National Board of Examinations - Journal of Medical Sciences
Volume 4, Issue 6, Pages 898–908, June 2026
DOI 10.61770/NBEJMS.2026.v04.i06.004

ORIGINAL ARTICLE

Study of Methylene Tetrahydrofolate Reductase (MTHFR C677T) Single Nucleotide Polymorphism in Preeclampsia

S. Vinitha,[1,*] B.Anitha,[2] Vasmathie S[1] and Saravanan T[3]
[1]*Assistant Professor, Department of Biochemistry, Government Medical College and ESI Hospital, Coimbatore*
[2]*Assistant Professor, Department of Biochemistry, Coimbatore Medical College, Coimbatore*
[3]*Professor & HOD, Department of Biochemistry, Government Medical College and ESI Hospital, Coimbatore*

Accepted: 1-May-2026 / Published Online: 1-June-2026

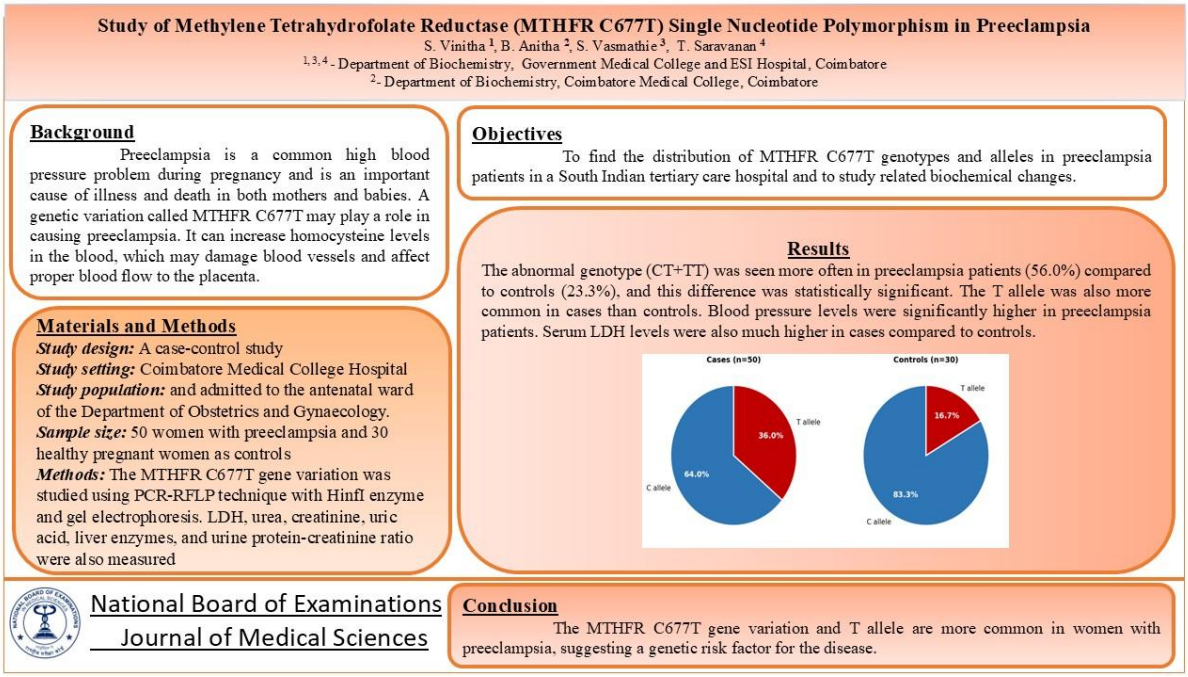
Abstract

Background: Preeclampsia is a common high blood pressure problem during pregnancy and is an important cause of illness and death in both mothers and babies. A genetic variation called MTHFR C677T may play a role in causing preeclampsia. It can increase homocysteine levels in the blood, which may damage blood vessels and affect proper blood flow to the placenta. **Methods:** This study was a hospital-based case-control study conducted at Coimbatore Medical College Hospital from May 2019 to February 2020. It included 50 women with preeclampsia and 30 healthy pregnant women as controls. The MTHFR C677T gene variation was studied using PCR-RFLP technique with HinfI enzyme and gel electrophoresis. Blood tests such as LDH, urea, creatinine, uric acid, liver enzymes, and urine protein-creatinine ratio were also measured. Statistical tests like Chi-square, Fisher's exact test, and odds ratio with confidence interval were used for analysis. **Results:** The abnormal genotype (CT+TT) was seen more often in preeclampsia patients (56.0%) compared to controls (23.3%), and this difference was statistically significant. The T allele was also more common in cases than controls. Blood pressure levels were significantly higher in preeclampsia patients. Serum LDH levels were also much higher in cases compared to controls. **Conclusion:** The MTHFR C677T gene variation and T allele are more common in women with preeclampsia, suggesting a genetic risk factor for the disease. Serum LDH can be used as a helpful marker to assess disease severity. Further studies with larger samples are needed to better understand the relationship between this gene variation and homocysteine levels.

