

Distribution of Dimethylarginine-Dimethylaminohydrolase-II (DDAH2) Gene Polymorphism in Hemodialysis Patients

Muhammad Thaha¹, Muhammad Yusuf^{2,3}, Maulana A. Empitu⁴, Achmad Bakarman⁵, Yasuhiko Tomino³

¹ Department of Internal Medicine, Faculty of Medicine, Airlangga University, Surabaya, Indonesia. ² Cardiology and Vascular Medicine Department, Faculty of Medicine, Airlangga University, Surabaya, Indonesia. ³ Department of Internal Medicine, Faculty of Medicine, Juntendo University, Tokyo, Japan. ⁴ Department of Pharmacology, Faculty of Medicine, Airlangga University, Surabaya, Indonesia. ⁵ Al-Irsjad Private Hospital, Surabaya, Indonesia.

Correspondence mail:

Nginden Intan Timur I no.2 Surabaya 60118, Indonesia. email: mochthaha@yahoo.com.

ABSTRAK

Tujuan: untuk mendeskripsikan distribusi single nucleotide polymorphisms (SNPs) di -449 (promoter region) DDAH2 pada pasien hemodialisis (HD). **Metode:** studi ini bersifat deskriptif, dilakukan pada 56 pasien HD dan 30 individu sehat. Pasien HD dikelompokkan berdasarkan etiologinya, yakni kelompok hipertensi dan non-hipertensi. Ekstraksi DNA dilakukan menggunakan kit isolasi DNA yang tersedia secara komersial, dengan menggunakan sampel whole blood. Penelusuran genotip dari polimorfisme dilakukan dengan metode PCR-based SNP detection (Applied Biosystems, Carlsbad, USA) berdasarkan penilaian aktivitas 5'-exonuclease terhadap rs805305 (-449 G/C). Studi ini juga menilai variasi allele pada setiap subyek studi. **Hasil:** ditemukan bahwa heterozigot merupakan genotip terbesar pada kedua kelompok subyek (70% pada individu sehat dan 55% pada pasien HD), diikuti genotip GG pada pasien HD. Sedangkan pada individu sehat, CC (27%) merupakan genotip terbanyak kedua pada individu sehat. **Kesimpulan:** terdapat perbedaan distribusi polimorfisme yang signifikan antara kelompok individu sehat dengan pasien HD ($p=0,01$).

Kata kunci: CKD, DDAH2 gen polimorfisme, hemodialisis, hipertensi.

ABSTRACT

Aim: to describe the distribution of single nucleotide polymorphisms (SNPs) at -449 (promoter region) DDAH 2 in hemodialysis (HD) patients. **Methods:** this study was a descriptive study, 56 HD patients and 30 healthy individuals were enrolled. Based on its etiology, the HD patient group was further divided into hypertension (HT) group and non-HT group. DNA was extracted from whole blood samples with a commercially available DNA isolation kit. Genotyping of the polymorphisms was performed using PCR-based SNP detection methods (Applied Biosystems, Carlsbad, USA) based on 5'-exonuclease activity assays for rs805305 (-449 G/C). Allelic variation was assessed in each participant. **Results:** heterozygotes were observed as the most abundant genotypes in both groups (70% in healthy individuals and 55% in HD patients), followed by GG genotype in the HD patients (30%), while CC (27%) was the second most common genotype polymorphism in the healthy individuals. **Conclusion:** there is a significant difference in distribution of DDAH 2 gene polymorphism among HD patients compared to healthy individuals ($p=0.01$).

Key words: CKD, DDAH2 gene polymorphism, hemodialysis, hypertension.

INTRODUCTION

Atherosclerotic cardiovascular diseases (CVD) are major causes of morbidity and mortality in patients receiving ongoing hemodialysis (HD).^{1,2} Despite its increasing incidence and the accelerated worsening of atherosclerosis in patients on chronic hemodialysis, the proportion of individuals with chronic kidney disease (CKD) receiving appropriate cardiovascular (CV) risk modification treatment is lower than that of the general population.³ In metabolic disorders associated with atherosclerosis (dyslipidemia, hypertension and diabetes mellitus), a reduced endothelium-mediated, nitric oxide (NO)-dependent vasodilation has been observed, which may contribute to the initiation and progression of atherosclerosis associated with these disorders.⁴

The mechanisms of endothelial vasodilator dysfunction (EVD) are likely multi-factorial.⁵ Evidence from both animal models and clinical studies suggests that accumulation of the endogenous nitric-oxide synthase (NOS) inhibitors, asymmetric dimethylarginine (ADMA) and NG methyl-L-arginine (L-NMMA) contributes to reducing nitric oxide (NO).^{6,7} Plasma levels of ADMA are inversely related to the vascular NO bioavailability and ADMA is an independent determinant of the vascular redox-state.^{8,9} ADMA is also a risk factor for EVD, CV mortality and progression of CKD.^{8,10}

