Rathod *et al.*
Egyptian Journal of Medical Human Genetics (2023) 24:5
https://doi.org/10.1186/s43042-023-00385-2

Egyptian Journal of Medical Human Genetics

RESEARCH

Open Access

Association of *MTHFR* C677T polymorphism with risk of preterm birth in Indian mothers: a case–control study

Pratibha Rathod[1], Ajesh Desai[2] and Divya Chandel[1*]

Abstract

Background Since *MTHFR* is the key enzyme in folate metabolism, its reduction can lead to hyperhomocysteinemia, which can have a negative impact on pregnancy outcome. Moreover, *MTHFR* polymorphism has also been linked with oxidative stress and genotoxicity. Identifying its ethnicity-specific association can help to reduce the incidence of preterm birth (PTB). Material and methods: Age-matched preterm birth mothers (< 37 weeks) and full-term mothers (> 37 weeks) were carefully selected for the study. The polymerase chain reaction–restriction fragment length polymorphism (PCR–RFLP) method was adopted to analyse *MTHFR* C677T polymorphism. Oxidative stress (OS) analysis was performed by measuring the levels of antioxidants (superoxide dismutase (SOD) and catalase (CAT)) and OS damage markers (lipid peroxidation (LPO), total protein). Genotoxicity was confirmed by the cytokinesis-block micronucleus cytome (CBMN-Cyt) assay. The statistical analysis was performed by Student's t test, chi-square test, and one-way ANOVA. The relevant risk of premature delivery was represented by odds ratios (ORs) with 95% confidence intervals (95% CIs).

Results The *MTHFR* polymorphism showed statistical significance for PTB outcome with CT and TT genotype frequencies at $p < 0.01$ and $p < 0.05$, respectively, between cases and controls. Within the PTB categories (extreme-, very-, moderate-PTB), TT genotype showed statistical significance at ($p < 0.05$), while CT genotype remained insignificant. Also, statistically high oxidative stress and DNA damage were observed in cases compared to controls for all genotypes. Furthermore, the T allele of the *MTHFR* gene was found to be linked with significantly increased OS and DNA damage on comparison within the groups.

Conclusions This study confirms the *MTHFR* C677T polymorphism, oxidative stress, and genotoxicity biomarkers are associated with the PTB outcome. Analysis of these biomarkers during pregnancy can be of clinical significance.

Keywords *MTHFR* polymorphism, Preterm birth, Pregnancy, Oxidative stress, DNA damage

Background

Preterm birth (PTB) (birth before 37 weeks of gestation) is the leading cause of neonatal mortality and is linked to long-term disabilities in survivors. Since it is a multifactorial condition, it is influenced by a variety of factors including environmental, physiological, and epigenetic [1]. Growing evidence suggests that the genes linked with the folate metabolism pathway are involved in PTB outcome. Among numerous genetic variants, the single nucleotide polymorphisms (SNPs) of the methylenetetrahydrofolate reductase (*MTHFR*) gene are gaining

*Correspondence:
Divya Chandel
divya_chandel@yahoo.com; divyachandel@gujaratuniversity.ac.in
[1] Department of Zoology, BioMedical Technology and Human Genetics, Gujarat University, Ahmedabad, India
[2] Obstetrics and Gynaecology Department, B J Medical College, Gujarat University, Ahmedabad, India

importance due to their involvement in hyperhomocysteinemia, which can lead to an increased risk of adverse birth outcomes.

Pregnant women may be exposed to oxidative stress-inducing situations on a daily basis, such as toxins in drinking water and food, cigarette smoking or air pollution. Monitoring oxidative stress in pregnant women is critical for understanding the link between oxidative stress and pregnancy outcome, and some studies have found links between oxidative stress and poor prenatal outcomes such as PTB [2, 3]. Antioxidant enzymes such as superoxide dismutase (SOD) and catalase (CAT) and vitamins such as vitamins C, E and folate actively eliminate reactive oxygen species (ROS) by transforming the noxious forms of oxygen into innocuous molecules [4, 5].

The role of the folate metabolism, antioxidant system, and *MTHFR* C677T polymorphism (rs1801133) with the PTB outcome has been investigated on a wide range of ethnicities across the world [6–9]. Unfortunately, even with high numbers of PTB cases and related complications in India, there is just one previous study from the northern region of India on *MTHFR* C677T polymorphism [10]. Therefore, this study aimed to analyse the effect of *MTHFR* C677T variants on pregnancy outcome and whether it has any correlation with oxidative stress and DNA damage markers.

