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MTHFR and ERVFRD-1 Polymorphisms and Preeclampsia Risk in Iran population: A Case-Control Study

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Association of MTHFR and ERVFRD-1 Polymorphisms with Preeclampsia in Iran population (Alborz province): A Case-Control Study

Abstract

Background and aims: Preeclampsia (PE) is a complicated disease during pregnancy that could be a risk factor for the mother's health and fetus. The mechanisms of PE pathogenesis might associate with some candidate gene polymorphisms. This study is aimed to evaluate the relation between MTHFR (C677T) MTHFR (A1298C) and ERVFRD-1 (rs9393931) single nucleotide polymorphisms (SNPs) and PE in our patients.

Material and Methods: The present study was a case-control study carried out between January 2019 and January 2021, in the Kamali hospital, Karaj, Iran. A total of 104 pregnant women who were diagnosed with Preeclampsia clinically as a case, and 100 healthy pregnant women as a control group were compared for the study.

Results: There was not a 39T allele of the ERVFRD-1 gene was observed in this studied population. There was a significant difference in frequency of 677CT genotype in PE pregnant women compared to controls (P = 0.002). Also, our result indicated that the frequency of the MTHFR 1298C allele was found to be significantly higher in the control group than in the case group (P = 0.021).

Conclusion: Our results suggested that C677T polymorphism in MTHFR might be related to an increased risk of Preeclampsia in pregnancy.

Keywords: Pre-Eclampsia; Pregnancy; Genes; Hypertension

1. Introduction

Preeclampsia (PE) is one of the complications of pregnancy that cause perinatal and maternal morbidity and mortality, particularly when early onset. The prevalence of this disease varies from 3% to 7% and causes over 50,000 maternal deaths, and more than 500,000 fetal deaths around the world (1-3); this rate is reported in Iran as about 4-7% (4). It causes 16% of maternal mortality in high-income countries, compared to 9% to 26% in low-income nations (5). Risk factors for PE include those with previous early onset preeclampsia, diabetes type one, , chronic kidney disease, and women with preexisting hypertension (6). Short-term maternal complications of PE include HELLP syndrome, retinal detachment, cerebrovascular bleeding, and eclampsia. Also, it is related to an increased risk of ischemic heart disease stroke, chronic hypertension, and death from cardiovascular events (7).

Single-nucleotide polymorphisms (SNPs) and genetic linkage have been studied until now, and various genes have been identified with an increased risk of PE. For example, SNP rs479200 in the EGLN1 gene and SNP rs4759314 in the HOTAIR gene are associated with increased susceptibility to PE (8, 9). Due to the multifactorial nature of PE and the low odds ratio of identified genes, more genetic traits and pathways remain to be identified.

The enzyme tetrahydrofolate reductase is encoded by the methylenetetrahydrofolate reductase (MTHFR) gene, which catalyzes the irreversible conversion of 10-,5-methylene tetrahydrofolate to 5-methyltetrahydrofolate. Subsequently, 5-methyltetrahydrofolate is used as a substrate to convert homocysteine to methionine (10). Methionine is used to synthesize s-adenosyl methionine (SAM), and it is the most important donor of methyl groups for the methylation of DNA, lipids, and proteins. The gene encoded by this enzyme is located on chromosomal region 1p36.3. The presence of two single nucleotide polymorphisms, rs1801133, and rs1801131, in this gene, influences its enzymatic activity (11). The rs1801133 mononucleotide polymorphism replaces the amino acid valine with alanine at point 222. In rs1801131, the amino acid valine replaces glutamic acid (Val429Ala). These mononucleotide polymorphisms lead to a 70% reduction in MTHFR activity compared to the standard type (12). A decrease in the activity of the MTHFR enzyme has been identified in association with several developmental and reproductive disorders. The presence of rs1801133 and rs1801131 variants in parents has been associated with abortion and neural tube defects in the fetus (13). Previous studies reported that

MTHFR rs1801133 and rs1801131 SNPs were implicated in several pregnancy-related complications such as placental abruption or infarction, and PE (14-16).

