JAMBI MEDICAL JOURNAL
Jurnal Kedokteran dan Kesehatan
Vol. 13 No. 1 May 2025
DOI: 10.22437/jmj.v13i1.41334
Journal homepage: https://online-journal.unja.ac.id/kedokteran
p-Issn : 2339-269x
e-Issn : 2580-6874

Original Article

The Genetic Variation of Ace Gene Rs4343 Has Lack Association With Pre-Eclampsia: Case-Control Study In Jambi Malay Population

[1]Anggelia Puspasari, [2]Herlambang, [1]Citra Maharani, [1]Tengku Arief Buana Perkasa1, [3]Rina Nofrienis, [3]Susan Tarawifa, [4]Astian Nathaniel Syukur

[1]Department of Biochemistry and Medical Biology, Faculty of Medicine and Health Sciences, Universitas Jambi, Jambi, Indonesia
[2]Fetomaternal division, Department of Obstetrics and Gynecology, Faculty of Medicine and Health Sciences, Universitas Jambi, Jambi, Indonesia
[3]Department of Anatomy, Faculty of Medicine and Health Sciences, Universitas Jambi, Jambi, Indonesia.
[4]Bachelor Of Medicine Degree,, Faculty of Medicine and Health Sciences, Universitas Jambi, Jambi, Indonesia.

E-mail Corresponding: anggelia.puspasari@unja.ac.id

Article History:
Submit: January 2025
Accepted: March 2025

Keyword:
Preeclampsia;
ACE;
rs4343;
Malay;
Indonesia

ABSTRACT

Background: The PE is leading cause of maternal and neonatal mortality and morbidity worldwide. While its etiology remains unclear, genetic factors contribute 20–40% to PE risk. The rs4343 variant of the ACE gene has been reported as a potential risk factor for PE, with varying effect sizes across studies. However, its association with PE in the Indonesian population, particularly among Jambi Malays, remains unstudied. This study investigates the association of ACE gene rs4343 variation with PE risk in Jambi Malays.

Methods: A case-control study included 78 pregnant women matched by age and parity. PE was diagnosed based on American College of Obstetricians and Gynecologists criteria. Genotyping was performed using tetra ARMS-PCR, and bivariate statistical analysis was applied.

Results: While the distribution of the AA and AG genotypes showed slight differences between groups, all GG genotypes were associated with PE. A recessive model revealed a higher, but statistically insignificant, PE risk for GG/AG genotypes compared to AA (p=0.784, OR=1.16, 95%CI=0.40–3.41). The G allele also showed a non-significant association with increased PE risk (p=0.338, OR=1.59, 95%CI=0.62–4.14).

Conclusion: The ACE gene rs4343 variant showed no statistically significant association with PE in this Jambi Malay population, yet all GG genotypes were observed in PE cases. This suggests a potential role warranting further research with larger, multi-ethnic cohorts to clarify genetic influences on PE risk and inform early screening strategies

INTRODUCTION

Preeclampsia (PE) is defined as a specific syndrome in pregnancy signed by hypertension in pregnancy older than 20 weeks with at least one of the following signs: proteinuria, increased creatinine, the sign of maternal organ dysfunction and the sign of uteroplacental dysfunction[1,2,3]. The PE is leading cause of maternal and neonatal mortality and morbidity worldwide. This condition affected 2-8% of pregnancy[4,5]. The aetiology of PE remains not yet identified clearly, genetics contributed 20-40% of PE[6].

One of the theories of PE reported PE started with the failure of spiral artery remodeling which caused a decrease in utero placenta blood flow and increased blood vessel resistance. This triggers inflammation in the endothelial including in maternal circulation which causes maternal hypertension and may lead to maternal organ damage[7,8]. Renin Angiotensin System (RAAS) is one system which plays a role in the regulation of blood pressure in pregnancy. Previous studies reported dysregulation of this system contributed to PE[8].