Keywords: MTHFR, C677T Polymorphism, Preeclampsia, Hyperhomocysteinaemia, PCR-RFLP, Hypertension in Pregnancy

*Corresponding Author: S. Vinitha
Email: vini.somasekar@gmail.com

Graphical Abstract



Introduction

Preeclampsia (PE) is a condition in pregnancy where a woman develops high blood pressure (systolic BP ≥140 mmHg or diastolic BP ≥90 mmHg) along with protein in urine after 20 weeks of pregnancy, and it is one of the most serious complications of pregnancy [1]. It affects about 5–8% of pregnancies worldwide and is responsible for 10–15% of maternal deaths globally [2]. In India, preeclampsia occurs in about 8–10% of pregnancies and is a major cause of illness and death in both mothers and babies [3].

The cause of preeclampsia involves problems in the blood vessels, including damage to the inner lining of blood vessels, poor invasion of placental cells into uterine arteries, and increased inflammation in the body [4]. It is caused by multiple factors, including genetics. Women whose mothers had preeclampsia have about a 3 times higher risk of developing the condition, showing the role of genetic factors [5]. Many genes related to blood clotting, blood vessel function, immune system, and metabolism have been studied in relation to preeclampsia [6].

The Methylene Tetrahydrofolate Reductase (MTHFR) gene, located on chromosome 1p36.3, produces an important enzyme involved in folate and homocysteine metabolism [7]. A common genetic change called C677T (change from cytosine to thymine at position 677 in exon 4) leads to a less active enzyme. In people with TT genotype, enzyme activity is reduced by about 75%, causing increased levels of homocysteine in the blood [8]. High homocysteine levels can damage blood vessels, increase clot formation, cause oxidative stress, and lead to thrombosis, all of which are important in the development of preeclampsia [9]. Many meta-analysis studies have shown a strong link between MTHFR C677T variation and preeclampsia, especially in Caucasian and Asian populations [10].

However, there is limited information about this genetic variation in

South Indian populations. Therefore, this study was done to find the distribution of MTHFR C677T genotypes and alleles in preeclampsia patients in a South Indian tertiary care hospital and to study related biochemical changes.

Materials and Methods

Study Design and Setting

This was a hospital-based case-control study carried out at Coimbatore Medical College & Hospital, Coimbatore, Tamil Nadu, India from May 2019 to February 2020. Approval was obtained from the Institutional Ethics Committee, and written informed consent was taken from all participants before including them in the study.

Study Population

As this was an exploratory hospital-based genetic association study, a formal sample size calculation was not performed. The sample size was based on the number of eligible participants recruited during the study period and feasibility considerations

Cases: 50 pregnant women diagnosed with preeclampsia and admitted to the antenatal ward of the Department of Obstetrics and Gynaecology.

Controls: 30 healthy pregnant women (first pregnancy) beyond 20 weeks of gestation with normal blood pressure and no other medical problems.

Inclusion and Exclusion Criteria

Inclusion: Pregnant women aged 15–45 years; first pregnancy; gestational age more than 20 weeks; diagnosis of preeclampsia based on blood pressure ≥140/90 mmHg recorded twice at least 6 hours apart, along with urine protein (protein-creatinine ratio >0.3 or ≥+1 on dipstick) after 20 weeks.

Exclusion: Women with chronic hypertension, multiple pregnancy, molar pregnancy, hydrops fetalis, diabetes, kidney disease, liver disease, autoimmune disorders, or other systemic illnesses were excluded.

Sample Collection

A total of 5 mL of venous blood was collected under sterile conditions. Out of this, 2 mL was collected in an EDTA tube for DNA extraction, and 3 mL was collected in a plain tube for biochemical tests.

DNA Extraction and MTHFR C677T Genotyping

DNA was extracted using the salting-out method. Red blood cells were first broken down using TKM1 buffer and Triton-X, followed by cell lysis using TKM2 buffer with SDS and sodium chloride. DNA was then precipitated using isopropanol, washed with ethanol, dried, and dissolved in buffer. The quality of DNA was checked using spectrophotometry.

