

Relationship Between Serum Total Testosterone Concentration and Augmentation Index at Radial Artery in Japanese Postmenopausal Patients

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Abstract

Background: The significance of testosterone as a risk factor for cardiovascular disease (CVD) in females is controversial. This cross-sectional study aimed to elucidate the relationship between serum total testosterone concentration (T-T) and augmentation index at the radial artery (r-AIx) as a marker of arterial function in Japanese postmenopausal patients.

Methods: A total of 447 postmenopausal patients with traditional cardiovascular risk factors and/or a history of CVD (age (mean \pm standard deviation (SD)), 73 ± 10 years) were enrolled. r-AIx was measured using tonometry, and the association between r-AIx and various clinical parameters, including T-T, was determined.

Results: r-AIx significantly increased (CVD vs. non-CVD: $99 \pm 11\%$ vs. $91 \pm 11\%$, $P < 0.001$) and T-T significantly decreased (CVD vs. non-CVD: 0.31 ± 0.13 ng/mL vs. 0.49 ± 0.23 ng/mL, $P < 0.001$) in patients with CVD than in those without CVD. A significant negative correlation ($r = -0.48$; $P < 0.001$) between r-AIx and T-T was observed. Furthermore, multiple regression analysis indicated that T-T (t value = -7.7 ; $P < 0.001$), height (t value = -5.3 ; $P < 0.001$), d-ROMs test as a marker of oxidative stress *in vivo* (t value = 3.2 ; $P < 0.001$), CVD (t value = 2.9 ; $P < 0.01$), and pulse rate (t value = -2.7 ; $P < 0.01$) were independent variables for r-AIx as a subordinate factor.

Conclusion: This study revealed that low T-T is an important determining factor for an increase in r-AIx in Japanese postmenopausal patients. A prospective multicenter study with a large sample size is required to confirm the results of this study.

Keywords: Testosterone; Augmentation index; Oxidative stress; Cardiovascular disease; Female

Introduction

Testosterone is an important sex hormone that influences

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various health problems in males. In particular, recent clinical studies indicated that low blood testosterone concentration in males is closely associated with the incidence of cardiovascular disease (CVD) [1]. Testosterone is also produced in females but at levels approximately 5-10% of the levels in males. Recent studies have indicated that testosterone is also associated with female health. The significance of blood testosterone concentration as a risk factor for CVD in females is controversial. Several clinical studies have indicated that high blood testosterone concentration in females is associated with the incidence of CVD [2, 3]. However, Sievers et al reported that low serum total testosterone concentration (T-T) in German female patients was associated with a higher incidence of CVD events compared with high T-T [4]. Such reports mainly involve analysis of the Caucasian population.

Augmentation index (AIx) is known to indicate arterial wave reflection [5]. Clinical studies have reported that elevated AIx and central blood pressure are important predictors of CVD [6-9]. However, few studies have reported the association between AIx and blood testosterone concentration in females [10, 11]. To the best of our knowledge, there are no studies reporting the association between AIx and blood testosterone concentration in the Asian female population. This study aimed to elucidate the association between T-T and AIx at radial artery (r-AIx) in Japanese postmenopausal patients.

Materials and Methods

Patients

This cross-sectional study was performed at the Hitsumoto Medical Clinic in Yamaguchi, Japan, between September 2014 and August 2016. T-T, r-AIx, and various clinical parameters were analyzed in 447 postmenopausal patients with traditional cardiovascular risk factors and/or a history of CVD (age (mean \pm standard deviation (SD)), 73 ± 10 years). Patients administered dehydroepiandrosterone, estradiol, and testosterone were excluded from the study. Clinical history of CVD was defined as previous ischemic heart disease, cerebrovascular disease, peripheral arterial disease, or heart failure according to medical records. All patients provided informed consent, and the study protocol was approved by the local ethics committee of the Hitsumoto Medical Clinic.

