

# SLC19A1 Gene Polymorphism; Risk Factor for Preeclampsia

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## ABSTRACT

**Objective:** To determine the genotypic and allelic frequency of *SLC19A1* (*rs1051296 G>T*) polymorphism in pre-eclamptic patients and normal pregnant females, and also the genetic association of *SLC19A1* (*rs1051296 G>T*) polymorphisms with the levels of homocysteine (Hcy) and folate in pre-eclamptic Pakistani population.

**Study Design:** A case-control study.

**Place and Duration of the Study:** Department of Biochemistry, Islamic International Medical College, Riphah International University, Rawalpindi, Pakistan, from September 2022 to 2023.

**Methodology:** Gene association of *SLC19A1* (*rs1051296 G>T*) polymorphism was studied in pre-eclamptic patients to elucidate its clinical significance. The study included 166 pre-eclamptic patients and 166 normotensive pregnant females as controls. Maternal venous blood samples were obtained between 20<sup>th</sup> and 40<sup>th</sup> weeks of gestation and DNA was extracted using the Chelex method. Allele-specific polymerase chain reaction (PCR) was used to analyse the *SLC19A1* (*rs1051296 G>T*) polymorphism. Data were analysed using the Chi-square and Student's t-test.

**Results:** A significant association in terms of susceptibility was found between *SLC19A1* (*rs1051296 G>T*), CA ( $p < 0.03$ ), and AA ( $p < 0.001$ ) genotype with preeclampsia (PE). A significant association in terms of protection was found between the *SLC19A1* (*rs1051296 G>T*) CC genotype and PE. Notably, low folic acid along with CA ( $p < 0.05$ ) genotype is a high-risk factor for PE. A prior family history of high blood pressure had not proved any significant association with *SLC19A1* (*rs1051296 G>T*),  $p < 0.6$ .

**Conclusion:** *SLC19A1* (*rs1051296 G>T*) polymorphism is a risk factor for PE in the Pakistani population.

**Key Words:** Preeclampsia, Blood pressure, Single nucleotide polymorphism, *SLC19A1*, Polymerase chain reaction.

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## INTRODUCTION

Preeclampsia (PE) accounts for one of the alarming heterogeneous gestational disorders influencing maternal and perinatal mortality and morbidity. As per the guidelines provided by the WHO and the International Society for the Study of Hypertension in Pregnancy (ISSHP), PE is defined as a systolic blood pressure (SBP) of 140 mmHg or more or diastolic blood pressure (DBP) of 90 mmHg or more on two occasions at least 4 hours apart after the 20<sup>th</sup> week of gestation or during the postpartum period in a patient who was previously normotensive. It is characterised by the presence of proteinuria exceeding 300 mg in a 24-hour urine collection, resulting in oedema and ultimately leading to major organ dysfunction (renal, hepatic, neurologic, haematological, or uteroplacental).<sup>1</sup>

The incidence of all HDP ranges from 4 to 25%. PE exhibits a high prevalence of 2-8% globally.<sup>2</sup> The worldwide incidence of the disease is reported to be 4.6%,<sup>3</sup> resulting in the unfortunate demise of approximately 50,000 infants and 76,000 women annually.<sup>4</sup> Recent studies conducted in Pakistan reported the prevalence of PE as 8.5%<sup>5</sup> and 9.2% among all hypertensive disorders of pregnancy.<sup>6</sup>

The incidence of PE ranges from 2-6% of all pregnancies in Pakistan.<sup>2</sup> Research conducted in Pakistan, specifically in Sukkar and Bahawalpur, indicated the incidence range falls between 5.6% and 9.3%.<sup>7,8</sup> Pre-eclamptic patients can be classified into two primary categories based on the severity of uteroplacental dysfunction and maternal organ dysfunction.<sup>1</sup>

