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Serum Homocysteine Level and Ankle-Brachial Index in Peripheral Arterial Disease

Yanna Indrayana✉, Herpan Syafii Harahap

[1]Department of Cardiology and Vascular Disease, Faculty of Medicine, University of Mataram
[2]Department of Neurology, Faculty of Medicine, University of Mataram

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Abstract

Patients with peripheral arterial disease have a higher risk of death compared to normal populations. There are several relatively new risk factors significantly increase the vulnerability to suffering from peripheral arterial disease, one of which is homocysteine. Studies investigating the role of serum homocysteine level as a biomarker of the severity of peripheral arterial disease based on an ankle-brachial index (ABI) in the different populations were still limited and it was never been studied in Mataram. This was a cross-sectional study aimed to investigate the correlation between serum homocysteine level and ABI in 77 peripheral artery disease (PAD) outpatients in Siti Hajar Hospital, Mataram. The diagnosis of PAD was based on ABI<0.9. Fasting serum homocysteine level was examined using the ELISA technique. Characteristic data collected were gender, age, hypertension, diabetes mellitus, and body mass index (BMI) category. There was a significant correlation between the increase of serum homocysteine levels and the decrease of ABI. The main risk factors for peripheral arterial disease in the subjects are hypertension, diabetes mellitus, and overweight/obesity. Serum homocysteine level is a predictor of peripheral arterial disease severity measured using ABI.

Introduction

At present, cardiovascular diseases (CVDs) are still becoming the main cause of mortality around the world. In 2016, 17.9 million people died due to CVDs, representing about 31% of total death with all causes (World Health Organization, 2017). The prevalence of cardiovascular diseases is predicted to continue to increase, especially in developing countries, since the prevalence of its risk factors is also increasing (Stewart, Manmathan, & Wilkinson, 2017). Basic Health Research (Riskesdas) survey conducted in 2013 showed that the prevalence of cardiovascular diseases in Indonesia, a representation of developing countries, is about 1.5% from the total population (Badan Penelitian dan Pengembangan Kesehatan, 2013). Similar to the number of death globally, about 37% of deaths in Indonesia is attributable to cardiovascular diseases (Cardiovascular Division and Health Services Research Centre, 2017).

Cardiovascular diseases are a group of diseases consisted of coronary heart disease, cerebrovascular disease, and peripheral arterial disease. Peripheral artery disease (PAD) is the common manifestation of systemic atherosclerosis especially involving arteries other than the coronary artery, most common in lower extremity arteries (Kohlman-Trigoboff, 2019). Globally, its prevalence is high, especially in low-to-middle income countries, estimated at about 28.7% (Criqui & Aboyans, 2015). Since most of the PAD are unfortunately asymptomatic, it is commonly underdiagnosed and undertreated (Olin & Sealove, 2010). Peripheral arterial disease, either symptomatic or asymptomatic, is associated with increased risk of cardiovascular mortality in the cohort study and its early diagnosis and reduction risk

✉ Correspondence Address:
Faculty of Medicine, Universitas Mataram, Indonesia
Email: yannaindrayana@unram.ac.id

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management is important (Sartipy, Sigvant, Lundin, & Wahlberg, 2018). Therefore, the diagnosis of PAD in the primary care setting has important prognostic value (Diehm, et al., 2009). In clinical practice, screening and diagnosis of PAD can be conducted using a simple and non-invasive instrument named *ankle-brachial index* (ABI) (Campia, Gerhard-Herman, Piazza, & Goldhaber, 2019).

The major risk factors of PAD are similar to those of coronary artery disease and cerebrovascular disease, including older age, male, hypertension, diabetes mellitus, overweight/obesity, and cigarette smoking (Bennett, Silverman, Gill, & Lip, 2009). The prevalence of those risk factors is quite high among Indonesian population (Mihardja, Soetrisno, & Soegondo, 2014; Rachmi, Li, & Baur, 2017; Rosjidi, Isro'in, & Wahyuni, 2017; Harahap & Indrayana, 2018; Peltzer & Pengpid, 2018). Optimal management of those risk factors is crucial in the management of PAD and it may reduce overall cardiovascular mortality (Shammas, 2007). In recent years, some relatively new risk factors for PAD have been proposed, including homocysteine (Bennett, Silverman, Gill, & Lip, 2009).

Homocysteine is an intermediate product of the amino acid methionine metabolism produced via the transmethylation pathway. In normal conditions, homocysteine that is formed will be metabolized either via remethylation or transsulfuration pathway. In the remethylation pathway, homocysteine is converted back to methionine either via folate-dependent or folate-independent remethylation pathway. In the transsulfuration pathway, homocysteine is converted to cysteine (Cacciapuoti, 2018). The remethylation pathway through the folate-dependent pathway is dependent on folic acid, B2, and B12 vitamins, while the transsulfuration pathway is dependent on B6 vitamin (Barroso, Handy, & Castro, 2017). Therefore, the total plasma concentration of homocysteine (tHcy) is influenced by blood concentrations of folic acid, B12 and B6 vitamins (Hankey, 2018). In the blood circulation, a level of homocysteine <13 μmol/L is considered normal, while a level ≥13 μmol/L is considered hyperhomocysteinemia (Cacciapuoti, 2018).