Measurement of r-AIx

r-AIx was measured in a temperature-controlled room maintained between 20 and 25 °C. Patients undergoing treatment with antihypertensive drugs ceased treatment more than 24 h before measurement. r-AIx of the patient was measured in the sitting position using an applanation tonometry-based device (HEM-9010AI, Omron Healthcare Co., Ltd, Kyoto, Japan), as previously described [4, 12]. The tonometry sensor is a pressure sensor composed of an array of 40 microtransducer elements. On placing the sensor on a patient's wrist, one of the microtransducers is automatically selected to obtain optimal radial pressure waveforms. The first and second systolic peaks are automatically detected and consequently r-AIx is measured. The validity and reliability of r-AIx measurement by this method are well established; several studies have indicated a close linear correlation between r-AIx and central AIx [5, 9, 12].

Evaluation of cardiovascular risk factors

Obesity was assessed using body mass index, which was calculated as the ratio of weight (kg) to square of height (m). A current smoker was defined as an individual who smoked at least one cigarette per day during the previous 28 days. Right brachial blood pressure was measured twice using a mercury sphygmomanometer with patients in the sitting position. An average of two readings was used to determine systolic and diastolic blood pressures. Hypertension was defined as systolic blood pressure ≥ 140 mm Hg and/or diastolic blood pressure ≥ 90 mm Hg and/or under antihypertensive treatment. Dyslipidemia was defined as a serum low-density lipoprotein cholesterol concentration ≥ 140 mg/dL, a serum high-density lipoprotein cholesterol concentration ≤ 40 mg/dL, and/or a serum triglyceride concentration ≥ 150 mg/dL and/or under antihyperlipidemic treatment. Diabetes mellitus was defined as fasting blood glucose level ≥ 126 mg/dL and/or under antidiabetic treatment.

Blood sampling

Blood samples were collected from the antecubital vein in the morning after 12 h of fasting. Serum lipid concentration, plasma glucose concentration, serum insulin concentration, serum high-sensitivity C-reactive protein (hs-CRP) concentration, reactive oxygen metabolites (d-ROMs) test as a marker of oxidative stress, serum estradiol concentration, and T-T were subsequently measured. Total cholesterol and triglyceride concentrations were measured by standard enzymatic methods. High-density lipoprotein cholesterol concentration was measured by selective inhibition. Low-density lipoprotein cholesterol concentration was calculated using the Friedewald formula [13]. Patients with a serum triglyceride concentration ≥ 400 mg/dL were excluded because the method is accurate only below this concentration. Plasma glucose concentration was measured by the glucose oxidase method, and serum in-

Table 1. Patient Characteristics

n	447
Age (years)	73 \pm 10
Height (m)	1.57 \pm 0.09
Body weight (kg)	56 \pm 10
Body mass index (kg/m ²)	22.8 \pm 3.8
Current smoker (%)	32 (7)
Hypertension (%)	321 (72)
Systolic BP (mm Hg)	146 \pm 8
Diastolic BP (mm Hg)	89 \pm 9
Pulse rate (/min)	68 \pm 18
Dyslipidemia (%)	268 (60)
Diabetes mellitus (%)	139 (31)
CVD (%)	143 (32)
Total cholesterol (mg/dL)	214 \pm 38
LDL-cholesterol (mg/dL)	134 \pm 35
Triglyceride (mg/dL)	132 \pm 56
HDL-cholesterol (mg/dL)	53 \pm 14
FBG (mg/dL)	112 \pm 24
IRI (μ g/mL)	7.3 \pm 4.6
HOMA-IR	2.0 \pm 1.4
Log hs-CRP (mg/L)	-1.3 \pm 0.6
d-ROMs test (U. Carr)	309 \pm 82
Detection of estradiol (%)	377 (84)
Estradiol (pg/mL)	7.8 \pm 3.3
T-T (ng/mL)	0.4 \pm 0.2
r-AIx(%)	94 \pm 12
Medications	
RAS inhibitor (%)	197 (44)
Statin (%)	160 (36)

