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Redox Imbalance in Patients With Coronary Artery Disease Showing Progression of Atherosclerotic Lesions

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Abstract

Objectives. To clarify the relationship between changes in redox balance and the development of new coronary lesions in patients with coronary artery disease (CAD).

Methods. We studied 82 CAD patients (70 males and 12 females, mean age 61.8 ± 9.2 years) who underwent repeated coronary angiography within 1 year after percutaneous coronary intervention. Levels of serum lipid peroxide, erythrocyte glutathione peroxidase activity, and the redox state of erythrocyte (ratio of reduced to oxidized glutathione, the GSH/GSSG ratio) were measured at the time of follow-up coronary angiography. According to the development of significant stenotic lesions, we divided the patients into two groups: 57 patients without the development of new stenotic lesions (group A) and 25 patients showing new significant stenotic lesions within 1 year (group B).

Results. The serum lipid peroxide level in group B was significantly higher than those of group A (2.61 ± 0.32 vs 1.74 ± 0.16 nmol/m*l*, $p < 0.01$). Erythrocyte glutathione peroxidase activity did not differ significantly between two groups. The erythrocyte GSH/GSSG ratio in group B was significantly lower than that of group A (83 ± 9.6 vs 126 ± 7.3, $p < 0.01$). The sensitivity and specificity of GSH/GSSG ratio to detect CAD patients with the development of significant coronary stenosis were 80.0% and 61.4%, respectively.

Conclusions. CAD patients who showed development of new coronary lesions within 1 year have increased oxidative stress and imbalanced erythrocyte redox state. The GSH/GSSG ratio, an indicator for redox balance, could be a useful marker to identify high-risk CAD patients.

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Key Words

■**Stress (oxidative)** ■**Atherosclerosis** ■**Antioxidants (enzyme)**
■**Interventional cardiology (percutaneous coronary intervention)**
■**Coronary artery disease**

INTRODUCTION

A growing body of evidence indicates that reactive oxygen species (ROS) play an important role in the development of cardiovascular diseases such as atherosclerosis,[1] heart failure,[2−4] and hypertension.[5,6] Oxidative stress is defined as an imbalance between production and degradation of ROS, such as the superoxide radical, hydrogen peroxide, the hydroxyradical, peroxinitrite and lipid peroxide (LPO). LPO, one of the free radicals generated in human tissues, may be important in the develop-

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ment of liver disease, atherosclerosis, degeneration and aging.[7–10] To eliminate ROS, a number of antioxidant enzymes such as superoxide dismutase, catalase and various reductases have been developed in higher organisms. Among antioxidant enzymes, glutathione peroxidase (GPX) has a critical role in quenching LPO.

In mammalian tissues, GPX and glutathione constitute a major antioxidant defense system. The reduced form of glutathione (GSH) is a major cellular antioxidant that provides a proton (H^+) for GPX to reduce LPO and hydrogen peroxide (H_2O_2). During this process, GSH is converted to oxidized glutathione (GSSG), and GSSG is reduced into GSH by glutathione reductase (GR) receiving H^+ from β-nicotinamide adenine dinucleotide phosphate (NADPH) and is recycled in the glutathione system (**Fig. 1**). Excess oxidative stress causes accumulation of GSSG, leading to a decrease in the GSH/GSSG ratio.

In the clinical situation, we often observe development of new coronary lesions in patients with coronary artery disease (CAD). Development of coronary stenosis may induce a recurrence of myocardial ischemia/infarction, critical ventricular arrhythmia, acute pump failure, and sudden cardiac death. Thus, the disease progression may be a critical problem in patients with CAD even after treatment with drug-eluting stents. In addition, we sometimes experience patients with rapid development of stenotic lesions in coronary arteries within 1 year after diagnosis.[11] To date, there are few clinical markers to identify these high-risk CAD patients.

In this study, we investigated the relationship between markers of redox system and the development of new lesions in CAD patients who had already undergone percutaneous coronary intervention (PCI). We found increased oxidative stress and imbalance of oxidant/antioxidant system in patients with CAD who showed new stenotic lesions within 1 year after PCI.

