HYPERTENSION IN PREGNANCY

Hypertension in Pregnancy

ISSN: (Print) (Online) Journal homepage: www.informahealthcare.com/journals/ihip20

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To cite this article: Sadia Mahmood, Amna Younus, Sammar Nathaniel & Hooria Younas (2023) MTHFR A1298C polymorphism: a predictor of reduced risk of preeclampsia in Punjab, Pakistan, Hypertension in Pregnancy, 42:1, 2187621, DOI: 10.1080/10641955.2023.2187621

To link to this article: https://doi.org/10.1080/10641955.2023.2187621

HYPERTENSION IN PREGNANCY
2023, VOL. 42, NO. 1, 2187621
https://doi.org/10.1080/10641955.2023.2187621

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MTHFR A1298C polymorphism: a predictor of reduced risk of preeclampsia in Punjab, Pakistan

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ABSTRACT

Objectives: This study aimed to investigate the genetic association between *MTHFR* (A1298C) SNP and preeclampsia (PE) in Punjab, Pakistan.

Methods: A sample of 80 pregnant women (40 healthy pregnant women and 40 with PE) was pooled for genotyping *MTHFR* A1298C polymorphism by using the tetra-primer amplification refractory mutation system (ARMS) PCR. The Genotypic and allelic assessments were performed using various statistical techniques.

Results: The AC genotype and C allele of *MTHFR* A1298C were found to be associated with decreased risk of PE (odds ratio [OR]: 0.31, risk ratio [RR]: 0.58, $p = 0.01$), and (odds ratio [OR]: 0.49, risk ratio [RR]: 0.61, $p = 0.04$), respectively.

Conclusion: In conclusion, genetic polymorphism A1298C in *MTHFR* may pose a protective effect in the studied population.

ARTICLE HISTORY
Received 28 September 2022
Accepted 1 March 2023

KEYWORDS
MTHFR; polymorphism; preeclampsia

Introduction

Preeclampsia (PE) represents a major cause of materno-fetal morbidity and mortality and is estimated to complicate almost 2–8% of all pregnancies (1). The WHO reported seven times higher figures in developing countries than developed countries; especially in Asian regions where 9.1% of maternal deaths are due to gestational hypertensive disorders (2,3). Perinatal mortality in developed countries remains five times higher for women experiencing preeclampsia than non-preeclamptic women, where 15% of preterm births are due to preeclampsia; however, maternal mortality has been greatly reduced by antenatal care and risk management (4). Pakistan reports that it has the third highest burden of maternal, fetal and child mortality, with one-third of maternal deaths specifically attributed to PE. Another study reports the incidence of PE in Pakistan to be 19% (4). Similar trends can also be cited in its neighboring South Asian countries, the prevalence of PE in India, for instance, is 2–15%, 14.4% in Bangladesh, 2.07% in China, 1.19% in Japan, 2.22% in Thailand, and 0.59% in Nepal (5).

PE is characterized by elevated levels of blood pressure (>140 mmHg/90 mmHg) and increased urine protein (300 mg/dl 24 h) in formerly normotensive women post-second trimester (6). The malady is multifactorial and thus the exact mechanism remains to be fully elucidated; however, a strong implication is linked with physiological and biochemical changes in placental metabolism. In the diseased state, the trophoblasts cease to invade effectively in the uterine wall, thus leading to defective remodeling of spiral arteries and obstructing the blood flow and oxygen supply to the placenta. This results in the release of cascades of factors posing ischemic symptoms and placental dysfunction (7,8).

There are many non-modifiable and modifiable risk factors for preeclampsia. Non-modifiable risk factors include advanced age, nulliparity, multi-pregnancy, family history, ethnic background, and preexisting conditions, such as diabetes, chronic hypertension, and other ailments (9–13). Obesity, the interval between pregnancies and nutrient status are among modifiable risk factors (14,15). Additionally, the prominent focus has been on genetic studies as the outcome of the disease has been strongly linked with the familial predisposition of genetic variants (16). The study has taken the Methylenetetrahydrofolate reductase (*MTHFR*) gene into account, which plays a central role in folate-mediated pathway.

The *MTHFR* enzyme is crucial in the removal of the excess buildup of homocysteine levels generated intermediately in the folate-mediated one-carbon metabolism (FOCM) pathway (17). However, its genetic

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Supplemental data for this article can be accessed online at https://doi.org/10.1080/10641955.2023.2187621.

alteration impairs the enzymatic function and perturbs the homocysteine metabolism leading to PE. Several studies have shown elevated levels of plasma homocysteine in women experiencing preeclampsia and a striking severity of the disease (18,19).