PE can be divided into two distinct stages. The initial stage is characterised by abnormal placentation due to spiral artery pathology, autoimmune, a genetic anomaly located on chromosome-13.<sup>4</sup> During the second stage, angiogenic imbalance and endothelial dysfunction cause proteinuria, thrombocytopenia in pre-eclamptic mothers, and 12 to 25% complications in newborns, such as foetal growth restriction (FGR), intrauterine foetal demise (IUID), and preterm births.<sup>9</sup>

As a multifactorial phenomenon, maternal age, race, and parity are also influencing factors. This process results in an elevation

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of oxidative stress. Vitamin deficiencies of folic acid and cobalamin are one of the factors for abnormal angiogenesis. Folic acid plays a role in one-carbon metabolism for *de novo* purine and pyrimidine synthesis.<sup>8,9</sup> The active form (tetrahydrofolate) causes the remethylation of homocysteine (Hcy) to methionine by methionine synthase (MS). If tetrahydrofolate is deficient, methionine levels decrease and Hcy levels rise, causing hyperhomocysteinaemia.<sup>10,11</sup>

*SLC19A1* is a reduced folate-carrier gene present on chromosome 19 and encodes reduced folate carrier protein. It provides major folate to systemic tissues, by endocytic mechanisms via folate membrane receptors (FOLR1, FOLR 2). Genetic defect of *SLC19A1* (*rs1051296 G>T*) results in decreased absorption of folic acid in serum and can cause hyperhomocysteinaemia.<sup>12</sup> *SLC19A1* correlation with rheumatoid arthritis<sup>13</sup> and idiopathic recurrent implant<sup>14</sup> has been proven with different SNP. *SLC19A1* (*rs1051296 G>T*) has not been studied with PE.

The study was conducted to assess the impact of *SLC19A1* (*rs1051296 G>T*) folate transporter gene polymorphisms in the pre-eclamptic population of Pakistan and explore their potential association. This research intended to contribute to the development of effective screening protocols, including cardiography, biophysical profiles, and amniotic fluid volume assessments. It will also support early diagnostic methods such as umbilical artery Doppler and ultrasound for foetal growth monitoring, ultimately enhancing prevention strategies for high-risk susceptible individuals.

The objective of this study was to determine the genotypic and allelic frequency of *SLC19A1* (*rs1051296 G>T*) polymorphism in pre-eclamptic patients and normal pregnant females; and also to determine the genetic association of *SLC19A1* (*rs1051296 G>T*) polymorphisms with the serum Hcy and folate levels.

## METHODOLOGY

This case-control study was conducted from September 2022 to 2023, at the Department of Biochemistry, Islamic International Medical College (IIMC), in collaboration with Railway Hospital Rawalpindi and Cantonment General Hospital Rawalpindi, Pakistan. Ethical approval for the study was obtained from the Ethical Review Committee of the Department of Biochemistry, Islamic International Medical College, Riphah International University, Rawalpindi, Pakistan. The study included 166 pre-eclamptic patients and 166 normal pregnant women as controls, all from the Department of Obstetrics and Gynaecology.

The inclusion criteria for the study were pregnant women aged 25-45 years, with a blood pressure exceeding 140/90 mmHg at 20 weeks of gestation or later, and who presented with proteinuria. The control group consisted of healthy, age-matched singleton pregnant women, at a gestational age of more than 20 weeks, who were willing to participate in the study. Exclusion criteria were women with a gestational age  $\leq$  20 weeks, chronic hypertension, gestational diabetes mellitus, multifetal, megaloblastic anaemia, those receiving anti-folate medicines,

eclamptic patients, and those in active labour.<sup>15</sup> Demographic and clinical characteristics of the participants including age, weight, gravidity, parity, and gestational age were obtained using electronic medical records. Maternal peripheral blood samples were taken with prior informed consent to quantify serum Hcy and folate levels. Each participant's demographics and sampling dates were recorded on a standard questionnaire, and the blood samples were labelled accordingly. Patient confidentiality was strictly maintained throughout the study.

