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Prooxidant–antioxidant balance as a new risk factor in patients with angiographically defined coronary artery disease

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Abstract

Objectives: Determination of the prooxidant–antioxidant balance (PAB) in patients with angiographically defined coronary artery disease (CAD+) by a modified PAB assay and presentation of PAB value as a novel cardiovascular risk factor.

Design and methods: For 61 patients with CAD+ and 63 healthy volunteers, the PAB were measured and its correlation was determined with anthropological and clinical parameters.

Results: A significant increase of the PAB value was observed in patients in comparison to control group. A correlation, which is not quite significant, was noted between angiographic finding (number of diseased vessel) and the PAB values in patients. A significant positive correlation was established between the PAB value and systolic blood pressure, diastolic blood pressure, smoking, fasting blood sugar and serum urea concentration; and a significant negative correlation was established between PAB value and serum creatinine and bilirubin.

Conclusions: This study shows that the PAB value may be considered as a cardiovascular risk factor. Further clinical research is needed to substantiate the potency of the PAB value as a cardiovascular risk factor.

Keywords: Prooxidant–antioxidant balance; Angiography; Coronary artery disease; Risk factor; Oxidative stress method

Introduction

Cardiovascular diseases (CVD)—coronary artery disease, hypertension, congestive heart failure, and stroke are leading causes of death and disability in the world [1].

The major and independent risk factors for CVD are cigarette smoking, elevated blood pressure, elevated serum total and low-density lipoprotein cholesterol (LDL-C), low high-density lipoprotein cholesterol (HDL-C), diabetes mellitus, and advanced age. These risk factors are usually used for the evaluation of an individual's predisposition to cardiovascular disease [2]. However, these typical risk factors can be accounted for no more than 25% to 30% of excess cardiovascular risk factors in patients [3]. This suggests that other factors might play a key role in the progression of atherosclerosis. Recently oxidative stress [4,5] and inflammation [6,7] have been considered as significant risk factors for CVD [8].

Several lines of evidence support a role for oxidative stress in atherogenesis [9,10]. Oxidative stress is defined as an imbalance between prooxidants and antioxidants in favor of prooxidants

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[11]. Growing evidence indicates that chronic and acute overproduction of reactive oxygen species (ROS) under pathophysiologic conditions is essential for the development of cardiovascular diseases (CVD). Clinical investigations support the oxidative stress hypothesis of atherosclerosis [12]. The reactive oxygen species leads to many proatherogenic events such as LDL oxidation, endothelial dysfunction, and vascular smooth muscle proliferation and migration. The oxidant species mediate various signaling pathways, which cause the initiation of fatty streak development through lesion progression to ultimate plaque rupture [9,10]. Oxidative stress is the unifying mechanism for many CVD risk factors [8].

Recently, we developed a method [13] which can measure the balance of oxidants and antioxidants simultaneously, by using 3,3′,5,5′-Tetramethylbenzidine (TMB) and two different kinds of reactions; one enzymatic reaction where the chromogen TMB is oxidized to a color cation by peroxides, and a chemical reaction where the TMB cation is reduced to a colorless compound by antioxidants; and given a redox index.

In this study, we determined the prooxidant–antioxidant balance in patients with angiographically defined coronary artery disease by a modified PAB assay; and its correlation with anthropological and clinical parameters.

Because now the measurement of this prooxidant–antioxidant balance could be done rapidly, easily and cost-effectively in a clinical laboratory, the aim of this study was to investigate and present the prooxidant–antioxidant balance as a risk factor that could be estimated along with other risk factors, for devising strategies to lessen or delay the progression and/or the complications of atherosclerosis in high-risk groups.

Materials and methods

Chemicals

TMB powder (3,3′,5,5′-Tetramethylbenzidine, Fluka), peroxidase enzyme (Applichem: 230 U/mg, A3791,0005, Darmstadt, Germany), chloramine T trihydrate (Applichem: A4331, Darmstadt, Germany), hydrogen peroxide (30%) (Merck). All the other reagents used were reagent grade and were prepared in double distilled water.