Methods

Study design

The current case–control study included a total of 200 individuals, who were further classified based on gestation age, with 100 full-term birth mothers (>37 weeks) and 100 preterm birth mothers (<37 weeks). All mothers delivered babies with a cephalic presentation at the GMERS Medical College and Hospital in Gujarat, India. The study was approved by the Institutional Ethical Committee of Gujarat University (GUJIEC_03_2017) and GMERS Medical College and Hospital, Sola (GMERSMCS/IEC/37/2018). Gestation age was confirmed by LMP (last menstrual period) and sonography. Mothers with vaginal infection, multiple gestations, pregnancy with Mullerian anomalies, caesarean delivery, and non-cephalic presentation were excluded. Inclusion and exclusion criteria were decided to make sampling more uniform for both the groups and eliminate conditions that are primarily known to be linked with PTB or related complications. The blood collection with consent was taken from the participants at the time of enrolment.

MTHFR genotype distribution

First, the isolation of DNA was carried out from 2 ml of EDTA blood by the method of John et al. [11] with slight modification. Then, the *MTHFR* 677C-T polymorphism was detected after PCR amplification using the primers 5'TGAAGGAGAAGGTGTCTGCGGGA3' and 5'AGG ACGGTGCGGTGAGAGTGY3' [12], followed by allele-specific restriction digestion using the Hinf I enzyme, and then restriction fragments were separated by 3% agarose gel electrophoresis and visualized under UV light. The *MTHFR* PCR amplification showed the presence of a 198 bp band, which after enzyme treatment generated fragments of the single uncut band of 198 bp (wild type-CC), two bands of 198 + 175 bp (heterozygote) or one band of 175 bp (homozygote mutant) characterized as heterozygous (CT), and homozygous mutant (TT) variants, respectively, which can be visualized through gel documentation system (Fig. 1).

Oxidative stress markers

The serum was separated at 2000 rpm for 15 min at 4 °C, and isolated serum was then collected in the microfuge tube, stored at − 20 °C, and processed within 12 h for all the assays.

Antioxidants, i.e. superoxide dismutase (SOD) and catalase (CAT), were measured using the method of Kakkar et al. [13] and Sinha [14], respectively. Furthermore, non-enzymatic markers, i.e. lipid peroxidation (LPO) and total protein, were analysed by the method of Ohkawa et al. [15] and Lowry et al. [16], respectively.

Cytokinesis-block micronucleus cytome assay markers (CBMN-Cyt assay) [17]

The cultures were set according to the standard protocol, and cytochalasin B (10 mg/mL) was added at 44 h of incubation. The cultures were harvested at the 72-h hypotonic treatment 0.075 M KCL (37 °C) for 20 min followed by fixative (1:3 acetic acid/methanol) treatment. The slides were prepared, stained with 2% Giemsa stain, and labelled with the code number and observations noted. The frequency of micronuclei (MN), nucleoplasmic bridges (NPB), or nuclear buds (NBUD) was determined by scoring 1000 BN cells from each duplicate culture.

Statistical analysis

MTHFR gene polymorphism was analysed by chi-square tests. The odds ratio and 95% CI were calculated to estimate the risk of different genotypes. The oxidative stress parameters and CBMN-Cyt assay were expressed as mean ± SEM. A comparison of oxidative stress and CBMN-Cyt assay markers with *MTHFR* genotypes for both the groups was made by unpaired Student's t test. Moreover, a comparison within the groups (controls/cases) was made using one-way analysis of variance (ANOVA). The GraphPad Prism 7.0 software was used