The base transition of adenine to cytosine at residue 1298 results in an amino acid substitution of glutamic acid to alanine at the C terminus of the *MTHFR* enzyme and thus is reported to be the cause of repressive enzymatic activity (20,21).

The *MTHFR* (A1298C) variant shows a difference in the prevalence of its allelic distribution among different populations and ethnicities. Its association with preeclampsia has been studied in Asians, Caucasians, Africans, and Americans where it correlated well with the Asian and Caucasian populations (5,21,22). Furthermore, *MTHFR* A1298C polymorphism has been earlier associated with PE among the Pakistani population, where the majority of cohorts were made up of participants from the Sindhi population (22). As there is a significant role of ethnicity and geography in the outcome of the disease, therefore, the study aims to explore the genotypic and allelic distributions of the *MTHFR* (A1298C) gene and its association with susceptibility to preeclampsia in Punjab, Pakistan. The role of the *MTHFR* has globally been well established for the disease, but meager reports from the Punjabi genetic pool of Pakistan make it a novel finding of its kind.

Materials and methods

Ethical statement

The study was approved by the Institutional Review Board of the Services Institute of Medical Sciences (SIMS), Ref No. (IRB/2021/812/SIMS). All the women who participated in the study were consented before the initiation of the study.

Selection of SNP and study design

The study design was a case control, involving 80 pregnant females, equally grouped as preeclamptic and normotensive. The patient samples were recruited from the tertiary care unit of the gynecology department of SIMS hospital, while the control subjects were those seeking routine antenatal care.

The inclusion criteria for the cases included a gestational period of ≥20 weeks and the presentation of blood pressure ≥140/90 mmHg, along with proteinuria of 0.3 g or more in a 24-hour urine collection. Patients suffering from conditions like HELLP syndrome, diabetes, fetal abnormalities, and kidney and autoimmune diseases were excluded from the study. Similarly, normotensive pregnant women were selected on similar anthropometric and gestational status, but did not show any symptoms of preeclampsia or its associated conditions.

Data collection

Information from each participant was obtained by conducting interviews and thoroughly checking medical records. A questionnaire was designed for this purpose, which included socio-demographic characteristics, personal and family history of preeclampsia, as well as BMI, blood pressure, and proteinuria levels.

DNA extraction

Peripheral blood samples (2–3 ml) were collected from each participant, transferred to EDTA vacationers, and stored at −20°C until further use. The stored EDTA-treated blood samples were then used to extract genomic DNA by the conventional phenol-chloroform method (23) and resuspended in TE buffer. All DNA samples were subsequently stored at−20°C. The DNA concentration was further quantified for PCR analysis using a nanodrop spectrophotometer (Bio-Rad) at a concentration of 50ng/dl.

Genotyping

The primers used for ARMS PCR were taken from previously reported literature and were optimized for this study (22). The desired fragments were amplified by PCR (Bio-Rad, Model no. T100 thermal cycler) using A-Forward (5′- TGTGGGGGGAGGAGCTG-ACCAGTGAGGA-3′) and Reverse outer (5′-TACCCTTCTCCCTTTGCCATGTCCACAG-3′) primer pairs, which specifically amplified the A allele with a product size of 230 bp. The C-Reverse (5′-GGTAAAGAACGAAGACTTCAAAGACACCTG-3′) and Forward outer primers were used to specifically amplify the C allele with a product size of 316 bp. A 488 bp internal control was amplified using forward outer and reverse outer primers. The PCR amplification system was performed in a 20 µl volume, including 3.75 µl ultrapure water, 1 µl primers (10 pM), 10 µl PCR master mix (2×) (Thermo Scientific), 0.25 µl Taq polymerase (5 U/µl) (Thermo Scientific) and 2 µl template DNA. The PCR conditions for detection of A1298C polymorphism were as follows: an initial denaturation at 95°C for 5 min, 35 cycles of denaturation at 94°C for 30 s, annealing at 60°C for 45 s, extension at

65°C for 2 min, and final extension at 72°C for 10 min. The amplicons were electrophoresed with a 50 bp ladder (Fermentas) on a 2% agarose gel stained with ethidium bromide at 120 V for 1 hr, followed by visualization under UV light through a Gel documentation system (Bio-Rad).