A non-probability convenient sampling (non-random) technique was used. Blood samples were collected under aseptic conditions after obtaining informed consent. The veins were made more prominent by applying a tourniquet 3-4 inches above the intended venipuncture site. To disinfect the venipuncture site, 70% isopropyl alcohol was used. The median cubital vein was venipunctured with a 20-gauge needle and syringe to extract a 5ml blood sample into two purple vacutainers (3cc and 2cc). Following the collection of the required volume of blood, a bandage was applied, and the tourniquet was relaxed. A 2cc blood sample was safely transported to the laboratory in EDTA-containing vacutainers and stored at 4-8°C. DNA extraction was performed using the Chelex method. The extracted DNA was then subjected to PCR, and the respective allelic frequencies were recorded. The DNA was stored at -70°C for safekeeping and further analysis. Additionally, a 3cc blood sample was transported to Wilson's Biochemistry Lab for measurement of folate and Hcy levels. Folate and Hcy levels were measured using competitive protein binding chemiluminescent assays, following the manufacturer's instructions: The Tosoh Kit for folic acid and the Architect Homocysteine Reagent Kit for Hcy. The assay detection ranges were 6-16  $\mu$ mol/L for Hcy and 3-18 ng/mL for folate.

*SLC19A1* (*rs1051296 G>T*) was genotyped by tetra-primer amplification refractory mutation system (T-ARMS) polymerase chain reaction. Primer for the genotype was designed from an article as reference<sup>16</sup> and available from (<https://primer1.soton.ac.uk/primer1.html>). The primer sequence used for genotyping in the current study is shown in Figure 1.

Primers of <i>SLC19A1</i> rs 1051296	Primer sequence 5 to 3	Primer Bp (18 to 30)	Target Size (bp)	GC content (40_600)	Melting temp Tm (55-65°C) Variation b/w Pp 3-5
RS96F1-A	AGTCCCCTCCTGGGCTGGGCGCA	21	187 bp	76%	77.7
RS96RI-C	GCGGGTCTCTCAGCTGCCACATTG	26	216 bp	65%	73.2
96-FO	TCACATCAGATGGTGCCGACCTGTGG	27	356 bp	59%	71.9
RS96-RO	GCCATCTCAGTTGGCGGAGACACA	26	356 bp	62%	71.9

**Figure 1: Primer sequence of *SLC19A1* (*rs1051296 G>T*).**

**FO: Forward outer primer, RO: Reverse outer primer, F1: Forward inner primer, R1: Reverse inner primer.**

Primers used for gene amplification: PCR reaction mixture constituents for *SLC19A1* (*rs1051296*) in which 7.75  $\mu$ l PCR water by Invitrogen was added with DMSO (1.75  $\mu$ l). 12.5  $\mu$ l thermoscientific master mix, which consisted of 0.05  $\mu$ l Taq DNA polymerase, reaction buffer, 4 ml magnesium chloride, and 0.4 Mm of each dTNP (dATP, dGTP, and dTTP). Each primer vial contained 1  $\mu$ l of the respective primer, which was diluted to 1.5

µl working solution. Primers were supplied by M/s Macrogen™ (Korea). A final volume of 1 µl primer mix was added to the reaction mixture, and finally, 2 µl of DNA was added from the sample to be genotyped. The total volume for PCR was 25 µl.

PCR amplification started with an initial denaturation at 95°C for 5 minutes, followed by 35 amplification cycles. Each cycle included denaturation at 90°C for 30 seconds, annealing at 62°C for 30 seconds, and extension at 72°C for 30 seconds. A final extension was performed at 72°C for 5 minutes, and the cycle was concluded by holding the samples at 4°C. PCR reactions were carried out in a thermal cycler (Major Cycler, Verti 96 Well Thermal Cycler by Thermo Fisher Scientific).