Angiotensin Converting Enzyme (ACE) is one of the enzymes which have a crucial role in RAAS, this enzyme converts inactive angiotensin I into active angiotensin II. ACE proteins are encoded by the ACE gene, the ACE gene is a gene with numerous genetic variants. Genetic variations in the ACE gene have played a role in the level, stability and activity of the translated proteins[9]. The rs4343 is a genetic variant located in Chromosome 17 and a synonymous mutation which has the strongest association with ACE level. Individuals with the G allele of rs4343 had a higher level and activity of ACE protein than individuals with the A allele[10,11]. Furthermore, genotype-phenotype association studies showed that the variation of the rs4343 gene was significant as a risk factor for PE and the severity of PE with effect sizes varying between studies[12,13]. These variations may be due to population differences.

Genetic risk assessment for PE was beneficial for the early detection of susceptibility of pregnant women to suffer PE. Genetics is an unmodified factor, but screening performed before and at the early stage of pregnancy can help for better pregnancy planning, and increase awareness during prenatal care and delivery[14,15]. This may have an impact on reducing maternal and neonatal mortality and morbidity due to PE. To the best of our knowledge, the association of rs4343 in the ACE gene for PE risk has not been reported to the population of Indonesia, especially the Jambi Malays.

METHOD

Study Design And Subject Recruitment

This recent study was a case-control, as many as 78 pregnant women who reside in Jambi Province and are listed as Jambi-Malay ethnicity participated in this study. The case group was pregnant women who suffered from PE and the control group was normotensive women. Between case and control match carefully based on maternal age and number of pregnancies. All the subjects gave birth in Raden Mattaher General Hospital, Jambi, Indonesia in periods March 2020 to December 2022. The racial background was determined based on interviews, subjects listed as Jambi Malay population if they had grandparents from both parents listed as Jambi-Malay. The exclusion criteria for both groups were multiple pregnancy, and pregnancy with in vitro fertilization.

The PE criteria of diagnosis are based on the American College of Obstetricians and Gynecologists (ACOG), 2020[16]. The measurement of each variable diagnosed for PE methods is based on our previous paper[17]. The ethical permission was approved by the Ethics Committee for Health Research of the Faculty of Medicine and Health Sciences Universitas Jambi, Jambi, Indonesia (ethical approval number 1892/UN21.8/PT.01.04/2024)

Genotyping

Venous blood buffy-coat was used for DNA extraction using solid-phase with proteinase K DNA extraction kit (FavorPrep from Fevorgene, Ping-tung, Taiwan). The Quality of DNA extracted is measured by staining DNA and then running unmodified using agarose gel. The DNA appear as a continuous band above 100 bp then used for genotyping with one-step tetra amplification refractory mutation system (ARMS) PCR. The methods of ARMS PCR used are based on protocol specific for rs4343[17].

Data Analysis

The continuous data scale was not normally distributed, the analysis used non-

parametric test (Mann-Whitney) and the data was presented as median (min-max). The categories data analysis used Pearson Chi-square and Fisher test based on expected value calculation.

RESULT AND DISCUSSION

This study was case-control with carefully matching age groups for pre-eclampsia risk and the number of pregnancies. As many as 78 pregnant women who fulfilled study criteria, participated in this study. Baseline subject characteristics are shown in Table 1.

Table 1. Baseline Subjects' Characteristic and Neonatal Outcome

Characteristic	Pre-eclampsia (n = 39)	Normotensive (n = 39)	P-value
Age (years old)	29 (19-41)	29 (19-42)	0.802[a]
< 20 years old (n, (%))	3 (7.7)	3 (7.7)	
20-35 years old (n, (%))	26 (66.7)	26 (66.7)	1.000[b]
> 35 years old (n, (%))	10 (25.6)	10 (25.6)	
Systolic blood pressure (mmHg)	170.0 (140.0-210.0)	110.0 (100.0-130.0)	<0.001[a]
Diastolic blood pressure (mmHg)	100.0 (80.0-150.0)	70.0 (60.0-80.0)	<0.001[a]
Severity of pre-eclampsia			
Mild	6 (15.4)		
Severe	33 (84.6)		
Number of Pregnancy			
First pregnancy (n, (%))	15 (38.5)	15 (38.5)	1.000 [b]
Later pregnancy (n, (%))	24 (61.5)	24 (61.5)	
Gestational age at birth			
Premature (n, (%))	17 (43.6)	14 (35.9)	0.003 [b]
Mature (n, (%))	22 (56.4)	25 (64.1)	
Fetal weight (gr)	2600 (1050-3800)	3030 (1400-3980)	0.037[a]
APGAR score			
Asphyxia (n, (%))	10 (34.5)	0 (0)	0.001
Normal (n, (%))	19 (65.5)	28 (100)	