Statistical analysis

Student's t-test and Chi-square test ($\chi 2$) were used to evaluate continuous and categorical variables, respectively. The *MTHFR* polymorphisms were assessed for deviation from Hardy-Weinberg equilibrium (HWE) using the $\chi 2$ test. The genotype and allele distributions of *MTHFR* were compared among different groups using Pearson's $\chi 2$ test. Moreover, the Chi-square test ($\chi 2$) was used to determine the risk of the disease associated with each genotype. The binary logistic regression method was used to estimate odds ratios (OR) with corresponding 95% confidence intervals (CI) to find the strength of association between each genotype and PE. All ORs were adjusted by considering age, body mass index, gestational age, parity, gravidity, and family history as confounding factors. To analyze the distribution of genotypes according to the clinical and demographic characteristics of both groups, ANOVA was used for continuous variables, whereas the $\chi 2$ test was applied for categorical data with regression analysis. We have also analyzed the association between different clinical and demographic variables using the Pearson correlation coefficient. A p-value of less than 0.05 was considered to be statistically significant. All of the statistical analyses were performed in the Statistical Package for Social Sciences (SPSS, version 17.0).

Results

Characteristics of subjects

A total of 40 healthy pregnant women (controls) and 40 women experiencing preeclampsia (cases) were recruited for this study. All of the controls and cases were from the Punjab population. The demographic and clinical characteristics of the overall population of cases and controls, including age, body mass index, systolic blood pressure (SBP), diastolic blood pressure (DBP), proteinuria (PU), parity, gravidity, family history, edema, headache, seizures, epigastric pain, and abortion were shown in Table 1. The studied population showed a significant increase in BMI ($p< 0.001$), SBP ($p < 0.001$), DBP ($p < 0.001$), and PU ($p < 0.001$) in preeclamptic women as compared to controls. Patients with PE showed a higher prevalence of edema ($p < 0.001$), headache ($p < 0.001$), epigastric pain ($p = 0.012$), and abortion ($p = 0.05$) than the asymptomatic controls. Age ($p = 0.809$), gestational age ($p = 0.175$), prevalence of parity ($p = 0.38$), gravidity ($p = 0.46$), family history ($p= 0.48$), and seizures ($p = 0.116$) showed an equal distribution between cases and controls suggesting that these variables were matched.

Correlation between demographic and clinical parameters

Correlation coefficients between different demographic and clinical parameters among controls and preeclamptic women were analyzed (supplementary table S1). SBP and DBP had a significant positive correlation with proteinuria among the preeclampsia group, with correlation coefficients of 0.358($p = 0.02$*) and 0.532($p = 0.000$**), respectively. . However, no such correlation was observed among the control group ($p > 0.05$) (supplementary table S1).

Supplementary table S2 presents the correlation coefficient between different studied parameters according to the individual's genotype. In both the AA and CC genotypes of the preeclampsia group, a significantly positive correlation was found between and SBP and proteinuria, as well as DBP and proteinuria. However, for the AC genotype, the correlation between blood pressure and proteinuria was not obtained.

Genotype and allele frequencies of the studied population

The distribution of genotypes and allele frequencies of *MTHFR* A1298C polymorphism in case and control groups are presented in Table 2. In the control group, the frequency of AA, AC and CC genotypes was 12 (30%), 25 (62.5%) and 3 (7.5%), respectively, whereas, in the patient group, it was 23 (57.5%), 15 (37.5%), and 2 (5%), respectively. The A allele frequency was 49 (61.3%) and 61 (76.3%) for the control and preeclampsia patients, respectively. The C allele frequency was 31 (38.8%) in controls and 19 (23.8%) in preeclampsia patients. A significant difference in genotypic and allelic frequencies was observed between both groups. According to the dominant model, the genotypic distribution was significant in both study groups. The distribution of *MTHFR* genotypes in the overall population was in accordance with HWE. The data indicate a lower prevalence of *MTHFR* 1298AC heterozygote genotype in PE patients as compared to controls (37.5% vs. 62.5%, $\chi 2 = 5.000$, $p = 0.02$). Similarly, in

Table 1. Comparison of demographic, anthropometric, and clinical parameters among controls and patients.