The ERVFRD-1 gene, located on chromosomal position 6p24.2, encodes a functional protein called syncytin-2. This gene is part of the human endogenous retrovirus (HERV) family, which is expressed at high levels in placental tissue and plays a functional role in reproduction (17, 18). The syncytin-2 protein is involved in the placenta's growth and development, the integration of cytotrophoblast mononuclear cells, and the formation of the syncytium, the last differentiated step in the trophoblast line. Histological placental abnormalities in pregnancy with decreased perfusion and oxidative stress are associated with PE (19, 20). Previous studies show a decrease in syncytin gene expression levels in pregnant women with PE compared with healthy women (21, 22). Ying Hua et.al in a newly published research reported that ERVFRD-1 (rs9393931) polymorphism was significantly associated with an increased risk of preeclampsia development (23).

In this study, we examined the association of rs1801133 and rs1801131 variants in the MTHFR gene and the rs9393931 variant in the ERVFRD-1 gene with PE in a population of Iranian women. We chose the rs9393931 variant in the ERVFRD-1 gene because it was a newly discussed polymorphism in association with PE in China population. Then to prove the result of this study we try to add polymorphisms related to PE like MTHFR (rs1801133) and MTHFR (rs1801131) which were previously discussed in many research.

2. Materials and Methods

2.1 Study design:

The Ethical Committee at the Alborz University of medical sciences approved this project, and all participants provided written informed consent. (Ethical number: IR.ABZUMS.REC.1397.132, IR.ABZUMS.REC.1397.159).

We used a 95% confidence interval and power of 90%, to estimate sample size. It is estimated that at least 200 participants are required. In this case-control study, 104 pregnant women with PE based on clinical and laboratory evidence and 100 healthy pregnant women with blood pressure less than 130/85 mmHg participated as a control group. This study was performed in Kamali Hospital, Karaj, Iran, and recruited from January 2019 to January 2020. Inclusion criteria were classified according to the ISSHP (The International Society for the Study of Hypertension

in Pregnancy) guideline in the group of cases with PE (17). The criteria for PE were severe PE and term PE, the patients who were admitted to ICU (Intensive Care Units), were enrolled in this study. All women with chronic diseases, including those with a history of thromboembolism, recurrent miscarriage, and premature birth, as well as those with a history of systemic diseases such kidney disease, diabetes mellitus, and connective tissue disorders, were excluded from the study. Because these pregnant women might have underlying diseases that could cause a bias in our study.

In the control group, pregnant women without a history of hypertension from the beginning of pregnancy up to twelve weeks after delivery were studied.

2.2 Data extraction:

A questionnaire was prepared to collect demographic information, delivery history, health history, and nutrition. Blood pressure was measured twice in a sitting position with a mercury sphygmomanometer 30 minutes apart, and mean blood pressure above 140/90 mmHg was considered hypertensive. A urinalysis was also done to check urine protein levels. Cases with detectable proteinuria and high blood pressure levels defined each pregnant woman with PE and were matched with one of the control items for age, gestational age, and parity status. Two cubic centimeters (cc) of venous blood containing EDTA (Ethylenediaminetetraacetic acid) anticoagulant was taken from cases and controls and sent to a genetic laboratory.

2.3 Molecular Methods:

Blood samples took from both patients with PE and healthy controls. We used the Qiagen extraction kit under the manufacturer's protocol for genomic DNA extraction. The concentration, purity, and quality of DNA were evaluated using nanodrop by measuring the rate of absorption at 260 nm and 280 nm. DNA samples were stored at -20°C until testing. Polymerase chain reaction (PCR) was performed by designing specific primers on each gene to determine the genotype of SNPs. Then their genetic sequence was sequenced and analyzed by Chromas software by the Sanger sequencing method.

