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High serum concentrations of pentosidine, an advanced glycation end product, are associated with low normal value of ankle-brachial index in apparently healthy men

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Abstract

The ankle-brachial index (ABI) is widely used for peripheral arterial disease screening and is associated with future cardiovascular events. Pentosidine, an advanced glycation end product, accumulates with age and in diabetes and end-stage renal disease; but the significance of elevated serum pentosidine is not well known. We investigated the relationship of the ABI to circulating pentosidine concentrations as well as other atherogenic factors in apparently healthy men. The study group consisted of a total of 170 apparently healthy men (age, 55 ± 9 years). Serum pentosidine concentrations were measured by enzyme-linked immunosorbent assay. The mean ABI and pentosidine concentrations of the whole study group were 1.16 ± 0.07 (range, 0.98−1.35) and 36.1 ± 10.6 ng/mL (range, 11.2−81.0), respectively. Univariate analysis showed that the ABI was inversely correlated with pentosidine ($P = .0004$), small low-density lipoprotein (LDL) cholesterol ($P = .017$), LDL cholesterol ($P = .019$), apolipoprotein B ($P = .024$), fasting insulin ($P = .028$), very small LDL cholesterol ($P = .036$), difference in ABIs between legs ($P = .037$), malondialdehyde-modified LDL ($P = .044$), and homeostasis model assessment of insulin resistance ($P = .047$). Stepwise multiple linear regression analysis revealed that increased pentosidine, fasting insulin, small LDL cholesterol, difference in ABIs between legs, difference in systolic blood pressure between arms, and reduced body mass index were independent determinants of reduced ABI (adjusted $R^2 = 0.237$, $P < .0001$). Serum pentosidine was an important, independent determinant of ABI in healthy men. Subjects with an ABI less than 1.10 showed higher pentosidine concentrations.

1. Introduction

The ankle-brachial index (ABI) is a very quick and easy measure and thus is widely used to screen for unrecognized peripheral artery disease, even in the context of a standard health checkup. In addition, many studies have shown that an abnormal ABI is associated with significant increases in cardiovascular morbidity and mortality, and is an independent predictor of incident cardiovascular events [1-3].

Advanced glycation end products (AGEs) are of interest in cardiovascular disease. Advanced glycation end products are formed by nonenzymatic glycation and oxidative reaction of the amino groups on proteins with reducing sugars to form stable structures that accumulate on long-lived proteins [4]. The formation and accumulation of AGEs occur during normal aging in tissue proteins and are accelerated in hyperglycemia, renal failure, atherosclerosis, and inflammatory conditions [5]. Increased AGE formation may be involved in the pathogenesis of atherosclerosis and cardiovascular dysfunction. Advanced glycation end products are postulated to oxidize low-density lipoprotein (LDL) [6] and form abnormal cross-links in collagen [7], resulting in the development of vascular and cardiac

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dysfunctions [8]. It is conceivable that there are non–receptor-dependent and receptor-mediated mechanisms by which AGEs contribute to the accelerated atherosclerosis and deleterious functions [7].

Pentosidine, a cross-link between arginine and lysine, is a well-characterized AGE [9]. Recently, the elevated concentrations of serum pentosidine have been shown to be strongly associated with reduced ABI in type 2 diabetes mellitus patients with peripheral artery disease [10]. In the present study, we evaluated circulating concentrations of pentosidine and compared them with the ABI in healthy men without apparent cardiovascular disease.

2. Methods

2.1. Study subjects

Participants in this study were apparently healthy subjects who visited Chunichi Hospital in Nagoya for an annual routine checkup. A total of 170 Japanese men (age, 33-82 years; mean, 55 years) with no history of cardiovascular disease and who were not taking any medication participated in this study. We recruited only men because of their advanced atherosclerosis compared with those for women. For blood pressure (BP) evaluation, sitting BP was measured using an oscillometric noninvasive BP monitor (BP-103iII; Colin, Komaki, Japan) after an appropriate rest of 15 minutes. Venous blood samples were collected for chemical analysis at approximately 8:00 AM after a 12- to 14-hour fasting period. The study was approved by the ethics committee of Chunichi Hospital and undertaken in accordance with the Declaration of Helsinki. Written informed consent was obtained from all subjects.

2.2. ABI measurement

Bilateral ABIs were measured using a noninvasive automatic oscillometric device (BP-203RPE; Colin), which incorporates an automatic oscillometer and can measure BP levels simultaneously in both arms and both legs by using 4 cuffs with the subject in the supine position. This device was also used to determine the brachial-ankle pulse wave velocity (baPWV). Ankle systolic pressures were measured at the posterior tibial artery. The ABI was calculated by dividing the ankle systolic pressure by the higher of the 2 brachial systolic BPs in the right and left arms because the higher BP might reflect the pressure of the aortic artery in case of the presence of subclavian or brachial artery stenosis. The ABI was calculated for each leg, and the lower of the 2 indices was taken as the individual ABI for analysis.