Parameter	Control group (n = 40)	Preeclampsia group (n = 40)	P- value
Age (years)	29.73 ± 2.76	29.95 ± 5.16	0.809
BMI (kg/m^2)	25.32 ± 2.24	35.00 ± 9.75	**<0.001****
GA (weeks)	34.13 ± 3.267	33 ± 3.74	0.175
SBP (mm Hg)	118.5 ± 3.61	154 ± 21.82	**<0.001****
DBP (mm Hg)	79.75 ± 6.19	98.73 ± 9.38	**<0.001****
PU (mg/dl)	9.78 ± 2.587	212.88 ± 284.476	**<0.001****
Parity			
nulliparous	13 (32.5)	16 (40)	0.38
primparous	20 (50)	14 (35)	
multiparous	7 (17.5)	10 (25)	
Gravidity			
primigravida	10 (25)	13 (32.5)	0.46
multigravida	30 (75)	27 (67.5)	
Family History			
No	27 (67.5)	24 (60)	0.48
Yes	13 (32.5)	16 (40)	
Edema			
No	4 (10%)	2 (5%)	**<0.001****
Moderate	32 (80%)	6 15%)	
Extreme	4 (10%)	32 (80%)	
Headache			
No	35 (87.5%)	3 (7.5)	**<0.001****
Yes	5 (12.5%)	37 (92.5)	
Seizures			
No	40 (100%)	36 (90%)	0.116
Yes	0 (0%)	4 (10%)	
Epigastric pain			
No	40 (100%)	33 (82.5)	**0.012***
Yes	0 (0%)	7(17.5)	
Abortion			
No	35 (87.5)	28 (70)	**0.05***
Yes	5 (12.5)	12 (30)	

Data is represented as Mean± SD for continuous variables and no. (%) for categorical variables. P- values for continuous variables were calculated using independent t-test or Mann-Whitney U test, and for categorical variables using Chi- square test or Fisher's exact test depending on the data distribution.
BMI; body mass index, GA; gestational age, SBP; systolic blood pressure, DBP; diastolic blood pressure, PU; proteinuria.
*Significant at the level of 0.05.
**Highly significant at the level of 0.01.

the dominant model, the frequency of the combined genotype (AC+CC) in the patient group was also reported to be lower than in the control (42.5% vs. 70%, $\chi2 = 6.146$, $p = 0.01$). The C allele had a markedly lower prevalence in the PE group as compared to controls (23.8% vs. 38.8%, $\chi2 = 4.189$, $p = 0.04$).

Relationship between A1298C MTHFR polymorphism and preeclampsia risk

Tables 2 and 3 further outline the effects of *MTHFR* A1298C on the risk of developing preeclampsia. Genotypic analysis revealed a significant association between the polymorphism and the reduced susceptibility toward PE in the co-dominant, AC vs. AA genetic model (OR = 0.31, 95% CI: 0.12–0.80, p = 0.01) (RR = 0.58, 95% CI = 0.12–0.80, p= 0.01), and the dominant genetic model (OR = 0.31, 95% CI: 0.12–0.79, p = 0.01) (RR = 0.60, 95% CI = 0.40–0.91 p= 0.01). Furthermore, the C allele conferred a decreased risk for developing the disease as compared to the A allele (OR = 0.49, 95% CI: 0.24–0.97, p = 0.04) (RR = 0.61, 95% CI: 0.37–0.99, p = 0.04).

The protective effect of A1298C polymorphism remained constant for the disease while keeping the confounding factors such as age, BMI, gestational age, parity, gravidity, and family history adjusted. The association was significant for the codominant model, AC vs. AA genetic model (AOR = 0.19, 95% CI: 0.04–0.87; adjusted p = 0.03), and the dominant genetic model (AOR = 0.18, 95% CI: 0.04–0.79; adjusted p = 0.02). However, the association was not significant for C vs. A allele (AOR = 0.42, 95% CI: 0.16–1.14, adjusted p = 0.09).

Analysis of genotype-phenotype association

The genotypic and phenotypic characteristics of the patient group are presented in Table 4. Patients with AA genotype had a higher SBP (164.7 ± 23.04 vs.152.6 ± 20.86

Table 2. Distribution of genotypic and allelic frequencies for *MTHFR* A1298C polymorphism in the studied population and association for preeclampsia.

Genotypes	Control group N (%)	Preeclampsia group N (%)	χ^2	p^a -value	OR (95% CI)	p^b -value	Adjusted OR (95% CI)[a]	p^c - value
A1298C genotype								
Codominant model								
AA	12 (30)	23 (57.5)	—		1.00[ref]		1.00[ref]	
AC	25 (62.5)	15 (37.5)	5.000	**0.02***	0.31 (0.12–0.80)	**0.01***	0.19 (0.04–0.87)	**0.03***
CC	3 (7.5)	2 (5)	0.213	0.644	0.34 (0.05–2.37)	0.28	0.14 (0.006–3.44)	0.23
Dominant model								
AA	12 (30)	23 (57.5)	—		1.00[ref]		1.00[ref]	
AC+CC	28 (70)	17 (42.5)	6.146	**0.01***	0.31 (0.12–0.79)	**0.01***	0.18 (0.04–0.79)	**0.02***
Recessive model								
AA+AC	37 (92.5)	38 (95)	—		1.00[ref]		1.00[ref]	
CC	3 (7.5)	2 (5)	0.213	0.644	0.64 (0.10–4.11)	0.64	0.40 (0.02–6.26)	0.52
Overdominant model								
AA+CC	16 (40)	24 (60)	—		1.00[ref]		1.00[ref]	
AC	24 (60)	16 (40)	3.200	0.074	0.44 (0.18–1.08)	0.07	0.39 (0.10–1.51)	0.17
Alleles								
A allele	49 (61.3)	61(76.3)	—		1.00[ref]		1.00[ref]	
C allele	31 (38.8)	19 (23.8)	4.189	**0.04***	0.49 (0.24–0.97)	**0.04***	0.42 (0.16–1.142)	0.09
HWE P	0.34							