SUBJECTS AND METHODS

Subjects

This study complied with the Declaration of Helsinki. The Ethics Committee of Jichi Medical University approved the protocol of this study. All patients enrolled in this study gave informed consent. Patients examined by coronary angiography in our university hospital between October 2002 and April 2005 were included in this study. We selected patients who had already undergone PCI of the coronary arteries within 1 year. We identified 82 patients who had undergone second coronary angiography for routine follow-up or examination for the recurrence of symptoms during the study period.

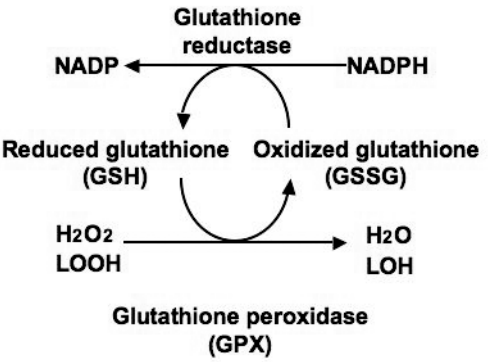

Fig. 1 Glutathione system
Glutathione, a tripeptide (γ-glutamylcysteinylglycine), is a major cellular antioxidant. Reduced glutathione (GSH) provides protons (H^+) for glutathione peroxidase (GPX) that reduces hydrogen peroxide (H_2O_2) and lipid peroxide (LOOH) to H_2O and LOH, respectively. During this process, GSH becomes oxidized to glutathione (GSSG). Then GSSG is reduced by glutathione reductase and nicotinamide adenine dinucleotide phosphate (NADPH). When cells are exposed to oxidative stress, the ratio of GSH/GSSG decreases as a consequence of GSSG accumulation.

Blood collection

Cardiac catheterization was performed according to the standard diagnostic procedure. Under 1% lidocaine hydrochloride local anesthesia, the femoral or radial artery was cannulated with a 5 Fr-sheath. Heparin (3,000 U) was administered intravenously before coronary angiography. Nonionic contrast media (ioxaglate) was used for all patients. The coronary stenosis was accurately evaluated by selective coronary angiography. Stenotic lesions in the coronary arteries treated by PCI (PCI-related stenosis) were excluded from the study. Patients with chronic renal failure, liver dysfunction, malignancy, and hematological disorders were also excluded from the study.

Arterial blood samples were obtained through a 5 Fr-catheter from the radial or femoral artery. After the initial 3 m*l* of blood was discarded, blood samples were gently obtained with syringes containing EDTA. Blood was centrifuged and the plasma was

stored at −80 ℃ until the assay. Erythrocyte GPX activity was measured as previously described.[12] Briefly, red blood cells were washed with ice-cold physiological saline three times and 5 μ*l* of the sample was lysed into 250 μ*l* of distilled water. Samples were then frozen until the assay. Our preliminary study revealed that freezing did not change GPX activity (data not shown).

Erythrocyte lysate, GSH, GR, and NADPH were mixed together in a 96-well plate. Enzyme reaction was initiated by adding t-butyl hydroperoxide and absorbance at 340 nm was monitored for 3 min at 30 ℃. Change in absorbance at 340 nm from 1 to 3 min was measured and GPX activity was calculated. Serum LPO levels were measured by the colorimetric method as described previously using methylcarbamoyl 1-3,7-dimethylamino-10 H-phenothiazine.[13] The redox state of erythrocyte (the GSH/GSSG ratio) was measured by 5,5-dithio-bis (2-nitrobenzoic acid)-enzyme recycling assay (GSH/GSSG-412 assay kit, Oxis Research) according to the manufacturer's instructions. The % coefficient of variance values of intra- and inter-assay kit were 3.11 and 3.18, respectively. Quantitative coronary angiography (QCA) was performed by QCA-CMS software (MEDIS).

Statistical analysis

Data are expressed as mean ± SEM unless otherwise indicated. Comparisons between the two groups were analyzed by unpaired *t*-test. Difference of distribution was analyzed by χ square test. Changes in stenosis rate of coronary arteries between two groups were analyzed by two-way ANOVA with repeated measurement. Multivariate stepwise regression analysis was performed by Stat-View version 5.0 (SAS Institute). Receiver-operating characteristic (ROC) curve analysis was performed by MedCalc (MedCalc Software, version 8.0.2.0). Values of $p < 0.05$ were considered significant.

