMA[2], KOICHI KAIKITA[2], SEIGO SUGIYAMA[2], HIROSHIGE YAMABE[2], IZURU SHIOJI[3], SHINYA SASAKI[3], YUTAKA KURODA[1], & HISAO OGAWA[2]

[1]*Department of Community Medicine, Kumamoto University Hospital, Kumamoto City, Japan,* [2]*Department of Cardiovascular Medicine, Graduate School of Medical Sciences, Kumamoto University, Kumamoto City, Japan, and* [3]*Shino-Test Corporation, Tokyo, Japan*

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**Abstract**

Various oxidative stress markers have been measured to evaluate the status of heart failure (HF). However, the relationships between these markers and the aetiology of HF have not been fully investigated. This study compared 8-hydroxy-2′-deoxyguanosine (8-OHdG) and biopyrrins levels in patients with ischemic and non-ischemic HF. Study subjects were divided into a coronary artery disease (CAD) group ($n$ = 70), a non-CAD group ($n$ = 61) and a control group ($n$ = 33). In the CAD group, 8-OHdG and biopyrrins levels increased with the severity of the New York Heart Association (NYHA) functional class and log BNP levels correlated with 8-OHdG and biopyrrins levels. However, non-CAD patients with NYHA class III/IV had significantly lower 8-OHdG levels than CAD patients with NYHA class III/IV and the levels did not correlate with log BNP levels. In the CAD group, 8-OHdG levels reflected the severity of atherosclerosis. These results indicate that the properties of oxidative stress markers should be carefully taken into consideration for the assessment of HF status.

**Keywords:** *Biopyrrins, oxidative stress, 8-hydroxy-2′-deoxyguanosine, heart failure*

## Introduction

In patients with chronic heart failure (HF), the presence of coronary artery disease (CAD) plays a crucial role in mortality and morbidity. Ischemic cardiomyopathy is the ultimate result of CAD. Patients with ischemic cardiomyopathy have a worse prognosis than patients with non-ischemic cardio-myopathy [1,2]. The aetiology of HF also influences the response to medical treatment such as amiodar-one, beta-blockers and calcium channel blockers [3]. Appropriate revascularization therapy should be considered to improve the prognosis in patients with ischemic cardiomyopathy. For these reasons, classification of HF patients based on the presence of CAD is important for risk stratification.

Recently, various studies have implicated oxidative stress in the progression of left ventricular (LV) remodelling and failure, and congestive heart failure is characterized by chronic and progressive LV systolic dysfunction. Accordingly, surrogate markers of oxidative stress are currently measured to evaluate the status of HF [4–9]. However, only a few studies have assessed the association of oxidative stress levels with the aetiology of HF. Nucleobases and their corresponding 2′-deoxyribonucleotides are highly susceptible to oxidation and subsequent damage, leading to specific replication errors. Urinary 8-hydroxy-2′-deoxyguanosine (8-OHdG) serves as a putative marker of systemic oxidatively generated damage to DNA

Correspondence: Yasuhiro Nagayoshi, MD, Department of Community Medicine, Kumamoto University Hospital, Kumamoto City, Japan. 1-1-1 Honjo, Kumamoto City 860-8556, Japan. Tel: +81-96-373-5175. Fax: +81-96-362-3256. Email: ynagayos@kumamoto-u.ac.jp

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[10,11]. In addition, excessive reactive oxygen species (ROS) activates hemeoxygen-ase, the rate-limiting enzyme in the heme degradative pathway, leading to the production of bilirubin [12,13]. Biopyrrins are oxidatively-modified metabolites of bilirubin and as such are potential markers of scavenged radicals [14,15]. The present study was designed to compare patients with an ischemic and non-ischemic aetiology of HF to determine whether these oxidative stress markers are different.

## Methods

The study subjects comprised 164 consecutive patients (115 men and 49 women; mean age 64 years) with reduced left ventricular systolic function (left ventricular ejection fraction [LVEF] $<$ 55%) and control subjects (19 men and 14 women; mean age 69 years). The diagnosis of heart disease was based on the clinical history and results of physical examination, electrocardiogram, chest X-ray, echocardiogram, left ventriculogram and coronary angiogram. The New York Heart Association (NYHA) functional classification was evaluated at the time of admission. Patients with NYHA functional class I had cardiac disease that did not limit physical activity; ordinary physical activity in these patients did not cause undue fatigue, palpitation, dyspnea or angina. The remaining patients with heart disease were symptomatic for heart failure. Patients in NYHA functional class III/IV had significant clinical findings (e.g. oedema, $S_3$ and pulmonary congestion). The cause of heart disease was ischemic cardiomyopathy in 70 patients, idiopathic dilated cardiomyopathy (DCM) in 46 patients, dilated phase of hypertrophic cardiomyopathy in two patients and hypertensive heart disease in 13 patients. We excluded patients with active autoimmune disease, cancer, severe renal insufficiency and severe liver dysfunction on admission. Renal insufficiency was defined by a serum creatinine of $>$2.0 mg/dl. We used the abbreviated MDRD equation to estimate the glomerular filtration rate [16]. We also excluded those patients who had undergone coronary artery bypass surgery or angio-plasty in the preceding 3 months. Risk factors for coronary artery disease included advancing age ($>$75 years of age), hypertension, dyslipidemia, diabetes mellitus, cigarette smoking and obesity. Diabetes mellitus was defined according to the criteria of the American Diabetes Association [17]. The control subjects had suspected angina pectoris, but showed no coronary stenosis or coronary spasm after intracoronary injection of acetylcholine. These subjects had preserved left ventricular systolic function (mean LVEF of 73.4%). In the control subjects, the prevalence of coronary risk factors was advancing age, 15% ($n$ = 5); hypertension, 48% ($n$ = 16); dyslipidemia, 52% ($n$= 17); current smoking, 21% ($n$ = 7); diabetes mellitus, 24% ($n$ = 8); and obesity, 45% ($n$ = 15).

