Biological Antioxidant Potential Negatively Correlates with Carotid Artery Intima-Media Thickness

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SUMMARY

Oxidative stress is a crucial factor in the pathogenesis and development of cardiovascular disease. Recently, simplified methods for the detection of reactive oxygen species (ROS) using the derivatives of reactive oxygen metabolites (d-ROMs) test as an index of ROS products and the biological antioxidant potential (BAP) test as an index of antioxidant potential have been utilized. These methods are easy to perform, quick, inexpensive by use of small equipment, and provide reliable results compared with established oxidative stress and antioxidant markers. Because oxidative stress has shown the balance of production of ROS and antioxidant capacity, it is more appropriate to evaluate ROS and antioxidant capacity simultaneously. However, no study has examined associations among d-ROMs, BAP values, and carotid artery intima-media thickness (IMT) concurrently. Therefore, we studied the associations among d-ROMs, BAP values, and the carotid artery IMT. Carotid artery IMT, blood pressure (BP), fasting circulating d-ROMs, BAP, glucose metabolism, lipid, and C-reactive protein levels were measured in 95 subjects (age: 49.5 ± 13.8 years; men: 41; women: 54), including 42 healthy subjects and 53 patients with hypertension, dyslipidemia, and diabetes mellitus who are not on medication. The results of multiple regression analysis revealed that dependent carotid artery IMT determinants remained significantly associated with age, systolic BP, total cholesterol, and BAP, whereas dependent BAP determinants remained significantly associated with body mass index, and carotid artery IMT. BAP strongly correlated with carotid artery IMT in our cohort. Our results suggest that BAP may be a useful risk marker for carotid atherosclerosis.

Key words: Antioxidant markers, Carotid atherosclerosis, Oxidative stress

Oxidative stress is involved as a crucial factor in the pathogenesis and development of a variety of chronic and degenerative diseases, including aging, cancer, and cardiovascular disease. An enhanced oxidative stress status, defined as an imbalance between oxidants and antioxidants in favor of the former, plays a significant role in the onset and progression of atherosclerosis.[1–3] Levels of oxidative stress markers, such as thiobarbituric acid-reactive substances (TBARSs),[4] 8-iso-prostaglandin (PG) $F_2\alpha$,[5] oxidized low-density lipoprotein (LDL) (oxLDL),[6] and myeloperoxidase (MPO),[7,8] independently predict an increased risk for coronary artery disease (CAD). In contrast, glutathione peroxidase (GPx), superoxide dismutase (SOD), catalase enzymes, and total antioxidant status (TAS) are antioxidant markers that constitute the first line of defense against oxidative stress by removing key reactive oxygen species (ROS).[9] Meta-analyses of observational studies have identified inverse associations of circulating SOD, GPx, and catalase activity levels with CAD.[10] Furthermore, another study has demonstrated that low GPx1 activity level was independently associated with an increased risk of cardiovascular events in patients with CAD.[11]

An increased carotid artery intima-media thickness (IMT) measured noninvasively by B-mode ultrasonography is considered a good surrogate marker for early atherosclerosis. It is reportedly associated with cardiovascular risk[12] and the severity of coronary atherosclerosis[13] and can predict cardiovascular events, such as myocardial infarction and stroke, in several populations.[14] Progression of carotid artery IMT is prevented by intensive lipid-lowering therapy.[15,16] With regard to the relationship between oxidative stress markers and carotid artery IMT, several clinical studies have reported that circulating levels of TBARS in chronic hemodialysis[17] and type 2 diabetic patients,[18] oxLDL in asymptomatic familial combined hyperlipidemia families,[19] and MPO in hemodialysis patients[20] positively correlated with carotid artery IMT. Furthermore, increased urinary excretion of 8-iso-PG F2α was also associated with increased carotid artery IMT.[21] In contrast, several clinical studies have demonstrated correlations between antioxidant markers and carotid artery IMT: SOD activity in diabetic patients,[18] catalase activity in chronic hemodialysis[17] and type 2 diabetic patients,[18] and serum TAS in workers occupationally exposed to mercury vapor[22] were negatively correlated with carotid artery IMT.

Nonetheless, assays used for the determination of abovementioned oxidative stress and antioxidant markers are complicated. Recently, several studies utilizing simplified methods for the detection of ROS using the derivatives of reactive oxygen metabolites (d-ROMs) test and the biological antioxidant potential (BAP) test have been published.[23–25] These methods are easy to perform, quick, inexpensive, utilize small equipment, and provide reliable results compared with established oxidative stress and antioxidant markers.[26,27]

Because oxidative stress has shown the balance of production of ROS and antioxidant capacity, it is more appropriate to evaluate ROS and antioxidant capacity simultaneously. However, only one study has reported an association between d-ROMs and carotid artery IMT in patients with hypercholesterolemia,[28] and, thus far, no study has examined associations among d-ROMs, BAP values, and carotid artery IMT concurrently. Therefore, we measured d-ROMs and BAP values as indices for the ROS production and the antioxidant potential, respectively, and carotid artery IMT within the same subjects to investigate the association between carotid artery IMT and clinical parameters values, including d-ROMs and BAP.