Data are expressed mean \pm SD. BP: blood pressure; CVD: cardiovascular disease; LDL: low-density lipoprotein; HDL: high-density lipoprotein; FBG: fasting blood glucose; IRI: immunoreactive insulin; HOMA-IR: homeostatic model assessment of insulin resistance; hs-CRP: high-sensitivity C-reactive protein; d-ROMs: derivatives of reactive oxygen metabolites; T-T: total testosterone; r-AIx: radial augmentation index; RAS: renin-angiotensin system.

sulin concentration was measured by enzyme immunoassay. To measure insulin resistance, the homeostatic model assessment of insulin resistance (HOMA-IR) was used as follows [14]: HOMA-IR = fasting glucose concentration (mg/dL) \times fasting immunoreactive insulin concentration (μ g/mL)/405. hs-CRP concentration was measured using high-sensitivity, latex-enhanced immunonephelometrics. The d-ROMs test, which reflects blood hydroperoxide concentrations, was performed using a commercial kit (Diacron, Grosseto, Italy) [15]. Serum estradiol concentration was measured using a commercial kit (ARCHITECT Estradiol II, Chicago, IL, USA). Detection limit of estradiol using this kit is 5 pg/mL. T-T was also

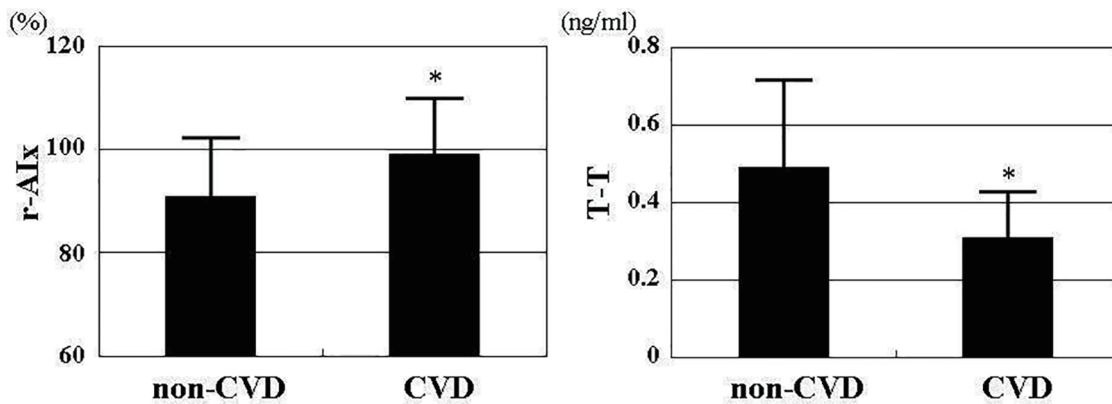


Figure 1. Comparisons of r-AIx or T-T between non-CVD and CVD patients. r-AIx significantly increased (CVD vs. non-CVD: $99 \pm 11\%$ vs. $91 \pm 11\%$, $P < 0.001$) and T-T significantly decreased (CVD vs. non-CVD: 0.31 ± 0.13 ng/mL vs. 0.49 ± 0.23 ng/mL, $P < 0.001$) in CVD patients than in non-CVD patients. r-AIx: radial augmentation index; T-T: serum total testosterone concentration; CVD: cardiovascular disease. * $P < 0.001$ vs. non-CVD.

measured using a commercial kit (ARCHITECT Testosterone II, Chicago, IL, USA).

Statistical analysis

A commercially available statistical software program (Stat-View-J 5.0, Hulinks, Inc., Tokyo, Japan) was used for all statistical analyses. Continuous variables were expressed as the mean \pm SD. Between-group comparisons were performed using the Student's *t*-test. The correlation coefficient was estimated by Spearman's rank correlation analysis. Multivariate analysis was performed using multiple regression analysis. A *P* value of < 0.05 was considered statistically significant.