Subjects

Sixty-one patients (33 males and 28 females) were chosen for this study. The presence of one or more stenoses ≥50% in diameter of at least one major coronary artery (Left main, Right coronary artery, Left anterior descending, Circumflex) was determined by angiographic assessment [14]. Coronary angiograms were performed using routine procedures. Analysis of the angiograms was performed offline by a specialist cardiologist. These patients were undergoing routine coronary angiography, mainly for stable angina, and were positive for at least one objective test of myocardial ischemia including exercise stress test, Dobutamin stress echocardiography, and Thallium SPECT. These tests and the angiogram were performed at the Ghaem Medical Education Hospital, Mashhad, Iran. The patients ranged from 36 to 80 years of age. Patients who were on lipid-lowering medication, oral contraceptives, or hormone replacement therapy as well as pregnant women were excluded from the study. None of our subjects had a prior history of coronary angioplasty or coronary artery bypass graft (CABG). None of the subjects had overt clinical features of infection, or chronic inflammatory disease, and all subjects were negative for HBS antigen, anti-HCV antibody, and anti-HIV antibody. Data about the smoking habit was collected from each subject. Also, 63 age-matched healthy volunteers (33 males and 30 females) were chosen as a control group. For all individuals, anthropometric parameters including weight, height, and waist circumference were measured using standard protocols. Each patient and healthy volunteer gave informed written consent to participate in the study, which was approved by the Mashhad University of Medical Science Ethics Committee.

Collection of serum samples

Blood samples were collected in the morning from each subject after an overnight fast. After being allowed to clot, blood was then centrifuged at 2500 rpm for 15 min at room temperature to obtain serum. Hemolyzed samples were excluded from analysis. Serum was stored at −20 °C prior to analysis. Three aliquots of 20 samples were prepared for the evaluation of the precision (inter-assay).

Routine biochemical analysis

A full fasted lipid profile, comprising of the total cholesterol, triglycerides, high-density lipoprotein cholesterol (HDL-C), and low-density lipoprotein cholesterol (LDL-C), was determined for each patient. Serum lipid and fasting blood sugar concentrations were measured by enzymatic methods. Serum ceruloplasmin was measured by turbidometry for 20 samples.

Prooxidant–antioxidant balance (PAB) assay

A modified PAB was applied based on a previously described method [13]. The standard solutions were prepared by mixing varying proportions (0–100%) of 250 μM hydrogen peroxide with 3 mM uric acid (in 10 mM NaOH).

Sixty mg TMB powder was dissolved in 10 mL DMSO; for preparation of TMB cation, 400 μL of TMB/DMSO was added in 20 mL of acetate buffer [0.05 M buffer, pH 4.5], and then 70 μL of fresh chloramine T (100 mM) solution was added into this 20 mL, mixed well, incubated for 2 h at room temperature in a dark place; 25 U of peroxidase enzyme solution was added into 20 mL TMB cation, dispensed in 1 mL and put at −20 °C; in order to prepare the TMB solution 200 μL of TMB/DMSO was added into 10 mL of acetate buffer [0.05 M buffer, pH 5.8]; the working solution was prepared by mixing 1 mL TMB cation with 10 mL of TMB solution, incubated for 2 min at room temperature in a dark place and immediately used. Ten microliters of each sample, standard or blank (distilled water) were mixed with 200 μL of working solution, in each well of a 96 well plate,

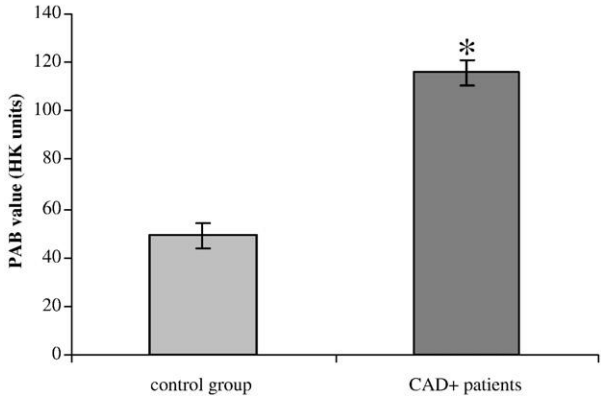

Fig. 1. The PAB value of control group and CAD+ patients. * Significant difference ($p<0.0001$) between the PAB value of the control group and the CAD+ patients.

which was then incubated in a dark place at 37 °C for 12 min; at the end of the incubation time, 100 μL of 2 N HCl was added to each well; and measured in an ELISA reader at 450 nm with a reference wavelength of 620 or 570 nm. A standard curve was provided from the values relative to the standard samples. The values of the PAB are expressed in arbitrary HK units, which are the percentage of hydrogen peroxide in the standard solution. The values of the unknown samples were then calculated based on the values obtained from the above standard curve.