The study protocol was approved by the Human Ethics Review Committee of Kumamoto University School of Medicine and a signed consent form was obtained from each subject.

All subjects supplied urine samples in the early morning after admission. All urine samples were immediately centrifuged at 2000 rpm for 10 min at 4°C and stored at –80° C for subsequent analysis. Biopyrrins were measured using an enzyme immunoassay kit based on a monoclonal antibody (Shinotest Co, Tokyo, Japan). A competitive enzyme-linked immunosorbent assay kit was used to measure 8-OHdG levels (8-OHdG check, Japan Institute for the Control of Aging, Shizuoka, Japan). The results were expressed relative to the urinary creatinine concentration and reported in μmol/g creatinine for biopyrrins and ng/mg creatinine for 8-OHdG. Blood samples were obtained from an antecubital vein immediately after admission. The plasma concentration of B-type natriureticpeptide (BNP) was measured with a specific radioimmunoassay for human BNP.

The hemodynamic state of subjects was measured using a Swan-Ganz catheter. LVEF was measured by left ventriculography. Coronary artery narrowing was visually assessed and reported as the percentage of luminal diameter stenosis. Angiograms were assessed by two cardiologists who were unaware that the patients were enrolled in the study. Significant CAD was defined as $>$75% narrowing of the internal diameter of the main vessels or their major branches and $>$50% narrowing of the left main coronary artery. The severity of CAD was represented by the number of diseased vessels.

All results except for urinary biopyrrins and plasma BNP are expressed as the mean ± SEM. Multiple group comparisons of continuous data were performed with a one-way analysis of variance, followed by Scheffe's method to compare individual groups. Other clinical characteristics were compared by either a chi-squared or Fisher's exact test. Plasma levels of BNP and urinary levels of biopyrrins were not distributed normally and thus expressed as the median value (25-75$^{th}$ percentile ranges). Non-parametric analysis was used to compare these variables using both a Kruskal-Wallis and Mann-Whitney U-test. Linear regression analysis was used to determine the correlation among urinary 8-OHdG levels, log BNP levels and log biopyrrins levels. A $p$-value $<$0.05 was considered significant in all statistical analyses.

## Results

The baseline characteristics of the patients with reduced left ventricular systolic function are listed in Table I. Patients in the CAD group were significantly

older than those in the non-CAD group (69 ± 1 vs 59 ± 2, respectively, $p < 0.01$). Half of the patients with CAD also had diabetes mellitus and the mean HbA1C level in the group was significantly higher than that in the non-CAD group (6.4 ± 0.2 vs 5.7 ± 0.1, respectively, $p < 0.01$). The prevalence of dyslipidemia was higher in CAD patients. The difference in BNP levels between CAD and non-CAD patients was not significant, although the mean LVEF was significantly lower in the non-CAD group than in the CAD group (33.5 ± 1.6 vs 40.5 ± 1.3, respectively, $p < 0.01$).

Patients in the CAD and non-CAD groups were further divided into three sub-groups based on their NYHA functional classification (Table II). The LVEF was reduced in NYHA class III/IV patients, compared with class I/II patients. The BNP levels were highest in NYHA class III/IV patients.

Urinary levels of biopyrrins for all subjects increased with the severity of NYHA class (Figure 1) and correlated with log BNP levels (r= 0.280, $p < 0.01$ in the CAD group and r= 0.465, $p < 0.01$ in the non-CAD group, respectively) (Figure 2). In contrast, urinary levels of 8-OHdG increased with the severity of NYHA class and correlated positively with log BNP levels ($r$= 0.338, $p < 0.01$) only in the CAD group. In the non-CAD patients, urinary 8-OHdG levels were higher than in the controls; however, the levels did not correlate with log BNP levels. There was no correlation between urinary 8-OHdG levels and biopyrrins levels in both the CAD and non-CAD groups. Urinary 8-OHdG levels in the CAD group correlated negatively with LVEF ($r$= –0.432, $p < 0.01$), but there was no significant relationship between urinary 8-OHdG levels and LVEF in non-CAD patients.

CAD patients were divided into three sub-groups based on analysis of the coronary angiogram; those with one vessel disease (1VD group, $n$= 16), two-vessel disease (2VD group, $n$ = 17) and three-vessel disease (3VD group, $n$ = 37). Urinary 8-OHdG levels increased along with the number of diseased vessels (Figure 3). The concentrations of urinary 8-OHdG and biopyrrins did not correlate with the number of risk factors for CAD. Urinary biopyrrins levels were not associated with the severity of vessel disease (data not shown).

