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Original article

Homocysteine is related to peripheral arterial disease only in male elderly with type 2 diabetes mellitus

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ABSTRACT

Purpose: To find out a novel risk factor for peripheral arterial disease (PAD) in the elderly with Type 2 diabetes mellitus (T2DM).
Methods: A cross-sectional study involving 70 out of 146 elderly (≥60 years) with T2DM whose ankle–brachial index (ABI) was measured at the Geriatric Out-patient Clinic, Sanglah Hospital.
Results: The overall prevalence of PAD was 30.8% (45 out of 146); in males, it was 25.88% (22 out of 85) and in females, 37.70% (23 out of 61). Only 70 out of 146 subjects who had complete data were further analyzed. By bivariate correlation test, it was found that age (right ABI: $r = -0.396$, $p < 0.001$; left ABI, $r = -0.509$, $p < 0.001$); lying systolic blood pressure (right ABI: $r = -0.268$, $p = 0.012$; left ABI: $r = -0.267$, $p = 0.013$); and concentration of 2-hours post-prandial (2-hpp) plasma glucose (right ABI: $r = -0.252$, $p = 0.018$) had inverse correlation with ABI, whereas waist circumference, body mass index, sitting and standing systolic and diastolic blood pressures, lying diastolic blood pressure, levels of total cholesterol, low-density-lipoprotein (LDL) cholesterol, high-density-lipoprotein (HDL) cholesterol, triglyceride, fasting plasma glucose, haemoglobin A1C (A1C), and duration of diabetes, had no correlation with ABI. The ages of subjects who had PAD were greater than those without PAD (71.5 years *vs.* 65.2 years, $p < 0.001$). Homocysteine tended to be correlated only with left ABI ($r = -0.198$; $p = 0.050$), but after assessment on sex factor, it showed that homocysteine had inverse correlation with ABI in males (right ABI: $r = -0.371$, $p < 0.026$; left ABI: $r = -0.358$, $p = 0.032$). Homocysteine also had a positive correlation with age ($r = 0.315$, $p = 0.004$). By multiple regression test, age, LDL cholesterol, and 2-hpp plasma glucose had a role in the PAD event.
Conclusions: Some traditional risk factors (age, lying systolic blood pressure, LDL cholesterol, and 2-hpp plasma glucose) were related with PAD in the elderly with T2DM. Homocysteine as a novel risk factor had a correlation with ABI only in the male subjects. Age was the most important risk factor for PAD, either directly or indirectly through homocysteine (only in males).
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1. Introduction

Aging is a process of gradual decrease of tissue's ability to rejuvenate and maintain its normal structure and function; thus, the tissues cannot resist lesions.[1,2] This condition results in the vulnerability of the elderly of getting ill or acquiring diseases. Some theories of aging process that currently exist and are related to the present study are glycosylation process; metabolism causes; free-radical exposure; and, most recently accepted, telomere shortening.

One of the diseases that is commonly related to aging is peripheral arterial disease (PAD), which is known as a marker of systemic atherosclerosis.[3] PAD is an independent risk factor for cardiovascular death.[4] Aging is associated with the increase of PAD incidence, especially among women. It commonly starts at 40 years of age and sharply increases after 70 years. The mechanism of PAD is similar to that of coronary atherosclerosis, which is influenced by many risk factors. These factors can be differentiated into traditional [such as hypercholesterolemia, hypertension, Type 2 diabetes mellitus (T2DM), and smoking] and nontraditional (or novel) risk factors.[5–7] Several studies reviewed by Kuswardhani[8] show the prevalence of PAD ranging from 16% to 30%.

Besides traditional risk factors, there are some PAD-related novel risk factors that are still being studied, such as transforming

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growth factor β, vascular cellular adhesion molecule, apoptotic inducer (Fas ligand), asymmetric dimethylarginine (ADMA), soluble vascular adhesion molecule 1, high-sensitivity C-reactive protein, and homocysteine.[4,9–17] To date, no data are available regarding prevalence and risk factors that influence the mechanism of PAD, especially among the elderly population with T2DM in Indonesia. Therefore, a study on the role of some novel or nontraditional risk factors in the occurrence of PAD in this group was carried out. The objective of this study was to find out the association of a novel risk factor (homocysteine) and PAD in the elderly with T2DM.