Comparison of modified PAB assay with the original PAB assay

The modified PAB assay and the original PAB assay were applied for 40 samples and their correlations were determined. Also the modified PAB assay was calibrated against these standards: vitamin C (0–800 μM), trolox (0–800 μM), uric acid (0–6 mM in 10 mM NaOH), glutathione (0–2500 μM), bilirubin (0–40 μM), albumin [0–1000 mM (68 g/L)], ceruloplasmin (0–32.5 mg/dL), hydrogen peroxide (0–1000 μM), *tert*-buthylhydroperoxide (0–1000 μM), chloramine T (0–200 μM) and HClO (0–200 μM).

Statistical analysis

For the statistical analysis, the GraphPad Instat statistical package was used (GraphPad Software, Inc). All parameters were given as mean±S.D. The group comparisons were assessed by the *t*-test. Parametric and non-parametric correlations were assessed using Pearson correlation coefficients and Spearman correlation coefficients, respectively. The level of statistical significance was set to $p<0.05$.

Results

PAB assay

The PAB values of control group and CAD+ patients were 49.0±41.4 (HK unit) and 115.9±39.7 (HK unit), respectively (Fig. 1). There was a significant difference ($p<0.0001$) between the PAB value of the control group and the CAD+ patients.

A not quite negative significant correlation ($r=-0.45$, $p=0.07$) was found between PAB and serum ceruloplasmin concentrations. A marginally significant correlation, ($r=0.18$, $p=0.09$), was noted between the severity of angiographic findings (number of diseased vessels) and the PAB values in CAD+ patients.

The clinical and biological parameters and their correlation with PAB values

The clinical and biological parameters of the CAD+ patients and the control group were presented in Table 1, along with the correlation of the clinical and biological parameters with the PAB values.

Comparison of modified PAB assay with the original PAB assay

A significant correlation was established between the results of the modified PAB and the original PAB assay ($r=0.81$, $p<0.0001$) with no significant differences between the values obtained by the two assays.

A linear decrease ($R^2>0.85$) of the modified PAB value was observed in response to increasing major antioxidants such as vitamin C, trolox, glutathione, uric acid, bilirubin, albumin and ceruloplasmin. On the other hand, increasing the concentration of the prooxidants such as hydrogen peroxide, *tert*-buthylhydroperoxide, chloramine T and HClO resulted in a linear increase ($R^2>0.86$) of the PAB value.

Precision and storage

For the determination of the precision of the modified PAB method, the intra- and inter-assay coefficients of variation (%CV)

Table 1
Correlation between PAB values and clinical–biological parameters of CAD+ patients and control group

	Control group ($n=63$)	CAD+ patients ($n=61$)	Correlation with PAB ($n=124$) ($p<0.05$)
Age, yrs	55.6±14.5	58.8±9.9	No
Gender, M/F	33/30	33/28	–
Waist circumference (cm)	94.7±10.5	95.0±11.04	No
Waist/Hip	0.96±0.10	0.98±0.08	No
BMI, kg/m^2	25.2±4.0	25.7±4.1	No
Systolic BP, mm Hg	116.3±9.1	128.7±18.8 [a]	Yes
Diastolic BP, mm Hg	71.1±10.1	83.1±10.0 [a]	Yes
Smokers *n* (%)	14 (22.2)	13 (21.3)	Yes
Fasting blood sugar, mg/dL	98.0±11.6	112.3±50.3 [a]	Yes
Triglycerides, mg/dL	130.5±72	134.1±65.2	No
Total cholesterol, mg/dL	182.3±36.7	185.3±38.6	No
LDL cholesterol, mg/dL	130.1±20.4	135.8±58.2	No
HDL cholesterol, mg/dL	43.3±10.4	42.04±8.2	No
Serum creatinine, mg/dL	0.89±0.15	1.1±1.4	Yes [b]
Urea, mg/dL	21.3±11.4	36.7±17.7 [a]	Yes
Bilirubin mg/dL	1.6±0.7	1.7±0.9	Yes [b]
PAB value (HK unit)	49.0±41.4	115.9±39.7	–

Values are mean±S.D.
M: male, F: female.
[a] Significant difference ($p<0.05$).
[b] Significant negative correlation.