METHODS

Subjects: One hundred and ninety-five consecutive Japanese subjects who visited our department for assessment of cardiovascular risk or disease participated in this study. Medical and familial histories were obtained from all subjects. Subjects with the following conditions that could potentially significantly influence carotid artery IMT, blood pressure (BP), circulating lipid, and glucose metabolism were excluded from the study: those with a history of cardiovascular disease, including stroke, CAD, thromboembolic disease, or congestive heart failure; peripheral arterial disease; malignancy; infectious disease; liver or renal disease; overt endocrine disease; and current smokers and those currently on medication. Subjects with hypertension, dyslipidemia, and diabetes mellitus (DM), as defined by the established diagnostic criteria,[29-31] were included. Written informed consent

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was obtained from each subject, and the study was approved by the institutional review board of Gunma University Hospital.

We performed physical examinations and carotid artery IMT measurements in the morning (after a 12-h fast) and simultaneously obtained blood samples from the antecubital vein for serum and plasma analyses.

Physical examination: The height and weight of subjects were measured, and the body mass index (BMI) was calculated (weight in kilograms divided by height in meters squared). BP was measured in the morning (after a 12-h overnight fast) by the same investigator with a sphygmomanometer on the right arm of the subject after a 10-min rest in the supine position.

Laboratory analyses: Serum total cholesterol, triglyceride, high-density lipoprotein (HDL) cholesterol, and LDL cholesterol concentrations were measured by enzymatic methods, and C-reactive protein concentrations were measured by latex immunoassay, respectively, using an automatic analyzer (LABOSPECT 008; Hitachi, Tokyo, Japan). Serum insulin concentrations were measured by chemiluminescence immunoassay using an automatic analyzer (AIA-2000 LA; Tosoh, Tokyo, Japan). Plasma glucose concentrations were measured by hexokinase method, and hemoglobin A1c (HbA1c) levels were measured by high-performance liquid chromatography, using automatic analyzers (ADAMS Glucose GA-1170 and ADAMS A1c HA-8180, respectively; Arkray, Tokyo, Japan).

Measurement of d-ROMs and BAP: Based on the recommendation from the manufacturer (Diacron International, Grosseto, Italy), all analyses were performed within 48 h of venous blood collection to avoid false high or false low results. To analyze serum levels of ROS and serum antioxidant potential, d-ROMs and BAP tests were performed, respectively.

The d-ROMs were measured using a free radical elective evaluator (FREE Carpe Diem; Diacron International) that included a spectrophotometric device reader, and measurement kits (d-ROMs test) (Wismerll Co. Ltd., Tokyo, Japan) were optimized to the FREE Carpe Diem System according to the manufacturer's instructions. Briefly, a 20 µl serum sample and 1 ml buffered solution (R2 kit reagent, pH 4.8) were gently mixed in a cuvette, and 20 µl chromogenic substrate (R1 kit reagent) was then added to the cuvette. After mixing well, the cuvette was immediately incubated in the thermostatic block of the analyzer for 5 min at 37°C, and absorbance at 505 nm was recorded.[23-25] The results are expressed in arbitrary units (U.CARR), one unit of which corresponds to 0.8 mg/l of hydrogen peroxide.[23-25]

The BAP was measured using a free radical elective evaluator (FREE Carpe Diem) that included a spectrophotometric device reader, and measurement kits (BAP test) (Wismerll Co. Ltd.) were optimized to the FREE Carpe Diem System according to the manufacturer's instructions. Briefly, a 50µl chromogenic substrate (R2 kit reagent) and 1 ml reactive solution (R1 kit reagent) were gently mixed in a cuvette, and absorbance at 505 nm was recorded. A 10 µl serum sample was then immediately added to the cuvette. After mixing well, the cuvette was immediately incubated in the thermostatic block of the analyzer for 5 min at 37°C, and absorbance at 505 nm was recorded.[23-25] The results are expressed in mmol/l of reduced ferric ions.