A 10X TBE buffer was prepared by dissolving the buffer in 1000 mL of water, and a 1X solution was made by diluting the 10X buffer with 9 parts distilled water. For example, 100 mL of 10X TBE buffer was dissolved in 900 mL of distilled water.

The PCR products were visualised on a 2% agarose gel premixed with 1% Ethidium Bromide solution in 1X TBE buffer, run at 700 amperes and 100 volts for 45 minutes. The gel was compared to a 50 bp DNA ladder (Gene Ruler 50 bp by Thermo Scientific).

The electrophoresis tank was filled with TBE buffer, and the gel was placed into the tank while still in its casting tray. The comb was carefully removed from the gel. Next, 8 µL of the 50 bp ladder was loaded into the appropriate well, followed by 12 µL of the final PCR reaction mixture into the wells of the gel. Electrophoresis was then initiated at 100 volts and 700 amperes of direct current for 45 minutes. The progress of the electrophoresis was monitored by observing the movement of the coloured dye, already present in the Master Mixture.

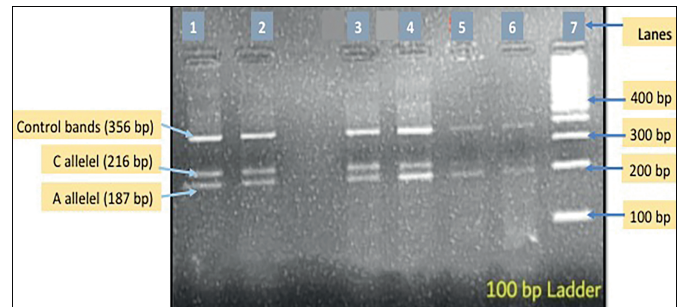
Statistical analysis was performed using the SPSS 25 software package for Windows. Descriptive statistics were presented as mean ± standard deviation. Frequencies and percentages were also calculated for descriptive statistics. The categorical variables were compared with Student's t-test. The potential association of the SLC19A1 (rs1051296 G>T) genetic polymorphism with PE in the Pakistani population was assessed by calculating the odds ratio (OR) and 95% confidence intervals (CIs). A significance threshold of p <0.05 denoted statistical significance. Differences between groups were analysed using the 2 x 2 contingency chi-square test. The association between genetic variants and factors such as folic acid, Hcy levels, positive family history, and alleles was evaluated using the chi-square test via an online calculator (<https://vassarstats.net/odds2x2.html>). Allelic frequencies were assessed for deviations from Hardy-Weinberg equilibrium using a significance threshold of p = 0.05.

## RESULTS

The gel images of the extracted DNA samples from the study participants were captured using a UV transilluminator (Gene Box™ by Gene Sys™). The images below (Figure 2-4) show the DNA fragments from both cases and controls, with the amplified

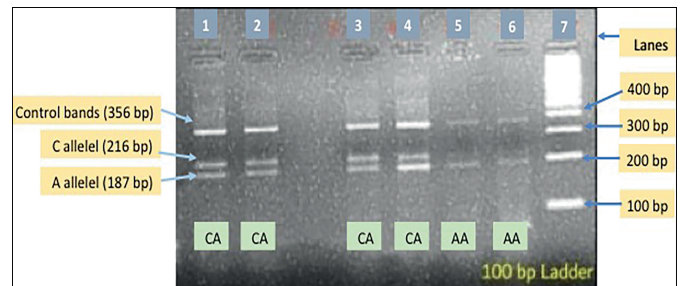
bands of interest visible alongside any non-specific bands. A 100 bp DNA ladder is included in the image as a reference to determine the size of the DNA fragments.

Table I shows that the mean age of participants was 29.07 ± 4.2 years, ranging from 25-45 years. A statistically significant difference was observed among patients and controls regarding weight (p <0.001), gestational weeks (p <0.001), parity (p <0.001), gravidity (p <0.007), and abortion (p <0.007).