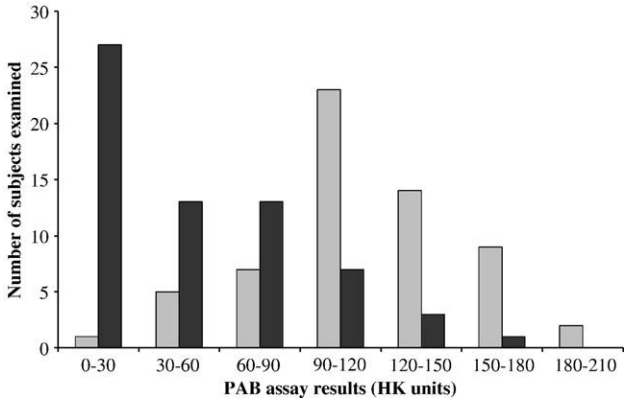

Fig. 2. Distribution of PAB values among healthy individuals (black columns) and CAD patients (gray columns).

were determined. The %CV of the intra-assay for 28 samples analyzed in triplicate was between 1.4 and 3.5%, with a mean of 2.1%. The %CV of the inter-assay for 20 samples, analyzed over 3 days, was between 4.1 and 8.5%, with a mean of 6.1%.

Serum PAB was not affected by storage at 4 °C for 1 day, or at −20 °C for 1 week, or at −80 °C for 3 months. Three times of repetitive freezing and thawing did not change the PAB value of serum samples (for 20 samples).

Interference, recovery and biological variation

Hemolysis interfered with the assay. Heparin, EDTA and citrate did not interfere. PAB values of plasma samples collected in heparin, EDTA and citrate were the same for the same individuals. PAB values of plasma samples were higher than those of serum samples in the same individuals. The difference between plasma and serum PAB values was related to the total levels of protein in the plasma and the serum samples.

The absorbance of plasma samples was higher than those of serum samples for the same individuals after adding HCl (because of turbidity). Therefore, the assay was performed with serum samples. Triglycerides up to 0–1000 mg/dL and cholesterol up to 900 mg/dL had no interfered on PAB assay.

The spiked samples were prepared by separately adding 200 μM of vitamins C, E, glutathione and hydrogen peroxide to serum samples, and the percentage recovery of the assay was then determined for each of them. The mean percentage recovery of added vitamins C, E, glutathione and hydrogen peroxide were; 91–111%, 91–125%, 96–111%, 90–91%, respectively.

In order to check diurnal variation, serum samples of five subjects were collected in the morning of the first day, 1 day after and 1 month after, in a fasting state. No significant variation was observed (data not shown). The distribution curves of PAB values of the controls and patients are presented in Fig. 2.

Discussion

In spite of the well documented role of oxidative stress in the atherosclerotic transformation, the determination of prooxidant–antioxidant balance is not yet a routine clinical laboratory test, mainly because of the lack of a universally accepted method. Many methods have been developed that can measure separately the total prooxidant and antioxidant capacities.

We presented recently a simple, rapid and cost-effective method (the PAB assay), which can measure the prooxidant burden and the antioxidant capacity in one assay, giving a redox index [13]. The assay uses TMB–TMB cation a known and well studied redox index [15,16].

The assay showed a linear response against a series of oxidants and antioxidants as well as serum proteins like albumin and ceruloplasmin. The oxidant moiety of the test consists of the well known peroxidase total oxidants assay that measures the total peroxides. Changes in hydroperoxides have been widely used in clinical laboratory settings as an indicator of total oxidant status (TOS) [17]. Hydrogen peroxide, although negligible in healthy people plasma, has been widely used as a representative of hydroperoxides in most TOS determination methods that express TOS as μM H_2O_2 equivalent [18].