The MTHFR gene (exon 4) was amplified using PCR with specific primers. The PCR product (198 base pairs) was then cut using HinfI enzyme and analysed on agarose gel. Based on the band pattern: normal (CC) showed one band at 198 bp; heterozygous (CT) showed three bands (198, 175, 23 bp); and mutant (TT) showed two bands (175 and 23 bp) [11].

Biochemical Parameters

Blood tests were done using an automated analyser (XL 640). These included urea, creatinine, uric acid, LDH, ALP, SGOT, and SGPT using standard laboratory methods. Urine protein-creatinine ratio was measured using

standard methods, and platelet count was assessed using an automated cell counter.

Statistical Analysis

Data were analysed using IBM SPSS version 20. Continuous data were expressed as mean ± standard deviation and compared using Student's t-test. Categorical data were analysed using Chi-square test or Fisher's exact test. Odds ratios with 95% confidence intervals were calculated. Hardy-Weinberg equilibrium was checked using Chi-square test. A p-value less than 0.05 was considered statistically significant.

Results

Demographic and Clinical Parameters

The average age of women in both groups was similar, with cases having a mean age of 27.26 ± 4.12 years and controls 26.37 ± 3.76 years (p = 0.33), showing that both groups were well matched for age. The average gestational age and haemoglobin levels were also similar in both groups.

However, systolic and diastolic blood pressure were significantly higher in women with preeclampsia compared to controls (SBP: 147.60 ± 5.55 vs 118.87 ± 5.72 mmHg; DBP: 86.32 ± 4.61 vs 74.33 ± 4.37 mmHg), and this difference was statistically significant (p = 0.001 for both). Clinical parameters between cases and controls are compared and displayed in Table 1.

Table 1. Comparison of Clinical Parameters between Cases and Controls

Parameter	**Cases Mean ± SD**	**Controls Mean ± SD**	**t value**	**P value**	**Sig.**
Age (years)	27.26 ± 4.12	26.37 ± 3.76	0.96	0.33	NS
Gestational Age (weeks)	28.14 ± 4.27	28.57 ± 3.70	0.45	0.65	NS
SBP (mmHg)	147.60 ± 5.55	118.87 ± 5.72	22.1	0.001	S
DBP (mmHg)	86.32 ± 4.61	74.33 ± 4.37	11.47	0.001	S
Hb (g/dL)	11.11 ± 1.24	11.14 ± 1.12	0.08	0.92	NS

S = Significant; NS = Not significant

Biochemical Parameters

Serum LDH levels were much higher in women with preeclampsia (553.66 ± 195.31 U/L) compared to controls (247.57 ± 53.77 U/L; p = 0.001), indicating damage to the placenta and blood vessel lining. Liver enzymes SGOT (p = 0.049) and SGPT (p = 0.05) were also significantly higher in cases.

Urea levels were significantly lower in preeclampsia cases (20.46 ± 4.18 vs 25.23 ± 5.41 mg/dL; p = 0.045). However, there was no significant difference between cases and controls in ALP, uric acid, creatinine, platelet count, and urine protein-creatinine ratio. Biochemical parameters between cases and controls are compared and displayed in Table 2.

Table 2. Comparison of Biochemical Parameters between Cases and Controls

Parameter	**Cases Mean ± SD**	**Controls Mean ± SD**	**t value**	**P value**	**Sig.**
LDH (U/L)	553.66 ± 195.31	247.57 ± 53.77	8.3	0.001	S
SGOT (U/L)	22.92 ± 9.36	27.33 ± 7.09	2.22	0.049	S
SGPT (U/L)	19.46 ± 6.66	24.67 ± 7.56	3.2	0.05	S
ALP (U/L)	159.20 ± 72.45	141.07 ± 29.77	1.3	0.19	NS
Urea (mg/dL)	20.46 ± 4.18	25.23 ± 5.41	4.42	0.045	S
Creatinine (mg/dL)	0.82 ± 0.15	0.77 ± 0.12	1.54	0.12	NS
Uric Acid (mg/dL)	5.30 ± 1.14	5.25 ± 0.90	0.20	0.83	NS
Platelet ($\times 10^3/\mu L$)	183.72 ± 57.82	203.93 ± 55.57	1.5	0.12	NS
Spot Urine PCR	0.20 ± 0.13	0.15 ± 0.05	1.8	0.07	NS

S = Significant; NS = Not significant; PCR = Protein:Creatinine Ratio

MTHFR C677T Genotype Distribution

Among the 50 women with preeclampsia, 22 (44.0%) had the normal CC genotype, 13 (26.0%) had CT, and 15 (30.0%) had TT. Among the 30 control women, 23 (76.7%) had CC, 4 (13.3%) had CT, and 3 (10.0%) had TT. Distribution of *MTHFR C677T* Genotype between cases and controls compared and displayed in Table 3.