Results

Baseline clinical characteristics are shown in Table 1. The mean value of r-AIx was $94 \pm 12\%$ (range, 60-134%), and the mean value of T-T was 0.43 ± 0.22 ng/mL (range, 0.06 - 1.55 ng/mL). These parameters indicated a nearly normal distribution. Comparisons of r-AIx or T-T between non-CVD and CVD patients are shown in Figure 1. r-AIx significantly increased and T-T significantly decreased in CVD patients than in non-CVD patients. The association between r-AIx and T-T is shown in Figure 2. A significant negative correlation between r-AIx and T-T was observed. The association between r-AIx or T-T and various clinical parameters is shown in Table 2. r-AIx showed a significant positive correlation with age, current smoking, hypertension, systolic blood pressure, hs-CRP concentration, and d-ROMs. Conversely, r-AIx showed a significant negative correlation with height, pulse rate, and serum estradiol concentration. T-T showed a significant positive correlation with hypertension, serum estradiol concentration. Conversely, T-T showed a significant negative correlation with age, diabetes mellitus, plasma glucose concentration, serum insulin concentration, HOMA-IR, hs-CRP concentration, and d-ROMs.

For further analysis of the independent association be-

tween r-AIx and T-T, multiple regression analysis was performed to evaluate the ability of 15 factors, including T-T, to explain r-AIx as a subordinate factor. Coefficient of determination (R^2) of this analysis is 0.32, indicating that 32% of r-AIx as a subordinate factor is explained by 15 explanatory factors. Variance ratio (*F* value) of this analysis is 25.9, and significance level is statistically significant ($P < 0.001$). In this analysis, five factors (T-T, height, d-ROMs test, history of CVD, and pulse rate) were selected as independent variables for r-AIx (Table 3).

Discussion

The significance of AIx in females as a risk factor for CVD is controversial. Janner et al reported that AIx is a predictor for CVD endpoint in males but not in females [16]. However, other researchers indicated the importance of AIx as a risk factor for CVD in females. Higashi et al reported that increase in carotid AIx reflected the impairment of left ventricular diastolic function in females but not in males [17]. Yasmin et al reported that AIx had a significant positive correlation with pulse-wave velocity as a marker of arterial stiffness in males and females [18]. Furthermore, other clinical researches also indicated a significant correlation between biomarker of inflammation or vascular calcification and AIx in female patients [19, 20]. These reports indicated that increase in AIx in female patients was associated with CVD. In the present cross-sectional study, r-AIx significantly increased in CVD patients than in non-CVD patients; furthermore, multivariate analysis indicated that CVD was an independent variable for r-AIx as a subordinate factor. In contrast, the results of this study indicated that T-T significantly decreased in CVD patients than in non-CVD patients. Thus, results of the present study and the previous study supported the increase in AIx and decrease in blood testosterone concentration as considerable risk factors for CVD not only in the Caucasian population but also in the Asian female population.