2.3. Measurement of biochemical variables

Standard assays were used to measure the serum concentrations of total cholesterol, high-density lipoprotein (HDL) cholesterol, LDL cholesterol, and triglycerides as well as insulin, glucose, and hemoglobin A_{1c}. For the estimation of insulin sensitivity, a homeostasis model assessment of insulin resistance (HOMA-IR) was calculated. The serum high-sensitivity C-reactive protein (hsCRP) concentration was measured by an automatic immunonephelometer with a sensitivity of 0.02 mg/dL. Apolipoproteins A-I and B were assayed by immunoturbidimetry. Serum pentosidine, adiponectin, and malondialdehyde-modified LDL were measured by immunosorbent assay (enzyme-linked immunosorbent assay) in duplicate. The intraassay coefficients of variation for serum pentosidine and malondialdehyde-modified LDL were 5.0% and 8.1%, respectively. Estimated glomerular filtration rates (eGFRs) were calculated by the simplified Modification of Diet in Renal Disease equation for Japanese [11]. Plasma small LDL cholesterol and very small LDL cholesterol were determined after separation of lipoproteins by high-performance liquid chromatography with a gel permeation column [12]. According to this method, small LDL cholesterol and very small LDL cholesterol were identified as particles of approximately 23.0 and 16.7 nm, respectively [13].

2.4. Statistical analysis

Data are reported as the means ± SD. Statistical analysis was performed using SPSS version 16.0 (SPSS, Chicago, IL). Continuous variables were tested for normal distribution by the Kolmogorov-Smirnov test. When data were not normally distributed (ie, triglycerides, fasting insulin, HOMA-IR, hsCRP, and adiponectin), they were logarithmically transformed before statistical analysis. Statistical analysis was adequately performed by unpaired Student *t* test or 1-way analysis of variance followed by Scheffe multiple-comparison post hoc test. Stepwise multiple linear regression analysis was used to test the independent relationship of the collected variables to ABI or pentosidine. Collinearity testing was used to avoid including the model variables that were interdependent. The levels for inclusion and exclusion of individual variables were 0.05 and 0.10, respectively. A value of $P < .05$ was considered statistically significant.

3. Results

Table 1 shows the clinical and biochemical characteristics of the study participants. Of the 170 subjects enrolled in the study, there were 22 with hypertension (systolic BP ≥140 mm Hg and/or diastolic BP ≥90 mm Hg), 53 with hypercholesterolemia (>5.7 mmol/L), 24 with LDL hypercholesterolemia (>3.6 mmol/L), 13 with HDL hypocholesterolemia (<1.0 mmol/L), and 66 with hypertriglyceridemia (>1.7 mmol/L). There were 33 subjects with metabolic syndrome according to the Japanese definition [14], 42 with overweight (body mass index [BMI] ≥25 kg/m^2), 97 with central obesity (waist circumference ≥85 cm), and 19 with diabetes mellitus diagnosed by an oral glucose tolerance test (OGTT).

Table 1
Clinical and biochemical characteristics of study subjects

Variables	Mean ± SD (N = 170)	Range
Age (y)	55 ± 9	33-82
Smoking (pack-y)	38 ± 59	0-450
BMI (kg/m^2)	23.6 ± 2.6	17.1-31.1
Waist circumference (cm)	85.3 ± 6.7	68.0-106.0
Systolic BP (mm Hg)	117 ± 17	83-162
Diastolic BP (mm Hg)	73 ± 12	40-107
Uric acid (μmol/L)	374 ± 70	36-547
eGFR (mL/[min 1.73 m^2])	74.0 ± 13.0	43.4-113.8
White blood cell count (/μL)	5482 ± 1464	2410-10620
hsCRP (mg/dL)	0.091 ± 0.151	0.005-1.710
Fasting plasma glucose (mmol/L)	5.88 ± 090	4.5-12.9
Hemoglobin A_{1c} (%)	5.02 ± 0.63	3.7-8.5
Fasting insulin (pmol/L)	31.4 ± 17.1	12.0-105.6
HOMA-IR	1.39 ± 0.84	0.20-4.78
Apolipoprotein A-I (mg/dL)	140 ± 23	84-213
Apolipoprotein B (mg/dL)	101 ± 22	42-157
Total cholesterol (mmol/L)	5.32 ± 0.93	2.12-7.92
LDL cholesterol (mmol/L)	2.89 ± 0.66	1.03-4.53
HDL cholesterol (mmol/L)	1.44 ± 0.37	0.66-2.60
Triglycerides (mmol/L)	1.69 ± 0.89	0.54-5.42
Small LDL cholesterol (mmol/L)	0.69 ± 0.18	0.23-1.23
Very small LDL cholesterol (mmol/L)	0.45 ± 0.14	0.13-0.92
Malondialdehyde-modified LDL (U/L)	139.4 ± 49.9	38.1-330.9
Adiponectin (μg/mL)	5.10 ± 3.16	0.60-17.60
Pentosidine (ng/mL)	36.1 ± 10.6	11.2-81.0
ABI	1.16 ± 0.07	0.98-1.35
baPWV (cm/s)	1488 ± 251	1056-2488
Difference in systolic BP between arms (mm Hg)	3 ± 3	0-19
Difference in ABIs between legs	0.04 ± 0.03	0.00-0.19