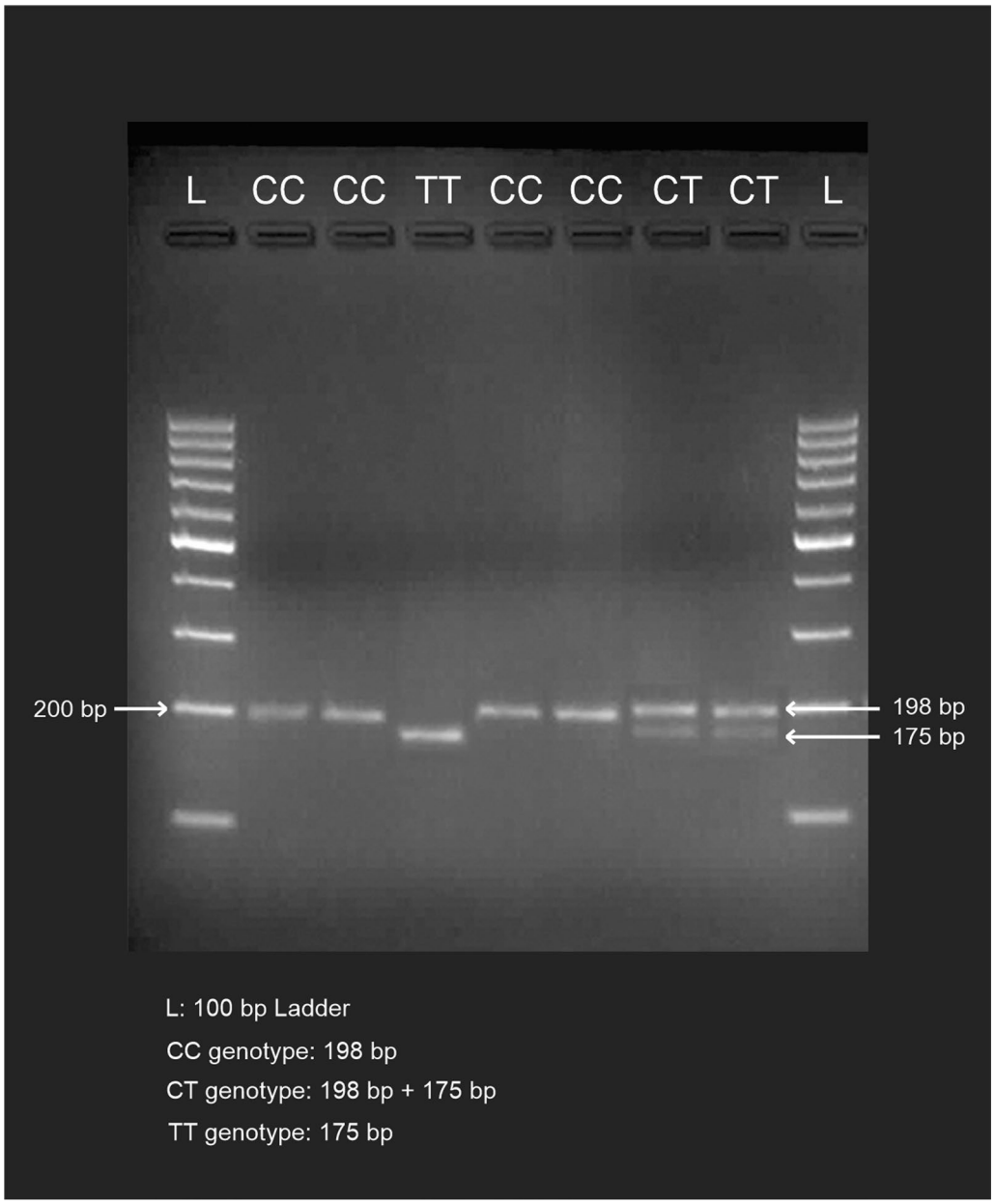

Fig. 1 Gel picture showing *MTHFR* C/C, C/T, and T/T polymorphism, where L: 100 bp Ladder; CC genotype: 198 bp; CT genotype: 198 bp + 175 bp; TT genotype: 175 bp

to verify values, and $p < 0.05$ was considered statistically significant.

Results

MTHFR genotype distribution

The polymorphism frequencies have shown statistical significance between preterm and full-term mothers (Table 1) with CT and TT genotype frequencies at $p < 0.01$ and $p < 0.05$, respectively. Moreover, T allele distribution was statistically higher in cases than in controls ($p < 0.05$). When we compared the intercategories (extremely, very, moderately PTB) relative to the wild homozygous genotype, TT genotype showed significance ($p < 0.05$), while CT genotype remained insignificant (Table 2).

MTHFR polymorphism and oxidative stress markers

For all the genotypes of the control, PTB, and PTB sub-categories, the levels of enzymatic (SOD, catalase) and non-enzymatic markers (LPO, total protein)

were compared. The levels of SODs were significantly decreased in all the genotypes of PTB compared to control (CC vs. CC-$p < 0.0001$; CT vs. CT-$p < 0.0001$; TT vs. TT-$p < 0.001$). Likewise, the levels of catalase were also significantly decreased in all the genotypes of PTB (CC vs. CC-$p < 0.0001$; CT vs. CT-$p < 0.05$; TT vs. TT-$p < 0.001$). The non-enzymatic markers of oxidative stress in all the genotypes between cases and controls showed significantly high levels of LPO (CC vs. CC-$p < 0.001$; CT vs. CT-$p < 0.0001$; TT vs. TT-$p < 0.0001$) and low levels of total protein (CC vs. CC-$p < 0.0001$; CT vs. CT-$p < 0.0001$; TT vs. TT-$p < 0.0001$) in all the PTB genotypes. Comparison of the genotypes (CC vs CT vs TT) within the control group showed high statistical significance for all the markers ($p < 0.0001$), likewise comparison within the PTB group (CC vs CT vs TT) that showed significance at $p < 0.0001$ for SOD and CAT, $p < 0.01$ for LPO, and remained non-significant for the total protein (Table 3).