Carotid artery IMT measurement: The wall thickness of the carotid artery was evaluated bilaterally by ultrasonography (LOGIQ 9; GE Healthcare Japan Corporation) using a 7.5-MHz linear type B-mode probe.[32-34] After the subject had rested for at least 10 min in the supine position with the neck in slight hyperextension, the visualization of bilateral common carotid arteries was optimized, and three measurements of end-diastolic IMT of the far wall of each artery were averaged: one at the thickest site and the other two measurements from 1 cm upstream and 1 cm downstream from the thickest site. A physician who was blinded to the clinical characteristics of subjects evaluated all scans. The variability of the ultrasonographic measurements was assessed by performing five measurements over a 1-month period in 12 volunteers. The intraobserver coefficient of variation for the IMT measurement was 5.5% ± 0.8%.

Statistical analyses: Data were expressed as means ± standard deviations. Pearson's correlation coefficient analyses were used to examine the relationships between carotid artery IMT values and clinical parameters values, including d-ROMs, BAP, and other clinical variables, and between BAP values and clinical parameters values, including carotid artery IMT and other clinical variables. We chose the clinical parameters of significant correlation with carotid artery IMT by Pearson's correlation coefficient analyses as candidate confounding factors. Multiple regression analysis for correlation between carotid artery IMT as a dependent variable and these clinical parameters values as independent variables was then performed. In addition, we chose clinical parameters of significant correlation with BAP values by Pearson's correlation coefficient analyses as candidate confounding factors. Multiple regression analysis for correlation between BAP values as a dependent variable and these clinical parameters values as independent variables was then performed. All the probability values were two tailed. $P < 0.05$ was considered statistically significant for all analyses performed. All statistical analyses were performed using the IBM SPSS software, version 21.0.

RESULTS

Clinical characteristics and complications: Of the 195 subjects available during the study period, 95 met the inclusion criteria. The average age was 49.5 ± 13.8 years (range, 23–77 years), and the cohort comprised 41 men (age: 52.3 ± 14.8 years; range, 24–77 years) and 54 women (age: 47.3 ± 12.8 years; range, 23–68 years). We included 42 healthy subjects, 23 subjects with essential hypertension, 44 with dyslipidemia, and 8 with type 2 diabetes mellitus. The characteristics of subjects are summarized in Table I.

Univariate and multivariate analyses for correlations with carotid artery IMT: The results of the univariate regression analysis revealed that carotid artery IMT positively correlated with age ($r = 0.730$, $P < 0.001$), systolic BP ($r = 0.586$, $P < 0.001$), diastolic BP ($r = 0.536$, $P < 0.001$), fasting plasma glucose ($r = 0.254$, $P = 0.013$), HbA1c ($r = 0.287$, $P = 0.005$), total cholesterol ($r = 0.474$, $P < 0.001$), triglycerides ($r = 0.281$, $P = 0.006$), and LDL cholesterol ($r = 0.506$, $P < 0.001$) and negatively correlated with HDL cholesterol ($r = -0.205$, $P = 0.047$) and BAP ($r = -0.484$, $P < 0.001$; Fig. 1). There were no significant associations between carotid artery IMT and clinical parameters, including d-ROMs (Fig. 2) and other clinical variables (Table II). In addition, results of the multiple regression analysis, including age, systolic

Table I. Subject Characteristics

Variable	Subjects
Number	95
Age (years)	49.5 ± 13.8
Sex (M/F)	41/54
Body mass index (kg/m^2)	22.4 ± 3.4
Normal healthy participants [n (%)]	42 (44)
Hypertension [n (%)]	23 (24)
Dyslipidemia [n (%)]	44 (46)
Diabetes mellitus [n (%)]	8 (8)
Systolic BP (mmHg)	121.4 ± 15.4
Diastolic BP (mmHg)	78.3 ± 10.8
Fasting plasma glucose (mg/dl)	100.0 ± 25.1
HbA1c (%)	5.8 ± 1.2
Insulin (µU/ml)	5.7 ± 4.5
eGFR (ml/min/1.73 m^2)	87.0 ± 15.7
C-reactive protein (mg/dl)	0.09 ± 0.15
Total cholesterol (mg/dl)	213.0 ± 41.7
HDL cholesterol (mg/dl)	59.3 ± 13.9
Triglyceride (mg/dl)	106.0 ± 54.4
LDL cholesterol (mg/dl)	125.2 ± 36.7
d-ROMs (U.CARR)	377.2 ± 82.1
BAP (µmol/l)	2395.4 ± 359.9
Carotid artery IMT (mm)	0.66 ± 0.14

All the results are presented as mean ± SD.
BAP indicates biological anti-oxidant potential; BP, blood pressure; d-ROMs, derivatives of reactive oxygen metabolites; eGFR, estimated glomerular filtration rate; HbAlc, hemoglobin A1c; HDL, high-density lipoprotein; IMT, intima-media thickness; and LDL, low-density lipoprotein.