RESULTS

Profiles of study subjects

The 82 CAD patients included 70 males and 12 females aged from 36 to 79 [mean 61.8 ± 9.2 (SD)] years. Fifty six patients had hypertension (68%), 29 patients had diabetes (35%), 49 patients had hyperlipidemia (60%), 47 patients were current smokers (57%), and 18 patients had a family history of CAD (22%). The baseline coronary artery diseases were acute coronary syndromes in 16 patients and stable angina pectoris in 66, including 41 patients with one vessel, 31 with two vessel and 10 with three vessel disease. Two patients had received coronary bypass surgery. Forty two (51%) patients had old myocardial infarction. Left ventricular ejection fraction ranged from 23 to 84% (mean 61.5 ± 1.6%).

Comparison between patients with or without progression of coronary stenosis

According to the development of coronary stenosis, we divided the patients into two groups: 57 patients without development of new lesions (group A) and 25 patients showing new significant stenotic lesions [over 75% by the American Heart Association (AHA) classification] within 1 year (group B). **Table 1** shows the clinical characteristics of the two groups. Mean observation period was not significantly different between two groups. Age, distribution of sex, incidence of major coronary risk factors, severity of the CAD, left ventricular ejection fraction, and incidence of previous myocardial infarction were not significantly different between the two groups. Body mass index in group B was significantly higher than that in group A. As shown in **Table 2**, systolic and diastolic blood pressure, total cholesterol, high-density lipoprotein cholesterol, low-density lipoprotein cholesterol, fasting blood glucose, and hemoglobin A_{1c} levels, and duration of diabetes mellitus were not significantly different between the two groups. High sensitive C-reactive protein level in group B was significantly higher than that in group A. The number of patients taking angiotensin receptor blocker (ARB)/angiotensin converting enzyme (ACE) inhibitor in group A was significantly higher than that in group B.

Table 3 summarizes the changes in stenosis of PCI-treated lesions and new lesions analyzed by QCA. Sixty two lesions were treated in group A and 25 in group B. There was no significant difference in changes in stenosis of PCI-treated lesions between the two groups ($F = 1.26$, $p = 0.26$).

As shown in **Fig 2**, the mean serum LPO level in group B was significantly higher than that in group A (2.61 ± 0.32 vs 1.74 ± 0.16 nmol/m*l*, $p < 0.01$). The mean erythrocyte GPX activity did not differ significantly between groups A and B (2.06 ± 0.08 vs 2.00 ± 0.08 U/g protein, $p = 0.66$). The mean erythrocyte GSH/GSSG ratio in group B was sig-

Table 1 Clinical characteristics of the study subjects (1)

	Group A (n=57)	Group B (n=25)
Observation period (months)	7.1±2.9	6.1±2.6
Age (yr)	61.7±8.5	62.0±10.8
Sex (male/female)	52/5	18/7
Body mass index (kg/m^2)	24.0±0.4	25.4±0.7*
Hypertension	40 (70%)	16 (64%)
Diabetes mellitus	19 (33%)	10 (40%)
Hyperlipidemia	31 (54%)	18 (72%)
Current smokers	35 (61%)	12 (48%)
Family history of coronary artery disease	13 (23%)	5 (20%)
1/2/3 vessel disease	34/16/7	7/15/3
Left ventricular ejection fraction (%)	62.1±1.9	60.3±3.1
Previous myocardial infarction	29 (51%)	13 (52%)

Continuous values are mean±SEM. *$p<0.05$ vs group A.
Group A : Patients with coronary artery disease without progression of coronary atherosclerosis.
Group B : Patients with coronary artery disease with progression of coronary atherosclerosis.