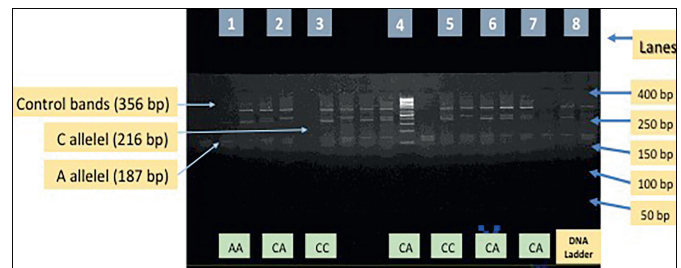
**Figure 2: Electrophoretogram of SLC19A1 (rs1051296 G>T) in cases showing amplified C and A bands with DNA ladder of 100 bp separated on gel electrophoresis.**

- **C allele at 216 bp are present in lane 1, 2, 3, and 4.**
- **A allele at 187 bp are present in lane 1, 2, 3, 4, 5, and 6.**
- **SLC19A1 (rs1051296 G>T) control gene band present at 356 bp is present in almost all lanes as internal control.**
- **Wild genotype is CC, while the mutant allele is found at lane 1, 2, 3, and 4.**
- **Homozygous mutant genotype AA is seen at 5 and 6 lanes.**



**Figure 3: Electrophoretogram of SLC19A1 (rs1051296 G>T) showing AA and CA genotypes in cases with DNA ladder of 100 bp.**

- **Lane 7 represents the DNA ladder of 100 bp as internal control in all cases and controls.**
- **Lane 1, 2, 3, and 4 represent CA genotype in cases.**
- **Lane 5 and 6 represent AA genotype in cases.**



**Figure 4: Electrophoretogram showing amplified PCR products of SLC19A1 (rs1051296 G>T) CA, AA, and CC genotypes in cases with DNA ladder of 50 bp.**

- **Control band (356 bp) is present in all lanes.**
- **Lane 1 represents AA.**
- **Lane 2, 4, 6, and 7 represent CA genotype in cases.**
- **Lane 5 represents the CC genotype (wild type).**
- **Lane 8 shows DNA ladder (50 bp) as an internal control in all cases and control.**



**Table I: Mean ± standard deviation of demographic characteristics.**

Parameters	Cases 166	Controls 166	p-value ≤0.05	
<b>Demographic characteristics of the study population</b>				
Mean age (years)	29.07 ± 4.2	28.97 ± 4.85	<0.847 <sup>a</sup>	
Weight	77.05 ± 11.34	72.50 ± 8.54	<0.001 <sup>s,a</sup>	
Gestational age (weeks)	31.84 ± 4.34	29.31 ± 4.50	<0.001 <sup>s,a</sup>	
Family history of HTN	No	162 (97.5)	87 (52.40)	<0.001 <sup>s,b</sup>
	Yes	4 (2.5)	79 (47.6)	
Eclamptic history	Present	0 (0)	11 (6.6)	<0.001 <sup>s,b</sup>
	Absent	166 (100)	155 (93.4)	
Parity (%)	Nulliparity	46 (27.7)	50 (30.1)	<0.628 <sup>b</sup>
	Multiparity	120 (72.3)	116 (69.9)	
Gravidity (%)	Primigravida	43 (25.9)	125 (75.3)	<0.001 <sup>s,b</sup>
	multigravida	123 (74.1)	41 (24.7)	
Abortion (%)	No	102 (61.4)	125 (75.3)	<0.007 <sup>s,b</sup>
	Yes	64 (38.5)	41 (24.7)	
<b>Pathological findings of the study population</b>				
Serum folic acid (ng/mL)	8.86 ± 3.30	13.47 ± 5.98	<0.001 <sup>s,b</sup>	
Serum homocysteine (µmol/L)	11.57 ± 2.97	7.54 ± 2.94	<0.001 <sup>s,b</sup>	

<sup>'S'</sup> denotes significant value. a: Chi-square test used for continuous variables. b: Student's t-test for categorical variables.

