International Journal of Reproduction, Contraception, Obstetrics and Gynecology
Sultana M et al. Int J Reprod Contracept Obstet Gynecol. 2024 Mar;13(3):564-569
www.ijrcog.org
pISSN 2320-1770 | eISSN 2320-1789

DOI: https://dx.doi.org/10.18203/2320-1770.ijrcog20240456

Original Research Article

Association of methylene tetrahydrofolate reductase gene mutation in unexplained recurrent pregnancy loss

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Received: 14 January 2024
Accepted: 03 February 2024

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ABSTRACT

Background: Recurrent pregnancy loss (RPL) without apparent causative factor which may be identified in about 50% of cases known as unexplained recurrent pregnancy loss. RPL is very distressing and can be heartbreaking for the couple. Among the many causes of RPL Methylene Tetrahydrofolate Reductase (MTHFR) gene mutation have been postulated as a possible cause. Aim of the study was to assess the association of methylene tetrahydrofolate reductase gene mutation (C677T and A1298C) in unexplained recurrent pregnancy loss.
Methods: This was a case-control study conducted at the Bangabandhu Sheikh Mujib Medical University (BSMMU), Dhaka, Bangladesh from May 2020 to April 2021. A total of 34 patients with unexplained recurrent pregnancy loss (RPL) and 34 age and BMI-matched controls were selected as study subjects. Data was analyzed using SPSS software, version 22.0.
Results: The frequency of heterozygous mutant genotype of MTHFR C677T and A1298C was statistically significantly higher in the case group than the control (38.2% vs 5.9%, p=0.001 and 55.9% vs 11.8%, p=0.000 respectively). No homozygous mutation for MTHFR C677T and only 1 for A1298C in the case group was found. The mutant T allele for MTHFR C677T and Mutant C allele for A1298C were found more frequently in cases compared to the controls (19.1% vs. 2.9% and 30.9% vs. 5.9%). Both the differences were statistically significant (p=0.003 and 0.000 respectively). Compound heterozygous mutant genotype CT/AC was found in 20.6% of RPL patients and not was found in the control.
Conclusions: MTHFR C677T and A1298C mutations pose a risk for unexplained recurrent pregnancy loss (RPL). Individuals with these mutations and a history of recurrent pregnancy loss may benefit from tailored management strategies, including low dose aspirin and low molecular weight heparin, to address potential risks.

Keywords: C677T mutation and A1298C mutation, MTHFR gene, Recurrent pregnancy loss, Unexplained

INTRODUCTION

Recurrent pregnancy loss (RPL) is emotionally distressing for the couple and requires sensitive and reassuring care, along with optimal management by the obstetrician. Various factors can contribute to RPL, including chromosomal anomalies, maternal thrombophilic defects, structural abnormalities of the uterus, endocrinological problems, and autoimmune disorders.[1,2] Despite thorough screening efforts, the underlying cause remains undetermined in up to 50% of RPL cases, leading to what

is known as unexplained recurrent pregnancy loss (URPL).[3-5] Thrombophilia is recognized as one of the primary causes of RPL.[6] Inherited thrombophilia encompasses Factor V Leiden, prothrombin and methylene tetrahydrofolate reductase (MTHFR) gene mutations, protein C, protein S, and antithrombin deficiencies, while hyperhomocysteinemia represents a form of acquired thrombophilia.[7,8] The primary underlying mechanism appears to involve the inhibition of trophoblast differentiation and thrombosis on the maternal side of the placenta.[9] Methylene tetrahydrofolate reductase (MTHFR) is a crucial enzyme responsible for the irreversible conversion of 5,10MTHF to 5-MTHF.[10] There are two common types of mutations in the MTHFR gene: C677T and A1298C. The MTHFR C677T mutation can exist in a homozygous form (when both copies of the gene carry the mutation) or a heterozygous form (when only one copy of the gene carries the mutation). The same applies to MTHFR A1298C. When one copy of the MTHFR gene carries the C677T mutation and the other copy carries the A1298C mutation, this is known as a compound heterozygous mutation. In the MTHFR C677T mutation, there is a cytosine-to-thymine transition at position 677.[7] This single nucleotide polymorphism leads to an alanine-to-valine substitution at codon 222 in the MTHFR enzyme, resulting in the MTHFR enzyme becoming thermolabile.[11] Consequently, enzymatic activity is decreased by 35% in the heterozygous state and by 70% in the homozygous state. Another common mutation is MTHFR A1298C, resulting in a glutamate-to-alanine substitution at codon 429. Although the decrease in enzyme activity in the case of MTHFR A1298C is less than MTHFR C677T, it contributes to the reduction of MTHFR enzyme activity.[1] This reduction decreases the concentration of 5-MTHF, ultimately elevating the Hcy level, which can result in unexplained recurrent pregnancy loss.[10,11] Moreover, in a recent study, mutations in the MTHFR gene leading to decreased enzyme activity and hyperhomocysteinemia have been postulated as a possible cause of RPL.[12]