Table II. Pearson's Correlation Coefficients between Carotid Artery Intima-Media Thickness Values and Clinical Parameters Values, including Derivatives of Reactive Oxygen Metabolites, Biological Antioxidant Potential, and Other Clinical Variables

Variable	*r*	*P* value
Age (years)	0.730	<0.001
Body mass index (kg/m^2)	0.193	0.061
Systolic BP (mmHg)	0.586	<0.001
Diastolic BP (mmHg)	0.536	<0.001
Fasting plasma glucose (mg/dl)	0.254	0.013
HbA1c (%)	0.287	0.005
Insulin (μU/ml)	−0.037	0.723
C-reactive protein (mg/dl)	0.078	0.452
Total cholesterol (mg/dl)	0.474	<0.001
HDL cholesterol (mg/dl)	−0.205	0.047
Triglyceride (mg/dl)	0.281	0.006
LDL cholesterol (mg/dl)	0.506	<0.001
d-ROMs (U.CARR)	0.005	0.962
BAP (μmol/l)	−0.484	<0.001

BAP indicates biological antioxidant potential; BP, blood pressure; d-ROMs, derivatives of reactive oxygen metabolites; HbAlc, hemoglobin A1c; HDL, high-density lipoprotein; and LDL, low-density lipoprotein.

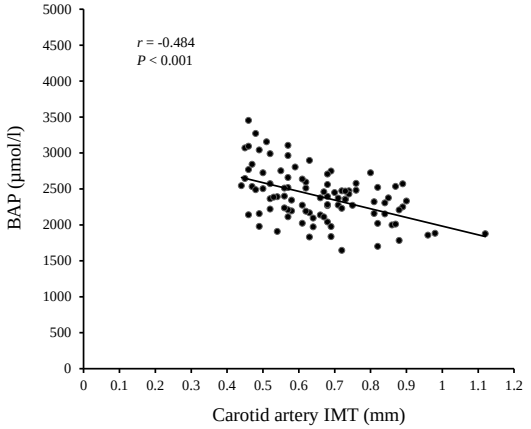

Figure 1. Correlation between carotid artery intima-media thickness (IMT) and biological antioxidant potential (BAP) in subjects. The results of the univariate regression analysis revealed a negative correlation between carotid artery IMT and BAP ($r = -0.484$, $P < 0.001$).

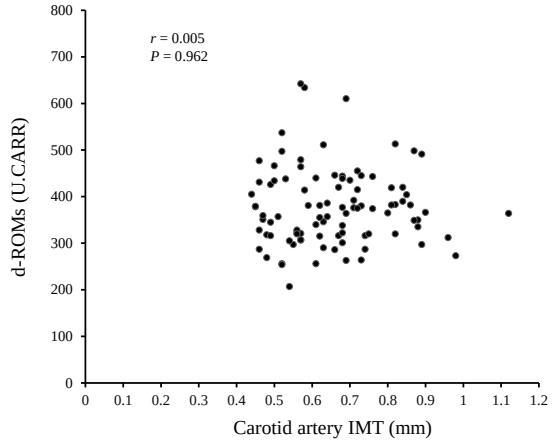

Figure 2. Correlation between carotid artery intima-media thickness (IMT) and derivatives of reactive oxygen metabolites (d-ROMs) in subjects. The results of the univariate regression analysis revealed no correlation between carotid artery IMT and d-ROMs ($r = 0.005$, $P = 0.962$).

BP, diastolic BP, fasting plasma glucose, HbA1c, total cholesterol, and BAP revealed that dependent carotid artery IMT determinants remained significantly associated with age, systolic BP, total cholesterol, and BAP (Table III).

Table III. Independent Predictors of Carotid Artery Intima-Media Thickness by Multiple Regression Analysis

Variable	*β*	*P* value
Age (years)	0.481	<0.001
Systolic BP (mmHg)	0.441	<0.001
Diastolic BP (mmHg)	−0.190	0.127
Fasting plasma glucose (mg/dl)	−0.019	0.894
HbA1c (%)	0.012	0.933
Total cholesterol (mg/dl)	0.151	0.041
BAP (μmol/l)	−0.176	0.015

BAP indicates biological antioxidant potential; BP, blood pressure; and HbAlc, hemoglobin A1c.