Since homocysteine was proposed to be associated with the progression of atherosclerosis, now it is considered to be the risk factor of PAD. A meta-analysis of the epidemiologic study showed that the homocysteine level in PAD patients was higher than those healthy subjects (Khandanpour, Loke, Meyer, Jennings, & Armon, 2009). The role of homocysteine in the pathogenesis of PAD can be influenced by ethnic variation. This was the first study investigating the correlation between serum homocysteine level and severity of PAD determined based on ABI in the sub-population of Mataram, West Nusa Tenggara.

Method

This was a cross-sectional study involving 77 asymptomatic peripheral artery disease outpatients visiting Siti Hajar Hospital, Mataram, during the period of May to October 2019. The diagnosis of peripheral artery disease in those patients was based on their ankle-brachial index (ABI) <0.9. The exclusion criteria are the coexistence of some medical conditions, i.e chronic kidney disease, liver disease and hypothyroid based on medical records, and prior history of consumption folic acid, B6 and B12 in the last three months. This study was approved by Komisi Etik Penelitian Kesehatan Universitas Mataram (No. 233/UN18.F7/ETIK/2019).

The characteristics data of the subjects collected in this study were age, gender, hypertension, diabetes mellitus, and cigarette smoking based on their medical records. The data of body mass index (BMI) of each subject was also collected by measuring weight in kilograms (kg) divided by height in square meters (m^2) (Hicks, et al., 2018). The BMI was categorized into three groups, namely underweight (BMI<18.5 kg/m^2), norm weight (BMI 18.5-24.9 kg/m^2), overweight/obese (BMI≥25 kg/m^2). Since overweight and obesity are risk factors for PAD, they are grouped into one group, the overweight/obesity group respectively (Murata, et al., 2015).

The ABI was measured by comparing the ratio of higher of the systolic blood pressure of the two ankle arteries of the limb and higher of the two systolic blood pressure of the upper arms using handheld vascular Doppler Bestman BF-520T. The ABI<0.9 was diagnostic for PAD (Rac-Albu, Iliuta, Guberna, & Sinescu, 2014).

The serum samples for the examination of homocysteine levels were obtained from 5cc of overnight (8 to 10 hours) fasting blood samples of the patients. The serum homocysteine level was measured in the Laboratory of Hepatika, Mataram, using FineTest® Human HCY (Homocysteine) ELISA Kit (Kit Product Code HCY161119).

The data of age was presented as mean (95%CI), while data of gender, hypertension, diabetes mellitus, cigarette smoking, and BMI category were presented as frequency. The statistical analysis of the correlation of the serum homocysteine level and ABI was based on the Spearman test. The result of statistical analysis was significant if $p<0.05$.

Result and Discussion

The incidence of peripheral artery disease (PAD) is mostly determined by the existence of both major and relatively new risk factors. Homocysteine is a representative of relatively new risk factors for PAD which are getting more attention from the researchers in recent years. In the present study, the characteristic data of the subjects representing the major risk factors as well as serum hocysteine level representing the relatively new risk factor for PAD were obtained (table 1). The results showed that most of the subjects are hypertensive, suffered from diabetes mellitus, overweight/obese, but with a small frequency of male gender and those who had cigarette smoking habits. The higher frequency of hypertension, diabetes mellitus, and overweight/obese among subjects with PAD in the present study was in accordance with the results of previous studies in different populations (Alvim, et al., 2018; Krishnan, Geevar, Mohanan, Venugopal, & Devika, 2018). The mean age of the subjects is 57 years old. The population of subjects aged 40 years and older in Indonesia is at high risk for cardiovascular disease (Maharani, Sujarwoto, Praveen, Delvac, Tampubolon, & Patel, 2019).