Table 2 Clinical characteristics of the study subjects (2)

	Group A (n=57)	Group B (n=25)
Blood pressure (mmHg)		
Systolic	135±3.1	130±1.9
Diastolic	78±1.9	71±3.3
Total cholesterol (mg/d*l*)	191±4.9	202±12.0
Triglyceride (mg/d*l*)	134±10.5	160±24.7
High-density lipoprotein cholesterol (mg/d*l*)	47.6±1.7	46.6±2.0
Low-density lipoprotein cholesterol (mg/d*l*)	120±4.3	131±9.5
Fasting blood glucose (mg/d*l*)	129±6.5	131±10.6
Hemoglobin A_{1c} (%)	6.0±0.16	6.1±0.24
History of diabetes mellitus (yr)	2.0±0.52	3.1±1.2
High sensitive C-reactive protein (ng/m*l*)	1,439±420	4,940±2,199*
Medication		
Beta blockers	25 (44%)	10 (40%)
ARB/ACE inhibitors	42 (74%)†	11 (44%)
Calcium blockers	23 (40%)	14 (56%)
Statins	30 (52%)	16 (64%)

Continuous values are mean±SEM. *$p<0.05$ vs group A. †$p<0.05$ vs group B.
ARB=angiotensin receptor blocker ; ACE=angiotensin converting enzyme.

nificantly lower than that in group A (83±9.6 vs 126±7.3, $p<0.01$).

Relationship between the GSH/GSSG ratio and coronary risk factors

To examine the factors that affect the erythrocyte GSH/GSSG ratio, we performed multivariate stepwise regression analysis to examine the factors that affect the GSH/GSSG ratio. We set the GSH/GSSG ratio as a responsible parameter and age, sex, severity of CAD (the number of stenotic major coronary arteries), left ventricular ejection fraction, blood pressure, total cholesterol, fasting blood sugar, hemoglobin A_{1c}, smoking, body mass index and the

drugs received(β blocker, ARB/ACE inhibitors, calcium blockers and statins)as explanatory variables. In this model, administration of ARB/ACE inhibitors was a significant independent predictor of the GSH/GSSG ratio($F = 7.64$, $p = 0.007$, $r^2 = 0.083$, $\beta = +0.31$).

ROC curve analysis

We found increased LPO levels and decreased GSH/GSSG ratio in CAD patients with progressive coronary lesions within 1 year after PCI. To analyze whether these markers can be used to detect these high-risk CAD patients, we performed ROC curve analysis. As shown in **Table 4**, the GSH/GSSG ratio predicted progression of coronary atherosclerosis in CAD patients within 1 year with a sensitivity of 80.0% and specificity of 61.4%. Serum LPO level also predicted the progression of new coronary lesions, but with lower sensitivity (72.0%)and higher specificity(64.9%)than the GSH/GSSG ratio. The erythrocyte GPX levels predicted progression of disease with a high sensitivity

Table 3 Changes in stenosis of coronary artery lesions analyzed by quantitative coronary angiography

	Group A (n=57)	Group B (n=25)
Number of PCI-treated lesions	62	25
PCI-treated lesions		
Before PCI (%)	91.0±1.5	94.2±1.9
Follow-up CAG(%)	26.0±1.9	16.1±5.8
New lesions		
Before PCI (%)		37.5±5.0
Follow-up CAG(%)		85.6±2.6

Continuous values are mean±SEM.
PCI=percutaneous coronary intervention; CAG=coronary angiography.