When CT and TT were grouped together as mutant types and compared with the normal CC type, the mutant genotype was seen more often in preeclampsia cases (56.0%) than in controls (23.3%). This difference was statistically significant (p = 0.004), and women with the mutant genotype had about 4 times higher risk of developing preeclampsia (OR = 4.18; 95% CI: 1.51–11.49) (Table 4). Distribution of *mutant and wild* Genotype between cases and controls compared and displayed in Table 4.

Table 3. Genotype Distribution of MTHFR C677T between Cases and Controls

Genotype	**Cases n (%)**	**Controls n (%)**	**Total**
Wild-type CC	22 (44.0%)	23 (76.7%)	45
Heterozygous CT	13 (26.0%)	4 (13.3%)	17
Homozygous TT	15 (30.0%)	3 (10.0%)	18
Total	50 (100%)	30 (100%)	80

Table 4. Mutant (CT+TT) vs Wild-Type (CC) Genotype — Cases vs Controls

Genotype	**Cases n (%)**	**Controls n (%)**	**P value**	**OR (95% CI)**
Wild-type CC	22 (44.0%)	23 (76.7%)	0.004	Reference
Mutant CT+TT	28 (56.0%)	7 (23.3%)	0.004	4.18 (1.51–11.49)

Allele Frequency Distribution

The T allele was found more often in women with preeclampsia (83.3%) compared to controls (16.7%), and this difference was statistically significant (p = 0.04). Women carrying the T allele had about 2.84 times higher risk of developing preeclampsia (OR = 2.84; 95% CI: 1.55–4.13).

The C allele frequency was 64% in cases and 36% in controls. The distribution of both alleles and genotypes followed Hardy-Weinberg equilibrium which is displayed in Table 5 and Figure 1.

Table 5. Allele Frequency Distribution of MTHFR C677T — Cases vs Controls

Allele	**Cases n (%)**	**Controls n (%)**	**P value**	**OR (95% CI)**
C allele	64 (64.0%)	36 (36.0%)	0.04	Reference
T allele	50 (83.3%)	10 (16.7%)	0.04	2.84 (1.55–4.13)

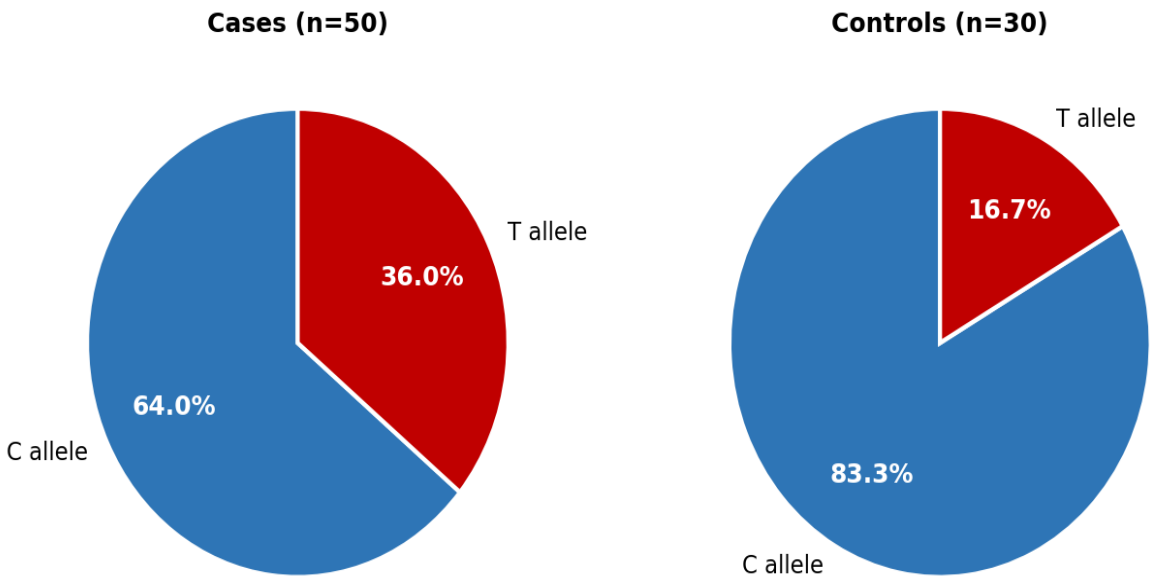


Figure 1. Allele Frequency Distribution (%) in Cases and Controls.