Primers F1: CTTGGCCTCTTGCTAGCTGT, F2: TGGGAAGGAACTTGGAAATG, and AM1: GGCCTGACATGTCCTATGCT were used for PCR conditions. Detection of SNPs is as follows:

- Initial denaturation at 95°C for 5 min.

- Thirty-five cycles (denaturation at 95°C for 30 seconds, annealing at 55°C for 30 seconds, and extension at 72°C).
- Final expansion at 72°C for 7 minutes.

The distribution of genotype was assessed by Hardy-Weinberg equilibrium (HWE). It is the situation that the genotypic frequency of two alleles of one autosomal gene locus after one discrete generation of random mating in an indefinitely large population: if the alleles are A and a with frequencies p and q(=1-p), then the equilibrium gene frequencies are simply p and q and the equilibrium genotypic frequencies for AA, Aa, and aa are p2, 2pq, and q2 (24).

2.4 Statistical analysis:

Statistical analysis was performed using SPSS statistical software version 23.0. A chi-squared test was used to assess qualitative variables (like comparing the genotypes in the two groups). qualitative variables were reported as percentages and frequency. We also used the accurate Fisher test and logistic regression to examine the relationship between variables.

3. **Results**

A hundred healthy individuals were included in the control group, whereas 104 patients with PE applied to the case group. The mean age of the control group and cases were 30.7 years and 28.8 years, respectively. Maternal age was significantly different between the two groups ($p = 0.037$). The history of hypertension in the case group was significantly higher ($p<0.001$) than in the control group. The two groups also differentiated significantly in terms of multiple gestations ($p=0.002$) and ages older than 35 ($p=0.042$). However, there was no significant difference between cases and controls in terms of body mass index ($p = 0.67$). There were no significant differences between the two groups in null parity, hypothyroidism, and neonate gender.

The genotype and allelic distribution of SNPs rs1801133 and rs1801131 in the MTHFR gene and rs9393931 in the ERVFRD-1 gene are presented in Table 2.

3.1 Allelic and genotypic distribution of MTHFR rs1801133 single nucleotide polymorphism between study and control groups

The frequency of MTHFR genotype for CC, CT, and TT among the study group was 84 (80.7%), 20.3% (19.3%), and 0 (0%), respectively. In contrast, the frequency of MTHFR genotype for CC, CT, and TT among the control group was (100%) 100, (0%) 0, and (0%) 0, respectively. CT

genotype was associated with a significant increase in the incidence of PE compared to the CC genotype (15.04 vs. 1.86 [95% CI = 95, OR = 5.29, p = 0.002]). There wasn't any with TT genotype in both case and control groups. Allelic frequencies for MTHFR rs1801133 C and T in the case group were 188 (90.4%) and 20 (9.6%), respectively, and in the control group were 208 (100%) and 0 (0%), respectively. The T allele was significantly associated with an increased risk of PE (p = 0.007).

3.2 Allelic and genotypic distribution of MTHFR rs1801131 single nucleotide polymorphism between study and control groups

The frequency of MTHFR genotype for AA, AC, and CC polymorphic variants in the study group was 104 (100%), 0 (0%), and 0 (0%), respectively. The frequency of AA, AC, and CC genotypes in the control group was 96 (96%), 4 (4%), and 0 (0%), respectively. There wasn't a significant relationship between the case and the control group.

3.3 Allelic and genotypic distribution of rs9393331 ERVFRD-1 single nucleotide polymorphism between study and control groups

The frequency of ERVFRD-1 gene genotype for TT, TC, and CC polymorphic variants in case group 104 (100%), 0 (0%), and 0 (0%), and in the control group was 100 (100%), 0% (0) and 0% (0), respectively. The electropherogram of reported polymorphisms were presented in Figure 1.