## Discussion

To the best of our knowledge, this study is the first report to reveal differences in the levels of oxidative

Table I. Clinical characteristics.

| | non-CAD group ($n$ = 61) | CAD group ($n$ = 70) | $p$-value |
|---|---|---|---|
| Age (years) | 59±1.9 | 69±1.2 | <0.01 |
| Men | 43 (69%) | 54 (77%) | NS |
| Coronary risk factors | | | |
| Hypertension | 25 (40%) | 39 (56%) | NS |
| Dyslipidemia | 24 (39%) | 43 (61%) | <0.01 |
| Smoking | 14 (23%) | 26 (37%) | NS |
| Diabetes mellitus | 17 (27%) | 35 (50%) | <0.01 |
| Obesity (BMI > 25 kg/m$^2$) | 19 (31%) | 19 (27%) | NS |
| T-Cho (mg/dl) | 186.8±5.5 | 178.1±4.0 | NS |
| LDL (mg/dl) | 117.2±4.3 | 109.1±3.5 | NS |
| Triglyceride (mg/dl) | 127.6±14.3 | 116.6±5.6 | NS |
| HDL (mg/dl) | 54.7±2.3 | 49.0±2.3 | NS |
| HbA1c (%) | 5.7±0.1 | 6.4±0.2 | <0.01 |
| CRP (mg/dl) | 0.55±0.14 | 0.47±0.10 | NS |
| BNP (pg/ml) | 523.1±94.4 | 404.0±66.1 | NS |
| LVEF (%) | 33.5±1.6 | 40.5±1.3 | <0.01 |
| serum creatinine (mg/dl) | 0.98±0.29 | 1.00±0.33 | NS |
| estimated GFR (ml/min/1.73m$^2$) | 60.9±1.9 | 60.7±2.3 | NS |
| urinary 8-OHdG | 19.7±1.4 | 24.0±1.9 | NS |
| urinary bioppyrins | 5.0±0.8 | 4.5±0.6 | NS |
| Concomitant medications on admission | | | |
| statins | 5 (8%) | 30 (43%) | <0.01 |
| ACE inhibitors/ARB | 50 (82%) | 61 (87%) | NS |
| β-blockers | 20 (32%) | 21 (30%) | NS |
| calcium antagonist | 7 (10%) | 27 (39%) | <0.01 |
| aspirin | 13 (21%) | 63 (90%) | <0.01 |
| warfarin | 22 (36%) | 15 (21%) | <0.05 |

8-OHdG = 8-hydroxy-2′-deoxyguanosine; ACE = angiotensin-converting enzyme; ARB = angiotensin II receptor blockers; BMI = body mass index; BNP = B-type natriuretic peptide; CRP = C-reactive protein; GFR = glomerular filtration rate; HDL = high density lipoprotein; LDL = low density lipoprotein; LVEF = Left ventricular ejection fraction.

Table II. New York Heart Association functional classification and haemodynamic variables.

| | Control group | Class I | Class II | Class III/IV |
|---|---|---|---|---|
| CAD group | $n = 33$ | $n = 26$ | $n = 19$ | $n = 25$ |
| HR (beats/min) | 67±2.3 | 69±2.2 | 70±2.4 | 88±3.8¶f* |
| BP (mmHg) | 134±3.2/77±1.7 | 126±3.6/72±2.2 | 122±4.9/70 + 2.8 | 123±5.4/73±2.7 |
| BNP (pg/ml) | 31.0 (16.3–56.6) | 53.5 (20.8–78.0) | 168.5 (120.0-286.0)¶‡ | |
| CI (I7min/m$^2$) | 2.7±0.1 | 2.4±0.1 | 2.4±0.1 | 2.3±0.1 |
| PCWP (mmHg) | 8.5±0.7 | 8.6±0.9 | 10.5±1.3 | 16.7±2. 1¶†* |
| LVEF (%) | 73.4±1.4 | 46.1±1.5¶ | 43.9±2.3¶ | 32.4±2.l¶†* |
| serum creatinine (mg/dl) | 0.84±0.06 | 0.86±0.04 | 1.04±0.08 | 1.12±0.08‡§ |
| estimated GFR (ml/min/1.73m$^2$) | 69.2±2.9 | 69.2±3.7 | 61.4±4.3 | 51.2±3.4¶† |
| urinary biopyrrins (mmol/g creatinine) | 2.09 (1.33–3.33) | 2.51 (1.58-2.98) | 2.25 (1.79-5.86) | 4.38 (2.50–9.75)¶† |
| urinary 8-OHdG (ng/mg creatinine) | 12.6±0.9 | 15.8±1.5 | 22.6±3.1‡ | 33.7±4.O¶†* |
| Non-CAD group | $n = 33$ | $n = 14$ | $n = 19$ | $n = 28$ |
| HR (beats/min) | 67±2.3 | 70±3 | 82±4 | 96±5¶† |
| BP (mmHg) | 134±3.2/77±1.7 | 127±4.9/79±3.8 | 127±9.0/79+5.7 | 120±5.9/76+3.7 |
| BNP (pg/ml) | 31.0 (16.3-56.6) | 63.5 (25.0-78.0)‡ | 144.0 (59.9-293.5)¶§ | |
| CI (I7min/m$^2$) | 2.7±0.1 | 2.3±0.1 | 2.7±0.2 | 2.2±0.1‡ |
| PCWP (mmHg) | 8.5±0.7 | 9.5±0.7 | 10.8±1.0 | 15.7±1.7¶†* |
| LVEF (%) | 73.4±1.4 | 41.4±1.4¶ | 34.6±3.2¶ | 30.4±2.5¶§ |
| serum creatinine (mg/dl) | 0.84±0.06 | 0.92±0.05 | 0.96±0.09 | 1.03±0.05 |
| estimated GFR (ml/min/1.73m$^2$) | 69.2±2.9 | 65.0±3.4 | 65.1±4.3 | 55.9±2.3† |
| urinary biopyrrins (mmol/g creatinine) | 2.09 (1.33–3.33) | 1.61 (1.23-2.21) | 3.15 (1.54–5.21)§ | 4.77 (3.58–8.62)¶†* |
| urinary 8-OHdG (ng/mg creatinine) | 12.6±0.9 | 16.9±2.7 | 19.6±1.8 | 20.3±2.3‡ |