[a] Non-parametric test for mean difference; [b] Pearson Chi-square; [c] Fisher Exact test

Based on age categories for PE risk this study match carefully subjects who are younger than 20 years old, aged between 20-35 years old and older than 35 years old. The most frequent subjects are between 20-35 years old. The PE patients have higher systolic and diastolic blood pressure than the control (normotensive pregnancy women). The frequency of subjects who have severe PE was higher than mild PE. The frequency of subjects who were in their first pregnancy was higher than in later pregnancy and the number between PE and the control group was same. (Table 1).The PE group have worse neonatal outcomes than normotensive pregnancy. The PE group have a higher frequency of premature birth, lower baby weight, and higher asphyxia babies than control. This difference is statistically significant. (Table 1).

Table 2. Genotype distribution of rs4343 in Jambi-Malay population

Genotype	Observed value	Expected value	X2 (DF)	P-value	MAF
AA	61	59			
AG	14	17	3.03	0.08	0.13
GG	3	2			

Chi-square with degree of freedom (DF)=1; MAF: minor allele frequency

The wild-type genotype for rs4343 in this study population was AA genotype. The minor allele was G, and the frequency of the minor allele was 0.13 (Table 2). The Hardy Weinberg equation calculation does not deviate from Hardy Weinberg equilibrium. The bivariate analysis between genotype and PE is shown in Table 3. In the additive model of genotype for PE, between AA and AG genotypes the frequency was slightly different but all subjects who had the GG genotype were suffering from PE. In the recessive (GG/AG vs AA) and in the dominant model (GG vs AA/AG) the frequency was slightly different. Allele analysis shows G allele has a higher risk for PE than the A allele. All the difference was not statistically significant.

Table 3. Bivariate Analysis between genetic variation of ACE rs4343 and pre-eclampsia

Genotype rs4343	PE N (%)	Control N (%)	OR (95% CI)	p-value
Genotype				
AA	30 (49.2)	31 (50.8)	*Ref*	*Ref*
AG	6 (42.8)	8 (57.2)	0.78 (0.24-2.50)	0.669[a]
GG	3 (100)	0 (0)	7.23 (0.36-145.90)	0.197[b]
Recessive/dominant				
GG/AG vs AA			1.16 (0.40-3.41)	0.784[a]
GG vs AA/AG			0.92 (0.84-1.01)	0.120[b]
Allele				
A	66	70	*Ref*	*Ref*
G	12	8	1.59 (0.62-4.14)	0.338[a]

[a]Chi-square; [b]Fisher exact test

DISCUSSION

The main finding in this recent study was that rs4343, a genetic variation of the ACE gene has a lack of association with PE in Jambi. But all the subjects with the GG genotype suffered from PE. In contrast with this recent study, previous studies reported this genetic variant rs4343 has an association with PE and severity of PE. Studies in Egypt and Iranian reported the GG genotype and G allele increase the risk of PE. In the genotype model, the effect size ranges between 2.21-10.31 and in the allele model effect size ranges from 1.9 to 3.47[12,13]. The variability in findings across different studies, such as the lack of significant association in some populations or otherwise, underscores the influence of ethnic, and geographical factors and the interaction of genetics with environmental on genetic predispositions for diseases[18,19].

A functional study of rs4343 reported that rs4343 has the strongest association with ACE activity among several SNP. Subjects who had AG and GG had higher ACE levels and activity than those who had A allele[11]. The ACE is the main component of the RAAS system which is well known to regulate blood pressure by converting AGT I to AGT II which plays the role of potent vasoconstrictor. In normal pregnancy, the sensitivity of AGT II is decreased despite the upregulation of the RAAS component. This causes a slight reduction in blood pressure, maintains normal vascular tone and supports optimal uteroplacental function. However, in PE there is dysregulation of AGT biosynthesis and signalling, leading to increased sensitivity to AGT II and disrupting the vasodilatory pathway leading to elevated blood pressure and endothelial dysfunction[20,21].