Values are expressed as the means ± SD.

Table 2
Correlation of ABI with other cardiovascular risk factors

Variables	r	P value
Age	0.085	.27
Smoking (pack-y)	0.098	.20
BMI	0.087	.26
Waist circumference	0.115	.14
Systolic BP	−0.057	.46
Diastolic BP	−0.072	.35
Uric acid	−0.107	.17
eGFR	0.030	.70
White blood cell count	0.113	.14
hsCRP[a]	−0.130	.090
Fasting plasma glucose	−0.129	.093
Hemoglobin A_{1c}	−0.049	.52
Fasting insulin[a]	−0.169	.028
HOMA-IR	−0.153	.047
Apolipoprotein A-I	−0.085	.27
Apolipoprotein B	−0.173	.024
Total cholesterol	−0.182	.017
LDL cholesterol	−0.180	.019
HDL cholesterol	−0.051	.51
Triglycerides[a]	−0.065	.40
Small LDL cholesterol	−0.183	.017
Very small LDL cholesterol	−0.161	.036
Malondialdehyde-modified LDL	−0.155	.044
Adiponectin[a]	0.116	.13
Pentosidine	−0.268	.0004
baPWV	0.035	.65
Difference in systolic BP between arms	−0.142	.065
Difference in ABIs between legs	−0.160	.037

[a] Logarithmically transformed.

Ankle-brachial index was defined as the lower of the 2 ankle systolic BPs divided by the higher of the 2 brachial systolic pressures and ranged from 0.98 to 1.35 (mean, 1.16 ± 0.07). Serum pentosidine concentrations ranged from 11.2 to 81.0 ng/mL (Table 1). Although it has been recommended that the presence of peripheral arterial disease be considered if the ABI is 0.90 or less [15-17], there were no subjects with such a low ABI in this study population. The difference in systolic BP between arms (right BP minus left BP) was 0.0 ± 4.0 mm Hg and ranged from −19 to 10 mm Hg.

The correlations of the ABI with other variables are indicated in Table 2. The ABI was inversely correlated with pentosidine (r = −0.268, P = .0004), small LDL cholesterol (r = −0.183, P = .017), LDL cholesterol (r = −0.180, P = .019), very small LDL cholesterol (r = −0.161, P = .036), apolipoprotein B (r = −0.173, P = .024), fasting insulin (r = −0.169, P = .028), difference in ABIs between legs (r = −0.160, P = .037), malondialdehyde-modified LDL (r = −0.155, P = .044), and HOMA-IR (r = −0.153, P = .047).

The scatter plot in Fig. 1 shows the correlation between the ABI and serum pentosidine concentrations. To further analyze the relationship, we divided the subjects into 5 subsets according to ABI levels (Fig. 2). The pentosidine concentrations in subjects with an ABI of 1.10 or less, the lowest subset, were significantly higher than those in the other ABI subsets (41.5 ± 12.9 vs 34.8 ± 9.6 ng/mL, P = .001) and in the subset of subjects with an ABI of 1.21 to 1.25 (P = .002).