Univariate and multivariate analyses for correlations with BAP: Accordingly, we performed univariate and multivariate analyses for correlations between BAP and clinical parameters, including carotid artery IMT and other clinical variables. The results of the univariate regression analysis revealed that BAP positively correlated with HDL cholesterol ($r = 0.216$, $P = 0.035$) and negatively correlated with age ($r = -0.416$, $P < 0.001$), BMI ($r = -0.305$, $P = 0.003$), systolic BP ($r = -0.247$, $P = 0.016$), diastolic BP ($r = -0.297$, $P = 0.004$), fasting plasma glucose ($r = -0.211$, $P = 0.040$), total cholesterol ($r = -0.382$, $P < 0.001$), triglycerides ($r = -0.392$, $P < 0.001$), LDL cholesterol ($r = -0.356$, $P < 0.001$), and carotid artery IMT ($r = -0.484$, $P < 0.001$; Fig. 1). BAP did not significantly correlate with HbA1c, insulin, and C-reactive protein (Table IV). Furthermore, results of the multiple regression analysis, including age, BMI, systolic BP, diastolic BP, fasting plasma glucose, total cholesterol, and carotid artery IMT, revealed that dependent BAP determinants remained significantly associated with BMI and carotid artery IMT (Table V).

Discussion

In the present study, we have demonstrated, for the first time, that carotid artery IMT positively correlated with age, systolic BP, and total cholesterol, negatively correlated with BAP, and did not correlate with d-ROMs and that BAP negatively correlated with BMI and carotid artery IMT. BAP strongly correlated with carotid artery IMT. The advantages of usage of BAP are that the method is easy to perform, quick, inexpensive, utilize small equipment, and provide reliable results compared with established antioxidant markers.[26,27]

Dursun *et al* reported that catalase activity was inversely correlated with carotid artery IMT in uremic and hemodialysis patients[17] and that erythrocyte Cu/Zn SOD and catalase activities were negatively correlated with carotid artery IMT in type 2 diabetic patients undergoing hemodialysis and those with normal renal function.[18] Skoczyńska *et al* also demonstrated a negative linear correlation between serum TAS and carotid artery IMT in workers occupationally exposed to mercury vapor.[22] These findings that have reported an inverse association between antioxidant markers and carotid artery IMT are compatible with the results of the present study.

In addition, Demirbag *et al* found a negative correlation between plasma TAS and thoracic aortic IMT in patients without a history of atherosclerotic cardiovascular disease.[35] Since there is a strong and statistically significant positive correlation between the assays for BAP and TAS,[36] BAP may potentially be negatively correlated with thoracic aortic IMT. In the present study, subjects with dyslipidemia, diabetes mellitus, and hypertension were included. Although there were differences in both the study populations and the methods used to assess antioxidant markers and IMT between the aforementioned studies and the present study, the findings of the aforementioned studies support the results of our study. Hence, BAP negatively correlated with carotid artery IMT in our subjects.

Conversely, Kotani *et al* reported that d-ROMs was positively correlated with carotid artery IMT in patients with hypercholesterolemia.[28] However, our findings showed that

d-ROMs were not correlated with carotid artery IMT in our cohort. Forty-four percent of the subjects in our cohort was either healthy participants or subjects with mild disease conditions, which is likely reflected in the relatively narrow range of carotid artery IMT values between 0.44 and 1.12 mm in our cohort and may be responsible for the lack of significant associations between d-ROMs and carotid artery IMT.

The mechanisms underlying the negative correlation between BAP and carotid artery IMT in subjects in this study are not fully understood. However, several credible mechanisms linking antioxidant markers with atherogenic processes should be considered. Among antioxidant enzymes, SOD, GPx, and catalase constitute the first line of defense against oxidative stress by removing the key ROS.[9] Several observational studies have demonstrated that low SOD, GPx, and catalase activity levels were associated with high risk of CAD.[10] Conversely, high GPx and SOD activity levels may confer protection against ROS production. Overexpression of GPx[37] or intracellular SOD[38] in transgenic mouse models of reperfusion injury prevents postischemic free radical injury and inhibits LDL oxidation in endothelial cells. Furthermore, overexpression of SOD[39] or administration of exogenous SOD[40] or catalase[40,41] has been shown to attenuate leukocyte adhesion to endothelial cells, while SOD or catalase administration has been shown to suppress vascular smooth muscle cells proliferation induced by oxLDL.[42,43] Thus, the levels of antioxidant markers may inversely correlate with the development of carotid atherosclerosis and, consequently, may underlie the strong inverse relationship between BAP levels and carotid artery IMT.

With regard to the relationship between carotid artery IMT and pro-atherosclerotic factors, such as BMI and HbA1c, Wang et al. demonstrated that carotid artery IMT had a positive correlation with age, gender, having DM, BMI, and HbA1c in middle-aged and elderly Chinese people and that BMI, HbA1c, and having DM predicted carotid artery IMT thickening remained significant after adjusting for age and gender.[44] However, the present study showed that HbA1c and BMI did not correlate with carotid artery IMT. The subjects of the present study had lower mean HbA1c (5.8 % versus 7.4 %) and mean BMI levels (22.4 kg/m^2 versus 25.6 kg/m^2) and fewer prevalence of DM (8 % versus 68.8 %) than the subjects of the aforementioned study, respectively. Therefore, the differences in the background of the subjects may contribute to the difference between the results of the present study and the results of the aforementioned study.