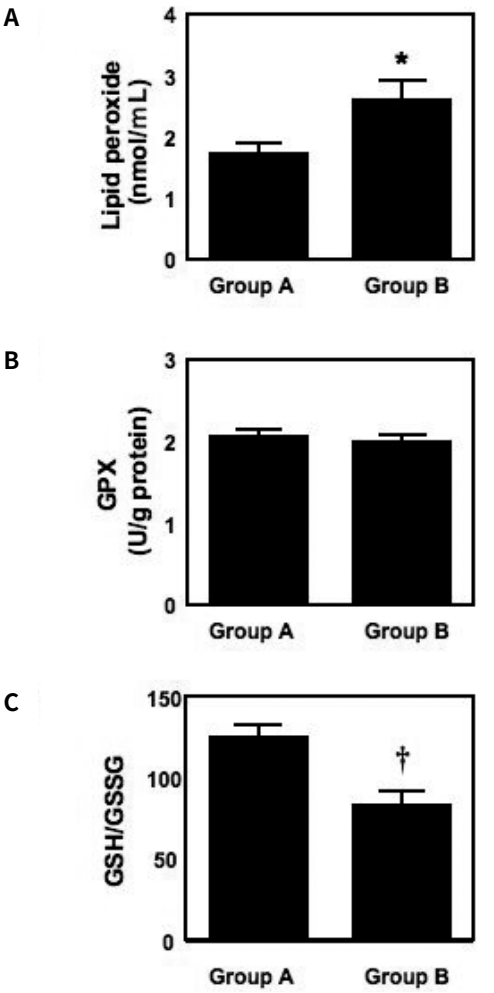

Fig. 2 Comparison of redox parameters between two groups of patients with coronary artery disease
$^{*}p < 0.01$ vs group A. $^{\dagger}p < 0.05$ vs group A.
A: Serum level of lipid peroxide in group B was significantly higher than that in group A .
B: Erythrocyte glutathione peroxidase activity did not differ significantly between groups A and B.
C : Ratio of reduced to oxidized glutathione (GSH/GSSG ratio)in group B was significantly lower than that in group A.
Abbreviations as in Fig. 1.

Table 4 Results of receiver-operating characteristic curve analysis

Parameters	Cut off value	Sensitivity	Specificity	Area under curve
GPX(U/g protein)	1.73	88.0%	29.8%	0.522
LPO(nmol/m*l*)	1.60	72.0%	64.9%	0.696
GSH/GSSG	107.4	80.0%	61.4%	0.728

LPO=lipid peroxide. Other abbreviations as in Fig. 1.

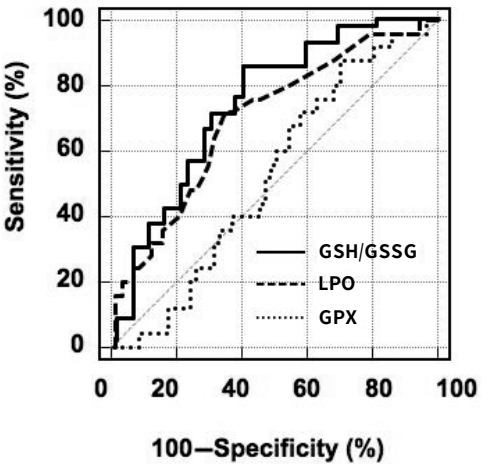

Fig. 3 Receiver-operating characteristic curve analysis of redox parameters
Receiver-operating curve analysis of redox parameters for the detection of new significant stenotic lesions. Horizontal axis indicates values of 100 − specificity (%). Vertical axis shows values of sensitivity (%). Abbreviations as in Fig. 1.

(88.0%) but a low specificity (29.8%) after 1 year of PCI. The highest area under the curve was observed in the ROC curve when the GSH/GSSG ratio was used for the parameter. **Fig. 3** shows the ROC curves of GSH/GSSG, LPO and GPX.

DISCUSSION

The present study investigated changes in redox states in patients with CAD. We hypothesized that CAD patients with progression of coronary atherosclerosis (" progressors ") have imbalanced redox state. Serum total cholesterol level was similar between the two groups, but progressors showed elevated serum LPO levels and their erythrocytes were more oxidized. These results suggest that disorder of redox balance is important in the progression of coronary atherosclerosis. The GSH/GSSG ratio may be a simple and useful marker to detect high-risk CAD patients such as progressors. The results of multivariate regression analysis showed that receiving ARB/ACE inhibitors increases the erythrocyte GSH/GSSG ratio, suggesting that inhibition of the renin-angiotensin system makes the cellular redox state less oxidative. The present study showed that the administration of ARB/ACE inhibitors was more common in non-progressors than in progressors. Angiotensin II activates NADPH oxidase, a ROS-producing enzyme, in vascular smooth muscle cells.[14] Thus, a more active renin-angiotensin system generates excess ROS that makes the cellular redox state oxidative. Although there is still no evidence that inhibition of the renin-angiotensin system prevents the progression of atherosclerosis, population-based studies might disclose the benefit of ARB/ACE inhibitors in the near future.

Our results suggest that regulation of the cellular redox state is critical to prevent rapid progression of atherosclerosis. Inhibition of the renin-angiotensin system might be one method to make the cellular redox state less oxidative by decreasing ROS generation. *In vitro* experiments showed that *N*-acetylcysteine is a potent substance to reduce intracellular glutathione. However, there are still no established drugs to regulate the cellular redox state. Our preliminary data indicate that anti-oxidant vitamins such as vitamin C and vitamin E did not change the cellular redox state (data not shown). In fact, there is no evidence that antioxidant vitamins reduce cardiovascular events.[15] Exercise increases antioxidant enzyme activities,[16] so physical training is one method to modulate the cellular redox state.