The minor allele of this study was the G allele, based on NCBI databased this finding was similar to other populations, but the minor alleles were less frequent in this recent study[22]. The HWE of this study was not a departure from the HWE equation. Instead of the genetic population assumption of HWE (random mating, the absence of natural selection, no gene flow and autosomal locus), HWE which is no departure from the equation may reflect the small bias for genotyping methods[23,24].

This recent study was performed with one-step tetra-ARMS PCR. Four specific primers were designed to yield product due to the specific allele of rs4343. The primer in silicon analysis was performed based on the NCBI database for estimation of the annealing accuracy of primer to area polymorphism[17]. The thermocycling setting and PCR mixture were optimized to yield high-resolution visualization by electrophoresis, this has been published in our previous study. This technique was simple, easier, faster, and relatively cost-effective but still reliable than high-resolution melting (HRM)-PCR or polymerase chain reaction–restriction fragment length polymorphism (PCR-RFLP) in resources without a sequencing machine[25,26]

Age and number of pregnancies is an unmodifiable risk factor for preeclampsia. Previous studies reported maternal age younger than 20 years old or older than 35 years old increased the risk of PE, and so does the first pregnancy. According to this, this study matches carefully based on age group and number of pregnancies to minimize the effect of the two unmodifiable risk factors influencing the association of rs4343 and preeclampsia. Due to disruption of placentation and uteroplacental blood flow, PE caused worse neonatal outcomes. Similar finding with this recent study the frequency of premature baby birth and asphyxia in PE was higher than control group. The baby's weight was lower in the PE group than in the control group[27-33].

The limitation of the study was the small sample size although it met the minimal sample size and performed in a single centre. Further studies with larger sample sizes, multiethnic, multi centre and comprehensive risk factor PE were needed to strengthen evidence of the role of genetics in PE and which genetics are robust as risk factors for PE. Genetics was an unmodifiable risk factor but this risk did not change over time, knowing it promises earlier screening for disease prevention, early treatment and minimize complications related to diseases.

CONCLUSION

The Genetic Variation of ACE gene rs4343 lacks a statistically significant association with PE in the Jambi Malay population. However, the observation that all individuals with the GG genotype presented with PE suggests a potential clinical relevance that warrants further investigation. This highlights the complex interplay of genetic factors in pre-eclampsia susceptibility and underscores the importance of continued research with larger sample sizes and diverse populations to better understand genetic predispositions for PE, ultimately contributing to earlier screening and improved pregnancy outcomes.

ACKNOWLEDGMENT

This research was fund by Faculty of Medicine and health sciences Universitas Jambi in grant number 198/UN21.11/PT.01.05/SPK/2024 for basic research scheme. We declare no conflict of interest regarding of this paper. This research was part of research theme of identification risk factor for preeclampsia in Jambi Malay pregnant woman. Thanks to all the research team who help sample collecting, handling and laboratory examination. We declare no conflict of interest regarding this paper. This research funding.