The present study showed that most of the subjects are female (79,2%). This result contrast with the existing reference which showed that male gender was the risk factor for PAD (Bennett, Silverman, Gill, & Lip, 2009;

Table 1. The Characteristics of the Subjects

Category	Mean (95%CI), unless otherwise stated
Gender, n(%)	
Male	16 (20.8)
Female	61 (79.2)
Age (years)	57.38(54.94 – 59.82)
Cigarette smoking, n(%)	
Yes	3 (3.9)
No	74 (96.1)
Hypertension, n(%)	
Yes	55 (71.4)
No	22 (28.6)
Diabetes mellitus, n(%)	
Yes	43 (55.8)
No	34 (44.2)
BMI category, n(%)	
Underweight (<18.5 kg/m^2)	4 (5.2)
Norm weight (18.5-24.9 kg/m^2)	29 (37.7)
Overweight/obese (≥25 kg/m^2)	44 (57.1)
ABI	0.79 (0.77 – 0.81)
Homocysteine **(µmol/L)**	4.79 (4.43 – 5.15)

CI=confidence interval; BMI=body mass index; ABI=ankle-brachial index
Source: Primary Data, 2019

Alzamora, et al., 2010; Aggarwal, Loomba, & Arora, 2012). However, another study conducted by Rafie *et al*. showed similar results with the present study in which the female gender had a higher frequency (Rafie, et al., 2010). These different results may be due to the differences ethnic of the subjects studied. The differences in ethnic studied affect the role of various vascular risk factors in the pathogenesis of PAD (Bennett, Silverman, Gill, & Lip, 2009). The small frequency of subjects who had the cigarette smoking habit is mostly due to the small frequency of male subjects.

All of the well-known risk factors mentioned above as well as hyperhomocysteinemia contribute to the abnormal ankle-brachial index (ABI) (Campia, Gerhard-Herman, Piazza, & Goldhaber, 2019). The present study showed that the mean of the serum homocysteine level was in the normal range. It is interesting that there was no subject which was PAD patients that showed an elevation in serum homocysteine level (table 1). In the context of the existence of frequency of subjects with PAD who had mild-moderate to severe hyperhomocysteinemia, this result is contradictive with the results of a meta-analysis and previous studies conducted in different populations (Khandanpour, Loke, Meyer, Jennings, & Armon, 2009; Weragoda, Seneviratne, Weerasinghe, & Wijeyaratne, 2016; Preethi & Hemachandran, 2019). It can be proposed that diverse ethnic groups may have different serum homocysteine profiles. However, statistical analysis showed that there was a significant correlation between the increase of serum homocysteine level and the decrease of the ankle-brachial index (ABI) (table 2). It suggested that even in the normal range, the increase of serum homocysteine level is correlated with the progression of peripheral artery disease, but it still needs further investigation.

Table 2. The Correlation between Serum Homocysteine Level and ABI

	Mean (95%CI)	r	p-value
Homocysteine	4.79	-0.323	0.004*
ABI	0.79		

*Significant ($p<0.05$)

The mechanism by which homocysteine is responsible for the progression of peripheral arterial disease is not well established yet. Theoretically, its role is mediated via its effect on the progression of atherosclerotic plaque of the arterial wall and the generation of hypertension by several mechanisms. First, homocysteine may induce oxidative stress via activation of NADPH oxidase resulting in the generation of reactive oxygen species (ROS) (Omae, Nagaoka, Tanano, & Yoshida, 2013). This ROS is responsible for the decrease of bioavailability of nitric oxide (NO), a molecule important for vasodilatation, and this will lead to vasoconstriction and hypertension (Lai & Kan, 2015). In addition, NO which reacts to superoxide (O_2^-), a kind of ROS, to form peroxynitrite ($ONOO^-$) will facilitate further atherosclerotic plaque progression and decrease of vascular compliance resulting in arterial hypertension (Mury, Chirico, Mura, Millon, Canet-Soulas, & Pialoux, 2018). Second, the homocysteine-induced oxidative stress is also responsible for the activation of matrix metalloproteinases (MMPs), zinc-containing endopeptidases secreted by connective tissue as well as proinflammatory cells. In the atherosclerotic plaque, MMPs degrade elastin matrix separating the intima from the media and induce collagen synthesis. These process will result in smooth-muscle cells migration from the media to the intima, the increase of intima-media thickness, and the decrease of elastin/collagen ratio which eventually induce vascular hypertrophy and endothelial dysfunction, the hallmark of hypertension (Vacek, Rehman, Neamtu, Yu, & Givimani, 2015). Third, homocysteine alters the transsulfuration pathway, a kind of its metabolism pathway, resulting in a decrease of hydrogen sulfide (H_2S) production (Yang & He, 2019). Hydrogen sulfide (H_2S) is well known as a vasorelaxant molecule produced by vascular tissue and a decrease of its production will lead to vascular constriction and hypertension (Sen, Mishra, Tyagi, & Tyagi, 2010). However, further study is still needed to investigate whether the theories described above have a significant negative correlation with ABI, as shown by the result of the present study.

Conclusion

The serum homocysteine level negatively correlates to the ankle-brachial index (ABI). The identifiable risk factors of peripheral arterial disease (PAD) were age, female gender, hypertension, diabetes mellitus, overweight/obesity, and serum homocysteine level, but not cigarette smoking. The role of ethnicity in influencing serum homocysteine level still needs further investigation.

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