4. Discussion

This study revealed that rs1801133 polymorphism in the MTHFR gene is associated with a risk of PE; however, there was no association of rs1801131 variants in the MTHFR gene. Which was reported in the previous studies in Iran and in meta-analyses.

Increased homocysteine levels are caused by a decrease in MTHFR protein levels or activity caused by distinct gene variations. The rs1801133 variant in the MTHFR gene converts an alanine to valine at aminoacid in the enzyme regulatory domain, causing a thermolabile enzyme with lower activity at 37C and so hyperhomocysteinemia happens. MTHFR activity may be lowered due to the MTHFR (rs1801133) polymorphism, which may impede the remethylation pathway. MTHFR (rs1801133) has been linked to altered methionine-homocysteine metabolism and elevated homocysteine levels. MTHFR gene polymorphism and hyperhomocysteinemia have been linked to gestational hypertension, recurrent pregnancy loss, placental abruption, and PE

(25). Therefore, it might demonstrate the reason that the MTHFR (rs1801133) polymorphism is related to the PE in this study.

Mohsen Azimi-Nezhad, in 2019 reported in a case-control study with 117 women with a diagnosis of PE and 103 healthy. They were referred to Hakim Hospital in Neishabour, Khorasan Province, in Iran. It is similar to our findings on rs1801133 polymorphism (26).

A meta-analysis study with 7398 patients with PE and 11230 healthy controls showed that genotype rs1801133 is associated with an increased risk of PE. At the same time, they didn't indicate an increased risk of PE associated with rs1801131 polymorphism (27).

Also, another meta-analysis study with 6403 patients and 11346 controls showed that MTHFR rs1801133 polymorphism was associated with an increased risk of PE (28).

On the other hand, Yuan Zhang, in 2018, in a meta-analysis study with 4536 cases and 4961 controls, reported that MTHFR rs1801133 and rs1801131 were not associated with PE(29).

Saeedeh Salimi et al. reported that in a case-control study on 192 preeclamptic and 196 healthy pregnant women, MTHFR rs1801131 polymorphism was associated with PE. However, they did not find any association between PE and MTHFR rs1801133 among patients and controls. They reported that the synergic (rs1801131+rs1801133) effect of MTHFR variants could increase PE (30).

Guifeng Ding et al investigated 26 SNPs in three genes including the MTHFR gene, associated with PE in the Chines population. They reported that rs1801131 and rs1801133 polymorphism in the MTHFR gene were not associated with an increased risk of PE (31).

Hua et al. conducted a case-control study in China of 120 pregnant women with PE and 180 control women. In this study, for the first time, they observed the association of several gene polymorphisms, including the rs9393931 polymorphism in the ERVFRD-1 gene. They reported a significant relationship between this polymorphism with PE (23, 32). However, we did not identify the ERVFRD-1 gene polymorphism associated with PE. Therefore, further investigations are needed to clarify the association between the ERVFRD-1 gene with PE.

Other SNPs were reported to increase or reduce the risk of PE. For example, HLA-G polymorphisms (rs17179101, rs9380142, and HLA-G 0106G) in the mother genotype, increased the risk of PE in pregnancy (33-35). However, SNPS like MTHFR (rs17367504), ATP2B1

(rs17249754a), PAX5 (rs16933812a), and PLCD3 (rs12946454a) reduced the risk of PE in pregnancy (36).

We can definitely use SNPs that are associated with disease diagnosis, prognosis, and risk assessment as candidate polymorphisms to be evaluated directly as the functional or causal mutations for a disease, which is more important than any other factor. After researching PE-related gene activities, we want to obtain more knowledge of the disease's basic causes and develop rational preventative and therapy strategies. These investigations offer a novel approach to identifying genes linked to diseases, and they can be the only means to investigate the molecular processes that underlie the onset of the disease. Additionally, these studies aid in our understanding of both the positive and negative effects of pharmaceutical responses (37, 38).