‡$p$ <0.05 vs control group, ¶$p$ <0.01 vs control group, †$p$ <0.01 vs class I, §$p$ <0.05 vs class NYHA I, *$p$ <0.05 vs class II 8-OHdG 5 8-hydroxy-2′-deoxyguanosine. BNP = B-type natriuretic peptide; BP = blood pressure; CI = cardiac index; GFR = glomerular filtration rate; HR = heart rate; LVEF = left ventricular ejection fraction; PCWP = pulmonary capillary wedge pressure.



stress markers according to the aetiology of LV systolic dysfunction. Urinary biopyrrins levels were elevated in association with NYHA classification severity in both the CAD and non-CAD groups. Urinary 8-OHdG levels were also associated with HF severity; however, 8-OHdG levels were higher in NYHA class III/IV patients with than without CAD. Furthermore, urinary 8-OHdG levels were related to the severity of CAD.

In the context of HF, excessive ROS are generated from many sources, including nicotinamide adenine dinucleotide phosphate oxidase, xanthine oxidase, auto-oxidation of catecholamines, nitric oxide synthase activation and mitochondrial leakage [18]. ROS are involved in the pathogenesis and progression of HF and many recent studies proposed a relationship between oxidative stress markers and functional indexes of HF [5–9]. However, increasing oxidative stress was not always specific to the HF state due to the presence of other conditions. The formation of ROS is also a critical event in the development of atherosclerosis [19,20]. In this study, urinary 8-OHdG levels significantly correlated with the number of diseased coronary arteries. Myocardial ischemia is one of the causes of ROS generation. Repetitive myocardial ischemia further depresses LV systolic dysfunction in a process called 'myocardial stunning' [21], resulting in the worsening of HF. Thus, increasing levels of 8-OHdG in the CAD patients in this study were induced, at least in part, by repetitive myocardial ischemia.

There was no correlation between the urinary biopyrrins levels and the severity of diseased coronary arteries. Urinary biopyrrins levels were correlated with the severity of HF in both the CAD and non-CAD groups. In a state of severe HF, impaired hemodynamics causes both liver congestion and hypoperfusion. The levels of bilirubin, an antioxidant, are often increased in decompensated HF [22]. These results might account for the elevation of urinary biopyrrins levels in both groups.

In the present study, urinary 8-OHdG levels in the control group were higher than previously reported levels measured by ELISA [23]. High inclusion rates of coronary risk factors might contribute to this difference. Oxidative stress is increased in the presence of risk factors for atherosclerosis such as diabetes mellitus, hypertension, dyslipidemia and smoking. These atherogenic risk factors additively increase systemic oxidative stress [24,25]. In the present study, the number of coronary risk factors showed no relationship with either urinary 8-OHdG or biopyrrins levels. This may have been due in part to the small sample size. In addition, both the degree of HF and severity of CAD might also influence these discrepancies.