MTHFR polymorphism and CBMN-Cyt assay markers

Comparison between control and PTB showed a significantly high frequency of MN in all the genotypes (CC vs. CC-$p < 0.01$, CT vs. CT-$p < 0.0001$, TT vs. TT-$p < 0.0001$). The frequency of NBUD for control CT vs PTB CT showed a significant difference at $p < 0.05$, while other genotypes were non-significant. The frequency of NPB remained non-significant for all the genotypes. Comparison of MN frequencies for all the genotypes within the groups (CC vs CT vs TT) also showed statistical significance (control $p < 0.0001$, PTB $p < 0.01$) (Table 4). Likewise, comparing NBUD frequencies of all genotypes within the groups showed a significance of $p < 0.05$ in the

Table 1 *MTHFR* genotype frequency distribution in cases and controls

Polymorphism	Genotype/allele	Control (full-term mothers) (N = 100) *n f* (%)	Cases (preterm mothers) (N = 100) *n f* (%)	Chi-square and *p* value	OR (95% CI)
C677T	CC	56 (56%)	36 (36%)	–	1
	CT	44 (44%)	61 (61%)	7.06**	2.16 (1.21–3.79)
	TT	0	3 (3%)	4.45*	Infinity (1.28-Infinity)
	C	156 (78%)	133 (66.5%)	–	1
	T	44 (22%)	67 (33.5%)	6.60*	1.79(1.13–2.81)

Statistical analysis is done by the chi-square test. *n*—number of genotypes, *f*—frequency, **$p < 0.01$; *$p < 0.05$

Table 2 *MTHFR* genotype frequency distribution among PTB categories

Polymorphism	Genotype/allele	Extreme PTB (< 28 weeks) (N = 8) *n f* (%)	Very PTB (28–32 weeks) (N = 16) *n f* (%)	Moderate PTB (32–37 weeks) (N = 76) *n f* (%)	Chi-square and *p* value
C677T	CC	2 (25%)	7 (43.75%)	27 (35.53%)	–
	CT	5 (62.5%)	9 (56.25%)	47 (61.84%)	0.53 ns
	TT	1 (12.5%)	–	2 (2.63%)	6.43*

Statistical analysis is done by the chi-square test. *n*—number of genotypes, *f*—frequency, *$p < 0.05$; ns—non-significant

Table 3 Correlation of oxidative stress markers with *MTHFR* polymorphism in cases and controls

ROS markers	Control (full-term mothers) N = 100			Cases (PTB mothers) N = 100		
	CC	CT	TT	CC	CT	TT
SOD	25.74 ± 0.24	22.53 ± 0.23####	00####	23.77 ± 0.14***	20.41 ± 0.25 ****####	18.67 ± 1.22 ***####
CAT	11.26 ± 0.27	10.39 ± 0.36####	00####	8.139 ± 0.25****	9.076 ± 0.36*####	6.457 ± 0.58***####
LPO	8.652 ± 0.20	8.858 ± 0.26####	00####	10.06 ± 0.30 ***	11.07 ± 0.15 *****##	12.05 ± 0.34 *****##
Total protein	29.11 ± 0.29	30.14 ± 0.34####	00####	24.11 ± 0.35 ****	25.17 ± 0.32 ****ns	24.37 ± 1.28 ****ns

ROS—reactive oxygen species, SOD—superoxide dismutase, CAT—catalase, LPO—lipid peroxidation. Results are expressed as the mean value ± SEM. Data were analysed by unpaired t test and one-way ANOVA

*Comparison between the group: Control vs Cases, CC vs CC, CT vs CT, TT vs TT: *$p < 0.05$; ***$p < 0.001$; ****$p < 0.0001$

#Comparison within each group

Control: CC vs CT vs TT: ####$p < 0.0001$

Cases: CC vs CT vs TT: ##$p < 0.01$; ####$p < 0.0001$; ns—non-significant

Table 4 Correlation of CBMN-Cyt assay markers with *MTHFR* polymorphism in cases and controls

CBMN-Cyt markers	Control (N = 100)			Cases (N = 100)		
	CC	CT	TT	CC	CT	TT
Micronucleus	4.48 ± 0.13	4.89 ± 0.19####	00####	5.17 ± 0.22**	5.90 ± 0.14 ****##	6.33 ± 0.33****##
Nuclear bridge	0.11 ± 0.04	0.20 ± 0.06 ns	00 ns	0.17 ± 0.06 ns	0.30 ± 0.06 ns; ns	0.67 ± 0.33 ns; ns
Nuclear bud	0.04 ± 0.03	0.11 ± 0.05 ns	00 ns	0.08 ± 0.05 ns	0.28 ± 0.06*; #	0.67 ± 0.33 ns; #

Results are expressed as the mean value ± SEM. Data were analysed by unpaired t test and one-way ANOVA

*Comparison between the group: Control vs Cases, CC vs CC, CT vs CT, TT vs TT: *$p < 0.05$; **$p < 0.01$; ****$p < 0.0001$; ns—non-significant

#Comparison within each group

Control: CC vs CT vs TT: ####$p < 0.0001$, ns—non-significant

Cases: CC vs CT vs TT: #$p < 0.05$; ##$p < 0.01$; ns—non-significant

PTB group, whereas controls remained non-significant ($p < 0.001$) (Table 4).

