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von Willebrand Factor Gene Polymorphism in Preeclampsia Pregnant at Medan, Indonesia

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Abstract

BACKGROUND: von Willebrand Factor (vWF) is a large glycoprotein mediating hemostasis and thrombosis. The roles of vWF are platelets adhesion to sites of vascular damage and stabilization of coagulation factor VIII.

AIM: This study aimed to analyze the polymorphism of the vWF gene on preeclampsia (PE) in pregnancy in Medan, Indonesia.

MATERIALS AND METHODS: DNA was amplified using the polymerase chain reaction and was electrophoresed in agarose 2%. Electrophoresis results were detected using Gel Doc 1000 (Biorad, USA). The sequencing method was used to identify polymorphism from vWF gene.

RESULTS: From 50 samples of PE patients, the g.93308C>T vWF gene polymorphism was found with the percentage of TT, CT, and CC genotypes as 50%, 42%, and 8%, respectively.

CONCLUSION: The c.93308C>T vWF gene polymorphism was found in the genotype percentage of homozygous TT, and heterozygote CT was greater than wild-type CC.

Introduction

von Willebrand Factor (vWF) is a large glycoprotein mediating hemostasis and thrombosis. vWF plays a role in platelets adhesion during vascular injury and stabilization of coagulation factor VIII. Deficiency of vWF in hemostasis leads to congenital bleeding disorder or von Willebrand disease (vWD). vWF also plays roles in blood vessels such as inflammation, permeability, and angiogenesis. A thrombotic event in thrombotic thrombocytopenic purpura is characterized by deposition of vWF and platelet thrombus in microvascular leading to vascular occlusion, tissue ischemia, organ failure, and even death [1], [2].

vWF is synthesized in endothelial cells and megakaryocytes and stored in Weibel-Palade bodies and granules [3]. The vWF gene is located on the short arm of chromosome 12, locus 12p13.3, spans 178 kilobases and consists of 52 exons and 51 introns [4]. Genetic variation is an alteration in the sequence of DNA nucleotides that causes changes in protein function. Genetic variation of vWF has been identified in 2728 single nucleotide polymorphisms (SNPs), 91 insertions and deletions in various ethnicity [5]. Genetic variation of vWF gene has been reported to be associated with several diseases of vascular occlusion such as deep vein thrombosis [6], heart disease [7], vWD types 1 and 2 [8], [9], hypertension, and preeclampsia (PE). A previous study by Sun *et al*. (2009) found Msp I vWF gene polymorphism in intron 19 associated with the prevalence and severity of PE [10]. In pregnancy, vWF levels increase together with factor VIII starting from the first trimester along with the gestational age [11].

PE is a pregnancy-specific complication characterized by hypertension, proteinuria, or other signs of organ dysfunction. This abnormality is seen after 20 weeks of gestation with previously normotensive patients [12]. PE is associated with extensive endothelial activation, increased inflammatory response, and placenta abnormalities. Endothelial cell activation causes increasing levels of soluble thrombomodulin, E-selectin, and vWF. Previous studies have shown increased levels of vWF antigen in PE patients characterized by endothelial cell activation [13]. In severe complicated PE cases, an increased level

of active vWF is associated with acute endothelial cell activation and endothelial dysfunction [14]. Increased level of vWF antigen is associated with genetic variation of vWF gene [5], [15]. Based on the description above, the researchers aimed to analyze the genetic variation of the vWF gene on PE in Medan, Indonesia. The researcher hopes this study can be used as a reference in determining several diseases for future studies.

Methods

Ethics

This research has been approved by Universitas Sumatera Utara (USU) Health Research Ethics Commission (No. 892/KEP/USU/2021).

Study design

This is a descriptive analytic study with a cross-sectional approach and conducted on 50 PE patients. Calculation of the number of sample was obtained using the prevalence value from the previous study in China. PE patients were picked from three different hospitals; H. Adam Malik Central General Hospital; Pirngadi Hospital and Sundari Hospital in Medan. The inclusion criteria for this study were pregnant women with clinically diagnosed PE and willing to provide informed consent which was complied with study procedures. The diagnosis of PE was determined by obstetricians with systolic blood pressure ≥140 mmHg and/or diastolic blood pressure ≥90 mmHg after 20 weeks of gestation until 2 weeks postpartum accompanied by proteinuria (24 h excretion ≥300 mg). In the absence of proteinuria, PE was diagnosed with new-onset hypertension and new onset of any severe features, namely thrombocyte count <100,000/μl, serum creatinine >1.1 mg/dl, or double the concentration in the absence of other renal diseases, serum liver transaminases 2× normal, pulmonary edema, cerebral/visual symptoms [16]. The exclusion criteria for this study were the presence of systemic disease, chronic disease, and previous abnormal conditions in the study participants.

