THE VARIATION OF THE NOS3 GENE RS1799983 IS NOT ASSOCIATED WITH PREECLAMPSIA IN MALAY POPULATION

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ABSTRACT

Background: *eNOS play role in preeclampsia pathophysiology due to disturbing spiral arteries re-modelling and enhanced maternal vasoconstriction. A genetic variant of NOS3 associated with eNOS level. In addition, the previous study reported a genetic variant of NOS3 rs1799983 as a risk of preeclampsia with conflicting results beyond the population. To the best of our literature search, this genetic variant as preeclampsia risk has never been reported in the Jambi Malay population. This study aims to determine the association of the NOS3 genetic variant with preeclampsia in the Jambi Malay population.*

Methods: *This study was a cross-sectional study that involved 43 pregnant women suffering preeclampsia and 53 normotensive pregnant women. All the subject resides in Jambi Province and was Jambi Malays. Genotyping was performed with tetra ARMS-PCR. Bivariate statistically analysis was performed.*

Result: *The frequency of GT genotype was lower in the preeclamptic group and GG genotype was higher in the preeclamptic group than the TT genotype, but the difference was not statistically different for NOS3 rs1799983.*

Conclusion: *Our findings indicate that the genetic variant of NOS3 rs1799983 is not associated with preeclampsia in the Jambi Malay population.*

Keywords: *NOS3, eNOS, rs1799983, genetic variant, preeclampsia, Malay population*

ABSTRAK

Pendahuluan: eNOS berperan dalam patofisiologi preeklamsia karena mengganggu *remodelling* arteri spiralis dan meningkatkan vasokonstriksi maternal. Varian gen NOS3 berkaitan engan peningkatan kadar eNOS. Penelitian sebelumnya melaporkan hasil yang bertentangan pada berbagai populasi terkait hubungan antara varian gen NOS3 rs1799983 dengan risiko kejadian preeklamsia. Sejauh pencarian literatur, varian gen NOS3 rs1799983 sebagai risiko preeklamsia belum pernah dilaporkan pada populasi Melayu Jambi. **Penelitian ini bertujuan** mengetahui hubungan varian gen NOS3 dengan preeklamsia pada populasi Melayu Jambi.

Metode: Penelitian ini menggunakan desain potong lintang, melibatkan 43 orang ibu hamil dengan preeklamsia dan 53 orang ibu hamil normotensif. Semua subjek berdomisili di Provinsi Jambi dan merupakan orang Melayu Jambi. Pemeriksaan genotip dilakukan dengan metode tetra ARMS-PCR. Analisis statistik dilakukan meggunakan uji bivariat.

Hasil: Hasil analisis didapatkan frekuensi genotip GT lebih rendah pada kelompok preeklampsia dan genotip GG lebih tinggi pada kelompok preeklampsia dibandingkan genotip TT, namun perbedaan tersebut tidak berbeda bermakna secara statistik.

Kesimpulan: Hasil penelitian ini mengesankan bahwa varian genetik NOS3 rs1799983 tidak meningkatkan risiko preeklampsia pada populasi Melayu Jambi.

Kata kunci: *NOS3, eNOS, rs1799983, varian genetik, preeklamsia, populasi Melayu*

INTRODUCTION

Preeclampsia is a life-threatening complication of pregnancy. The incidence of preeclampsia increases morbidity and mortality in both mother and fetus.[1] Impaired placentation and endothelial dysfunction have been widely recognized to play an important role in the pathogenesis of preeclampsia. Endothelial dysfunction occurs due to the reduced availability of nitric oxide due to decreased production and increased use of nitric oxide in oxidative stress. Nitric oxide is known to play a role in the process of placental formation in early pregnancy because it has play role in placental vasodilation and determined uteroplacental blood flow adequacy.[2,3]

The role of genetics as a risk factor for preeclampsia is associated with genetic variations in the Nitric Oxide Synthase 3 (NOS3) gene. Variations of the NOS3 gene are known to decrease endothelial Nitric Oxide Synthase (eNOS) causing a decrease in nitric oxide synthesis. The low availability of nitric oxide increases the risk of endothelial disorders that have the potential to increase blood pressure in preeclampsia.[4,5]

Several studies have reported the association between NOS3 gene variation and preeclampsia in various populations and obtained varying results.[4,6,7,8] Based on the differences in results and research objectives in each population, this present research aim to determine the association between the variation of the NOS3 gene rs1799983 with the risk of preeclampsia in the Jambi Malay population.