**Table II: Genotype and allelic frequencies of SLC19A1 (rs1051296 G>T) in cases and controls.**

Genotype	Cases n (%)	Controls n (%)	OR (95% CI)	p-value ≤0.05
<b>SLC19A1 (rs1051296 G&gt;T)</b>				
CC	56 (33.7)	30 (18.3)	Ref. 1	
CA	103 (62.0)	97 (59.1)	0.5 (0.33-0.959)	0.03 <sup>s</sup>
AA	7 (4.20)	39 (22.6)	0.09 (0.03-0.24)	<0.001 <sup>s</sup>
<b>Allele</b>				
C	215 (64.75)	157 (47.28)	Ref. 1	
A	117 (35.24)	175 (52.71)	0.48 (0.3-0.6)	<0.001 <sup>s</sup>
<b>Association of SLC19A1 (rs1051296 G&gt;T) with positive family history in cases and controls</b>				
SLC19A1(rs1051296 G>T)	75 (45.18)	4 (2.4)	-	-
CC	24 (14.45)	1 (0.6)	Ref. 1	-
CA	48 (28.91)	3 (1.80)	Ref. 1 0.6 (0.06-6.75)	0.6*
AA	3 (1.80)	0	-	-

<sup>'S'</sup> denotes significant value. p-values were determined using Chi-square test.

**Table III: Association of SLC19A1 (rs1051296 G>T) with folic acid and Hcy levels in cases and controls.**

Serum folic acid levels (ng/mL)	Cases	Controls	OR (95% CI)	p-value ≤0.05
<b>Folic Acid (ng/mL)</b>				
Below normal (<3.5)	63 (37.7)	0 (0)		
CC	21 (33.33)	0	Ref. 1	
CA	40 (63.49)	0	-	-
AA	2 (3.17)	0	-	-
Low normal (3.5-7.7)	96 (57.8)	10 (6)		
CC	33 (34.37)	1 (10%)	Ref. 1	
CA	61 (63.54)	9 (90%)	0.20 (0.02-1.69)	0.05 <sup>s</sup>
AA	2 (2.08%)	0	-	-
Medium normal (7.8-11.9)	5 (3.0)	60 (36.1)		
CC	1 (20)	4 (6.66%)	Ref. 1	
CA	2 (40)	56 (93.33)	0.14(0.01-1.935)	0.22
AA	2 (40)	0	-	-
High (12-18)	2 (1.2)	96 (57.8)		
CC	1 (50%)	25 (26.04%)	Ref. 1	
CA	0	32 (33.33%)	-	-
AA	1 (50%)	39 (40%)	0.6 (0.03-10.72)	0.6
<b>Serum Hcy levels (µmol/L)</b>				
<b>Hcy (µmol/L)</b>				
Normal (<6.3)	0 (0)	62 (37.7%)		
CC	0	11 (17.74)	Ref. 1	
CA	0	42 (67.74%)	-	-
AA	0	9 (14.51%)	-	-
Mild-to-moderate elevated (6.13-16.75)	120 (71.9%)	104 (62.65%)		
CC	34 (28.33)	17 (16.34%)	Ref. 1	
CA	82 (68.33)	51 (49.03%)	0.8 (0.407-1.58)	0.527
AA	4 (3.33)	30 (28.84%)	0.07 (0.02-0.24)	<0.001 <sup>s</sup>
Severely elevated (>16.75)	46 (27.5%)	0 (0)		
CC	22 (47.82)	0	Ref. 1	
CA	21 (45.65)	0	-	-
AA	3 (6.5)	0	-	-

<sup>'S'</sup> denotes significant value. p-values were determined using Chi-square test.

However, the difference in relation to age and parity was not significant ( $p = 0.8$  and  $0.628$ , respectively). Significant difference was observed in pre-eclamptics compared to healthy controls regarding serum folic acid ( $p < 0.001$ ) and serum Hcy ( $p < 0.001$ ) levels.