The co-localization of dimethylarginine-dimethylaminohydrolase (DDAH) enzyme and NOS at several sites supports the hypothesis that DDAH may regulate NOS activity by controlling the metabolism of ADMA.¹¹ As DDAH2 regulates ADMA levels, it is believable that functional DDAH2 gene polymorphisms may account for the variation in ADMA levels.¹² However, the distribution of DDAH 2 polymorphism among HD patient has not been clearly described. Therefore, the current study was undertaken to investigate the distribution of single nucleotide polymorphisms (SNPs) at -449 (promoter region) DDAH2 among HD patient. Furthermore, this study is looking into whether there are any allele distribution differences between regularly hemodialyzed patient with hypertension compared to without hypertension

at -449 (promoter region) DDAH2.

METHODS

Study Population and Dialysis Protocol

Fifty-six patients with a creatinine clearance of less than 15 ml/min per 1.73 m² who were on chronic HD treatment for more than 3 months were recruited for the present study. Thirty-four HD patients (60.7%) were male. Clinical practice was not changed or modified for the purpose of the study. As for the control group, 30 healthy individual paramedics who had no cardiovascular clinical risk factors (HT, diabetes, dyslipidemia, smoking, family history of premature coronary artery disease or CKD), were enrolled. Five healthy individual controls (16.7%) were male.

All participants (patients and controls) signed the informed consent forms and the study was approved by the ethics committee of Al-Irsjad Hospital, Surabaya, Indonesia. The present study was a descriptive study. Clinical and demographic data were also collected.

All patients underwent dialysis twice a week for four (4) weeks using a commercially available dialyser (type F6-8 HPS polysulfone semi-synthetic, fresenius GMBH, Germany). Each HD session lasted 4 hours.

Blood pressure (BP) was measured using a standard mercury sphygmomanometers after participants have been sitting for at least 10 minutes. Hypertension was defined as having a blood pressure of $\geq 140/90$ mmHg at the time of recruitment, history of hypertension before HD, or the use of antihypertensive medication to have a BP of $\leq 130/80$ mmHg. Hypertension was considered as an etiology of CKD if a patient had chronic hypertension before HD, and still had hypertension after HD.

Blood Sampling

Venous blood samples were drawn from the arterial-venous fistula 10 minutes before the first HD session in a week. Peripheral whole blood was obtained from 30 healthy individuals. Blood samples were 3 ml for each participant without additives. Plasma was immediately separated by centrifugation at 800 g for 15 minutes, and then stored at -20°C prior to use.

Genomic DNA

Genomic DNA was extracted from whole blood with a commercially available DNA isolation kit (QIAmp DNA blood Mini kit, Qiagen GmbH, Crawley, West Sussex, UK). Genotyping of the polymorphisms was performed using PCR-based SNP detection methods (Applied Biosystems, Carlsbad, USA) based on 5'-exonuclease activity assays for rs805305 (-449 G/C).^{13,14} Allelic variation was assessed in each participant.

Statistics

Distribution differences of gene polymorphisms between the groups were analyzed using the chi-square test. Distribution differences of gene polymorphism between subgroups were also analyzed using chi-square test.

RESULTS

Clinical Features

The clinical features of HD patients are shown in **Table 1**. The predominant causes of CKD were hypertension (35 patients, 62.5%) and diabetes (10 patients, 17.9%). Other etiologies such as infection, glomerulonephritis and kidney stone were less than 10% (**Figure 1**).

Table 1. Clinical features of HD patients

Variables	Mean±SD
Age	43.52±10.93
BMI	21.88±3.73
Hb	10.07±0.85
Serum creatinine	9.03±1.11
Systolic blood pressure	146.61±15.29
Diastolic blood pressure	90.71±7.83
ADMA	0.84±0.21

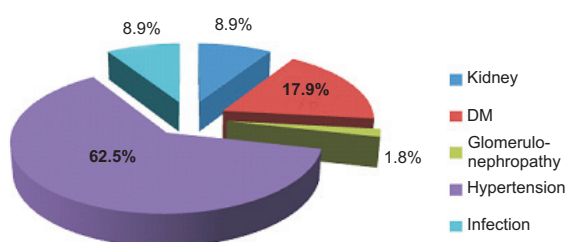


Figure 1. Etiologies of CKD in HD patients

Gene Polymorphism Distribution HD Patients and Healthy Individuals

Gene polymorphisms were assessed among healthy individuals and HD patients. Heterozygotes were observed as the most abundant genotypes in both groups (70% in healthy individuals and 55% in HD patients), followed by GG genotype in the HD patients (30%), while CC (27%) was the second most common genotype polymorphism in the healthy individuals (**Table 2**). A significant difference of polymorphism distribution between both groups was sought by using the chi-square test, and the result was $p=0.01$.