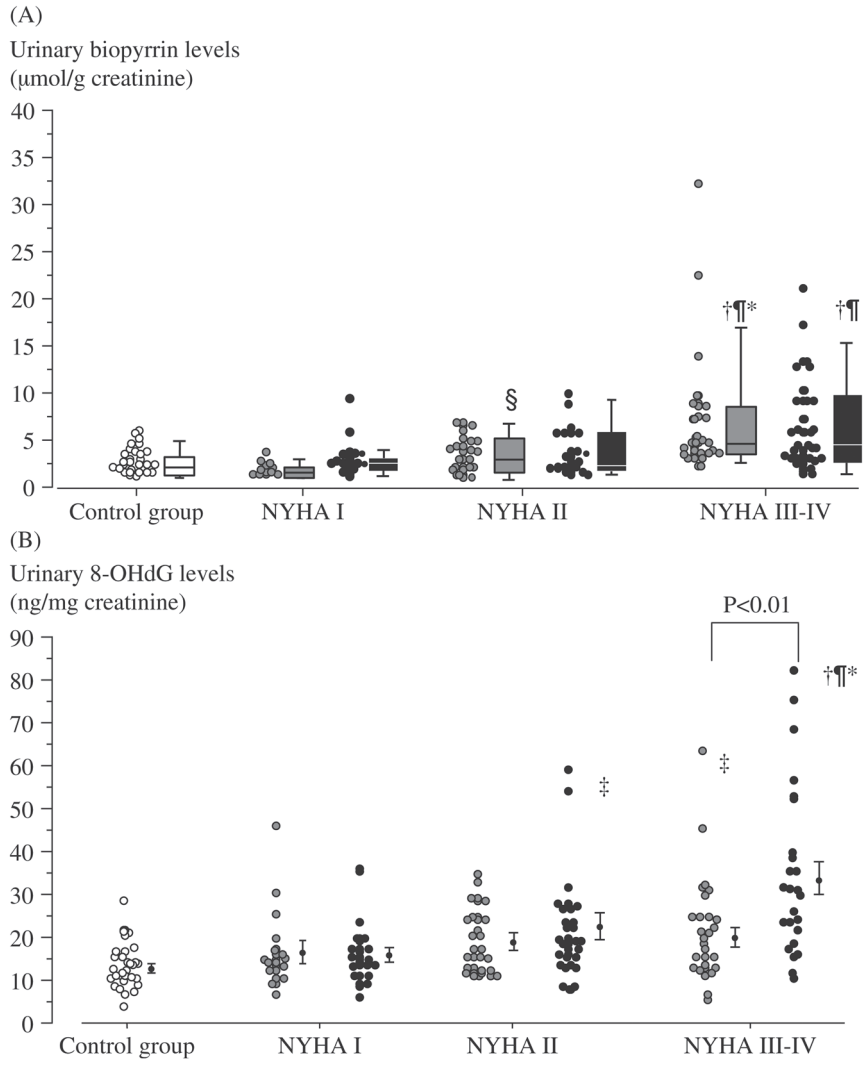


Figure 1. Comparison of urinary biopyrrins (A) and 8-OHdG levels (B) among patients with New York Heart Association (NYHA) functional classes I, II and III/IV, compared with controls. (A) The horizontal line in the box represents the median value; the boxed area is the inter-quartile range; and the whiskers are the 10–90% range. (B) Data are mean ± SEM. ‡$p < 0.05$ vs control group, ¶$p < 0.01$ vs control group, †$p < 0.01$ vs class I, §$p < 0.05$ vs class NYHA I, *$p < 0.05$ vs class II. The urinary 8-OHdG levels in NYHA class III/IV patients of the non-CAD group were significantly lower than those in the CAD group. CAD = coronary artery disease; NYHA = New York Heart Association; control group (white dots); CAD group (black dots); non-CAD group (grey dots).



In the chronic phase of myocardial infarction, ROS generated from mitochondria cause further mitochondrial dysfunction, which plays an important role in the progression of LV remodelling and failure [26]. ROS modify the DNA precursors in the nucleotide pool. The formation of 8-oxo-dGTP is one of the major sources of urinary 8-OHdG. 8-oxo-dGTP is degraded by an 8-oxo-2′-deoxyguanosine 5′-triphosphate pyrophosphohydrolase (hMTH1) to 8-oxodGMP [27,28]. This pathway was activated in mitochondria isolated from post-MI hearts [29]. These results might partly explain the association of urinary 8-OHdG levels with LVEF in CAD patients. In the present study, there was no correlation between urinary 8-OHdG levels with LVEF in non-CAD patients. Kono et al. [30] also reported elevated 8-OHdG levels in patients with DCM, but no correlation of these levels with the degree of LV dysfunction. Further investigations are needed to clarify the relationship between oxidative DNA damage and the severity of LV remodelling.

Urinary 8-OHdG has been analysed in the past by either ELISA or high-performance liquid chromatography (HPLC) [27]. Urinary 8-OHdG levels measured by these two methods showed a good correlation [31]. ELISA has been widely employed because of its ease-of-use [23,32,33]. However, urinary 8-OHdG levels analysed by ELISA are 4-10-times higher compared with the levels measured by HPLC [34]. This discrepancy is explained by the recognition of additional compounds with ELISA. Recently, Song et al. [35] reported that urea was one of the important