For multivariate reevaluation of univariate correlations, a stepwise multiple linear regression analysis was used to examine the significant contributors to the distribution of the ABI. As a result, the significant contributors were determined

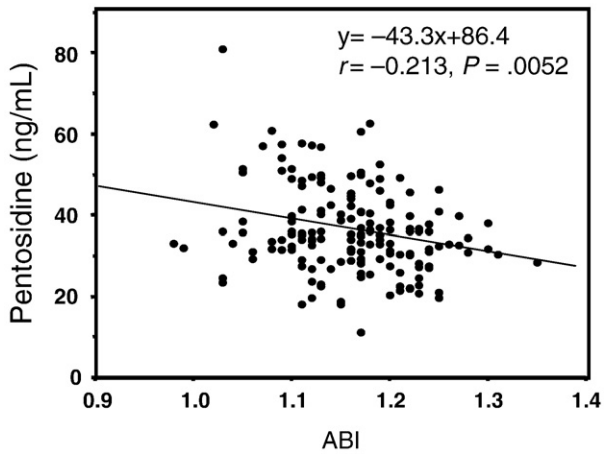

Fig. 1. Scatter plot of ABI and serum pentosidine concentrations. r = −0.268, P = .0004, y = −43.3× + 86.4.

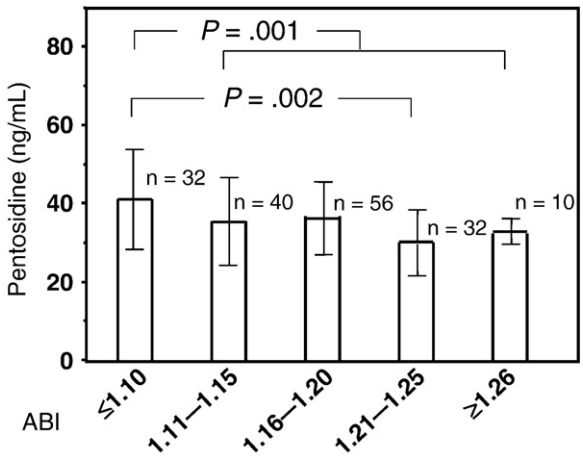

Fig. 2. Serum pentosidine concentrations in 5 subsets according to ABI concentrations. Bars represent means ± SD. n indicates the number of subjects in each subset.

to be pentosidine ($\beta = -0.282$, $P < .0001$), fasting insulin ($\beta = -0.291$, $P = .0003$), BMI ($\beta = 0.322$, $P < .0001$), small LDL cholesterol ($\beta = -0.207$, $P = .004$), difference in ABIs between legs ($\beta = -0.159$, $P = .023$), and difference in systolic BP between arms ($\beta = -0.145$, $P = .042$) (Table 3). It should be noted that the values of the standardized partial regression coefficient (β) were negative, except for that of BMI.

On the other hand, the serum pentosidine concentrations were inversely correlated with the white blood cell count ($r = -0.213$, $P = .0052$) and positively correlated with the systolic BP ($r = 0.170$, $P = .027$) in addition to ABI, whereas all other variables were not significantly correlated. By a stepwise multiple linear regression analysis, ABI ($\beta = -0.247$, $P = .001$) and white blood cell count ($\beta = -0.185$, $P = .013$) were determined to be independent predictors of the serum pentosidine concentrations (Table 4). We did not find a significant difference in the pentosidine concentrations between the subjects with normal OGTT results (n = 111, 35.8 ± 10.4 ng/mL) and those with diabetes (n = 19, 37.6 ± 11.4 ng/mL) diagnosed by OGTT ($P = .50$).

Table 3
Stepwise multiple linear regression analysis with ABI as the dependent variable

Variables	Regression coefficient	SE	β	P value
Pentosidine	−0.002	0.0004	−0.282	<.0001
Fasting insulin[a]	−0.036	0.010	−0.291	.0003
BMI	0.008	0.002	0.322	<.0001
Small LDL cholesterol	−0.035	0.012	−0.207	.004
Difference in ABIs between legs	−0.304	0.133	−0.159	.023
Difference in systolic BP between arms	−0.003	0.002	−0.145	.042

β indicates the standardized partial regression coefficient. The P value to enter was set at .05, and the P value to remove was set at .10. Adjusted $R^2 = 0.209$, $P < .0001$.

[a] Logarithmically transformed.

Table 4
Stepwise multiple linear regression analysis with pentosidine as the dependent variable

Variables	Regression coefficient	SE	β	P value
ABI	−39.9	11.9	−0.247	.001
White blood cell count	−0.0013	0.001	−0.185	.013

β indicates the standardized partial regression coefficient. The P value to enter was set at .05, and the P value to remove was set at .10. Adjusted $R^2 = 0.095$, $P < .0001$.