In clinical situations, we often experience patients with rapid progression of coronary atherosclerosis, sometimes occurring within months. The present study tried to detect these high-risk CAD patients because progression of coronary atherosclerosis may induce critical ischemic events. Serum LPO levels are increased in patients with CAD.[17,18] The present study clarified that progressors are exposed to excess oxidative stress and consequently their cellular redox state is more oxidized than that of non-progressors. Experimental studies have also shown that intracellular signal transduction is enhanced when cells are oxidized.[19] In fact, progressors showed significantly higher high sensitive C-reactive protein levels. Thus the inflammatory responses induced by atherogenic stimuli are more pronounced if cells or tissues are more oxidized. Therefore, vascular tissues in progressors are thought to be susceptible to inflammatory stimuli and this inflammatory response may induce the progression of stenosis.[20] In addition, the redox states of macrophages are critical role in their activation, which initiates the formation of atheromatous plaques in blood vessels.[21–23]

A number of studies have reported decreased GPX activity in erythrocytes or platelets in CAD patients.[24–28] Recently, decreased erythrocyte GPX

activity was independently associated with an increased risk of cardiovascular diseases.[29] In the present study, we did not find a significant difference in erythrocyte GPX activity between progressors and non-progressors. We have already observed decreased erythrocyte GPX activity of CAD patients with disease progression more than 1 year after the first PCI(unpublished observation). We speculate that progression of coronary atherosclerosis is more closely related to oxidative stress than changes in the antioxidant system within 1 year after PCI. In contrast, the decreased antioxidant system provides a greater contribution to disease progression after the first year of PCI. Thus disease mechanisms might be different within and after 1 year. The increased oxidative stress might accelerate accumulation of macrophages to atheromatous plaques, transformation of vascular smooth muscle cells and rapid proliferation of plaque composing cells, leading to the progression of atherosclerosis. In contrast, cellular antioxidant enzyme might have a central role in the prevention of chronic progression of atherosclerosis caused by aging. The balance of redox states appears to have critical roles in both situations. Previously, we reported that GSH/GSSG ratio is related to left ventricular function after acute myocardial infarction.[30] It is possible that cellular redox state is critical in the prognosis of cardiovascular diseases. Interestingly, changes in stenosis in PCI-treated lesions were not dependent on the cellular redox state. The clinical application of the drug eluting stent might be responsible for these results.

This study investigated the relationship between disease progression and redox markers in a retrospective design. We should investigate the relationship between redox parameters and the prognosis of CAD patients in a prospective study with a larger population. We still do not know the relationship between redox imbalance and atheromatous coronary plaque rupture, fatal arrhythmic events, and deterioration of heart failure in CAD patients. Further studies are needed to clarify these points. The results of the present study suggest that therapies reducing serum lipid levels and controlling redox balance may improve the prognosis of high-risk CAD patients.

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要　　約

病変進行を示す冠動脈疾患患者における酸化還元状態の不均衡

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水野　修　　勝木　孝明　　島田　和幸

目　的: 冠動脈疾患患者において新規冠動脈病変の進行と酸化還元状態の不均衡との関連を明らかにする．

方　法: 経皮的冠動脈形成術を施行後，1年以内に再冠動脈造影を受けた冠動脈疾患患者82例(男性70例，女性12例，平均年齢61.8±9.2歳)を対象とした．血清過酸化脂質と，再冠動脈造影の際，赤血球グルタチオンペルオキシダーゼ活性および赤血球酸化還元状態の指標である還元型/酸化型グルタチオン比(GSH/GSSG比)を測定した．新規病変進行の有無により対象を2群に分けた(A群: 病変進行のみられなかった患者57例，B群: 新規有意狭窄病変が出現した患者25例)．

結　果: B群の血清過酸化脂質値はA群よりも有意な高値を示した(2.61±0.32 vs 1.74±0.16 nmol/m*l*，$p<0.01$)．赤血球グルタチオンペルオキシダーゼ活性では2群間で有意な差はなかった．B群の赤血球GSH/GSSG比はA群と比べ有意な低値を示した(83±9.6 vs 126±7.3，$p<0.01$)．新規冠動脈病変出現検出におけるGSH/GSSG比の感度および特異度はおのおの80.0%と61.4%であった．

結　論: 新規病変が出現する冠動脈疾患患者では赤血球酸化還元状態の不均衡がみられた．酸化還元状態の指標であるGSH/GSSG比はこのようなハイリスク冠動脈疾患患者の検出に有用である

と考えられた.