2. Methods

The first step of the study was to measure ankle–brachial index (ABI) to confirm the diagnosis of PAD among 146 elderly patients (age ≥60 years) with T2DM, who visited the Geriatric Outpatients Clinic, Sanglah Hospital, Denpasar, Bali, Indonesia. The prevalence of PAD in the elderly with T2DM could be calculated from this population. By using inclusion–exclusion criteria, 70 out of 146 subjects were included for analysis in the cross-sectional study. The inclusion criteria included age between 60 years and 80 years, being Indonesian, and willingness to participate in the study with an informed consent. The exclusion criteria were smoking (last 1 year); acute coronary syndrome or recent heart attack; recent stroke; acute infection; liver function abnormalities or diseases (aspartate aminotransferase (AST) and/or alanine aminotransferase (ALT) ≥2× normal range, chronic liver disease or liver cirrhosis); malignancy; acute and chronic kidney diseases with creatinine serum greater than 3 mg/dL or creatinine clearance test (CCT) < 60 μmol/L (calculated by formula of Kockcroft–Gault); crisis hypertension; and technical inability to measure ABI because of certain conditions, such as extremity amputation.

The variables measured included age; waist circumference; body mass index (BMI); blood pressure (lying, sitting, and standing position); lipid plasma; fasting and 2-hpp plasma glucose; A1C; duration of diabetes; and homocysteine concentration. To determine the body height (for BMI calculation), we used the following formula: corrected height = 59.01 + (2.08 × knee height) for men, and 75.00 + (1.91 × knee height) × (0.17 × age) for women, in centimeters. Body weight was measured with automatic weight scale Camry EB6171 (Guangdong, China). Blood pressure was measured with a sphygmomanometer in lying, sitting, and standing positions, with intervals longer than 2 minutes. ABI was measured using automatic Vasera VS-1000 (Fukuda, Bunkyo-ku, Tokyo, Japan); homocysteine was measured using IMX Homocysteine Pack with fluorescence polarization immunoassay method (Abbott Park, Illinois, United States). Criteria for diabetes were based on those by American Diabetes Association (2009),[18] and PAD was confirmed by ankle–brachial index (ABI < 0.9). Minimal number of sample requirement by calculation with cross-sectional formula was 60 subjects.

Statistic tests used in the study were descriptive analysis, nonparametric one-sample Kolmogorov–Smirnov test, bivariate correlation test (Pearson or Spearman test), and stepwise linear regression test, with each significant value confirmed at $p < 0.05$.

3. Results

3.1. Proportion of PAD in the elderly with T2DM

One hundred and forty-six patients were included in the study. The number of samples in group with age 60–69 years was almost 2.3 times higher than the group with age greater than or equal to 70 years, and male-to-female ratio was 58.2:41.8. The overall proportion of PAD was 30.8% (45 out of 146). The proportion of PAD in the male subjects was 25.88% (22 out of 85), a little lower than that in female subjects, which was 37.70% (23 out of 61), but statistically, they were not significantly different. It was found that the subjects aged 70 years or older had a significant higher proportion of PAD than the subjects aged 60–69 years (48.89% *vs.* 20.79%; $p = 0.001$). With this finding, it can be concluded that age is an important risk factor for PAD.