4. Discussion

In the present study, we recruited apparently healthy men who visited our hospital for an annual routine checkup. They had no current cardiovascular diseases and did not take medications for hypertension, diabetes, or dyslipidemia. They had ABIs ranging from 0.98 to 1.35, which were within the reference range [18]. We demonstrated that serum pentosidine concentrations, fasting insulin concentrations, BMI, and small LDL cholesterol concentrations are all major contributors to the ABI. In addition, the difference in ABIs between legs and difference in systolic BP between arms were also significantly associated with the ABI. The differences in systolic BP between arms are reproducible and may carry prognostic information in terms of mortality prediction [19]. Among the significantly related variables to contribute to the ABI, only BMI was positively associated with the ABI; all the other factors were found to be associated with a lower ABI. The reason that there was no statistically significant relation with age may be attributable to apparently healthy subjects without claudication.

Although a meta-analysis of around 50 000 subjects suggested that the normal ABI range is 1.11 to 1.40 [3], the normal distribution of the ABI is more usually accepted as being from 0.90 to 1.40 [20]. In symptomatic individuals, an ABI of 0.9 or less is approximately 95% sensitive for detecting arteriogram-positive peripheral arterial occlusive disease [17]; and individuals with a borderline ABI of 0.90 to 0.99 have a higher incidence of mobility loss due to future atherosclerotic diseases compared with those with an ABI greater than 1.00 [21]. In addition, lower levels of ABI are associated with higher rates of concomitant coronary and cerebrovascular disease [22]. These increased relative risks have been shown to be independent of baseline cardiovascular disease and conventional risk factors [23], indicating that the ABI might be an independent risk factor for incident cardiovascular events. In contrast, individuals with a high ABI of greater than 1.40 have also been shown to be associated with all-cause and cardiovascular disease mortality [21] and often have foot ulcers and neuropathy [24].

Advanced glycation end products accumulate in tissue proteins during aging, and their formation is accelerated by hyperglycemia [4,5,25]. Circulating concentrations of AGEs have not been suggested to predict cardiovascular complications in patients with chronic kidney diseases [26,27] and hypertension [27]. Our results clearly show that high serum pentosidine, one of the AGEs, is the predominant predictor of lower ABI. Recently, the serum pentosidine concentrations have been shown to be strongly linearly related to ABI in type 2 diabetes mellitus patients with peripheral artery disease [10]. We had 19 subjects with diabetes diagnosed by OGTT, and they showed modest concentrations of fasting glucose (7.56 ± 1.62 mmol/L) and hemoglobin A_{1c} (6.1% ± 1.0%). The reason for the lack of a significant difference in pentosidine or ABI between our diabetic and healthy subjects could be due to undiagnosed and short-term diabetes.

Even with respect to normal concentrations of ABI, the individuals with an ABI of 1.10 or less had higher pentosidine concentrations; and they were suggested to have latent peripheral artery diseases because their pentosidine levels were also elevated. We expect that morphologic alterations in the atherosclerotic process of the peripheral arteries concomitantly activate the formation and accumulation of AGEs, and thereby increase the release of pentosidine into the circulation. Previously, increased serum pentosidine concentrations were reported in rheumatoid arthritis [28].

We also observed that fasting insulin concentrations, BMI, and small LDL cholesterol concentrations were the major predictors of the ABI. Fasting insulin [29] and small LDL cholesterol [30] are established risk factors for atherosclerotic diseases. Interestingly, in contrast to fasting insulin and small LDL cholesterol, higher BMI was associated with increased ABI in the present study. At present, we have no explanation for this discrepancy. The previous report regarding the relationship of ABI with mortality and cardiovascular risk also demonstrated a strong, positive correlation between ABI and BMI ($P < .0001$) in the subjects involving an ABI less than 0.61 or greater than 1.40; but these authors did not pay any attention to this finding [31]. Despite no relation of ABI to BMI by univariate analysis, we also found a significant positive relation between them by stepwise multiple regression analysis. We speculate that systolic BP could not be measured appropriately in obesity because of thick adipose tissue, which may attenuate the power with oscillometer technique. Serum pentosidine concentrations were significantly influenced by white blood cell count in the present study. Miyata et al [32] have reported that pentosidine was associated with inflammatory status, as reflected in such parameters as increased CRP and white cell count, in patients with rheumatoid arthritis. Our subjects did not show any association of pentosidine with hsCRP presumably because of the relatively small range of hsCRP. This study is limited by the small number of subjects and by lack of information concerning the amount of atherosclerotic lesions in the legs.

In conclusion, the distribution of the ABI in healthy men without apparent cardiovascular disease was associated with serum pentosidine concentrations as well as fasting insulin concentrations, small LDL cholesterol concentration, and BMI. Based on our findings, close clinical follow-up is recommended if serum pentosidine concentrations are elevated. Future arteriosclerosis obliterans may progress in such individuals. The circulating pentosidine concentrations may be worth taking into account in the diagnosis of peripheral artery disease.

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