This is the first study to have investigated the relationship between BAP and pro-atherosclerotic factors, such as BMI, total cholesterol, fasting plasma glucose, and blood pressure. The results of the multiple regression analysis revealed that BAP significantly correlated with BMI and carotid artery IMT, but did not significantly correlate with blood pressure and fasting plasma glucose. Although total cholesterol tended to correlate with BAP (P = 0.101), but it was not significant.

Table IV. Pearson's Correlation Coefficients between Biological Antioxidant Potential Values and Clinical Parameters Values, including Carotid Artery Intima-Media Thickness and Other Clinical Variables

Variable	r	P value
Age (years)	−0.416	<0.001
Body mass index (kg/m^2)	−0.305	0.003
Systolic BP (mmHg)	−0.247	0.016
Diastolic BP (mmHg)	−0.297	0.004
Fasting plasma glucose (mg/dl)	−0.211	0.040
HbA1c (%)	−0.197	0.056
Insulin (μU/ml)	−0.112	0.280
Total cholesterol (mg/dl)	−0.382	<0.001
HDL cholesterol (mg/dl)	0.216	0.035
Triglyceride (mg/dl)	−0.392	<0.001
LDL cholesterol (mg/dl)	−0.356	<0.001
C-reactive protein (mg/dl)	−0.098	0.344
Carotid artery IMT (mm)	−0.484	<0.001

BP indicates blood pressure; HbAlc, hemoglobin A1c; HDL, high-density lipoprotein; IMT, intima-media thickness; and LDL, low-density lipoprotein.

The d-ROMs and BAP as oxidative stress and antioxidant markers have advantages and disadvantages compared with other oxidative stress and antioxidant markers, respectively.[26,27] The advantages of these oxidative stress and antioxidant markers are that the methods are easy to perform (do not require skilled operators or complex instrumentation), quick, inexpensive, utilize small equipment, and provide reliable results compared with established oxidative stress and antioxidant markers.[26,27] In contrast, the disadvantages of these markers are that the specificity is not established and that these markers are probably not suitable for samples stored for prolonged periods compared with established ones.[26,27] In the present study, we used simpler methods such as d-ROMs as an indicator of ROS production and BAP as a readout for antioxidant potential. However, BAP alone significantly correlated with carotid artery IMT. Thus, the measurements of BAP may be superior to the measurements of GPx, SOD, catalase, and TAS for the assessment of antioxidant potential due to their simplicity, quickness, convenience, and reproducibility.

Table V. Independent Predictors of Biological Antioxidant Potential by Multiple Regression Analysis

Variable	β	P value
Age (years)	−0.094	0.510
Body mass index (kg/m^2)	−0.252	0.013
Systolic BP (mmHg)	0.146	0.395
Diastolic BP (mmHg)	0.000	0.999
Fasting plasma glucose (mg/dl)	−0.060	0.522
Total cholesterol (mg/dl)	−0.172	0.101
Carotid artery IMT (mm)	−0.356	0.017

BP indicates blood pressure; and IMT, intima-media thickness.

The present study has several limitations. First, the number of subjects in this study was relatively small, and a larger cohort will be necessary to confirm our findings. Second, the study may have had a bias in the enrollment of study subjects. Finally, as a cross-sectional study, it was not possible to determine a cause-and-effect relationship between BAP and carotid artery IMT.

In conclusion, BAP strongly correlated with carotid artery IMT. Our results suggest that BAP may be a useful risk marker for carotid artery atherosclerosis. Further studies are needed to determine whether the correlation between BAP and carotid artery IMT exists not only in subjects with coronary risk factors or CAD but also in subjects with no health issues.

DISCLOSURE

There are no conflicts of interest to declare.