N= number of individuals. % Frequency is shown in parentheses. Differences in the genotype and allele frequencies of *MTHFR* polymorphism between control and preeclampsia patients were compared using the χ2 test. Odds ratios (OR) with a 95% confidence interval (95% CI) were calculated using logistic regression. Odds ratios are adjusted as OR (95% CI)[a] with other co-variates (Age, BMI, gestational age, parity, gravidity, and family history). p^a –value (Chi-square p –value); p^b –value (odds ratio p-value) and p^c -value(Odd ratio p-value after adjustment with other co-variates), HWE; Hardy-Weinberg Equilibrium.
*Significant at the level of 0.05.

Table 3. *MTHFR* A1298C polymorphism and risk assessment for preeclampsia.

Genotypes	Control group N (%)	Preeclampsia group N (%)	RR (95% CI)	P- value
A1298C genotype				
Codominant model				
AA	12 (30)	23 (57.5)	1.00[ref]	
AC	25 (62.5)	15 (37.5)	0.58 (0.12–0.80)	**0.01***
CC	3 (7.5)	2 (5)	0.40 (0.07–2.12)	0.28
Dominant model				
AA	12 (30)	23 (57.5)	1.00[ref]	
AC+CC	28 (70)	17 (42.5)	0.60 (0.40–0.91)	**0.01***
Recessive model				
AA+AC	37 (92.5)	38 (95)	1.00[ref]	
CC	3 (7.5)	2 (5)	0.66 (0.11–3.77)	0.64
Over dominant model				
AA+CC	16 (40)	24 (60)	1.00[ref]	
AC	24 (60)	16 (40)	0.66 (0.42–1.05)	0.07
Alleles				
A allele	49 (61.3)	61 (76.3)	1.00[ref]	
C allele	31 (38.8)	19 (23.8)	0.61 (0.37–0.99)	**0.04***

N= number of individuals. % Frequency is shown in parentheses. Differences in the genotype and allele frequencies of MTHFR polymorphism between control and preeclampsia patients were compared using the χ2 test. Similarly, Risk ratios (RR) with 95% confidence interval (95% CI) and P- values were calculated using the χ2 test.
*Significant at the level of 0.05.

or vs. 135 ± 7.07; p= 0.03) and proteinuria than AC or CC genotypes. However, age, BMI, gestational age, and DBP showed no significant differences among these genotypes (p> 0.05). Binary Logistic regression was performed for categorical variables, and the AA genotype depicted a stronger familial association with the occurrence of the disease, while the remaining variables showed no significant distribution among all three genotypes. Among the control group, there was an insignificant distribution of all the studied variables among all three genotypes, except for SBP (supplementary table S3).

Discussion

The study assessed the genetic variation in the gene of *MTHFR* and further evaluated the modulatory effect of other socio-demographic factors in the etiology of the disease. The main finding of the current study reflects the protective role of the *MTHFR* (A1298C) SNP in the population.

Although many studies have elucidated the mechanisms of PE, the cause of the disease still remains a dilemma. Identification of the candidate genes for PE

Table 4. Associations between *MTHFR* A1298C genotype and characteristics among PE patients.