Table 2. Distribution of DDAH2 gene polymorphism among healthy individuals and HD patients

Groups	Frequency			Total
	GG	Heterozygote	CC	
Healthy individuals	1	21	8	30
HD patients	17	31	8	56
HT subgroups	11	21	3	35
Non-HT subgroup	6	10	5	21

Subgroups Within HD Patients

The HD patients were divided into two subgroups. The subgroups were categorized based on the etiology of CKD in HD patients. One subgroup was a hypertension subgroup (35 of 56 HD patients samples), another was a non-HT subgroup, a subgroup of HD patients in which the etiologies of CKD were DM, infection, glomerulonephritis and kidney stone (21 of 56 HD patients samples). There was no significant difference in the distribution of DDAH gene polymorphism among subgroups.

DISCUSSION

There are considerable data on the association of DDAH gene polymorphism and ADMA in certain populations. In this study, we described the distribution of a novel genetic polymorphism DDAH 2 in HD patients. The study revealed a significant distribution difference in DDAH 2 gene polymorphism between healthy individuals and hemodialysis patients. They shared the same most abundant genotype, which was G/C, followed by GG in the HD group and CC in the

healthy individuals group. The polymorphisms were in the non-coding part of the gene, putatively affecting promoter activity, designated by DDAH2 -449.

In this study, the HD patients group was divided into a HT subgroup and a Non-HT subgroup. This study revealed that the average blood pressure of containing -449 G allele (GG and G/C) was higher than that of CC allele. O'Dwyer et al. recently reported that the -449G allele of the DDAH 2 gene was associated with increased ADMA concentrations.¹⁵ Higher prevalence of hypertension with an odds ratio of 1.80 (1.29–2.49 95% CI; $p < 0.001$) for individuals homozygous for the -449G allele was also reported in another study.¹⁴

DDAH is most likely a key determinant of plasma ADMA concentration.¹⁶ Thus, DDAH plays a crucial role in the regulation of NO synthesis via modulating endogenous ADMA levels.¹⁷ Alterations in DDAH activity may effect vascular structure as well as vascular reactivity.¹⁸ As research has progressed, DDAH has emerged as an important regulator of NO bioavailability and the integrity of renal and vascular function.¹⁰

In higher organisms, including humans, two isoforms of DDAH exist which have distinct tissue distributions but similar enzymatic activity.¹⁹ Based on tissue messenger ribonucleic acid (mRNA) levels, it has been suggested that the expression pattern of DDAH1 overlaps more closely with the expression pattern of neuronal NOS, whereas DDAH2 expression had greater similarities with endothelial NOS expression.^{20,21} However, it is still unclear which DDAH isoform represents the principal methylarginine-metabolizing enzyme.

Oxidative stress is defined as tissue damage caused by the disequilibrium between pro-oxidants and anti-oxidants.²² Vascular oxidative stress is a major factor in the pathogenesis of atherosclerosis.²³ One of the main effects of oxidative stress is the decrease in the biological activity of NO.²² Among the mechanisms for impaired NO synthesis is the accumulation of the endogenous NOS inhibitor asymmetric ADMA.⁷ ADMA inhibits all three isoforms of nitric oxide synthase and therefore has the potential to produce diverse biological effects, particularly

in the cardiovascular system.^{20,24} Some studies of human subjects with essential HT report elevated plasma levels of ADMA.^{25,26} Investigations of ADMA concentration by independent methods in patients with renal failure, have identified plasma ADMA concentration as a strong independent predictor of disease progression. A particular interest was the finding that a 0.1 $\mu\text{mol/L}$ increase in plasma ADMA was associated with a 20% increase in cardiovascular event rate.^{7,27} Kuswardhani et al. also found that the increase of ADMA and the decrease of NO activity were included in non traditional risk factor for peripheral arterial dysfunction.²⁸

The enzyme DDAH specifically hydrolyzes ADMA to citrulline and methylamines.^{29,30} Several groups have demonstrated that modulating DDAH activity can have a profound effect on endothelial NO production.^{7,31,32} Two isoforms of DDAH have been identified. Since DDAH2 is the predominant isoform expressed in the cardiovascular system and since hypercholesterolemia impaired endothelial DDAH activity while DDAH2 protein expression remained unchanged,^{17,33,34} DDAH2 gene polymorphism was studied in the present study. However, the association between DDAH2 gene polymorphism and ADMA has not been investigated in this study. Further studies are required to determine the pathophysiological significance of elevated serum ADMA in HD patients and to understand better about how DDAH gene variation influences ADMA levels.

CONCLUSION

There is a significant difference in distribution of DDAH 2 gene polymorphism among HD patients compared to healthy individuals. Heterozygotes were observed as the most abundant genotypes in both HD and healthy groups. While there is no significant difference observed in DDAH gene polymorphism among HT and non-HT subgroups.

This study had several limitations. The number of patients was relatively small, and we did not evaluate the marker of oxidative stress nor other factors potentially involved in endothelial dysfunction.

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