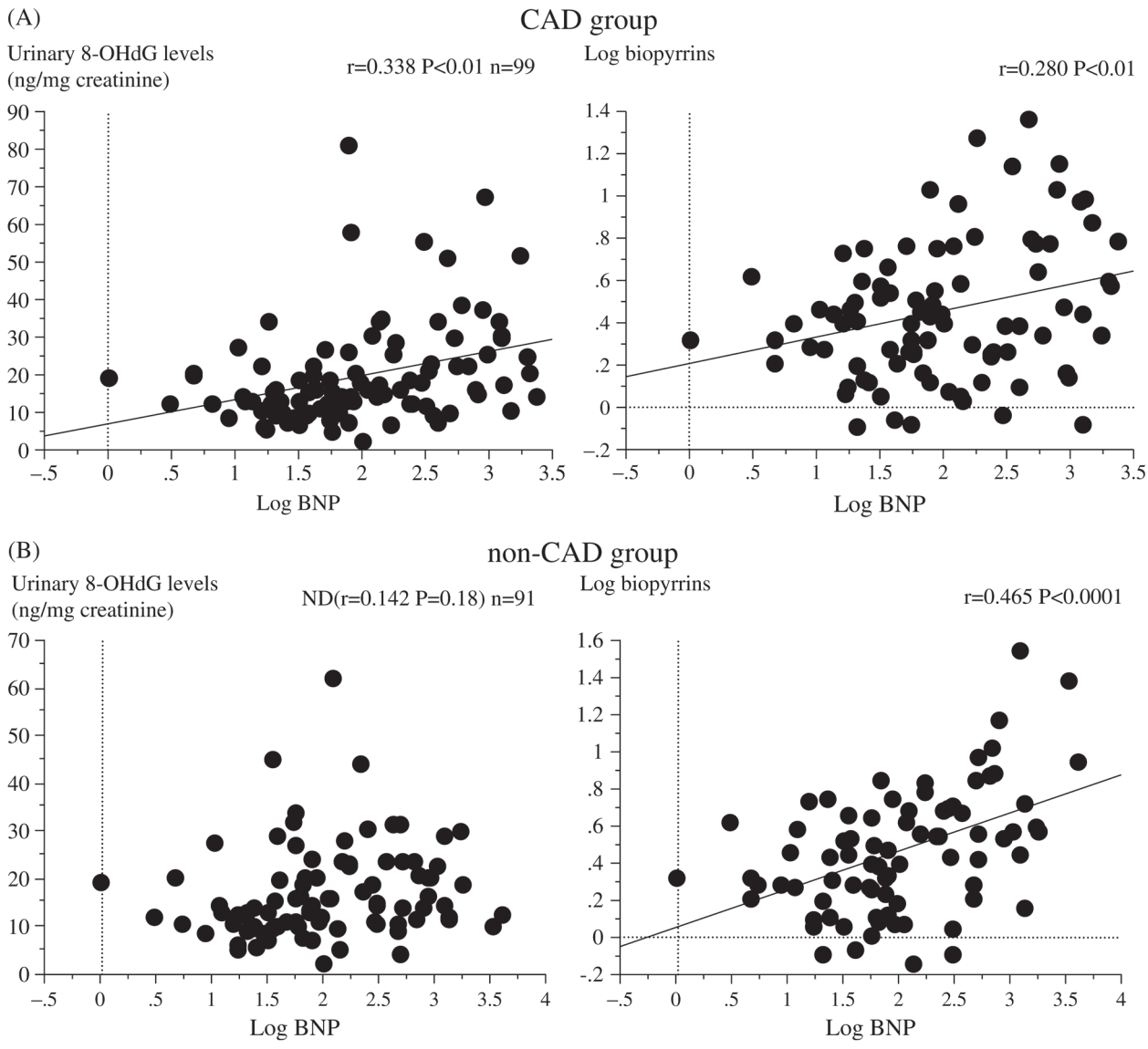


Figure 2. Relationship between oxidative stress markers and BNP levels. (A) In the CAD group, the urinary biopyrrins and 8-OHdG levels correlated positively with log BNP levels. (B) In the non-CAD group, urinary biopyrrins correlated positively with log BNP levels, but 8-OHdG levels did not correlate with log BNP levels. BNP = B-type natriuretic peptide; CAD = coronary artery disease.



compounds causing over-estimation of urinary 8-OHdG by ELISA. 8-oxodGMP and 8-oxodG-containing oligomers are also recognized by ELISA [34,36]. A limitation of our study is that we did measure 8-OHdG and biopyrrins levels by HPLC. Cooke et al. [37] reported an inconsistency of urinary 8-OHdG levels between these two methods and questioned the accuracy of ELISA. Performance of the ELISA at 4°C was recommended to improve the accuracy of the 8-OHdG analysis [34,35]. Since ELISA is a convenient and useful method, further development of monoclonal antibodies and improvement of the analytic method are necessary.

In conclusion, urinary oxidative stress marker levels were increased in patients with chronically reduced left ventricular function; however, those levels were influenced by the aetiology of HF. Urinary biopyrrins levels were strongly associated with the severity of HF. In contrast, urinary 8-OHdG levels were not associated with the severity of HF in patients with non-ischemic HF. However, in patients with ischemic cardiomyopathy, urinary 8-OHdG levels were increased according to the severity of HF and the levels were closely associated with the progression of atherosclerosis. Our findings suggest that the properties of oxidative stress markers should be carefully taken into consideration when assessing the severity of HF.

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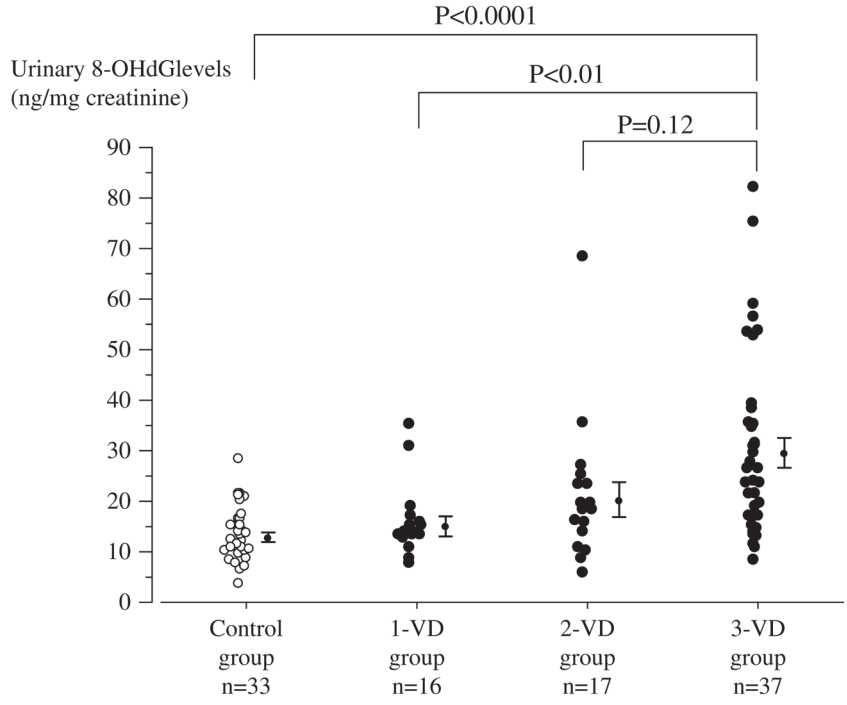


Figure 3. Relationship between urinary 8-OHdG levels and the severity of CAD. Urinary 8-OHdG levels increased with the number of diseased vessels. 1VD = one-vessel disease; 2VD = two-vessel disease; 3VD = three-vessel disease.