db SNP ID	(1) 1/1	(2) 1/2	(3) 2/2	(1) vs. (2) vs. (3)	(1) vs. (2)	(1) vs. (3)	(2) vs. (3)	(1) + (2) vs. (3)	(1) vs. (2) +(3)	(1) + (3) vs. (2)
				p-value	p- value	p-value	p-value	p-value	p-value	p-value
(allele1/allele2)	(n)	(n)	(n)	OR (95% CI)	OR (95% CI)	OR (95% CI)	OR (95% CI)	OR (95% CI)	OR (95% CI)	OR (95% CI)
rs1801133 (A/C)	23	15	2							
Age (years)	30.09 ± 5.67	29.60 ± 4.05	31.00 ± 9.89	0.923	0.959	0.970	0.934	0.772	0.848	0.745
BMI (kg/m^2)	35.66 ± 11.23	34.37 ± 8.01	32.05 ± 0.07	0.845	0.919	0.875	0.949	0.666	0.621	0.756
GA (weeks)	32.57 ± 4.25	33.00 ± 4.40	28.00 ± 2.82	0.310	0.950	0.328	0.279	0.131	0.913	0.576
SBP (mm Hg)	164.7 ± 23.04	152.6 ± 20.86	135 ± 7.07	**0.03***	0.145	0.09	0.425	0.08	**0.02***	0.142
DBP (mm Hg)	99.9 ± 10.87	97.33 ± 7.03	95.0 ± 7.07	0.606	0.686	0.761	0.943	0.571	0.341	0.475
PU (mg/dl)	363.4 ± 314.2	151.6 ± 112.4	157.5 ± 201.5	**0.04***	**0.04***	0.521	0.999	0.538	**0.01***	**0.02***
Parity										
Nulliparous	15/23	5/15	1//2	0.06	1.00^{ref}	1.00^{ref}	1.00^{ref}	1.00^{ref}	1.00^{ref}	1.00^{ref}
Primparous	2/23	7/15	0/2		**0.01***	**0.000***	**0***	**0***	**0.02***	**0.01***
					0.09 (0.01–0.61)	—	—	—	0.11 (0.01–0.71)	0.08 (0.01–0.57)
Multiparous	6/23	3/15	1/2		0.64	0.54	0.74	0.58	0.600	0.71
					0.66 (0.12–3.70)	0.40 (0.02–7.48)	0.60 (0.02–13.5)	0.45 (0.02–8.02)	0.124–2.914	0.72 (0.13–3.93)
Gravidity										
Primigravida	12/23	4/15	1/2	0.291	1.00^{ref}	1.00^{ref}	1.00^{ref}	1.00^{ref}	1.00^{ref}	1.00^{ref}
Multigravida	11/23	11/15	1/2		0.126	0.953	0.508	0.826	**0.04***	**0.032***
					3.0 (0.73–12.24)	0.91(0.13–55.1)	2.75 (0.13–55.1)	1.37 (0.08–23.6)	0.23 (0.05–0.9)	0.19 (0.04–0.87)
Family History										
No	7/23	10/15	2/2	**0.02***	1.00^{ref}	1.00^{ref}	1.00^{ref}	1.00^{ref}	1.00^{ref}	1.00^{ref}
Yes	16/23	5/15	0/2		**0.03***	0.999	0.999	0.999	**0.01***	0.06
					4.57 (1.13–18.41)	—	—	—	5.48 (1.39–21.59)	3.55 (0.99–13.69)
Edema										
No	1/23	1/15	1/2	0.227	1.00^{ref}	1.00^{ref}	1.00^{ref}	1.00^{ref}	1.00^{ref}	1.00^{ref}
Moderate	3/23	2/15	0/2		0.810	**0.000***	**0***	**0***	0.472	0.85
					1.50 (0.05–40.6)	—	—	—	3.00 (0.15–59.8)	0.75 (0.03–14.9)
Extreme	19/23	12/15	1/2		0.753	0.09	0.157	0.08	0.40	0.88
					1.58 (0.09–27.7)	19.0 (0.6–583.3)	12.0 (0.38–374.8)	15.5 (0.68–350.6)	2.92 (0.23–35.6)	0.83 (0.06–10.20)
Headache										
No	2/23	1/15	0/2	0.894	1.00^{ref}	1.00^{ref}	1.00^{ref}	1.00^{ref}	1.00^{ref}	1.00^{ref}
Yes	21/23	14/15	2/2		0.821	0.999	0.999	0.999	0.740	0.877
					0.75 (0.06–9.08)	0.00 (0.0–0)	0.00 (0.0–0)	0.00 (0.0–0)	0.65 (0.05–7.89)	0.821 (0.06–9.91)
Seizures										
No	19/23	15/23	2/2	0.193	1.00^{ref}	1.00^{ref}	1.00^{ref}	1.00^{ref}	1.00^{ref}	1.00^{ref}
Yes	4/23	0/15	0/2		1.000	0.999	1.000	0.999	0.998	0.998
					—	—	—	—	—	—
Epigastric pain										
No	19/23	12/15	2/2	0.783	1.00^{ref}	1.00^{ref}	1.00^{ref}	1.00^{ref}	1.00^{ref}	1.00^{ref}
Yes	4/23	3/15	0/2		0.839	0.999	0.999	0.999	0.983	0.748
					0.84 (0.16–4.43)	—	—	—	0.98 (0.18–5.10)	0.76 (0.14–3.99)
Abortion										
No	15/23	12/15	1/2	0.510	1.00^{ref}	1.00^{ref}	1.00^{ref}	1.00^{ref}	1.00^{ref}	1.00^{ref}
Yes	8/23	3/15	1/2		0.331	0.671	0.373	0.538	0.445	0.291
					0.46 (0.10–2.16)	0.57 (0.02–9.70)	0.25 (0.01–5.26)	0.40 (0.02–7.10)	1.73 (0.42–7.11)	2.25 (0.49–10.14)