Discussion

Mothers' folate metabolism plays a critical role in keeping a healthy pregnancy, and studies have shown mixed results regarding its association with PTB [6, 18–20]. The *MTHFR* is the key enzyme in the folate pathway metabolism and converts 5,10-methylenetetrahydrofolate into 5-methyltetrahydrofolate, the main form of circulatory folate, which converts methionine from homocysteine, thus providing a methyl group for many biological reactions, including DNA methylation [21]. Many genetic mutations in *MTHFR* can reduce enzyme activity by increasing homocysteine concentration, which is also influenced by genetic and physiological factors, lifestyle, and consumption of B vitamins such as folates, cobalamin, vitamin B6, and riboflavin [22, 23]. The most prevalent mutation in the homocysteine metabolism pathway is the *MTHFR* C677T polymorphism, located at the folate binding site, where cytosine (C) is replaced by thymine (T) at nucleotide 677 and in the *MTHFR* enzyme that converts alanine to valine [24]. This polymorphism causes the expression of a thermolabile enzyme, in less specific activity in heterozygous (CT) and abnormal homozygous (TT) carriers [25]. Previous reports have shown that *MTHFR* C677T and A1298C can be associated with an elevated risk of PTB [6, 7]. Recently, the C/T genotype has been significantly associated with PTB and low birth weight in mothers of Down syndrome children [20]. The *MTHFR* gene mutation increases the need for folic acid, and low folate levels during pregnancy adversely affect the normal foetal growth and increase the risk of adverse pregnancy outcome [6, 7, 20].

Our results are consistent with several findings, which suggest a correlation between the *MTHFR* C677T polymorphism and the increased risk of preterm delivery as we reported T allele significantly increased the risk for PTB in the cases. Furthermore, we observed the homozygous mutant TT genotype only among the cases. From several other studies, it is evident that TT genotype carriers have been linked to an elevated risk of PTB [8, 9] due to low metabolic folate level. A study among Asian carriers also observed a prominent risk of PTB with maternal TT genotype [26]. However, some reports did not find any clear association between the *MTHFR* C677T polymorphism and the chances of preterm delivery [27, 28]. The diverse genetic backgrounds, geographical and regional variations could be the reasons behind such unequivocal data [29, 30]. Women in our study belonged to the low socio-economic background and mostly lacked sufficient nutritional demand and lived a stressful life which is likely to have higher oxidative stress (OS) and DNA damage. There is a scarcity of literature linking *MTHFR* gene polymorphism to oxidative stress and genotoxicity, and no allele-specific stratification is available. Therefore, our study also emphasized the correlation of genotype-specific *MTHFR* polymorphism with OS and CBMN-Cyt assay.

The rise in homocysteine levels can also lead to oxidative stress, arteriolar constriction, endothelial damage, and placental thrombosis [31]. All of these conditions may be linked to impaired uteroplacental circulation and prothrombotic changes in the vessel wall, insufficient trophoblast invasion into the uterine vasculature, and placental hypoperfusion, which trigger poor pregnancy outcomes such as PTB and low birth weight (LBW) [32, 33]. All the genotypes of the cases showed statistically high oxidative stress compared to the controls, suggesting that PTB mothers had comparatively lower antioxidant capacity. Further, the high statistical significance for the SOD, CAT, and LPO within the cases establishes an association of the T allele with the higher OS. Similar observations within control group genotypes clearly indicate that the T allele is indeed associated with the elevated OS during pregnancy. The mechanisms underlying the link between folate deficiency/hyperhomocysteinemia and oxidative stress and its association with

oxidation and auto-oxidation of homocysteine together with a reduction in antioxidant enzyme activities are not fully understood [34].

Recent studies have shown an association between micronucleus (MN) formation and folate levels. As folate is crucial in the DNA methylation process, DNA hypomethylation, a marker of folic acid deficiency, has been linked to chromosome loss, most likely due to pericentromeric heterochromatin under condensation [35]. Chromosome loss results in the formation of MN and aneuploidy, which is recognized as a potential risk indicator for PTB in our study. Studies have linked folate deficiency to genomic damage, the formation of MN, and other nuclear abnormalities in human lymphocytes [36, 37]. Low folate levels or high homocysteine levels due to C677T *MTHFR* polymorphism increased genotoxicity and risk for PTB several folds. Although our study confirms relatively high genotoxic damage through higher MN frequencies in PTB for all the genotypes, the comparison within the genotypes of the cases showed significantly increased genotoxicity due to the mutant T allele. Similar observations in control mothers suggest that the DNA damage increases when coupled with the T allele of the *MTHFR* gene. Another genomic instability marker, NPB, which is formed by mis-repair of DNA breaks or telomere end fusions showed no significant difference between both the groups also within the groups. Likewise, for the NBUD, which is predictive of gene amplification, significance was observed only in the CT variants of both groups but not in the other genotypes (CC, TT). However, comparisons within genotypes of cases revealed statistical significance for NBUD frequency, indicating that the genomic damage markers had an association with the T allele for the PTB risk. A study by Leopardi et al. [38] confirmed NBUD and NPB as novel biomarkers other than MN indicating low folate levels in women in their study. In addition to that, they observed higher NPB specifically in TT genotype of *MTHFR*. Our study shows that *MTHFR* polymorphism, oxidative stress, and DNA damage play a role in PTB outcome although the correlation between them could not be established definitely here.