DNA extraction and sequencing

The 5 ml blood sample was taken and labeled in a vacuum tube, then centrifuged at 2500 rpm for 15 min to obtain a leukocyte buffy coat. Extraction of DNA from leukocytes buffy coat was done by using DNA extraction and purification kit (The Wizard Genomic DNA Purification Kit, Promega, USA). DNA purification was measured using spectrophotometer nano drops. This process was carried out at the Integrated Laboratory of USU, Faculty of Medicine. The ratio of purity DNA must be from 1.8 to 2.0; concentration results were at least 30 μl. Other than that was excluded from the study.

The polymerase chain reaction (PCR) reaction was done. The concentration of forward primer (5'-TGGCCGCG- TGCACCCTCACTCCACC-3') and reverse primer (5'-AGGGCTTTAG ATCAGT- CACT GTGGCCCT-3') each 1 uL, GoTaq® Green Master Mix (Promega, USA) 12.5 uL, DNA sample 2 uL, and dH2O 8.5 uL. The primers used referred to a previous study [10]. PCR was carried out with an initial denaturation step at 94°C for 5 min, continued with 35 cycles of denaturation at 94°C for 30 s, elongation at 72°C for 30 s, annealing at 57°C for 30 s, and final cycle at 72°C for 5 min. DNA was electrophoresed in agarose 2% at 70 volts for 90 min to determine isolation quality. Furthermore, electrophoresis results were detected using Gel Doc 1000 (Biorad, USA) to be visualized with ultraviolet light.

Pure DNA samples were sent to Malaysia's 1st Base laboratory. The sequencing results of vWF polymorphism gene were analyzed using 4Peaks program (A. Griekspoor and Tom Groothuis, nucleobytes.com). The sequencing results were forward and reverse nucleotide base pairs in the form of ab1 and seq format. vWF gene sequence data were compared with gene bank number NG_009072.2 using the BLAST program from NCBI. The homology assessment of the sample isolates against the reference sequence can be seen from the percentage value of its identity, a value of 100% indicating that the sample isolate sequence is exactly the same as the reference isolate, while the lowest percentage (95%) is still considered good.

Statistical analyses

The obtained data were analyzed using SPSS version 25 computer software. Data were presented in the form of figures and tables of frequency distribution. The χ^2 test was used to determine whether the observed genotype distributions confirm to the Hardy-Weinberg equilibrium expectation (HWE).

Results

This study identified gene variations of vWF in 50 PE patients in Medan, Indonesia. Characteristics of PE patients based on age, systolic blood pressure, diastolic blood pressure, platelets, platelet distribution width and mean platelet volume are shown in the median, minimum and maximum values in Table 1.

The DNA electrophoresed results from 50 samples showed in Figure 1.

One of the vWF gene polynucleotide sequencings showed in Figure 2. The positioning of mutations in vWF samples was carried out using the gene reference NG_009072.2. Sequencing was found