Several mechanisms explaining the vasoprotective effects

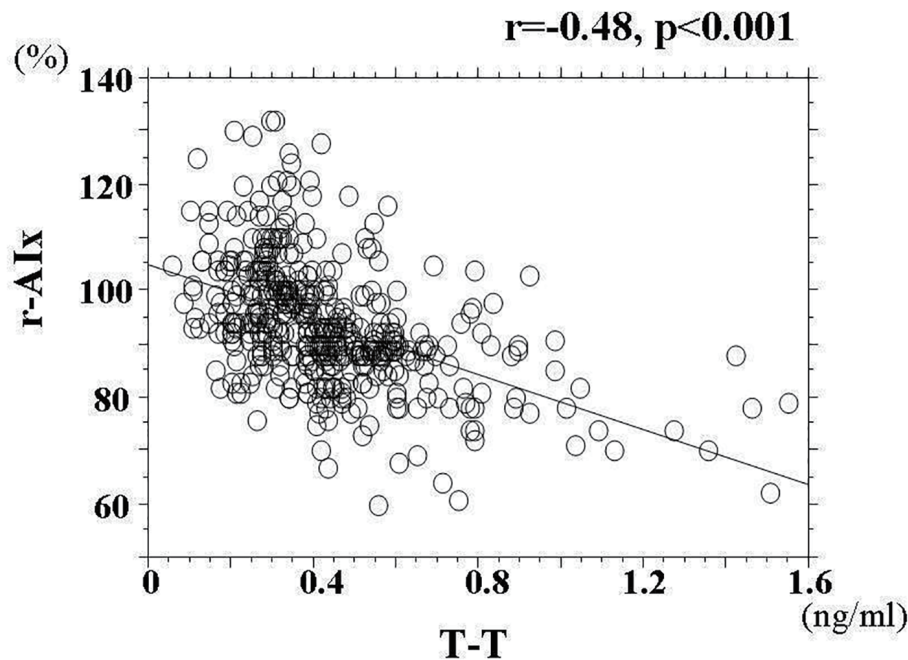


Figure 2. The association between r-AIx and T-T. A significant negative correlation between r-AIx and T-T ($r = -0.48, P < 0.001$) was observed. r-AIx: radial augmentation index; T-T: serum total testosterone concentration.

of testosterone such as vasodilatory effects, retention of endothelial function, and inhibition of vascular calcification have been reported [21, 22]. Clinical studies have indicated a significant association between low blood testosterone concentration and the impairment of markers for arterial function such as pulse-wave velocity, flow-mediated dilatation, and AIx in males [23-25]. Weiss et al reported a significant decrease in AIx and increase in blood testosterone concentration by oral administration of dehydroepiandrosterone in older adults, of whom 96.7% were Caucasian and 54.3% were female [11]. Furthermore, the results of the present study indicated that low blood testosterone concentration is an important determining factor for increase in AIx in the Asian postmenopausal population. AIx is known to be influenced by arterial stiffness or endothelial function in addition to height and pulse rate [26, 27]. In contrast, clinical studies reported that low T-T had a significant negative correlation with cardio-ankle vascular index as a marker of arterial stiffness or administration of testosterone improved endothelial function in postmenopausal patients [28, 29]. Therefore, a significant association between low T-T and r-AIx in the present study may be partly attributed to increased arterial stiffness or endothelial dysfunction owing to low testosterone levels. Decrease of estrogen in postmenopausal patients is widely known to be associated with various health problems, including arterial dysfunction. In this study, serum estradiol concentration had a significant negative correlation with r-AIx in univariate analysis. However, multivariate analysis indicated that serum estradiol concentration was not an independent variable for r-AIx. Weiss et al also reported that there was no association between the decrease in AIx and increase in blood estradiol concentration by oral administration of dehydroepiandrosterone [11]. Therefore, the results of

the present study and those of Weiss et al's study indicated that testosterone is a more important sex hormone than estrogen for AIx in postmenopausal patients.

A number of basic and clinical studies have illustrated that the increase in oxidative stress contributes to the development of atherosclerosis [30, 31]. Furthermore, some clinical studies have reported that there is a significant association between oxidative stress and AIx [32, 33]. The results of the present study also indicated that d-ROMs test, as a marker of oxidative stress *in vivo*, is selected as an independent factor for r-AIx in postmenopausal patients. In contrast, low blood testosterone concentration is reported to be associated with oxidative stress in males [34]. The results of the present study also indicated a significant association between oxidative stress and testosterone in postmenopausal patients. The association between oxidative stress and testosterone is controversial. Some basic studies have indicated that testosterone suppresses oxidative stress [35, 36]. In contrast, other studies reported that testosterone promotes oxidative stress [37-39]. Skogastierna et al reported that supraphysiological dose of testosterone induced oxidative stress *in vitro* and *in vivo* [40]. Therefore, the physiological levels of testosterone in postmenopausal patients in this study may have suppressed oxidative stress *in vivo*. Further studies concerning the association between oxidative stress and testosterone in postmenopausal patients, including intervention therapy such as the administration of dehydroepiandrosterone or testosterone, are warranted.