Discussion

Preeclampsia is a condition in pregnancy caused by a combination of genetic and environmental factors [12]. It affects about 2–8% of pregnancies worldwide, and its genetic causes have been widely studied in recent years [13]. One important genetic factor is the MTHFR C677T variation, which can increase homocysteine levels in the blood due to reduced enzyme activity, especially in people with the TT type [8].

In this study, the abnormal genotype (CT+TT) was found in 56.0% of preeclampsia cases compared to 23.3% of controls, showing a significantly higher risk (OR = 4.18; p = 0.004). This finding is similar to the results of Wu et al. [10], who showed a significant association between MTHFR C677T and preeclampsia in both Caucasian and Asian populations. They found that this mutation was about 1.37 times more common in preeclampsia cases. Merviel et al. [14] also reported a higher frequency of this mutation in pregnant women with hypertension, especially in early-onset cases. Yang et al. [15] supported these findings in a large meta-analysis of over 15,000 cases, confirming the link between MTHFR polymorphism and hypertension in pregnancy.

In our study, the T allele was much more common in cases (83.3%) than in controls (16.7%), with about 2.84 times higher risk (p = 0.04). El Baz et al. [16] reported a much higher risk (OR = 21.7), which may be due to differences in population, sample size, and genetic background. Salimi et al. [17] also found a strong association between MTHFR C677T and early-onset preeclampsia in an Iranian population, supporting its role in Asian groups.

The link between MTHFR C677T and preeclampsia is mainly due to increased homocysteine levels [18]. High homocysteine can reduce nitric oxide, increase oxidative stress, promote clot formation, and affect normal placental development, all of which contribute to preeclampsia.[9] This mutation may also lead to poor blood vessel formation in the placenta by causing small clots and affecting trophoblast invasion [19].

Serum LDH levels were much higher in preeclampsia cases (553.66 ± 195.31 vs 247.57 ± 53.77 U/L; p = 0.001), indicating cell damage, liver involvement, and placental injury. LDH is an important marker of disease severity and is also used in diagnosing HELLP syndrome.[20] The slight increase in SGOT and SGPT may suggest early liver involvement that has not yet progressed to severe disease.

In South India, Radha Rama Devi et al. [21] reported the presence of MTHFR polymorphism and its association with pregnancy-related complications, showing its importance in this region. The present study adds further evidence by studying this gene variation specifically in preeclampsia patients from Tamil Nadu.

One limitation of this study is that homocysteine levels were not measured, so a direct link between gene variation, homocysteine levels, and disease severity could not be established. The small sample size, single-centre design, and inclusion of only first-time pregnant women also limit how widely these results can be applied. Future studies should include larger and more diverse populations, measure homocysteine levels over time, assess enzyme activity, and study other related genetic factors.

Conclusion

The MTHFR C677T mutant genotype (CT+TT) and T allele were found more often in women with preeclampsia compared to healthy pregnant women, with risks increased by about 4.18 times and 2.84 times respectively. This shows that this gene variation may play a crucial role in preeclampsia in the South Indian population. Serum LDH levels were also significantly higher in preeclampsia patients, making it a useful test to assess how severity of the disease. Further large studies involving multiple centres are needed to better understand the role of this gene variation by studying its relationship with homocysteine levels in preeclampsia.

Limitations

Single-centre study; small sample size; serum homocysteine not measured; restricted to primigravidae. A formal sample size estimation was not performed prior to study initiation, which may limit the statistical power and generalisability of the findings.

Future Scope

Multicentre studies correlating MTHFR C677T genotype with serum homocysteine, folate, and vitamin B12 levels; extension to multigravidae and other hypertensive disorders of pregnancy; pharmacogenomic studies examining periconceptional folate supplementation in T allele carriers.

Conflicting Interest

The authors declare that they do not have conflict of interest.

Funding

No funding was received for conducting this study.

Data availability statement

The datasets generated and analysed in this study are available from the corresponding author on reasonable request. They are not publicly shared because they contain sensitive information that could indirectly identify participants.

Ethical considerations

This study has been approved by the Institutional Human Ethics Committee of Coimbatore Medical College, Coimbatore carrying certificate number 0172/2018, dt 15.12.2018

Informed Consent

Written informed consent was obtained from all participants after explaining the study procedures, potential risks and benefits. Consent covered both participation and publication of anonymised findings, with assurance of confidentiality and data privacy.

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