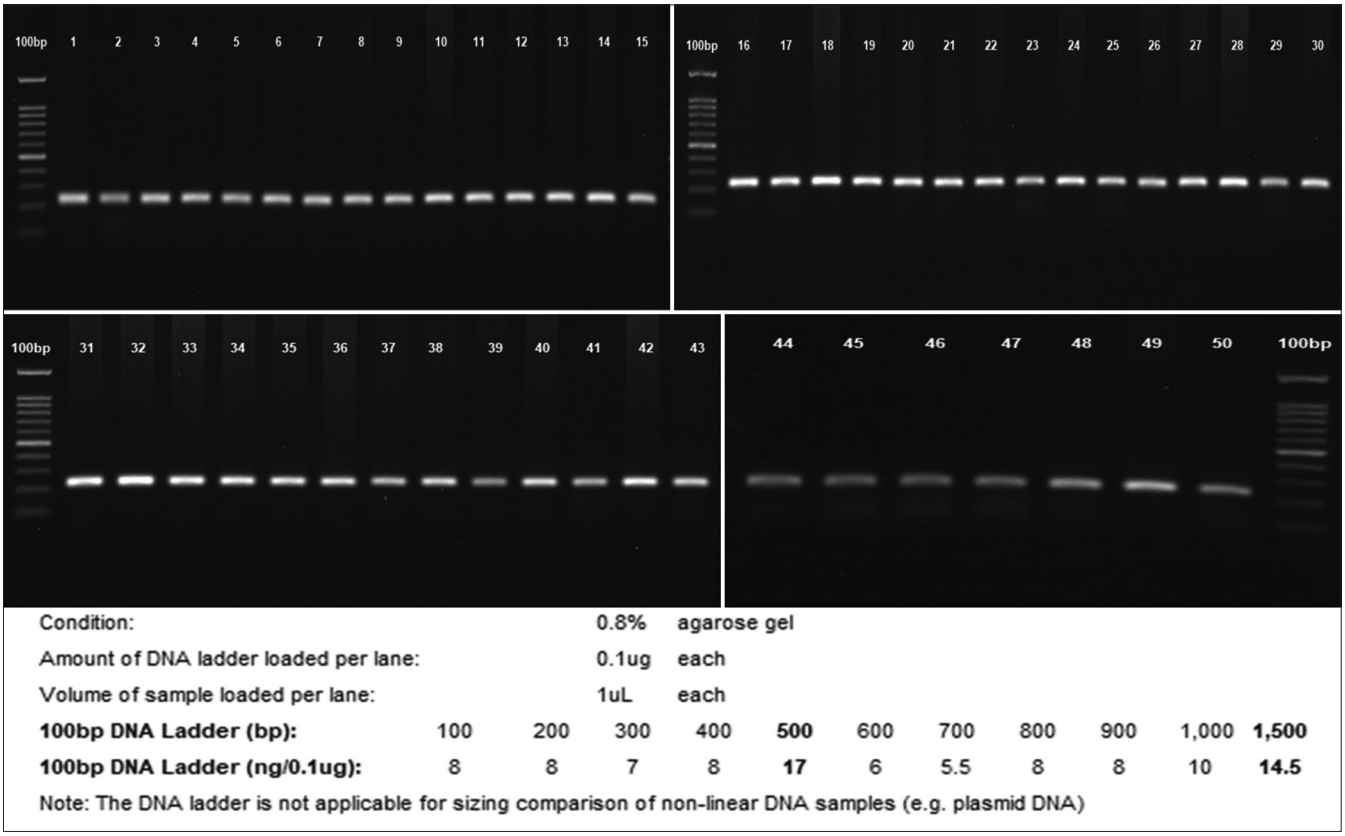

Figure 1: von Willebrand Factor gene amplification results from 50 samples (226 bp)

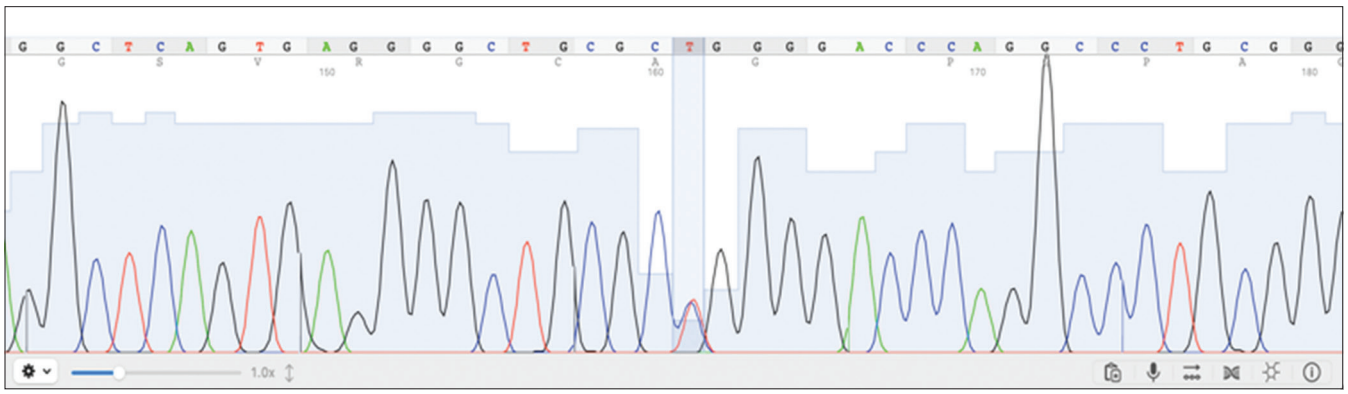

Figure 2: Double peak in von Willebrand Factor gene

double peaks coding for substitution of cytosine (C) to thymine (T) at nucleotide number 93308 indicated as heterozygous subjects.

Table 1: Characteristic of study participants

Characteristics	n	Median	Min-Max
Age (years)	50	30.5	21–42
Systolic blood pressure (mmHg)	50	160	128–200
Diastolic blood pressure (mmHg)	50	100	80–120
Platelet count ($\times 10^3/mm^3$)	50	299.5	150–1098
PDW (fl)	50	13	8.4–18.7
MVP (fl)	50	11.05	8.5–13.1

In this study, the vWF gene mutation g.93308C>T was found in 46 samples. The results of vWF gene sequencing in this study can be seen in Table 2.