REFERENCES

1. *Chaudhary BK, Shi J. Current Concept and Research Progress of Pre-Eclampsia (Early-Onset and Late-Onset Pre-Eclampsia). International Journal of Science, Engineering and Management 2022. https://doi.org/10.36647/ijsem/09.09.a005 .*
2. *Dimitriadis E, Rolnik DL, Zhou W, Estrada-Gutierrez G, Koga K, Francisco RPV, et al. Pre-eclampsia. Nature Reviews Disease Primers 2023. https://doi.org/10.1038/s41572-023-00417-6 .*
3. *Rupakala BM, Sudha GS, Lakshmi MPAS. Microalbuminuria and spot protein creatinine ratio in early pregnancy as a predictor of preeclampsia. International Journal of Reproduction, Contraception, Obstetrics and Gynecology 2021. https://doi.org/10.18203/2320-1770.IJRCOG20211119 .*
4. *Tanner RM, Bello NA. In Search of a Predictive Model for Preeclampsia: The Beat Goes On. Hypertension 2020. https://doi.org/10.1161/HYPERTENSIONAHA.120.15160 .*
5. *Birdir C, Tercanli S. Preeclampsia in Fetal Medicine. Ultraschall in Der Medizin 2017. https://doi.org/10.1055/S-0043-121859 .*
6. *Parada-Niño L, Castillo-León LF, Morel A. Preeclampsia, Natural History, Genes, and miRNAs Associated with the Syndrome. Journal of Pregnancy 2022. https://doi.org/10.1155/2022/3851225 .*
7. *Albrecht ED, Pepe GJ. Regulation of Uterine Spiral Artery Remodeling: a Review. Reproductive Sciences 2020. https://doi.org/10.1007/S43032-020-00212-8 .*
8. *Spradley FT, George EM, Palei AC, Warrington JP, Granger JP. Preeclampsia: Angiogenic Factors, Blood Pressure, and the Kidney 2015. https://doi.org/10.1007/978-1-4939-1982-6_13 .*
9. *Kanugula AK, Kaur J, Batra J, Ankireddypalli AR, Velagapudi R. Renin-Angiotensin System: Updated Understanding and Role in Physiological and Pathophysiological States. Cureus. 2023 Jun 21;15(6):e40725. https://doi.org/10.7759/cureus.40725 .*
10. *Chung CM, Wang RY, Chen JW, Fann CS, Leu HB, Ho HY, et al. A genome-wide association study identifies new loci for ACE activity: Potential implications for response to ACE inhibitor. Pharmacogenomics J. 2010; 10(6): 537-44 .*
11. *Firouzabadi N, Shafiei M, Bahramali E, Ebrahimi SA, Bakhshandeh H, Tajik N. Association of angiotensin-converting enzyme (ACE) gene polymorphism with elevated serum ACE activity and major depression in an Iranian population. Psychiatry Res. 2012;200(2-3):336-42. https://doi.org/10.1016/j.psychres.2012.05.006 .*
12. *Amal, A., Morsy., Alshaymaa, A, Abd, Elalim., Nashwa, Mohammed, Abd, Elbaky., H., A., Abdel, Halim. 1. Association between angiotensin converting enzyme gene polymorphism (rs4343) and susceptibility to gestational hypertension and preeclampsia in pregnant women. Egyptian Journal of Immunology, (2023). https://doi.org/10.55133/eji.300214 .*
13. *Atieh, Abedin, Do., Emran, Esmaeilzadeh., Mona, Amin-Beidokhti., Reihaneh, Pirjani., Milad, Gholami., Reza, Mirfakhraie. 2. ACE gene rs4343 polymorphism elevates the risk of preeclampsia in pregnant women. Journal of Human Hypertension, (2018). https://doi.org/10.1038/S41371-018-0096-4 .*
14. *Andrey, S., Glotov., Yulia, A., Nasykhova., N., M., Dvoynova., Olesya, N., Bespalova., I., Kogan. 8. Prospects for preconception genetic screening at the pregnancy planning stage. (2023). https://doi.org/10.17816/jowd622752 .*
15. *Marija, Majda, Perišić., Klemo, Vladimir., Sarah, Karpov., Mario, Štorga., Ali, Mostashari., Raya, Khanin. 6. Polygenic Risk Score and Risk Factors for Preeclampsia and Gestational Hypertension. Journal of Personalized Medicine, (2022). https://doi.org/10.3390/jpm12111826 .*
16. *ACOG. Gestational hypertension and preeclampsia. Obstet Gynecol. 2020;135:e237-60. https://doi.org/10.1097/AOG.0000000000003891 .*
17. *Elfiani E, Puspasari A, Arif CW, Maharani C, Ali Z, Suhaimi N, Effendi I, Suprapti S, Shafira NN. Angiotensin-Converting Enzyme Genetic Polymorphism rs4343 as Risk of Diabetic Nephropathy in Jambi-Malay Population. Molecular and Cellular Biomedical Sciences. 2024 Mar 22;8(1):37-43. https://doi.org/10.21705/mcbs.v8i1.410 .*
18. *An empirical Bayes approach to improving population‐specific genetic association estimation by leveraging cross‐population data. Genetic Epidemiology, (2022). https://doi.org/10.1002/gepi.22501 .*
19. *Michelle, S, Kim., Kane, P, Patel., Andrew, K, Teng., Ali, J., Berens., Joseph, Lachance. 9. Genetic disease risks can be misestimated across global populations. Genome Biology, (2018). https://doi.org/10.1186/S13059-018-1561-7 .*