Limitations

There are several limitations of this study. First, patients un-

Table 2. Relationships Between r-AIx, T-T and Various Clinical Parameters

	r	
	r-AIx	T-T
Age	0.22*	-0.28*
Height	-0.29*	-0.05
Body weight	-0.05	-0.07
Body mass index	-0.09	-0.07
Current smoker (yes = 1, no = 0)	0.15**	0.04
Hypertension (yes = 1, no = 0)	0.13**	0.13**
Systolic BP	0.11***	0.08
Diastolic BP	0.07	0.06
Pulse rate	-0.21*	0.08
Dyslipidemia (yes = 1, no = 0)	0.03	0.02
Diabetes mellitus (yes = 1, no = 0)	0.09	-0.16**
Total cholesterol	0.03	-0.04
LDL-cholesterol	0.02	-0.03
Triglyceride	-0.04	-0.03
HDL-cholesterol	0.03	0.02
FBG	0.09	-0.11***
IRI	0.08	-0.12**
HOMA-IR	0.09	-0.12**
Log hs-CRP	0.21*	-0.28*
d-ROMs test	0.40*	-0.41*
Detection of estradiol (yes = 1, no = 0)	-0.21*	0.43*
Estradiol	-0.13**	0.24*
RAS inhibitor (yes = 1, no = 0)	-0.09	-0.09
Statin (yes = 1, no = 0)	-0.08	-0.07

r-AIx: radial augmentation index; T-T: total testosterone; BP: blood pressure; LDL: low-density lipoprotein; HDL: high-density lipoprotein; FBG: fasting blood glucose; IRI: immunoreactive insulin; HOMA-IR: homeostatic model assessment of insulin resistance; hs-CRP: high-sensitivity C-reactive protein; d-ROMs: derivatives of reactive oxygen metabolites; RAS: renin-angiotensin system.

dergoing treatment with antihypertensive drugs ceased treatment more than 24 h before measurement of r-AIx to avoid the influence. However, 24 h was not sufficient to avoid the effect of long-acting drugs such as amlodipine. Second, angiography, computed tomography, and magnetic resonance imaging were not performed. Therefore, asymptomatic CVD may have been undetected. Third, this study was cross-sectional in a single unit, and the sample size was relatively small. A prospective multicenter study with a large sample size is necessary to confirm the significance of r-AIx or blood testosterone concentration as a risk factor for CVD in postmenopausal subjects. Furthermore, an extensive examination of basic and clinical studies investigating the significance of r-AIx or T-T as a risk factor for CVD in females is required in the future.

Table 3. Multiple Regression Analysis for r-AIx

Explanatory factor	β	t value	P value
T-T	-0.38	-7.7	< 0.001
Height	-0.22	-5.3	< 0.001
d-ROMs test	0.17	3.2	< 0.001
CVD	0.15	2.9	< 0.01
Pulse rate	-0.14	-2.7	< 0.01
Age	0.09	1.9	0.053
Hypertension	0.08	0.7	0.104
FBG	0.07	1.5	0.158
Diabetes mellitus	0.07	1.4	0.177
IRI	0.05	1.1	0.223
HOMA-IR	0.03	0.6	0.573
Estradiol	0.02	0.4	0.693
Log hs-CRP	-0.03	-0.6	0.552
Systolic BP	-0.04	-0.7	0.521
Current smoker	-0.05	-0.8	0.379

$R^2 = 0.32$, F value = 25.9, $P < 0.001$, $n = 447$. r-AIx: radial augmentation index; T-T: total testosterone; d-ROMs: derivatives of reactive oxygen metabolites; CVD: cardiovascular disease; FBG: fasting blood glucose; IRI: immunoreactive insulin; HOMA-IR: homeostatic model assessment of insulin resistance; hs-CRP: high-sensitivity C-reactive protein; BP: blood pressure; β : standardized regression coefficient; R^2 : coefficient of determination; F value: variance ratio.

Conclusion

In conclusion, the results of this study indicated that low T-T is one of the important determining factors for increasing r-AIx in Japanese postmenopausal patients. A prospective multicenter study with a large sample size is required to confirm the results of this study.

Competing Interests

Author has no competing interests.

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Financial Disclosure

None.

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