Our results suggested that the genotype distribution of vWF gene polymorphism g.93308C>T did not significantly deviate from the HWE ($p > 0.05$). The genotype of the polymorphism was CC type 8% (n = 4), CT type 42% (n = 21) and TT type 50% (n = 25). The T allele frequencies are 71% (Table 2).

Table 2: Genotype distribution of vWF in preeclampsia pregnant

Name	Genotypes			Allele frequencies	
	CC	CT	TT	C	T
Observed number (%)	4 (8)	21 (42)	25 (50)	0.29	0.71
Expected number	4.2	20.6	25.2		

Chi-squared test, $p = 0.888$

Discussion

PE is a multisystem disorder in pregnancy that complicates 3–5% of pregnancies. It is the

leading cause of mortality and morbidity in pregnancy worldwide. Genetic factors play an essential role in PE, but the exact pattern of inheritance is still unknown. One of the genetic factors known to play a role in PE is the genetic variation of vWF. The vWF gene in human is an important component of the hemostasis system. There are at least 33 types of polymorphisms of the vWF gene. One of the polymorphisms of the vWF gene is Msp1 which is found in several diseases [6], [7], [8], [9]. Msp I vWF gene polymorphism is located in intron 19 associated with platelets and coagulation factor VIII. This leads to a genetic mutation of vWF that has the potential to cause PE [10], [17].

The present study found a substitution of cytosine (C) to thymine (T) at vWF gene that indicates heterozygous mutant CT and homozygous mutant TT. In this study, we have not been able to determine whether there is an amino acid change in the g.93308C>T substitution. This is due to the incomplete reference sequence showing the exon and intron regions of the vWF gene.

In this study, the homozygous TT and heterozygous CT were higher than the homozygous wildtype CC (50% vs. 42% vs. 8%). This shows that in patients with PE, the percentage of mutant genotypes is higher than the wildtype. This result is different from the previous study conducted by Sun *et al*., 2009 where the genetic variation of the Msp1 vWF gene was taken from 70 samples of PE patients who had homozygous mutations (M+/M+), heterozygous mutations (M+/M-), homozygous wildtype (M-/M-) were 22.9% versus 45.7% versus 31.4%, respectively [10].

This study suggests that for PE pregnant women in Medan-Indonesia, the allele and genotype frequencies of the vWF gene polymorphism are in HWE. Thus, we can expect these allele frequencies to remain constant over time, ensuring genetic variation of vWF gene polymorphism in the population. Distribution of T allele was found higher than the C allele (71% vs. 29%).

The distribution of the polymorphism of the vWF gene in other populations around the world is shown in Table 3.

Based on Table 3, it was found that several polymorphism genes of vWF in populations of various countries in several diseases. The polymorphism gene of vWF in that population was not found to have Msp1 polymorphisms and PE population. Until today, we have only found Msp1 SNPs in patients with PE based on Sun *et al*. 2009 research [10]. Wang *et al*. (2013) stated that allelic diversity and polymorphisms gene of the vWF are very complex in various populations [5]. A novel vWF gene polymorphism was found in the Turkish population with SNP 4483C>T [23].

This study was an initial study of the polymorphism gene of vWF in PE patients in Indonesia, especially in Medan. It is known that data on the polymorphism gene of vWF in Indonesia is still very limited. Further research is needed to confirm the findings of the current study and address the study's genetic-related weaknesses.

Table 3: Distribution of vWF gene polymorphism in various populations and diseases

vWF gene polymorphism	Population	Disease	Genotype	%
4975C>T [18]	Pakistan	von Willebrand's disease	CC	38
			CT	38
			TT	24
3445T>C [18]	Pakistan	von Willebrand's disease	TT	19
			TC	45
			CC	36
7603C>T [18]	Pakistan	von Willebrand's disease	CC	19
			TC	38
			TT	43
1185A>G [19]	Brazil	Coronary artery disease	AA	18.1
			AG	50
			GG	31.9
SmaI [20]	China	Ischemic stroke	CC	26.4
			CT	41.5
			TT	32.1
SmaI [20]	China	Myocardial infarction	CC	13.6
			CT	54.5
			TT	31.8
1381A>T [21]	China	Coronary heart disease	GG	70
			AG	30
1185A>G [22]	Gaza	Coronary heart disease	AA	32.9
			AG	42.4
			GG	24.7

Conclusion

This study concluded that from fifty pregnant subjects with PE, mutations of vWF were found in genotype percentage of homozygous TT and heterozygote CT was greater than wild type CC (92% vs. 8%) and the allele frequency was 71%. We have not been able to determine whether there is an amino acid change in the g.93308C>T due to the incomplete reference showing the exon and intron regions of the vWF gene.

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