Due to the polymorphism and low folate levels, pregnancy outcome gets affected majorly. Interestingly, it has been found that various dietary conditions can alter the functions of *MTHFR* genotypes [39]. Further, in our previous report, we observed a significantly high number of healthier full-term births in the group of mothers who maintained regularity in folic acid intake [40]. Thus, to reduce the risk of a poor obstetric outcome, pregnant women should be evaluated for their metabolic folate levels and advised folic acid dosages according to their condition to minimize the effects of *MTHFR* polymorphism for the better pregnancy outcomes. The finding of the present study is limited to a smaller population and a specific ethnicity; therefore, it needs to be validated in a larger cohort.

Conclusions

Better knowledge of the mechanisms behind PTB complications would help identify mothers who are at risk and deal with this crucial pathology more effectively. The present analysis confirms the role of maternal *MTHFR* C677T polymorphism with PTB risk. The mutant T allele of *MTHFR* gene, in particular, is thought to increase the risk of PTB by altering gene function and causing faulty folate metabolism.

Abbreviations

PTB	Preterm birth
SNPs	Single nucleotide polymorphisms
MTHFR	Methylenetetrahydrofolate reductase
SOD	Superoxide dismutase
CAT	Catalase
ROS	Reactive oxygen species
LMP	Last menstrual period
LPO	Lipid peroxidation
CBMN-Cyt assay	Cytokinesis-block micronucleus cytome assay
MN	Micronuclei
NPB	Nucleoplasmic bridges
NBUD	Nuclear buds
LBW	Low birth weight

Acknowledgements

Not applicable.

Author contributions

PR carried out the practical work and drafted the manuscript. AD was the obstetrician for all the cases. DC conceived the study, monitored practical work, and helped to draft the manuscript. All authors read and approved the final manuscript.

Funding

This research did not receive any specific grant from funding agencies in the public, commercial, or not-for-profit sectors.

Availability of data and materials

The data sets used and/or analysed during the current study are available from the corresponding author upon reasonable request.

Declarations

Ethics approval and consent to participate

This study was approved by the Institutional Ethical Committee of Gujarat University (GUJIEC_03_2017) and GMERS Medical College and Hospital, Sola (GMERSMCS/IEC/37/2018), and an informed written consent has been taken from all the women while enrolling them for this study.

Consent for publication

The consent to publish has been taken from each participant at the start of this work.

Competing interests

The authors declare that they have no competing interests.

Received: 22 August 2022 Accepted: 2 January 2023
Published online: 10 January 2023