METHODS

Design and subjects

This study was a cross-sectional design and blood samples from 43 pregnant women with preeclampsia (case group) and 53 normotensive pregnant women (control group) who carried out pregnancy examinations and childbirth at hospitals in the Jambi City area was taken. Pregnant women with preeclampsia were defined by systolic blood pressure 140 mmHg or diastolic blood pressure 90 mmHg at pregnancies over 20 weeks and had met the inclusion and exclusion criteria of the study. The inclusion criteria for this study were having complete obstetric data, single fetus and live birth, while the exclusion criteria were the mother had a history of high blood pressure outside of pregnancy, was suffering from or had a history of metabolic disease, kidney disease and infectious disease.

This research has obtained research ethics permit from the Ethics *Committee* of the Faculty of Medicine and Health Sciences, Universitas Jambi (FKIK UNJA). The genotype examination was carried out at the Biomedical Laboratory, FKIK UNJA.

Genotyping

Genotyping used DNA isolates extracted from buffy coat blood samples. The DNA isolates were then amplified using the Amplification Refractory Mutation System - Polymerase Chain Reaction (ARMS-PCR) method as well as to identify variations in the NOS3 gene. The primers used in this study are shown in Table 1 below.

Table 1. Primer rs1799983[9]

Variant of NOS3 gene	*Primer Sequence*	*PCR Products*
rs1799983	*FO: 5'-AGCCTCGGTGAGATAAAGGATG-3'* *RO: 5'-CCTGGACCTGCTCTGATTGTC-3'* *FI: 5'-GCTGCTGCAGGCCCCAGAT**A**AG-3'* *RI: 5'-GCAGAAGGAAGTTCTGGG**A**GA-3'*	*GG: 475 bp, 701 bp* *GT: 271 bp, 475 bp, 701 bp* *TT: 271 bp, 701 bp*

The PCR cycle was 95^{o}C for 7 minutes for initial denaturation, 64^{o}C for 1 minute for the annealing and 72^{o}C for 1 minute for the extension process. Visualization of PCR products using electrophoresis on 2% agarose gel.

Statistical analysis

Normality test was performed using the Shapiro-Wilk test on all research data. Normally distributed data were analyzed using independent t-test and the data were presented in the form of the mean (±SD). Abnormally distributed data were analyzed using the Mann-Whitney test and the data presented was the median (min-max). Bivariate analysis using the Chi-Square test and Fisher's Exact test was chosen to analyze the relationship between variations in the NOS3 rs1799983 gene and the incidence of preeclampsia based on the Odds Ratio value. Hardy Weinberg Equilibrium (HWE) analysis was performed on all research samples. The significance value was $p<0.05$.

RESULTS AND DISCUSSION

Baseline characteristics of subjects

Subject characteristics based on maternal age and gravida showed no significant difference between pregnant women with preeclampsia and normotensive pregnant women. The frequency of preterm birth, low birth weight and asphyxia (Apgar score <7) increased statistically in the group of pregnant women with preeclampsia (Table 2).

Table 2. Baseline subjects characteristic

Characteristics of subjects	PE (n=43)	Normotensive (n=53)	p value
Maternal age			
<18 years	*0*	*1*	*0.823*
18-35 years	*13*	*16*	
>35 years	*30*	*36*	
Gravida			
Primigravida	*18*	*20*	*0.522*
Multigravida	*25*	*33*	
Gestational age			
<36 weeks (premature)	*14*	*3*	*0.001*
36 weeks (mature)	*29*	*50*	
Baby's birth weight			
< 2500 gr (LBW)	*19*	*6*	*<0.001*
2500 gr (normal)	*24*	*47*	
APGAR Score			
< 7 (asphyxia)	*13*	*0*	*<0.001*
7 (fit)	*30*	*52*	

Genotyping

Identification of the variation of the NOS3 gene rs1799983 was successfully carried out using the ARMS-PCR method. The PCR product of the NOS3 gene variation on 2% agarose gel is shown in Figure 1.

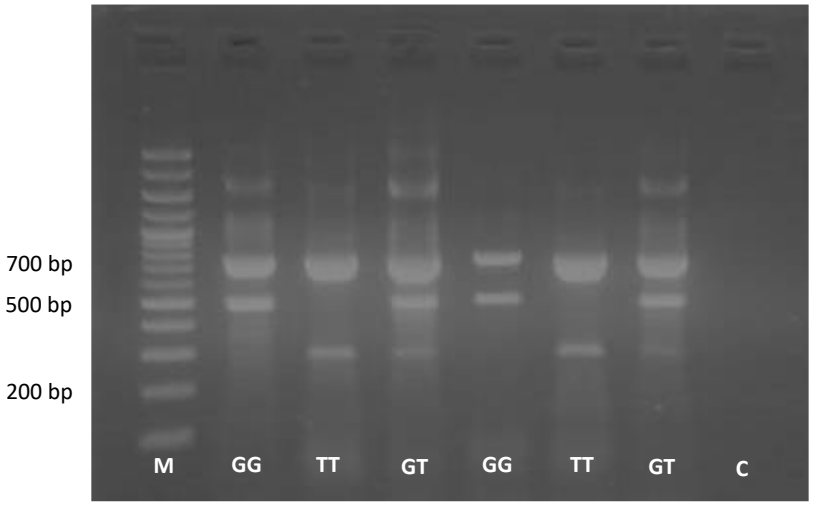

Figure 1. Genotyping of variations in the NOS3 1799983 gene using the Tetra ARMS-PCR method.