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## References

[1] Felker GM, Thompson RE, Hare JM, Hruban RH, Clem-etson DE, Howard DL, Baughman KL, Kasper EK. Underlying causes and long-term survival in patients with initially unexplained cardiomyopathy. N Engl J Med 2000; 342:1077–1084.

[2] Bart BA, Shaw LK, McCants CB Jr, Fortin DF, Lee KL, Califf RM, O'Connor CM. Clinical determinants of mortality in patients with angiographically diagnosed ischemic or nonischemic cardiomyopathy. J Am Coll Cardiol 1997;30: 1002–1008.

[3] Follath F, Cleland JG, Klein W, Murphy R. Etiology and response to drug treatment in heart failure. J Am Coll Cardiol 1998;32:1167–1172.

[4] Braunwald E. Biomarkers in heart failure. N Engl J Med 2008;358:2148–2159.

[5] Nishiyama Y, Ikeda H, Haramaki N, Yoshida N, Imaizumi T. Oxidative stress is related to exercise intolerance in patients with heart failure. Am Heart J 1997;135:115–120.

[6] Keith M, Geranmayegan A, Sole MJ, Kurian R, Robinson A, Omran AS, Jeejeebhoy KN. Increased oxidative stress in patients with congestive heart failure. J Am Coll Cardiol 1998;31:1352–1356.

[7] Mallat Z, Philip I, Lebret M, Chatel D, Maclouf J, Tedgui A. Elevated levels of 8-iso-prostaglandin F2alpha in pericardial fluid of patients with heart failure: a potential role for in vivo oxidant stress in ventricular dilatation and progression to heart failure. Circulation 1998;97:1536–1539.

[8] Hokamaki J, Kawano H, Yoshimura M, Soejima H, Miyamoto S, Kajiwara I, Kojima S, Sakamoto T, Sugiyama S, Hirai N, Shimomura H, Nagayoshi Y, Tsujita K, Shioji I, Sasaki S, Ogawa H. Urinary biopyrrins levels are elevated in relation to severity of heart failure. J Am Coll Cardiol 2004;43:1880–1885.

[9] Watanabe E, Matsuda N, Shiga T, Kajimoto K, Ajiro Y, Kawarai H, Kasanuki H, Kawana M. Significance of 8-hydroxy-2′-deoxyguanosine levels in patients with idiopathic dilated cardiomyopathy. J Card Fail 2006;12:527–532.

[10] Kasai H, Nishimura S. Hydroxylation of deoxyguanosine at the C-8 position by ascorbic acid and other reducing agents. Nucleic Acids Res 1984;12:2137–2145.

[11] Wu LL, Chiou CC, Chang PY, Wu JT. Urinary 8-OHdG: a marker of oxidative stress to DNA and a risk factor for cancer, atherosclerosis and diabetics. Clin Chim Acta 2004; 339:1–9.

[12] Stocker R. Antioxidant activities of bile pigments. Antioxid Redox Signal 2004;6:841–849.

[13] Morita T. Heme oxygenase and atherosclerosis. Arterioscler Thromb Vasc Biol 2005;25:1786–1795.

[14] Yamaguchi T, Shioji I, Sugimoto A, Komoda Y, Nakajima H. Chemical structure of a new family of bile pigments from human urine. J Biochem 1994;116:298–303.

[15] Shimomura H, Ogawa H, Takazoe K, Soejima H, Miyamoto S, Sakamoto T, Kawano H, Suefuji H, Nishikawa H, Arai H, Hokamaki J, Kajiwara I, Kugiyama K, Yoshimura M. Comparison of urinary biopyrrins levels in acute myocardial infarction (after reperfusion therapy) versus stable angina pectoris and their usefulness in predicting subsequent cardiac events. Am J Cardiol 2002;90:108–111.

[16] Imai E, Matsuo S, Makino H, Watanabe T, Akizawa T, Nitta K, Iimuro S, Ohashi Y, Hishida A. CKD-JAC Study Group. Chronic Kidney Disease Japan Cohort (CKD-JAC) study: design and methods. Hypertens Res 2008;3: 1101–1107.