References

1. Rathod P, Patel T, Desai A, Chandel D (2020) Socioeconomic, biological and genetic factors influencing preterm birth. Asian Pac J Reprod 9:215–222. https://doi.org/10.4103/2305-0500.294663
2. Menon R, Fortunato SJ, Milne GL, Brou L, Carnevale C, Sanchez SC et al (2011) Amniotic fluid eicosanoids in preterm and term births: effects of risk factors for spontaneous preterm labor. Obstet Gynecol 118:121–134. https://doi.org/10.1097/AOG.0b013e3182204eaa
3. Ferguson KK, McElrath TF, Chen YH, Loch-Caruso R, Mukherjee B, Meeker JD (2015) Repeated measures of urinary oxidative stress biomarkers during pregnancy and preterm birth. Am J Obstet Gynecol 212:208.e1-208.e8. https://doi.org/10.1016/j.ajog.2014.08.007
4. Ademuyiwa O, Odusoga OL, Adebawo OO, Ugbaja RN (2007) Endogenous antioxidant defences in plasma and erythrocytes of pregnant women during different trimesters of pregnancy. Acta Obstet Gynecol Scand 86:1175–1180. https://doi.org/10.1080/00016340701515357
5. Zhang PY, Xu X, Li XC (2014) Cardiovascular diseases: oxidative damage and antioxidant protection. Eur Rev Med Pharmacol Sci 18:3091–3096 (**PMID: 25392110**)
6. Engel SM, Olshan AF, Siega-Riz AM, Savitz DA, Chanock SJ (2006) Polymorphisms in folate metabolizing genes and risk for spontaneous preterm and small-for-gestational age birth. Am J Obstet Gynecol 195(1231):e1-11. https://doi.org/10.1016/j.ajog.2006.07.024
7. Chen DF, Hu YH, Yang F, Wu BY, Chen L, Fang ZA, Wang LH (2004) Mother's and child's methylenetetrahydrofolate reductase C677T polymorphism is associated with preterm delivery and low birth weight. Health Sci 36:248–53
8. Gargano JW, Holzman CB, Senagore PK, Reuss ML, Pathak DR, Friderici KH et al (2009) Polymorphisms in thrombophilia and renin-angiotensin system pathways, preterm delivery, and evidence of placental hemorrhage. Am J Obstet Gynecol 201:317.e1-e9. https://doi.org/10.1016/j.ajog.2009.05.060
9. Nan Y, Li H (2015) MTHFR genetic polymorphism increases the risk of preterm delivery. Int J Clin Exp Pathol 8:7397–7402 (**PMID: 26261642**)
10. Tiwari D, Bose PD, Das S, Das CR, Datta R, Bose S (2015) MTHFR (C677T) polymorphism and PR (PROGINS) mutation as genetic factors for preterm delivery, fetal death and low birth weight: A Northeast Indian population based study. Meta Gene 3:31–42. https://doi.org/10.1016/j.mgene.2014.12.002
11. John SW, Weitzner G, Rozen R, Scriver CR (1991) A rapid procedure for extracting genomic DNA from leukocytes. Nucleic acids Res 19:408. https://doi.org/10.1093/nar/19.2.408
12. Cyril C, Rai P, Chandra N, Gopinath PM, Satyamoorthy K (2009) MTHFR Gene variants C677T, A1298C and association with Down syndrome: A Case-control study from South India. Indian J Hum Genet 15:60–64. https://doi.org/10.4103/0971-6866.55217
13. Kakkar P, Das B, Viswanathan PN (1984) A modified spectrophotometric assay of superoxide dismutase. NIScPR Online Period Reposit 21:130–132 (**PMID: 6490072**)
14. Sinha AK (1972) Colorimetric assay of catalase. Anal Biochem 47:389–394. https://doi.org/10.1016/0003-2697(72)90132-7
15. Ohkawa H, Ohishi N, Yagi K (1979) Assay for lipid peroxides in animal tissues by thiobarbituric acid reaction. Anal Biochem 95:351–358. https://doi.org/10.1016/0003-2697(79)90738-3
16. Lowry OH, Rosebrough NJ, Farr AL, Randall RJ (1951) Protein measurement with the Folin phenol reagent. J Biol Chem 193:265–275 (**PMID: 14907713**)
17. Fenech M (2000) The in vitro micronucleus technique. Mutat Res- Fundam Mol Mech Mutagen 455:81–95. https://doi.org/10.1016/S0027-5107(00)00065-8
18. Johnson WG, Scholl TO, Spychala JR, Buyske S, Stenroos ES, Chen X (2005) Common dihydrofolate reductase 19-base pair deletion allele: a novel risk factor for preterm delivery. Am J Clin Nutr 81:664–668. https://doi.org/10.1093/ajcn/81.3.664
19. Stonek F, Hafner E, Philipp K, Hefler LA, Bentz EK, Tempfer CB (2007) Methylenetetrahydrofolate reductase C677T polymorphism and pregnancy complications. Obstet Gynecol 110:363–368. https://doi.org/10.1097/01.AOG.0000270122.13198.6f
20. Kedar R, Chandel D (2019) MTHFR gene polymorphism and associated nutritional deficiency in the etiology and pathogenesis of Down syndrome. Egypt J Med Hum Genet 20:1–10. https://doi.org/10.1186/s43042-019-0010-9
21. Wang L, Shangguan S, Chang S, Yu X, Wang Z, Lu X et al (2016) Determining the association between methylenetetrahydrofolate reductase (MTHFR) gene polymorphisms and genomic DNA methylation level: a meta-analysis. Birth Defects Res A Clin Mol Teratol 106:667–674. https://doi.org/10.1002/bdra.23511
22. Huang T, Hu X, Khan N, Yang J, Li D (2013) Effect of polyunsaturated fatty acids on homocysteine metabolism through regulating the gene expressions involved in methionine metabolism. Sci World J. https://doi.org/10.1155/2013/931626
23. Ueland PM, Hustad S, Schneede J, Refsum H, Vollset SE (2001) Biological and clinical implications of the MTHFR C677T polymorphism. Trends Pharmacol Sci 22:195–201. https://doi.org/10.1016/S0165-6147(00)01675-8
24. Li WX, Lv WW, Dai SX, Pan ML, Huang JF (2015) Joint associations of folate, homocysteine and MTHFR, MTR and MTRR gene polymorphisms with dyslipidemia in a Chinese hypertensive population: a cross-sectional study. Lipids Health Dis 14:1–11. https://doi.org/10.1186/s12944-015-0099-x
25. Ribeiro MR, Lima RP, Lisboa JV, Chaves TR, Luna RC, do Nascimento RA, et al. Influence of the C677T polymorphism of the MTHFR gene on oxidative stress in women with overweight or obesity: response to a dietary folate intervention. J Am Coll Nutr. 2018; 37: 677–84
26. Wu H, Zhu P, Geng X, Liu Z, Cui L, Gao Z et al (2017) Genetic polymorphism of MTHFR C677T with preterm birth and low birth weight susceptibility: a meta-analysis. Arch Gynecol Obstet 295:1105–1118. https://doi.org/10.1007/s00404-017-4322-z
27. Uvuz F, Kilic S, Yilmaz N, Tuncay G, Cakar E, Yuksel B et al (2009) Relationship between preterm labor and thrombophilic gene polymorphism: A prospective sequential cohort study. Gynecol Obstet Invest 68:234–238. https://doi.org/10.1159/000237743
28. Kim SJ, Lee BH, Kim YM, Kim GH, Yoo HW (2013) Congenital MTHFR deficiency causing early-onset cerebral stroke in a case homozygous for MTHFR thermolabile variant. Metab Brain Dis 28:519–522. https://doi.org/10.1007/s11011-013-9398-y
29. Alizadeh S, Djafarian K, Moradi S, Shab-Bidar S (2016) C667T and A1298C polymorphisms of methylenetetrahydrofolate reductase gene and susceptibility to myocardial infarction: a systematic review and meta-analysis. Int J Cardiol 217:99–108. https://doi.org/10.1016/j.ijcard.2016.04.181
30. Liang N, Deng Y, Zhou Y (2016) Study of polymorphisms of genes related to folic acid metabolism among women of child-bearing age from Shanxi. Chinese J Med Genet. 33:801–5. https://doi.org/10.3760/cma.j.issn.1003-9406.2016.06.012
31. Chen H, Yang X, Lu M (2016) Methylenetetrahydrofolate reductase gene polymorphisms and recurrent pregnancy loss in China: a systematic review and meta-analysis. Arch Gynecol Obstet 293:283–290. https://doi.org/10.1007/s00404-015-3894-8
32. Valdez LL, Quintero A, Garcia E, Olivares N, Celis A, Rivas F Jr et al (2004) Thrombophilic polymorphisms in preterm delivery. Blood Cells Mol Dis 33:51–56. https://doi.org/10.1016/j.bcmd.2004.04.011
33. ULUKIŞ M, EROĞLU FZ, YENİEL AÖ, TOPRAK E, KOSOVA B, TURAN ÖD, et al. Frequency of factor V leiden (G1691A), prothrombin (G20210A) and methylenetetrahydrofolate reductase (C677T) genes mutations in women with adverse pregnancy outcome. J Turk Ger Gynecol Assoc. 2006;7:195–201. (Google scholar: http://scholar.google.com/scholar?q=Frequency+of+factor+V+leiden+%28G1691A%29%2C+prothrombin+%28G20210A%29+and+methylenetetrahydrofolate+reductase+%28C677T%29+genes+mutations+in+woman+with+adverse+pregnancy+outcomea+Ulukus+2006)
34. Pravenec M, Kožich V, Krijt J, Sokolová J, Zídek V, Landa V et al (2013) Folate deficiency is associated with oxidative stress, increased blood pressure, and insulin resistance in spontaneously hypertensive rats. Am J Hypertens 26:135–140. https://doi.org/10.1093/ajh/hps015