Table 1
The characteristics of the 70 study subjects

Variables	Range	Mean ± standard deviation
Age, yr	60–80	67.30 ± 5.59
Waist circumference, cm	35.5–116.5	88.65 ± 14.29
Body mass index	16.44–35.98	24.88 ± 3.71
Lying systolic blood pressure, mmHg	110–194	142.54 ± 19.53
Sitting systolic blood pressure, mmHg	107–194	145.10 ± 18.84
Standing systolic blood pressure, mmHg	100–196	141.66 ± 18.28
Lying diastolic blood pressure, mmHg	54–95	74.57 ± 8.82
Sitting diastolic blood pressure, mmHg	47–106	78.01 ± 9.93
Standing diastolic blood pressure, mmHg	50–100	78.18 ± 10.00
Total cholesterol, mg/dL	108–300	198.00 ± 44.57
Low-density-lipoprotein cholesterol, mg/dL	51–213	127.16 ± 36.29
High-density-lipoprotein cholesterol, mg/dL	25–75	48.68 ± 9.77
Triglyceride, mg/dL	53–521	150.96 ± 90.25
Fasting plasma glucose, mg/dL	50–419	137.53 ± 71.01
2-hpp plasma glucose, mg/dL	109–379	187.01 ± 53.53
A1C, %	5–13.7	7.58 ± 1.94
Duration of diabetes, yr	0.5–37	9.79 ± 8.30
Homocysteine, μmol/L	7.10–24.40	12.41 ± 3.72

3.2. Correlation between ABI and some risk factors for PAD

Seventy out of 146 patients were involved in the cross-sectional study, for whom all parameters or biomarkers were completely measured. The characteristics of the 70 subjects are presented in Table 1. By bivariate correlation test, it was found that age, lying systolic blood pressure, and concentrations of 2-hpp plasma glucose had inverse correlation with ABI (Table 2). It is noted that, the lower the ABI, the smaller the diameter of peripheral artery. From the analysis, it was found that age ($r = -0.509$) had the strongest correlation, followed by lying systolic blood pressure ($r = -0.268$), and 2-hpp plasma glucose ($r = -0.252$).

This study also showed a tendency of inverse correlation between homocysteine and ABI, and after being analyzed by sex, it was found that homocysteine had inverse correlation with ABI in the male subjects (right ABI: $r = -0.371$, $p < 0.026$; left ABI: $r = -0.358$,

Table 2
Correlation between some risk factors for peripheral arterial disease and ABI

Variables	Right ABI		Left ABI	
	r	*p*	*r*	*p*
Age	−0.396	<0.001*	−0.509	<0.001*
Waist circumference	0.106	0.388	0.054	0.660
Body mass index	0.169	0.082	0.158	0.097
Lying systolic blood pressure	−0.268	0.012*	−0.267	0.013*
Sitting systolic blood pressure	−0.131	0.139	−0.103	0.199
Standing systolic blood pressure	−0.179	0.069	−0.193	0.054
Lying diastolic blood pressure	−0.064	0.299	−0.081	0.252
Sitting diastolic blood pressure	0.144	0.177	0.096	0.215
Standing diastolic blood pressure	0.097	0.213	0.120	0.161
Total cholesterol	−0.104	0.195	−0.012	0.460
Low-density-lipoprotein cholesterol	−0.164	0.087	0.039	0.376
High-density-lipoprotein cholesterol	−0.130	0.141	0.052	0.334
Triglyceride	0.050	0.340	0.094	0.218
Fasting plasma glucose	0.100	0.204	0.180	0.068
2-hpp plasma glucose	−0.252	0.018*	−0.128	0.146
A1C	0.007	0.477	0.010	0.469
Duration of diabetes	−0.114	0.179	−0.173	0.081
Homocysteine	−0.167	0.083	−0.198	0.050

*Statistically significant, $p < 0.05$.
ABI = ankle–brachial index.

$p = 0.032$), whereas, in the female subjects, no correlation was found between them (right ABI: $r = -0.057$, $p = 0.750$; and left ABI: $r = -0.071$, $p = 0.689$) (Fig. 1).

It is widely known, as also found in this study, that age is an important risk factor for PAD. Besides being directly associated with PAD, age may likely be indirectly associated with PAD in relation to other risk factors. To know the association, a correlation test between age and some other risk factors, both traditional (which have correlation with ABI) and nontraditional (homocysteine), was conducted. The result showed that homocysteine was significantly correlated with age ($r = 0.315$, $p = 0.004$) (Table 3).

Table 3
Correlation between age and some other risk factors for peripheral arterial disease

Variable	Age	
	r	*p*
Lying systolic blood pressure	0.140	0.125
2-hpp plasma glucose	0.059	0.313
Homocysteine	0.315	0.004*

*Statistically significant, $p < 0.05$.