[17] American Diabetes Association. Report of the expert committee on the diagnosis and classification of diabetes mellitus. Diabetes Care 1997;20:1183–1197.
[18] Giordano FJ. Oxygen, oxidative stress, hypoxia, and heart failure. J Clin Invest 2005;115:500–508.
[19] Martinet W, Knaapen MW, De Meyer GR, Herman AG, Kockx MM. Elevated levels of oxidative DNA damage and DNA repair enzymes in human atherosclerotic plaques. Circulation 2002;106:927–932.
[20] Botto N, Masetti S, Petrozzi L, Vassalle C, Manfredi S, Biagini A, Andreassi MG. Elevated levels of oxidative DNA damage in patients with coronary artery disease. Coron Artery Dis 2002;13:269–274.
[21] Bolli R, Zughaib M, Li XY, Tang XL, Sun JZ, Triana JF, McCay PB. Recurrent ischemia in the canine heart causes recurrent bursts of free radical production that have a cumulative effect on contractile function. A pathophysiological basis for chronic myocardial 'stunning'. J Clin Invest 1995;96:1066–1084.
[22] Shinagawa H, Inomata T, Koitabashi T, Nakano H, Takeuchi I, Naruke T, Ohsaka T, Nishii M, Takehana H, Izumi T. Prognostic significance of increased serum bilirubin levels coincident with cardiac decompensation in chronic heart failure. Circ J 2008;72:364–369.
[23] Sakano N, Takahashi N, Wang DH, Sauriasari R, Takemoto K, Kanbara S, Sato Y, Takigawa T, Takaki J, Ogino K. Plasma 3-nitrotyrosine, urinary 8-isoprostane and 8-OHdG among healthy Japanese people. Free Radic Res 2009;43:183–192.
[24] Schwedhelm E, Bartling A, Lenzen H, Tsikas D, Maas R, Brummer J, Gutzki FM, Berger J, Frolich JC, Boger RH. Urinary 8-iso-prostaglandin F2alpha as a risk marker in patients with coronary heart disease: a matched case-control study. Circulation 2004;109:843–848.
[25] Vassalle C, Petrozzi L, Botto N, Andreassi MG, Zucchelli GC. Oxidative stress and its association with coronary artery disease and different atherogenic risk factors. J Intern Med 2004;256:308–315.
[26] Ide T, Tsutsui H, Hayashidani S, Kang D, Suematsu N, Nakamura K, Utsumi H, Hamasaki N, Takeshita A. Mito-chondrial DNA damage and dysfunction associated with oxidative stress in failing hearts after myocardial infarction. Circ Res 2001;88:529–535.
[27] Cooke MS Olinski R Loft S European Standards Committee on Urinary (DNA) Lesion Analysis. Measurement and meaning of oxidatively modified DNA lesions in urine. Cancer Epidemiol Biomarkers Prev 2008;17:3–14.
[28] Kasai H, Kawai K, LiYS. Analysis of 8-OH-dG and 8-OH-Gua as biomarkers of oxidative stress. Genes and Environment. 2008;30:33–0.
[29] Tsutsui H, Ide T, Shiomi T, Kang D, Hayashidani S, Suematsu N, Wen J, Utsumi H, Hamasaki N, Takeshita A. 8-Oxo-dGTPase, which prevents oxidative stress-induced DNA damage, increases in the mitochondria from failing hearts. Circulation 2001;104:2883–2885.
[30] Kono Y, Nakamura K, Kimura H, Nishii N, Watanabe A, Banba K, Miura A, Nagase S, Sakuragi S, Kusano KF, Matsubara H, Ohe T Elevated levels of oxidative DNA damage in serum and myocardium of patients with heart failure. Circ J 2006;70:1001–1005.
[31] Shimoi K, Kasai H, Yokota N, Toyokuni S, Kinae N. Comparison between high-performance liquid chromatography and enzyme-linked immunosorbent assay for the determination of 8-hydroxy-2′-deoxyguanosine in human urine. Cancer Epidemiol Biomarkers Prev 2002;11: 767–770.
[32] Matsubasa T, Uchino T, Karashima S, Kondo Y, Maruyama K, Tanimura M, Endo F Oxidative stress in very low birth weight infants as measured by urinary 8-OHdG. Free Radic Res 2002;36:189–193.
[33] Su H, Gornitsky M, Velly AM, Yu H, Benarroch M, Schipper HM. Salivary DNA, lipid, and protein oxidation in non-smokers with periodontal disease. Free Radic Biol Med 2009;46:914–921.
[34] Evans MD, Singh R, Mistry V, Sandhu K, Farmer PB, Cooke MS. Analysis of urinary 8-oxo-7,8-dihydro-purine-2′-deoxyr-ibonucleosides by LC-MS/MS and improved ELISA. Free Radic Res 2008;42:831–840.
[35] Song MF, Li YS, Ootsuyama Y, Kasai H, Kawai K, Ohta M, Eguchi Y, Yamato H, Matsumoto Y, Yoshida R, Ogawa Y Urea, the most abundant component in urine, cross-reacts with a commercial 8-OH-dG ELISA kit and contributes to overestimation of urinary 8-OH-dG. Free Radic Biol Med 2009; 47:41–6.
[36] Patel PR, Bevan RJ, Mistry N, Lunec J. Evidence of oligonucleotides containing 8-hydroxy-2′-deoxyguanosine in human urine. Free Radic Biol Med 2007;42:552–558.
[37] Cooke MS, Singh R, Hall GK, Mistry V, Duarte TL, Farmer PB, Evans MD. Evaluation of enzyme-linked immunosorbent assay and liquid chromatography-tandem mass spectrometry methodology for the analysis of 8-oxo-7,8-dihydro-2′-deox-yguanosine in saliva and urine. Free Radic Biol Med 2006;41:1829–1836.

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