35. Luzhna L, Kathiria P, Kovalchuk O (2013) Micronuclei in genotoxicity assessment: from genetics to epigenetics and beyond. Front Genet 4:131. https://doi.org/10.3389/fgene.2013.00131
36. Bull CF, Mayrhofer G, Zeegers D, Mun GL, Hande MP, Fenech MF (2012) Folate deficiency is associated with the formation of complex nuclear anomalies in the cytokinesis-block micronucleus cytome assay. Environ Mol Mutagen 53:311–323. https://doi.org/10.1002/em.21688
37. Lu L, Ni J, Zhou T, Xu W, Fenech M, Wang X (2012) Choline and/or folic acid deficiency is associated with genomic damage and cell death in human lymphocytes in vitro. Nutr Cancer 64:481–487. https://doi.org/10.1080/01635581.2012.660671
38. Leopardi P, Marcon F, Caiola S, Cafolla A, Siniscalchi E, Zijno A et al (2006) Effects of folic acid deficiency and MTHFR C677T polymorphism on spontaneous and radiation-induced micronuclei in human lymphocytes. Mutagenesis 21:327–333. https://doi.org/10.1093/mutage/gel031
39. Wook Hwang I, Dan Kang Y, Na Kwon B, Ho Hong J, Hun Han S, Soo Kim J et al (2017) Genetic variations of MTHFR gene and their association with preterm birth in Korean women. Medicina 53:380–385. https://doi.org/10.1016/j.medici.2018.01.001
40. Rathod P, Desai A, Chandel D (2022) Evaluation of risk factors for preterm birth outcome in Gujarat. Int J Health Sci Res. 12:161–174. https://doi.org/10.52403/ijhsr.20220123

Publisher's Note