On the other hand, the antioxidant capacity is usually expressed in μM uric acid. It should be noted that hydrogen peroxide and uric acid do not directly interact with each other. Accordingly, PAB assay has been calibrated using a series of mixtures of hydrogen peroxide and uric acid. The results are expressed as the percentage of hydrogen peroxide in the calibration mixture and PAB value shows the oxidative stress index and defined as an arbitrary unit (HK unit).

The only way to estimate prooxidant–antioxidant balance, if not by PAB assay, is to perform two separate tests. This procedure is more laborious and needs more time and cost since two separate methods should be applied to determine the balance. Balance is then calculated indirectly by the data of two assays. By doing these calculations the imprecision increases significantly. Sugherini et al. [19] proposed a novel integrated parameter, called “redox compensation index”, obtained by combining the results of the Fox-2 assay for plasma lipid hydroperoxides and the ferric reducing/antioxidant power (FRAP) assay for total antioxidant potential of plasma. Prior and Cao mentioned that no single measurement of antioxidant status is going to be sufficient, but a “buttery” of measurements, will be necessary to adequately assess oxidative stress in biological systems [20]. Trotti et al. [21] used the chromogen *N*, *N*,-diethylparaphenylen-diamine in two different assays one for estimating prooxidant burden and a second to estimate antioxidant capacity (oxy-absorbent test). Although the same chromogen was used in both tests the two tests cannot be combined in one because of the nature of the second test that necessitates the addition of prooxidants into the assay admixture.

The modified PAB assay, as applied in the present study, performed equally to the PAB assay that can measure the total prooxidants and the total antioxidants in one experiment. PAB assay was previously compared to widely utilized and documented methods that aim to estimate permanent oxidative damage such as carbonyl assay (by immunoassay), advanced glyclation end product assay (AGEs) (by spectrofluorometry), and advanced oxidative protein products (AOPP) assay (by spectrophotometry) [13]. However it should be noted that comparison of PAB assay with methods that estimate permanent oxidative stress damage (including isoprostane), is a comparison

of unequal matters since PAB as well as other tests (the total oxidant status or the total antioxidant status) are immediate indicators of oxidative stress.

Accordingly it remains within the future scope of our laboratories to compare the results of PAB with the results of additional assays.

In the present study, a modification of the PAB method is presented that further simplifies the test without compromising its validity. The method was applied to patients with angiographically proven coronary artery disease as compared to healthy volunteers.

A significant increase of the PAB value was noted in patients in comparison with the control group. A marginally significant correlation, with the severity of angiographic findings was also observed. The results are consistent with other reports, indicating a correlation of CAD with increased oxidative stress levels.

Vassalle et al. [22] reported that elevated oxidative stress is an independent predictor for cardiac death. Botto et al. [23] evidenced elevated levels of oxidative DNA damage in patients with angiographically documented coronary artery disease (CAD).

It has been also reported that isoprostanes, markers of lipid peroxidation, and reduced antioxidant capacity are related to increased risk for cardiovascular disease, and correlated with the number of cardiovascular risk factors [24,25].

In addition, a recent study conducted in a large cohort of patients, with stable CAD, indicates that the oxidative stress level represents a strong and independent prognostic predictor of cardiovascular events [26]. Kotur-Stevuljevic et al. [8] revealed in CAD patients elevated levels of both oxidative stress status parameters [superoxide anion ($O_2^{\bullet -}$) and malonaldehyde (MDA)] and reduced protective superoxide dismutase (SOD) activities which were independent of the extent of CAD. Also a greater number of CAD patients with a higher degree of stenosis (more vessels with occlusion >50%) tended (which is not statistically significant) to have higher levels of both $O_2^{\bullet -}$ and MDA in combination with lower activities of plasma SOD. Jung [27] and Bridges [28] independently found significantly higher levels of MDA in patients with angiographically diagnosed CAD when compared with healthy controls.

Nojiri et al. [29] found that the total antioxidant status levels in CAD patients were significantly lower than those in the control subjects, and the total antioxidant status levels, carotenoids and GSH-Px inversely correlated with the number of diseased vessels in the CAD patients. Kadota [30] found that mercaptalbumin and total SH contents were reduced in proportion to the severity of CAD.