REFERENCES

1. Griendling KK, FitzGerald GA. Oxidative stress and cardiovascular injury: Part I: basic mechanisms and in vivo monitoring of ROS. Circulation 2003; 108: 1912-6.
2. Madamanchi NR, Vendrov A, Runge MS. Oxidative stress and vascular disease. Arterioscler Thromb Vasc Biol 2005; 25: 29-38.
3. Harrison D, Griendling KK, Landmesser U, Hornig B, Drexler H. Role of oxidative stress in atherosclerosis. Am J Cardiol 2003; 91: 7A-11A.
4. Walter MF, Jacob RF, Jeffers B, *et al*. 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.
5. Patrono C, FitzGerald GA. Isoprostanes: potential markers of oxidant stress in atherothrombotic disease. Arterioscler Thromb Vasc Biol 1997; 17: 2309-15.
6. Holvoet P, Mertens A, Verhamme P, *et al*. Circulating oxidized LDL is a useful marker for identifying patients with coronary

artery disease. Arterioscler Thromb Vasc Biol 2001; 21: 844-8.
7. Zhang R, Brennan ML, Fu X, *et al*. Association between myeloperoxidase levels and risk of coronary artery disease. JAMA 2001; 286: 2136-42.
8. Brennan ML, Penn MS, Van Lente F, *et al*. Prognostic value of myeloperoxidase in patients with chest pain. N Engl J Med 2003; 349: 1595-604.
9. Stocker R, Keaney JF Jr. New insights on oxidative stress in the artery wall. J Thromb Haemost 2005; 3: 1825-34.
10. Flores-Mateo G, Carrillo-Santisteve P, Elosua R, *et al*. Antioxidant enzyme activity and coronary heart disease: meta-analyses of observational studies. Am J Epidemiol 2009; 170: 135-47.
11. Blankenberg S, Rupprecht HJ, Bickel C, *et al*.; AtheroGene Investigators. Glutathione peroxidase 1 activity and cardiovascular events in patients with coronary artery disease. N Engl J Med 2003; 349: 1605-13.
12. Raitakari OT, Juonala M, Kähönen M, *et al*. Cardiovascular risk factors in childhood and carotid artery intima-media thickness in adulthood: the Cardiovascular Risk in Young Finns Study. JAMA 2003; 290: 2277-83.
13. Burke GL, Evans GW, Riley WA, *et al*. Arterial wall thickness is associated with prevalent cardiovascular disease in middle-aged adults. The Atherosclerosis Risk in Communities (ARIC) Study. Stroke 1995; 26: 386-91.
14. O'Leary DH, Polak JF, Kronmal RA, Manolio TA, Burke GL, Wolfson SK Jr. Carotid-artery intima and media thickness as a risk factor for myocardial infarction and stroke in older adults. Cardiovascular Health Study Collaborative Research Group. N Engl J Med 1999; 340: 14-22.
15. Yamazaki T, Nohara R, Daida H, *et al*. Intensive lipid-lowering therapy for slowing progression as well as inducing regression of atherosclerosis in Japanese patients: subanalysis of the JART study. Int Heart J 2013; 54: 33-9.
16. Yokoi H, Nohara R, Daida H, *et al*. Change in carotid intima-media thickness in a high-risk group of patients by intensive lipid-lowering therapy with rosuvastatin: subanalysis of the JART study. Int Heart J 2014; 55: 146-52.
17. Dursun B, Dursun E, Suleymanlar G, *et al*. Carotid artery intima-media thickness correlates with oxidative stress in chronic haemodialysis patients with accelerated atherosclerosis. Nephrol Dial Transplant 2008; 23: 1697-703.
18. Dursun B, Dursun E, Suleymanlar G, *et al*. The effect of hemodialysis on accelerated atherosclerosis in diabetic patients: correlation of carotid artery intima-media thickness with oxidative stress. J Diabetes Complications 2009; 23: 257-64.
19. Liu ML, Ylitalo K, Salonen R, Salonen JT, Taskinen MR. Circulating oxidized low-density lipoprotein and its association with carotid intima-media thickness in asymptomatic members of familial combined hyperlipidemia families. Arterioscler Thromb Vasc Biol 2004; 24: 1492-7.
20. Honda H, Ueda M, Kojima S, *et al*. Assessment of myeloperoxidase and oxidative alpha1-antitrypsin in patients on hemodialysis. Clin J Am Soc Nephrol 2009; 4: 142-51.
21. Yoon JH, Kim JY, Park JK, Ko SB. Oxidative damage markers are significantly associated with the carotid artery intima-media thickness after controlling for conventional risk factors of atherosclerosis in men. PLoS One 2015; 10: e0119731. doi: 10.1371/journal.pone.0119731. eCollection 2015.
22. Skoczyńska A, Poreba R, Steinmentz-Beck A, *et al*. The dependence between urinary mercury concentration and carotid arterial intima-media thickness in workers occupationally exposed to mercury vapour. Int J Occup Med Environ Health 2009; 22: 135-42.