20. *Eugenie, R., Lumbers., Sarah, J., Delforce., Anya, L, Arthurs., Kirsty, G., Pringle. 1. Causes and Consequences of the Dysregulated Maternal Renin-Angiotensin System in Preeclampsia. Frontiers in Endocrinology, (2019). https://doi.org/10.3389/FENDO.2019.00563 .*
21. *Claudia, Bezerra, da, Silva. 5. Regulation of the Placental Renin-Angiotensin-Aldosterone System in Early- and Late-Onset Preeclampsia. Doklady Biochemistry and Biophysics, (2022). https://doi.org/10.1134/s1607672922060011 .*
22. *National Center for Biotechnology Information [Internet]. Reference SNP (rs) Report rs4343 [updated 2022 Sep 1; cited 2023 Dec 15]. Available from: https://www.ncbi.nlm.nih.gov/snp/rs4343 .*
23. *Hosking L, Lumsden S, Lewis K, Yeo A, McCarthy L, Bansal A, et al. Detection of genotyping errors by Hardy-Weinberg equilibrium testing. Eur J Hum Genet. 2004; 12(5): 395-9. https://doi.org/10.1038/sj.ejhg.5201164 .*
24. *Chen J, Chatterjee N. Exploiting Hardy-Weinberg equilibrium for efficient screening of single SNP associations from case-control studies. Hum Hered. 2007; 63(3-4): 196-204. https://doi.org/10.1159/000101064 .*
25. *Ehnert S, Linnemann C, Braun B, Botsch J, Leibiger K, Hemmann P, et al. One-step ARMS-PCR for the detection of SNPs-using the example of the PADI4 gene. Methods Protoc. 2019; 2(3): 63. https://doi.org/10.3390/mps2030063 .*
26. *Medrano RF, de Oliveira CA. Guidelines for the tetra-primer ARMS-PCR technique development. Mol Biotechnol. 2014; 56(7): 599- 608. https://doi.org/10.1007/s12033-014-9744-3 .*
27. *Sharami SH, Kabodmehri R, Sorouri ZR, Aski SK, Chamani NA, Sabetghadam S (2023) Neonatal Outcomes of Severe Preeclampsia in Neonates Under 34 Weeks of Gestation. Journal of Kermanshah University of Medical Sciences. https://doi.org/10.5812/jkums-13913644 .*
28. *Badshah F (2023) Neonatal Outcomes in Women with Pre-Eclampsia in a Tertiary Care Hospital. Pakistan journal of medical & health sciences. https://doi.org/10.53350/pjmhs202317468945 .*
29. *Arulparithi CS, Manjani S (2023) Outcome of neonates born to mothers with pregnancy induced hypertension/preeclampsia: a systematic review. International Journal of Contemporary Pediatrics. https://doi.org/10.18203/2349-3291.ijcp2023150146 .*
30. *Rahman F, Ahmed M, Afroze N, Rahman MZ (2023) Study of Fetal Outcome in Preeclampsia and Eclampsia Syndrome. KYAMC Journal. https://doi.org/10.3329/kyamcj.v14i01.6747347*
31. *Jakovljevic TS, Kontic-Vucinic O, Miković Ž, Lazic-Mitrovic T (2024) Differences in neonatal outcomes between normal and preeclampsia complicated pregnancies. Global pediatrics. https://doi.org/10.1016/j.gpeds.2024.10016348 .*
32. *Kalambe M, Soni NN, Ali S, Bankar N (2024) An Observational Study of Maternal and Perinatal Outcome in Preeclampsia Cases in a Tertiary Care Center. Cureus. https://doi.org/10.7759/cureus.5935249 .*
33. *Peguero A, Fernandez-Blanco L, Mazarico E, Benitez L, Gonzalez A, Boada D, Youssef L, Crispi F, Hernández S, Figueras F (2023) Prediction of adverse neonatal outcome at admission for early-onset preeclampsia with severe features. Pregnancy Hypertension. https://doi.org/10.1016/j.preghy.2023.04.004 .*