Comparative analysis revealed a strong association between the SLC19A1 (rs1051296 G>T) CA and AA genotypes and PE (Table II). Calculation of the allelic distribution revealed a higher frequency of the C allele (64%) among controls; however, the A allele was more prevalent in the pre-eclamptic group (35%,  $p < 0.001$ ).

Table III indicates the strong association of CA genotype with low serum folic acid levels in cases ( $p = 0.05$ ) as compared to healthy controls, and a noteworthy significant association of rs1051296 A allele with an increased risk of hyperhomocysteinaemia shown in cases.

## DISCUSSION

The current study evaluated the levels of maternal plasma folate and Hcy in women diagnosed with PE. The outcomes of the study highlighted several significant observations in PE. The study revealed a significant association between both CA and AA genotypes of SLC19A1 (rs1051296 G>T) and PE. Serum folate levels were lower in pre-eclamptic individuals with the CA genotype of the SLC19A1 (rs1051296 G>T) polymorphism. Serum Hcy levels were elevated in pre-eclamptic patients with the AA genotype compared to healthy pregnant women. No significant association was found between the SLC19A1 (rs1051296 G>T) polymorphism and a positive family history of PE.

A genetic defect in the folate transporter gene SLC19A1 (rs1051296) leads to serum hyperhomocysteinaemia, which produces reactive oxygen species and free radicals. These cause oxidative stress and damage to vascular endothelial cells, contributing to arterial spasm, a key factor in PE.<sup>17</sup> Genetic studies in Asian women with PE are quite limited. The SLC19A1 (rs1051296 G>T) polymorphism, which is being explored in this study, has not been previously established as a risk factor for PE.<sup>18</sup>

The SLC19A1 gene plays a crucial role in the transport of folate across cell membranes, and the rs1051296G>T variant has been independently associated with hyperhomocysteinaemia in various other conditions, including Non-Hodgkin lymphoma, acute lymphoblastic leukaemia, osteoporosis, IBD (inflammatory bowel disease), and methotrexate uptake by cancer cells.<sup>15,17,18</sup>

In contrast, a previous study found a correlation between PE and the MTHFR C677T polymorphism.<sup>9</sup> In this case-control study, the SLC19A1 (rs1051296 G>T) polymorphism was genotyped to explore its association with PE and other study variables. A total of 332 participants were recruited, including 166 pre-eclamptic patients and 166 healthy pregnant

controls. Eight demographic factors were assessed for their potential relationship with PE.

The mean age of cases and controls was non-significant ( $p = 0.847$ ) in the present study, consistent with findings by Portelli *et al.* in her study on biomarkers for hypertensive disorders of pregnancy (HDP).<sup>19</sup> However, gestational week comparisons between cases and controls were highly significant ( $p < 0.001$ ). Serrano's study revealed a substantial difference in gestational weeks between the pre-eclamptic and control groups, thereby reinforcing the validity of current findings.<sup>20</sup>

A family history of hypertension was linked to PE in Tesfa *et al.*'s study on maternal serum zinc levels in African women, though a recent study did not find a consistent association ( $p = 0.6$ ).<sup>21</sup> No association was found between parity and PE in the current study ( $p = 0.628$ ), contrasting with Poon *et al.*'s findings, which indicated reduced risk in parous women without prior PE.<sup>8</sup>

In the present study, gravidity did impact disease risk ( $p \leq 0.001$ ), with multifoetal gestations increasing PE risk, aligning with Bergman *et al.*'s study.<sup>22</sup> Previous abortion history showed no significant difference between cases and controls in this study ( $p = 0.007$ ), consistent with Banerjee *et al.*'s findings.<sup>23</sup> Comparable results of strong positive associations between the study population's cases and controls, mirroring the outcomes of previous randomised controlled trials.<sup>5,6,15,20</sup>