3.3. The role of risk factors for PAD on ABI

By multiple regression with stepwise linear regression test, only age, low-density-lipoprotein (LDL) cholesterol, and 2-hpp plasma glucose, out of the all risk factors (age, waist circumference, BMI, lying systolic blood pressure, sitting systolic blood pressure, standing systolic blood pressure, lying diastolic blood pressure, sitting diastolic blood pressure, standing diastolic blood pressure, total cholesterol, LDL cholesterol, high-density-lipoprotein (HDL) cholesterol, triglyceride, fasting plasma glucose, 2-hpp plasma glucose, A1C, duration of diabetes, and homocysteine), had an effect on right ABI, and only age had an effect on left ABI (Tables 4 and 5).

4. Discussion

Prevalence of PAD varies depending on population and geographical factors. Prevalence of PAD among patients with T2DM is commonly not accurately confirmed because of the absence of symptoms and the use of different methods for diagnosis. Using ABI, the prevalence of PAD in diabetics older than 40 years was found to be 20%,[19] and in those older than 50 years, it was 29%.[20] Several other studies found that the prevalence of PAD in diabetes varied from 16% to 30%.[20,21] Based on surveys in the United States in 1999–2000, the prevalence of PAD by age and sex showed that, the older the patients, the greater the prevalence of PAD, especially increasing sharply after the age of 70 years. No significant difference was found between both sexes. The prevalence of PAD in the general population was lower than that among diabetics.[22] Race also has an influence on the prevalence of PAD. Data from The National Health and Nutrition Examination Survey in 1999–2000 showed that the prevalence of PAD was 11.7% among non-Hispanic whites, 19.5% among non-Hispanic blacks, 15.6% among Hispanics, as adapted from Steffen et al. (2008).[23] There are no certain genetic abnormalities related to PAD.[24] In this study, the proportion of PAD among the elderly with T2DM was similar with those of other studies. Age, especially greater than 70 years, was the most important risk factor for PAD. Sex had no relationship with the proportion of PAD; it can be understood that postmenopausal women have similar chances of getting cardiovascular diseases as men. Generally, the proportion of PAD in this study was somewhat higher than the findings of other studies, which might be because of the fact that the samples were a specific high-risk population, that is, the elderly with T2DM.

In the study, only lying systolic blood pressure, among other measurements, had a correlation with ABI. The reason behind this is not explained yet.

Hyperhomocysteinemia was related with atherosclerosis and thrombosis.[15,16] High homocysteine levels inhibit enzyme dimethylarginine dimethylaminohydrolase (DDAH), an enzyme to cleavage ADMA, and induces ADMA concentration.[25] In this study, it was shown that homocysteine had a relationship with PAD, especially in men. There is no explanation as yet on why is it only in male that the association exists. The difference in the prevalence and degree of PAD between the right and left limbs might reflect that atherosclerosis developed in blood vessels to different degrees. It can explain why homocysteine is related only with right ABI.

As only a few numbers of risk factors were analyzed in this study, there are still many other risk factors that are not yet evaluated, and this contributed to one of the weaknesses of this study. Interrelationship among risk factors also induced complexity in analyzing the role of each risk factor. This problem results in different findings concerning the role of each risk factor in different studies. In this study, we had difficulty tracing the family histories of cardiovascular diseases, and no reliable data were available about