PE development may be influenced by interactions between genes and environmental factors. The main findings of this case-control study indicate a possible association between PE and the heterozygous MTHFR rs1801133 genotype. These results might offer a few suggestions for identifying the genetic factors contributing to PE's pathogenesis. Further genetic association studies should be conducted in different populations to investigate other possible genetic factors. Because PE has the potential to be fatal for both the mother and the fetus, our study was crucial and significant. It is important to demonstrate the association between preeclampsia and MTHFR SNPs because our study revealed that the genetic features of the mother generation have a significant influence on the disease development.

This study has some limitations. The relationship between the ERVFRD-1 gene (rs9393931) with PE did not observe in previous studies so, we lack information to discuss it. Also, there were some potential sources of bias, such as population stratification or selection bias, and other challenges we faced during the study, such as difficulties in patient recruitment, and data collection.

In the study, we did not match the case and control group for demographics such as the mean age, parity, and socioeconomic factors. Still, it will help to ensure that the comparison between the case and control groups is valid and reduces the risk of confounding factors affecting the results. Therefore, we suggest further research using these matching to reduce the confounding factors.

Conclusion:

The results of this study demonstrate that the MTHFR rs1801133 polymorphism may be involved in the development of PE. Additional genetic research should be performed to determine other genes or polymorphisms involved in the pathogenesis of the disease. It could be helpful in the earlier recognition of PE in pregnant women, hence reducing the disease's consequences.

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Declarations of interest: none

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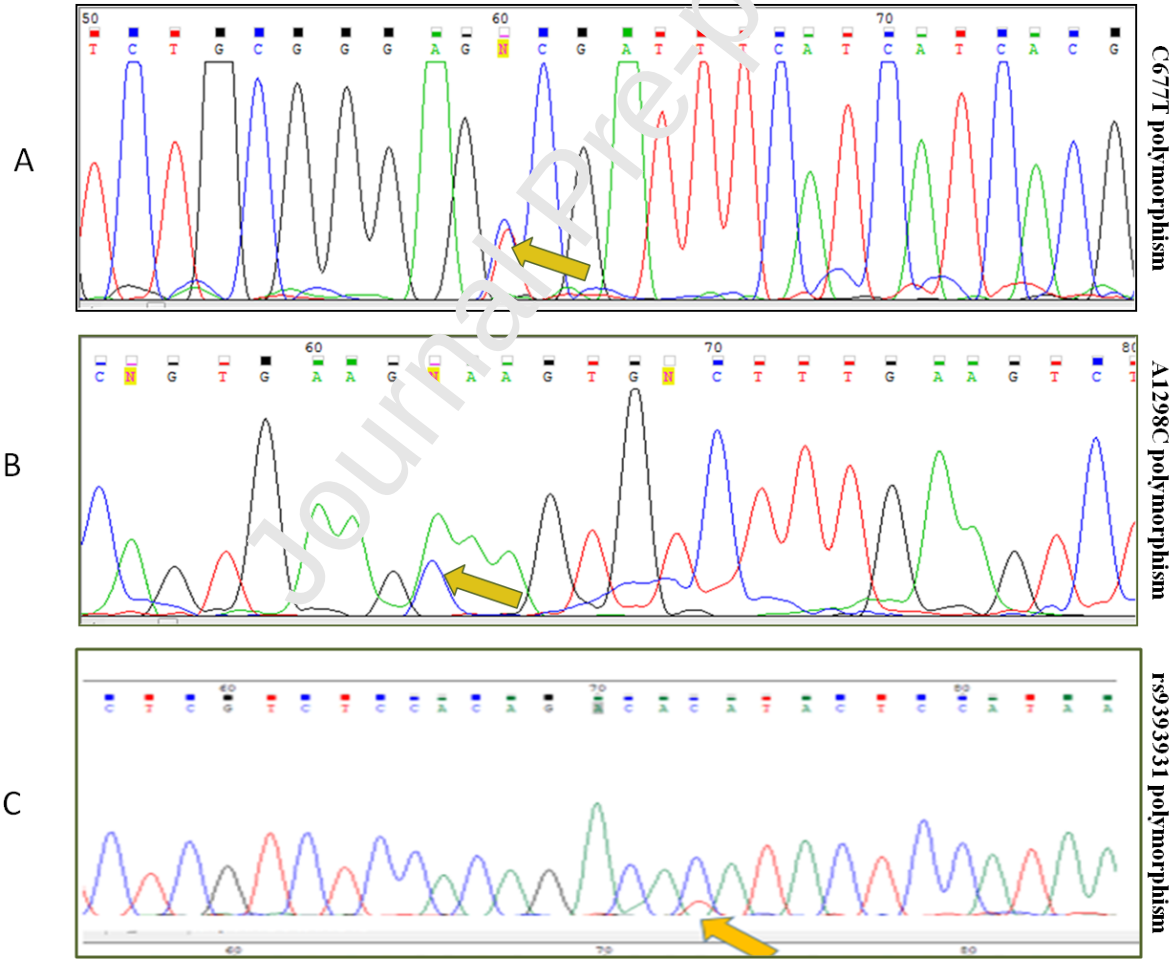
Figures:

Figure 1. Analysis of single nucleotide polymorphism (SNP) using Sanger sequencing. (A) A heterozygous variant of rs1801133 Polymorphism in the MTHFR Gene. (B) A heterozygous variant of rs1801131 Polymorphism in the MTHFR Gene. (C) A heterozygous variant of rs9393931 polymorphism in the ERVFRD-1 gene



Tables:

Table 1. Clinical and demographic characteristics in case and control groups and investigation of their role in the occurrence of preeclampsia.

variable	Odds Ratio,95%CI	p-value
history of hypertension	361 (1.75-7.44)	0.001
nulliparity	0.73 (0.5-1.05)	0.096
Age > 35	2.50 (1.03-6.06)	0.042
hypothyroidism	1.75 (0.51-5.99)	0.369
Multiple gestations	10.98 (1.86-103-33)	0.002
Gender of the neonate	0.656 (0.370-1.162)	0.149

Table 2. Genotype and Alleles frequencies of MTHFR polymorphism, ERVFRD-1 polymorphism in case and control groups.

Gene	Polymorphism	Genotype	Studied groups		P-value
			Case (n= 104)	Control (n=100)	
MTHFR	rs1801133	CC	84 (80.7%)	100 (100%)	-
		CT	20 (19.%)	0(0%)	0.002
		TT	0 (0%)	0(0%)	-
	rs1801131	AA	104 (100%)	96 (96%)	-
		AC	0 (0%)	4 (4%)	0.021
		CC	0 (0%)	0(0%)	-
ERVFRD-1	rs9393931	TT	104 (100%)	100 (100%)	-
		TC	0 (0%)	0 (0%)	-
		CC	0 (0%)	0 (0%)	-

Abbreviations

Preeclampsia (PE)

Single-nucleotide polymorphisms (SNPs)

methylenetetrahydrofolate reductase (MTHFR)

synthesize s-adenosyl methionine (SAM)

human endogenous retrovirus (HERV)

ICU (Intensive Care Units)

centimeters (cc)

EDTA (Ethylenediaminetetraacetic acid)

Polymerase chain reaction (PCR)

Hardy-Weinberg equilibrium (HWE)

Credit Author Statement

Masoumeh Farahani: Conceptualization, Investigation, Resources, Visualization, Supervision, Project administration, Funding acquisition

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Highlights

This Case-Control Study trial determined:

- rs 1801133 polymorphism in the MTHFR gene is associated with risk of preeclampsia
- rs 1801131 variant in the MTHFR gene is not associated with risk of preeclampsia
- rs 9393931 variant in the ERVFRD-1 gene is not associated with risk of preeclampsia