Continuous data are expressed as Mean ± Standard deviation and categorical data are expressed as number of genotypes. P- values for continuous variable (Age, BMI, GA, SBP, DBP, and PU) were calculated by Tukey's HSD (ANOVA) test and P- values for categorical variables (parity, gravidity, family history, edema, headache, seizures, epigastric pain, abortion) were calculated by the Chi- square test. Additionally, P- values for odds ratios and confidence interval were determined by logistic regression.

— Odds ratio cannot be interpreted due to limited sample size.

*significant at the level of 0.05 and **highly significant at the level of 0.01.

could substantially evaluate the mechanistic ways by which the disease progressed and may indicate the risk for the development of the disease in the future. Hence, treatment and prevention of the disorder can be personalized and tailored according to an individual's genetic profile. The *MTHFR* gene has received much attention in various physiological studies because of its contribution to a major metabolic pathway called folate-mediated one carbon metabolism (FOCM). *The MTHFR* enzyme is central to this pathway and plays a critical role in converting homocysteine to methionine, which otherwise gets accumulated and affects the other physiological functions. The reason for the accumulation of this toxic metabolite can potentially be a less functional *MTHFR* enzyme due to the presence of polymorphism in the gene (24). Many studies have reported an association between this polymorphism and hypertensive disorders (25–27).

Based on the observed genotypic and allelic frequencies in our population, it can be explicitly said that the AC genotype is prevalent among the control population, while the AA genotype has higher a frequency among cases. Overall, the A allele is more strongly associated with the PE group , while, the C allele is more frequently observed in the healthy group. A Chinese study has explained that the healthy individuals from southernChina have a higher incidence of the 1298CC genotype and 1298 C allele, with a decreasing trend observed from the southern to the northern population of China (28). Another study on a healthy population in east and south India reported a prevalence of the 1298 C allele among the Dravidian-speaking tribe (29). A different study indicated that the healthy Lebanese population harbors the highest frequency of the *MTHFR* AC genotype (30). These studies highlight the unique genetic feature of various ethnicities that may have been shaped by various factors, for example, mating patterns, migratory histories, ethnic background, and environment.

Calculated odds and risk ratios for the present study suggested the association of the AC genotype with a decreased risk factor for PE. In fact, the AC genotype had 31% fewer odds (OR = 0.31, 95% CI: 0.12–0.80, p = 0.01), and 58% less risk (RR = 0.58, 95% CI = 0.12–0.80, p = 0.01) toward developing the diseased state. After adjusting for confounding factors, it was predicted that the AC genotype was 19% less likely to cause PE (OR = 0.19, 95% CI: 0.04–0.87; adjusted P = 0.03). The results were also significant in the dominant model with 31% fewer odds (OR = 0.31, 95% CI: 0.12–0.79, p = 0.01) and 60% less risk (RR = 0.60, 95% CI: 0.40–0.91, p = 0.01) for the occurrence of PE. Similarly, for the C allele, it was predicted that it has 49% fewer odds and 61% less risk for PE. These results suggest that the AC genotype or the mutant C allele may act as an independent protective factor against the disease among the population of Punjab, Pakistan. Although an independent study is warranted to evaluate the biological mechanism of this association, it is known that the polymorphism is present in the SAM binding regulatory domain of an enzyme, and SAM inhibits MTHFR, preferably for methyl group biogenesis and prevention of 5, 10-methylene-THF depletion (31). It is then speculated that the SAM-insensitive MTHFR directs the conversion of a single carbon unit to 5-methyl-THF and hence methionine and SAM synthesis. In the context of this mechanism, the A1298C polymorphism may prevent the accumulation of homocysteine and its related harmful effects that may lead to the disease phenotype (32,33). Much attention has also been attributed to the nutritional or folate status of the women, which may counteract the reduction in enzymatic activity (34).