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References

1) Halliwell B: Free radicals, reactive oxygen species and human disease: A critical evaluation with special reference to atherosclerosis. Br J Exp Pathol 1989; **70**: 737−757
2) McCord JM: Oxygen-derived radicals: A link between reperfusion injury and inflammation. Fed Proc 1987; **46**: 2402−2406
3) Dhalla NS, Temsah RM, Netticadan T: Role of oxidative stress in cardiovascular diseases. J Hypertens 2000; **18**: 655−673
4) Lopez Farre A, Casado S: Heart failure, redox alterations, and endothelial dysfunction. Hypertension 2001; **38**: 1400−1405
5) Orie NN, Zidek W, Tepel M: Reactive oxygen species in essential hypertension and non-insulin-dependent diabetes mellitus. Am J Hypertens 1999; **12**: 1169−1174
6) Cai H, Harrison DG: Endothelial dysfunction in cardiovascular diseases: The role of oxidant stress. Circ Res 2000; **87**: 840−844
7) Suematsu T, Kamada T, Abe H, Kikuchi S, Yagi K: Serum lipoperoxide level in patients suffering from liver disease. Clin Chim Acta 1977; **79**: 267−270
8) Sato Y, Hotta N, Sakamoto N, Matsuoka S, Ohishi N, Yagi K: Lipid peroxide level in plasma of diabetic patients. Biochem Med 1979; **21**: 104−107
9) Harman D: The free-radical theory of aging. *in* Free Radicals in Biology(ed by Pryor WA), Vol. 5. Academic Press, New York, 1982; pp 255−275
10) Mezzetti A, Pierdomenico SD, Costantini F, Romano F, De Cesare D, Cuccurullo F, Imbastaro T, Riario-Sforza G, Di Giacomo F, Zuliani G, Fellin R: Copper/zinc ratio and systemic oxidant load: Effect of aging and aging-related degenerative diseases. Free Radic Biol Med 1998; **25**: 676−681
11) Tuzcu EM, Kapadia SR, Sachar R, Ziada KM, Crowe TD, Feng J, Magyar WA, Hobbs RE, Starling RC, Young JB, McCarthy P, Nissen SE: Intravascular ultrasound evidence of angiographically silent progression in coronary atherosclerosis predicts long-term morbidity and mortality after cardiac transplantation. J Am Coll Cardiol 2005; **45**: 1538−1542
12) Paglia DE, Valentine WN: Studies on the quantitative and qualitative characterization of erythrocyte glutathione peroxidase. J Lab Clin Med 1967; **70**: 158−169
13) Ohishi N, Ohkawa H, Miike A, Tateno T, Yagi K: A new assay method for lipid peroxides using a methylene blue derivative. Biochem Int 1985; **10**: 205−211
14) Griendling KK, Minieri CA, Ollerenshaw JD, Alexander RW: Angiotensin II stimulates NADH and NADPH oxidase activity in cultured vascular smooth muscle cells. Circ Res 1994; **74**: 1141−1148
15) Brown BG, Zhao X-Q, Chait A, Fisher LD, Cheung MC, Morse JS, Dowdy AA, Marino EK, Bolson EL, Alaupovic P, Frohlich J, Albers JJ: Simvastatin and niacin, antioxidant vitamins, or the combination for the prevention of coronary disease. N Engl J Med 2001; **345**: 1583−1592
16) Linke A, Adams V, Schulze PC, Erbs S, Gielen S, Fiehn E, Mobius-Winkler S, Schubert A, Schuler G, Hambrecht R: Antioxidative effects of exercise training in patients with chronic heart failure: Increase in radical scavenger enzyme activity in skeletal muscle. Circulation 2005; **111**: 1763−1770
17) Dincer Y, Akcay T, Konukoglu D, Hatemi H: Erythrocyte susceptibility to lipid peroxidation in patients with coronary atherosclerosis. Acta Med Okayama 1999; **53**: 259−264