The laboratory findings of the present study demonstrated a positive association with serum folic acid ( $p < 0.001$ ) and serum Hcy levels ( $p < 0.001$ ). These results are consistent with those of a recent cohort study by Hassen *et al.*, which assessed serum folic acid, Hcy, and uric acid levels in the Ethiopian population.<sup>24</sup> Serum folic acid levels were lower in 95.2% ( $8.86 \pm 3.30$ ) cases as compared to 10% controls ( $13.47 \pm 5.98$ ), serum Hcy was higher in 46% ( $11.57 \pm 2.97$ ) cases, while no control subject had high Hcy levels ( $7.54 \pm 2.94$ ) in the present study.

The AA genotype and A allele were significant risk factors for PE in the Pakistani population ( $p < 0.001$ ), while the CA genotype also showed a strong association with pre-eclamptic patients ( $p < 0.03$ ). These results may reflect demographic and genetic diversity. The findings are consistent with studies by Serrano *et al.*,<sup>20</sup> and Ahn *et al.*,<sup>15</sup> This study also found a strong association between low serum folate levels and the CA genotype of the SLC19A1 gene (rs1051296 G>T), and elevated Hcy levels with the AA genotype, supporting previous research.<sup>25</sup>

Genetic predispositions, lifestyle, and medical interventions all impact Hcy expression. This study found a strong link between the SLC19A1 (rs1051296 G>T) gene polymorphism and PE in the Pakistani population. The CA and AA genotypes were significant risk factors, with lower serum folate levels

associated with the CA genotype and mild-to-moderate increases in Hcy linked to the AA genotype. Although a family history did not directly correlate with PE, it was strongly associated with various clinicopathological parameters. Other genetic variants should be studied with PE in multiple ethnic groups of Pakistan in relation to biomarkers. The effect of a mother's pre-pregnancy weight can be correlated with the polygenic inheritance of PE. Studies should be conducted on genes playing a predominant role in HELLP syndrome. Besides this, genetic variation in genes responsible for spiral artery remodelling can be studied in high-risk populations.

The limitations of this study include its brief duration and small sample size. Additionally, the collection of only a single sample during the second and third trimesters restricts the scope of the findings. The study also does not consider the effects of neonatal post-delivery outcomes or the influence of early screening and preventive measures on patient health. Future research with a larger sample size, extended study period, and multiple serial samples would offer more comprehensive and robust insights.

### CONCLUSION

The current study has contributed to finding a significant association of *SLC19A1* (*rs1051296G>T*) polymorphism with PE. In the Pakistani population, the CA genotype had an increased risk of developing PE. No association of *SLC19A1* (*rs1051296G>T*) was found with a previous family history of hypertension among cases and controls of the study population. The findings of this research are useful for better clarifying the aetiology, timely screening, and diagnostic modalities of pre-eclamptic patients of the Pakistani population.

### ETHICAL APPROVAL:

Ethical approval was obtained from the Ethical Review Committee of the Islamic International Medical College, Riphah International University, Rawalpindi, Pakistan (Ref. No: Riphah IRC/ 22/2084; Dated: 5 October 2022).

### PATIENTS' CONSENT:

All participants signed a general research consent form, approved by the Ethical Review Committee of Medical College.

### COMPETING INTEREST:

The authors declared no conflict of interest.

### AUTHORS' CONTRIBUTION:

IN: Concept of the study, study design, data collection, draft of the manuscript, statistical analysis, data interpretation, and literature search.

AR: Contributed to the conception and design of work, analysis, drafting, and reviewing of the content critically.

MA: Helped evaluate the collected data and its co-relations and facilitated the experimental procedure.

SA: Assisted in study design and participated in the review of existing literature.

ZB: Contributed to critical review and interpretation of the content and final drafting of the document.

NZ: Contributed to data collection, analysis, and drafting of the manuscript.

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