In the present study, a significant positive correlation was observed between the PAB values and systolic and diastolic blood pressure, smoking, fasting blood sugar and serum urea concentration; and a significant negative correlation was established between PAB value and serum creatinine and bilirubin.

A marginal negative correlation between PAB and ceruloplasmin was observed. In vitro ceruloplasmin as well as albumin behaved as antioxidants. The antioxidant effect of proteins can be attributed to the SH groups of these molecules. In addition it should be noted that ceruloplasmin ferroxidase activity has been considered as an antioxidant activity in vivo since ferroxidase oxidizes ferro into ferric cations inhibiting thus Fenton reaction [31]. The inhibition of Fenton reaction does not interact with the oxidant moiety of PAB since peroxidase acts independently of ferrous cations. Therefore participation of ceruloplasmin as an antioxidant to the PAB can be rather attributed to similar factors that consider the antioxidant activity of any protein and less as an enzyme. The presence of lipids such as cholesterol and triglycerides did not influence the assay, accordingly no correlation was established between serum cholesterol, triglycerides, LDL or HDL concentrations and PAB values.

It has been reported that reactive oxygen species such as superoxide anion, hydrogen peroxide and hydroxyl radicals may play a role in the development of organ damage associated with cardiovascular disease in general and hypertension in particular [32,33].

Chrysohoou [34] found a positive correlation of oxidized LDL, with hypertension status and revealed an association of pre-hypertension with oxidative stress markers linking to the atherosclerotic process.

All this data and other reports, jointly support that oxidative stress could be used as a significant risk predictor in the atherosclerotic process; and evaluation of the PAB, along with other risk factors, might help in the prediction of the risk for cardiovascular events; and an effective primary prevention could reduce progression of the cardiovascular disease by appropriate interventions.

Further clinical research is needed based on a larger healthy population, as well as on various physiological and pathological states related to oxidative stress, and by multiple laboratories in order to substantiate the potency of the assay to become a clinical laboratory test; and the potency of the PAB value that could be presented as a cardiovascular risk factor.

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References

[1] Thom TJ. International mortality from heart disease: rates and trends. Int J Epidemiol 1989;18:S20–8.
[2] Wilson PW, D'Agostino RB, Levy D, et al. Prediction of coronary heart disease using risk factor categories. Circulation 1998;97:1837–47.
[3] Bierman EL. George Lyman Duff Memorial Lecture. Atherogenesis in diabetes. Atherioscler Thromb 1992;12:647–56.
[4] Cai H, Harrison DG. Endothelial dysfunction in cardiovascular disease. The role of oxidant stress. Circ Res 2000;87:840–4.
[5] Landmesser U, Spiekermann S, Dikalov S, et al. Vascular oxidative stress and endothelial dysfunction in patients with chronic heart failure. Circulation 2002;106:3073–8.