18) Miquel J, Ramirez-Bosca A, Soler A, Diez A, Carrion-Gutierrez MA, Diaz-Alperi J, Quintanilla-Ripoll E, Bernd A, Quintanilla-Almagro E: Increase with age of serum lipid peroxides: Implications for the prevention of atherosclerosis. Mech Ageing Dev 1998; **100**: 17−24
19) Hojo Y, Saito Y, Tanimoto T, Hoefen RJ, Baines CP, Yamamoto K, Haendeler J, Asmis R, Berk BC: Fluid shear stress attenuates hydrogen peroxide-induced c-Jun NH2-terminal kinase activation via a glutathione reductase-mediated mechanism. Circ Res 2002; **91**: 712−718
20) Tousoulis D, Davies G, Stefanadis C, Toutouzas P, Ambrose JA: Inflammatory and thrombotic mechanisms in coronary atherosclerosis, Heart 2003; **89**: 993−997
21) Gieche J, Mehlhase J, Licht A, Zacke T, Sitte N, Grune T: Protein oxidation and proteolysis in RAW264.7 macrophages: Effects of PMA activation. Biochim Biophys Acta 2001; **1538**: 321−328
22) Antonicelli F, Brown D, Parmentier M, Drost EM, Hirani N, Rahman I, Donaldson K, MacNee W: Regulation of LPS-mediated inflammation in vivo and in vitro by the thiol antioxidant Nacystelyn. Am J Physiol Lung Cell Mol Physiol 2004; **286**: L1319−L1327
23) Svensson PA, Asea A, Englund MC, Bausero MA, Jernas M, Wiklund O, Ohlsson BG, Carlsson LM, Carlsson B: Major role of HSP70 as a paracrine inducer of cytokine production in human oxidized LDL treated macrophages. Atherosclerosis 2006; **185**: 32−38
24) Buczynski A, Wachowicz B, Kedziora-Kornatowska K, Tkaczewski W, Kedziora J: Changes in antioxidant enzymes activities, aggregability and malonyldialdehyde concentration in blood platelets from patients with coronary heart disease. Atherosclerosis 1993; **100**: 223−228
25) Yegin A, Yegin H, Aliciguzel Y, Deger N, Semiz E: Erythrocyte selenium-glutathione peroxidase activity is lower in patients with coronary atherosclerosis. Jpn Heart J 1997; **38**: 793−798
26) Lapenna D, de Gioia S, Ciofani G, Mezzetti A, Ucchino S, Calafiore AM, Napolitano AM, Di Ilio C, Cuccurullo F: Glutathione-related antioxidant defenses in human atherosclerotic plaques. Circulation 1998; **97**: 1930−1934
27) Kesavulu MM, Rao BK, Giri R, Vijaya J, Subramanyam G, Apparao C: Lipid peroxidation and antioxidant enzyme status in Type 2 diabetics with coronary heart disease. Diabetes Res Clin Pract 2001; **53**: 33−39

28) Hamanishi T, Furuta H, Kato H, Doi A, Tamai M, Shimomura H, Sakagashira S, Nishi M, Sasaki H, Sanke T, Nanjo K: Functional variants in the glutathione peroxidase-1(GPx-1)gene are associated with increased intima-media thickness of carotid arteries and risk of macrovascular diseases in Japanese type 2 diabetic patients. Diabetes 2004; **53**: 2455−2460
29) Blankenberg S, Rupprecht HJ, Bickel C, Torzewski M, Hafner G, Tiret L, Smieja M, Cambien F, Meyer J, Lackner KJ; AtheroGene Investigators: Glutathione peroxidase 1 activity and cardiovascular events in patients with coronary artery disease. N Engl J Med 2003; **349**: 1605−1613
30) Ohsawa M, Tsuru R, Hojo Y, Mizuno O, Fukazawa H, Mitsuhashi T, Katsuki T, Shimada K: Relationship between redox state of whole arterial blood glutathione and left ventricular function after acute myocardial infarction. J Cardiol 2004; **44**: 141−146(in Jpn with Eng abstr)