Earlier studies from Egypt, Netherlands, Iran, and Tunisia have reported no association of this polymorphism with PE (25,30–33,35,36). Comparatively, other association studies have found that polymorphism may increase the risk factor for PE (37–39). In line with the present study, a previous study from Pakistan found that the A1298C polymorphism decreases the risk for PE (22). There are also studies that have supported the notion of *MTHFR* A1298 C polymorphism acting as a protective factor against other diseases, such as coronary artery disease and leukemia (40,41). Conflicting results between different studies are concluded because of differences in ethnicity and geography. In addition, the discrepancy can also exist because of methodological heterogeneity in terms of study design, sample size, and demographic features.

Correlation analysis observed a linear trend between blood pressure and proteinuria, which is due to the fact that blood pressure disturbs the glomerular filtration rate of kidneys, leading to an enhanced secretion of proteins in urine (42).

Demographic characteristics, such as age and gestational age, showed no significant difference between the cases and control. Stratified analyses of these phenotypes, according to different genotypic groups, showed an insignificant distribution ($p > 0.05$), similar to a Pakistani study (22). The mean BMI recorded at the time of presentation was found to be higher among cases than controls, but the stratification according to genotype precluded its significance. Nevertheless, the implication of higher BMI as a causative role in the pathogenesis of PE in our population cannot be ruled out. A study by Beloglovkin et al. (43) suggests that low or normal BMI

protects women from developing hypertension during pregnancy. The mechanisms of the influence of obesity on the higher risk of PE or gestational hypertension (GH) are not fully understood; however, obesity in the mother has been linked to insulin resistance, inflammation (including in the placenta), and restriction of placental blood flow. The increased inflammation and oxidative stress associated with obesity may exacerbate the underlying disorder (44). Mean SBP, DBP, and proteinuria levels were significantly higher among variants of the case group as compared to control, which again is reasoned by the fact that *MTHFR* inactivation leads to higher homocysteine levels and hence the mentioned clinical characteristics are probable consequences of homocysteinemia (45). There exists a contrast between the results of studies that explored the link between the *MTHFR* A1298C genotype and homocysteine. Few studies have found increased homocysteine levels with the mutant genotype, and others reported no association. A study in this regard described no association of this polymorphism with homocysteine; however, it indicated that among controls the genotype has a high methionine-to-homocysteine ratio (46). Furthermore, case stratification according to different phenotypic characteristics showed that the AA genotype had a higher mean SBP and proteinuria as compared to other genotypes. A binary Logistic regression has been performed for variables with categories (Table 4). Although the overall results depicted an insignificant distribution, stratification according to individual genotypes showed that preeclamptic women in our sample population were less likely to constitute the primiparous group as compared to the group of women with nulliparity. Moreover, preeclamptic women in our population were less likely to fall into the multigravida group in both dominant and over-dominant models. On this subject, a study has shown that nulliparity becomes a risk factor for preeclampsia, as nulliparous women have a more anti- angiogenic state in the placenta (47). Lastly, as positive family history is associated with PE (48) therefore history of the disease was also taken into account; it was revealed that a positive family history may increase the risk for the development of PE.

The study on a specific ethnic group allowed for avoiding biases because of the ethnic differences. The major limitation of the present study is a relatively small sample size, which should be expanded in both the case and control group with more genetic factors from the same and different loci to develop an optimized predictive model for the disease. Considering the individual's genetic background and demographic features, the regulation of environmental, dietary, and lifestyle risk factors may provide a better clue for personalized diagnosis and treatment measures. Further, in silico and functional analyses also need to be conducted to better understand the mechanism behind the linkage between *MTHFR* A1298C polymorphism and decreased susceptibility to PE.

Conclusion

Conclusively, our study indicates that the prevalence of variation in the *MTHFR* gene (A1298C) is less common in preeclamptic women as compared to the control cases from Punjab, Pakistan. Investigations into the genetic grounds of the disease revealed that the polymorphism in the *MTHFR* gene plays a preventive role against the development of preeclampsia. Our study establishes the need for investigating into the other genetic variants of the gene toward developing an in-depth understanding of the pathophysiological mechanisms underpinning the development of preeclampsia and identifying the efficacious drug targets.

Acknowledgments

The authors are thankful to all participants of the study.

Disclosure statement

No potential conflict of interest was reported by the authors.

Funding

The author(s) reported there is no funding associated with the work featured in this article.

Data availability statement

The data that support the findings of this study are available from the corresponding author, (AY), upon reasonable request.

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