[6] Ostend B, Bjorklid E. Role of monocytes in atherogenesis. Phisiol Rev 2003;83:1069–112.
[7] Pearson TA, Mensah GA, Alexander RW, et al. Markers of inflammation and cardiovascular disease: application to clinical and public health practice: a statement for healthcare professionals from the Centers for Disease Control and Prevention and the American Heart Association. Circulation 2003;107:499–511.
[8] Kotur-Stevuljevic J, Memon L, Stefanovic A, et al. Correlation of oxidative stress parameters and inflammatory markers in coronary artery disease patients. Clin Biochem 2007;40:181–7.
[9] Glass CK, Witztum JL. Atherosclerosis: the road ahead. Cell 2001;104: 503–16.
[10] Jay D, Hitomi H, Griendling KK. Oxidative stress and diabetic cardiovascular complications. Free Radical Biol Med 2006;40:183–92.
[11] Halliwell B, Gutteridge JMC. Free radicals in biology and medicine. 3rd edn. Oxford: Oxford Science Publications; 1999.
[12] Singh U, Jialal I. Oxidative stress and atherosclerosis. Pathophysiology 2006;13:129–42.
[13] Alamdari DH, Paletas K, Pegiou T, et al. Novel assay for the evaluation of the prooxidant–antioxidant balance, before and after antioxidant vitamin administration in type II diabetes patients. Clin Biochem 2007;40:248–54.
[14] Marroquin O, Kip K, Kelley DE, et al. Metabolic syndrome modifies the cardiovascular risk associated with angiographic coronary artery disease in women: a report from the Women's Ischemia Syndrome Evaluation. Circulation 2004;109:714–721.
[15] Josephy PD, Mason RP, Eling T. Cooxidation of the clinical reagent 3,5, 3′ 5′-tetramethylbenzidine by prostaglandin synthase. Cancer Res 1982;42:2567–2570.
[16] Misono Y, Ohkata Y, Morikawa T, Itoh K. Resonance Raman and absorption spectroscopic studies on the electrochemical oxidation processes of 3,3′,5,5′-tetramethylbenzidine. J Electroanal Chem 1997;436:203–12.
[17] Gutteridge, Halliwell B. The measurement and mechanism of lipid peroxidation in biological systems. Trends Biochem Sci 1990;15:129–35.
[18] Erel O. A new automated colorimetric method for measuring total oxidant status. Clin Biochem 2005;38:1103–11.
[19] Sugherini L, Valentini M, Cambiaggi C, et al. Determination of a redox compensation index and its relationships to glycaemic control in type 2 diabetes mellitus. Clin Chem Lab Med 2000;38:983–987.
[20] Prior RL, Cao G. In vivo total antioxidant capacity: comparison of different analytical methods. Free Radic Biol Med 1999;27:1173–81.
[21] Trotti R, Carratelli M, Barbieri M, et al. Oxidative stress and a thrombophilic condition in alcoholics without severe liver disease. Haematologica 2001;86:85–91.
[22] Vassalle C, Boni C, Cecco PD, Landi P. Elevated hydroperoxide levels as a prognostic predictor of mortality in a cohort of patients with cardiovascular disease. Intern J Cardiol 2006;110:415–6.
[23] Botto N, Masetti S, Petrozzi L, et al. Elevated levels of oxidative DNA damage in patients with coronary artery disease. Coron Artery Dis 2002;13: 269–74.
[24] 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;256:308–15.
[25] Schwedhelm E, Bartling A, et al. Urinary 8-iso-prostaglandin F2alpha as a risk marker in patients with coronary heart disease: a matched case–control study. Circulation 2004;109:843–8.
[26] Walter MF, Jacob RF, Jeffers B, et al. PREVENT Study. Serum levels of thiobarbituric acid reactive substances predict cardiovascular events in patients with stable coronary artery disease: a longitudinal analysis of the PREVENT study. J Am Coll Cardiol 2004;44:1996–2002.
[27] Jung HH, Choi DH, Lee SH. Serum malondialdehyde and coronary artery disease in hemodialisis patients. Am J Nephrol 2004;24:537–42.
[28] Bridges AB, Scott NA, Pringle TH, et al. Relationship between the extent of coronary artery disease and indicators of free radical activity. Clin Cardiol 1992;15:169–74.
[29] Nojiri S, Daida H, Mokuno H, Iwama Y, Mae K, Ushio F, Ueki T. Association of serum antioxidant capacity with coronary artery disease in middle-aged men. Jpn Heart J 2001;42:677–90.
[30] Kadota K, Yui Y, Hattori R, Murohara Y, Kawai C. Decreased sulfhydryl groups of serum albumin in coronary artery disease. Jpn Circ J 1991;55: 937–41.
[31] Healy J, Tipton K. Ceruloplasmin and what it might do. J Neural Transm 2007;114:777–81.
[32] Swei A, Lacy F, DELano FA, Schmid-Schonbein GW. Oxidative stress in the Dahl hypertensive rat. Hypertension 1998;16:291–303.
[33] Griendling KK, Sorescu D, Ushio-Fukai M. NAD(P)H oxidase: role in cardiovascular biology and disease. Circ Res 2000;86:494–501.
[34] Chrysohoou C, Panagiotakos DB, Pitsavos C, et al. The association between pre-hypertension status and oxidative stress markers related to atherosclerotic disease: the ATTICA study. Atherosclerosis 2007;192: 169–76.