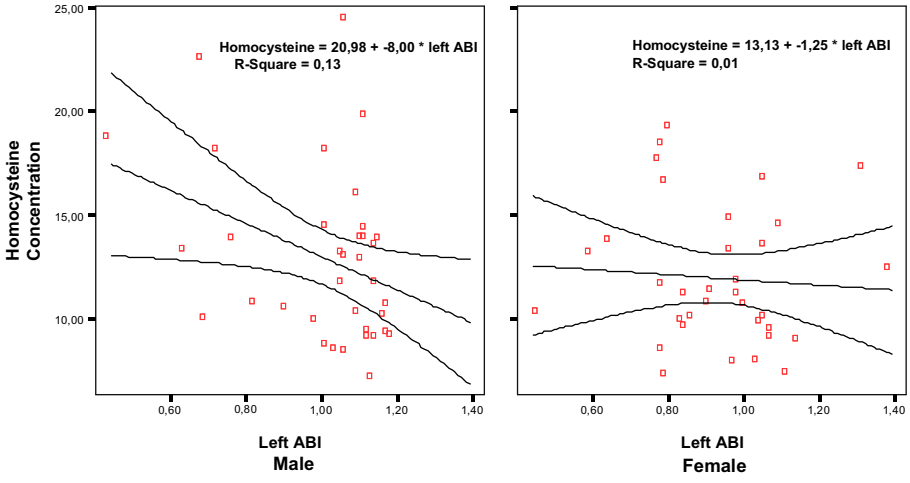

Fig. 1. Correlation between homocysteine concentration and left ankle–brachial index by sex. ABI = ankle–brachial index.

Table 4
The role of risk factors of PAD on right ABI

Model	Unstandardized coefficients		Standardized coefficients	*t*	Significance (*p*)
	β	Standard error	β		
1. Constant	1.869	0.249		7.492	<0.001
Age	−0.013	0.004	−0.395	−3.436	0.001
2. Constant	2.150	0.269		7.999	<0.001
Age	−0.014	0.004	−0.447	−3.948	<0.001
LDL cholesterol	−0.001	0.001	−0.267	−2.361	0.021
3. Constant	2.243	0.265		8.475	<0.001
Age	−0.014	0.004	−0.427	−3.869	<0.001
LDL cholesterol	−0.001	0.001	−0.244	−2.207	0.031
2-hpp plasma glucose	−0.001	0.000	−0.236	−2.175	0.033

LDL = low-density lipoprotein.

smoking (detailed report about the smoking history and the amount of cigarettes), although we tried to exclude smokers from the study samples. It is well known that smoking itself is an important risk factor for PAD and that it can induce an increase in the homocysteine concentration. No noted history of smoking can probably explain why the correlation between homocysteine and ABI exists only in men. Not all risk factors in this study showed a correlation with ABI, therefore confirming the complexity of the interrelationship among risk factors and the mechanism of PAD. Bias in the analysis may also result from incomplete reports about use of antithrombotic, antidyslipidemic, or oral antidiabetic drugs, which can influence the results. This latter limitation may have resulted in the fact that some risk factors previously expected to have relationship with ABI or PAD proved to be otherwise.

In our study, no correlation was found between obesity and long-term glycemic control (A1C) and ABI. Obesity generally leads to T2DM, but in the long term, uncontrolled diabetic patients will induce reduced body weight. Hence, in longstanding diabetic patients, BMI or obesity cannot be used as a consistent marker for cardiovascular diseases. Moreover, a part of the elderly patients has loss of appetite (impairment of taste). It may explain why no correlation between obesity and ABI was found. Reversed association concerning obesity and cardiovascular disease was shown in an epidemiological study in Nusa Ceningan by Suastika et al. (2006),[26] in which the slim group had a greater prevalence of coronary heart disease as compared with that in those with greater body weight (normal to obese). It may be because of the fact that all of the samples were diabetics; glycemic control was not significantly related with ABI. As already known and according to the finding of Haffner et al.,[27] T2DM was considered an equivalent risk factor for cardiovascular diseases. Probably, all elderly with T2DM already have some degree of atherosclerosis. Of course, the diagnosis of PAD by ABI cannot detect the occurrence of mild or early atherosclerosis.[27] No analysis of composite risk factors (glycemic control and duration of diabetes) and other unstudied risk factors as a cause of failure in proving the relationship between A1C and ABI is available.

How age induces other risk factors for PAD has not yet been understood completely. At least, aging itself can cause reduced insulin sensitivity (increased insulin resistance) and beta cell function.[28,29] Insulin resistance is now considered as central to cardiometabolic syndrome. Both age and T2DM (insulin resistance), as shown in the subjects in this study, may concomitantly induce higher concentrations of homocysteine in the population, especially in men.

Table 5
The role of risk factors of PAD on left ABI

Model	Unstandardized coefficients		Standardized coefficients	*t*	Significant (*p*)
	β	Standard error	β		
1. (Constant)	2.149	0.248		8.669	<0.001
Age	−0.017	0.004	−0.510	−4.764	<0.001

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