23. Nakayama K, Terawaki H, Nakayama M, Iwabuchi M, Sato T, Ito S. Reduction of serum antioxidative capacity during hemodialysis. Clin Exp Nephrol 2007; 11: 218-24.
24. Cesarone MR, Belcaro G, Carratelli M, *et al*. A simple test to monitor oxidative stress. Int Angiol 1999; 18: 127-30.
25. Pasquini A, Luchetti E, Marchetti V, Cardini G, Iorio EL. Analytical performances of d-ROMs test and BAP test in canine plasma. Definition of the normal range in healthy Labrador dogs. Vet Res Commun 2008; 32: 137-43.
26. Vassalle C. An easy and reliable automated method to estimate oxidative stress in the clinical setting. Methods Mol Biol 2008; 477: 31-9.
27. Ridker PM, Brown NJ, Vaughan DE, Harrison DG, Mehta JL. Established and emerging plasma biomarkers in the prediction of first atherothrombotic events. Circulation 2004; 109 (25 Suppl 1): IV6-19.
28. Kotani K, Koibuchi H, Miyamoto M, Yamada T, Taniguchi N. Relationship between reactive oxygen metabolites and carotid intima-media thickness in subjects with hypercholesterolemia. Med Princ Pract 2010; 19: 496-8.
29. Ogihara T, Kikuchi K, Matsuoka H, *et al*. Japanese Society of Hypertension Committee. The Japanese Society of Hypertension Guidelines for the Management of Hypertension (JSH 2009). Hypertens Res 2009; 32: 3-107
30. Teramoto T, Sasaki J, Ishibashi S, *et al*. Japan Atherosclerosis Society. Executive summary of the Japan Atherosclerosis Society (JAS) guidelines for the diagnosis and prevention of atherosclerotic cardiovascular diseases in Japan -2012 version. J Atheroscler Thromb 2013; 20: 517-23
31. American Diabetes Association. Diagnosis and classification of diabetes mellitus. Diabetes Care 2012; 35: S64-S71
32. Sumino H, Ichikawa S, Kasama S, *et al*. Effect of transdermal hormone replacement therapy on carotid artery wall thickness and levels of vascular inflammatory markers in postmenopausal women. Hypertens Res 2005; 28: 579-84.
33. Pignoli P, Tremoli E, Poli A, Oreste P, Paoletti R. Intimal plus medial thickness of the arterial wall: a direct measurement with ultrasound imaging. Circulation 1986; 74: 1399-406.
34. Yun L, Xu R, Zhang L, *et al*. The role of microalbuminuria in arterial endothelial dysfunction in hypertensive patients with carotid plaques. Int Heart J 2014; 55: 153-9.
35. Demirbag R, Yilmaz R, Kunt AS, Gur M, Ulucay A, Unlu D. Relationship between plasma total antioxidant capacity and thoracic aortic intima-media thickness. Echocardiography 2006; 23: 183-8.
36. Jansen EH, Ruskovska T. Comparative Analysis of Serum (Anti)oxidative Status Parameters in Healthy Persons. Int J Mol Sci 2013; 14: 6106-15.
37. Yoshida T, Watanabe M, Engelman DT, *et al*. Transgenic mice overexpressing glutathione peroxidase are resistant to myocardial ischemia reperfusion injury. J Mol Cell Cardiol 1996 ; 28: 1759-67.
38. Wang P, Chen H, Qin H, *et al*. Overexpression of human copper, zinc-superoxide dismutase (SOD1) prevents postischemic injury. Proc Natl Acad Sci U S A 1998; 95: 4556-60.
39. Stokes KY, Clanton EC, Russell JM, Ross CR, Granger DN. NAD(P)H oxidase-derived superoxide mediates hypercholesterolemia-induced leukocyte-endothelial cell adhesion. Circ Res 2001; 88: 499-505.
40. Lehr HA, Hübner C, Nolte D, *et al*. Oxidatively modified human low-density lipoprotein stimulates leukocyte adherence to the microvascular endothelium in vivo. Res Exp Med (Berl) 1991; 191: 85-90.
41. Alvarez A, Sanz MJ. Reactive oxygen species mediate angiotensin II-induced leukocyte-endothelial cell interactions in vivo. J Leukoc Biol 2001; 70: 199-206.
42. Stiko A, Regnström J, Shah PK, Cercek B, Nilsson J. Active oxygen species and lysophosphatidylcholine are involved in oxidized low density lipoprotein activation of smooth muscle cell DNA synthesis. Arterioscler Thromb Vasc Biol 1996; 16: 194-200.
43. Li PF, Dietz R, von Harsdorf R. Differential effect of hydrogen peroxide and superoxide anion on apoptosis and proliferation of vascular smooth muscle cells. Circulation 1997; 96: 3602-9.
44. Wang HM, Chen TC, Jiang SQ, Liu YJ, Tian JW. Association of conventional risk factors for cardiovascular disease with IMT in middle-aged and elderly Chinese. Int J Cardiovasc Imaging 2014; 30: 759-68.