The GG genotype (wildtype homozygous) is shown as 475 and 701 bp DNA fragments; GT genotype produced 271, 475 and 701 bp DNA fragments; the TT genotype produced 271 and 701bp DNA fragments; M is a marker and C control.

Genotypic distribution

The Hardy-Weinberg equilibrium of the rs1799983 variant has a significance value <0.05 (Table 3), this indicates that this variant deviated from the Hardy-Weinberg equilibrium.

Table 3. Hardy-Weinberg equilibrium

Genotype	Observed frequency	Expected frequency	X^2 (df)	*p* value
GG	89	87,2		
CT	5	8,6	16,70	<0.05
TT	2	0,2		

Statistical analysis of genetic variation with preeclampsia

The rs1799983 variant showed a higher frequency of the GG genotype in the normotensive group than the TT and GT genotypes. The frequency of GG was almost the same in both groups, and the frequency of GT was higher in the normotensive group than in the preeclampsia group. The TT genotype increased the risk of preeclampsia in the additive model but was not statistically significant with OR (95% CI) values of 1.17 (0.07-19.31); *p*-value = 1.000. (Table 4).

Table 4. Association NOS3 variant Rs1799983 with preeclampsia

Genotype	*PE (n=43)*	*Normotensive (n=53)*	*p value*	*OR (95% CI)*
Additive model				
GG	*41*	*48*	*ref*	
GT	*1*	*4*	*0.376**	*0.29 (0.03-2.72)*
TT	*1*	*1*	*1.000**	*1.17 (0.07-19.31)*
Recessive model				
GG	*41*	*48*	*ref*	
GT+TT	*2*	*5*	*0.454**	*0.46 (0.08-2.54)*

Discussion

The baseline characteristics of the two groups showed that there was no significant difference between maternal age and gravida. The risk of preeclampsia is increased in pregnant women older than 40 years and primigravida.[10,11] In this study, many subjects were less than 40 years old in the preeclampsia group and were multigravida. Pregnant women with preeclampsia are at risk of maternal and neonatal complications. The risk of premature birth affects the baby's low birth weight and increases the risk of asphyxia. This is because, in premature infants, the respiratory system is not fully developed so the risk of asphyxia increases.[10] The complications of preeclampsia in this study were premature birth, low birth weight and asphyxia were found to be more common in the preeclampsia group.

Variation of the NOS3 gene rs1799983 showed that deviated from the Hardy-Weinberg equilibrium. This may be related to the limited sample size. The association between the variation of the NOS3 gene rs1799983 with preeclampsia in the Malay population observed in this study

showed a non-significant association. Similar results were also shown in previous genetic studies in the UK population. A case-control study conducted on 438 women of various ethnicities in the UK showed no significant association in the recessive model and no significant differences between ethnicities in the case and control groups. Meta-analysis studies on recessive and dominant models also did not show a significant association.[2]

A significant association between the variation of the NOS3 gene rs1799983 and the risk of preeclampsia has been reported in a study of a Pakistani population. This study reported that the variation of the NOS3 gene rs1799983 causes functional changes in the eNOS gene, thereby reducing the bioavailability of nitric oxide which causes endothelial dysfunction in preeclampsia.[12] A genetic study in the Columbian population has also shown that variations in the NOS3 gene rs1799983 increase the risk of preeclampsia in bivariate analysis and after adjusting for another covariate variable.[13]

The NOS3 gene is encoding eNOS, which is an important regulator of vascular tone and contributes to the reduction of uteroplacental resistance in normal pregnancy through the production of nitric oxide. The presence of variations in the NOS3 gene rs1799983 can cause a decrease in the synthesis of eNOS, thereby reducing the production of nitric oxide. The variant gene of NOS3 rs1799983 or G894T, guanine/thymine substitution occurs at position 894 in exon 7, causing a change from glutamate to aspartate at codon position 298.[6,7] Decreased nitric oxide triggers endothelial damage so that the vasodilation process is disrupted. This situation can underlie the occurrence of endothelial dysfunction which results in vasoconstriction in preeclampsia.[12,14,15]

CONCLUSION

Our results suggest that variation of the NOS3 gene rs1799983 is not a risk factor for the incidence of preeclampsia in the Jambi Malay population. However, the results of the current findings need confirmation with a larger study.

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