METHODS

This case-control study was conducted in the outpatient department of Feto-maternal Medicine, BSMMU, Dhaka, Bangladesh during the period from May 2020 to April 2021. Following the inclusion and exclusion criteria of this study, 34 patients with unexplained recurrent pregnancy loss (RPL) and 34 age and BMI-matched controls were selected from the outpatient department (OPD) during the one-year study period as the study population. The study received approval from the ethical committee of the mentioned hospital. A convenient sampling technique was applied in sample selection, and proper written consent was obtained from all participants before data collection. The entire intervention was conducted following the principles of human research specified in the Helsinki Declaration and executed in compliance with currently applicable regulations and the provisions of the General Data Protection Regulation (GDPR).[13,14]

Inclusion criteria

Patients who attended the Feto-maternal OPD for preconceptional counseling for RPL and had a history of consecutive two or more failed clinical pregnancies were included as cases. On the other hand, age and BMI-matched women who had at least one successful pregnancy with no history of spontaneous pregnancy loss from both the outpatient and inpatient departments of Feto-maternal Medicine, BSMMU were included as control group participants.

Exclusion criteria

Women diagnosed with a known cause for RPL, parental chromosomal abnormalities (except for gene mutation), type-I and type-II diabetes mellitus, thyroid disorders, chronic renal disease, chronic HTN, PCOS, anatomic defects of the uterus, autoimmune disorders, and cases that received folate and Vit B12 supplementation within the previous three months were excluded.

From each participant, a 3ml blood sample was collected, and DNA was extracted using a genomic DNA extraction kit. The extracted DNA was amplified, and MTHFR gene mutation was detected by adding a specific primer from SNP Biotechnology R & D Ltd, Ankara, Turkey, using a 7500 Fast Dx Real-time PCR Instrument. The results were noted in the questionnaire, and data were analyzed using SPSS software. P-values were obtained from the chi-square test.

RESULTS

Table 1 presents that the majority of patients in the RPL (Case) group (64.7%) and the control group (67.7%) were aged between 25 to 34 years. Only 5 (14.7%) patients in the RPL group were between 35-40 years old. The mean age of patients with RPL was (28.44±5.25), while the mean age of the control group was (29.15±4.72). There was no significant difference between these two groups in terms of age (p=0.562).

Table 1: Distribution of participants as per age (n=68).

Age group	Case (n=34)		Control (n=34)		P value
	N	%	N	%	
18-24 yrs.	7	20.6	5	14.7	
25-34 yrs.	22	64.7	23	67.7	
35-40 yrs.	5	14.7	6	17.6	0.562
Mean±SD	28.44±5.25		29.15±4.72		

Table 2 illustrates the use of an independent sample t-test to compare the mean BMI between the RPL and control groups. The mean difference was not statistically significant (P=0.208). Therefore, it can be concluded that the BMI of the RPL group (24.95±3.48) was comparable

to that of the control group (23.69±4.07). The range of BMI for the RPL group was 20-32.4 (kg/m^2), and for the control group, it was 18-31.2 (kg/m^2).

Table 2: Distribution of participants as per BMI (N=68).

BMI (Kg/m^2)	Case (n=34)		Control (n=34)		P value
	N	%	N	%	
>18.5	0	0	1	2.9	0.208
18.5-24.9	18	52.9	21	61.8	
25-29.9	12	35.3	9	26.5	
≥30	4	11.8	3	8.8	
Mean±SD	24.95±3.48		23.69±4.07		
Range	20-32.4		18-31.2		

Figure 1 depicts a comparison of the number of previous conceptions in the study sample between the RPL and control groups. In the RPL group, the number of pregnancies ranged from 2 to 7, with the majority experiencing three pregnancies. Conversely, in the control group, the number of pregnancies ranged from 1 to 4, with most participants having been pregnant once or twice and no history of spontaneous abortion.

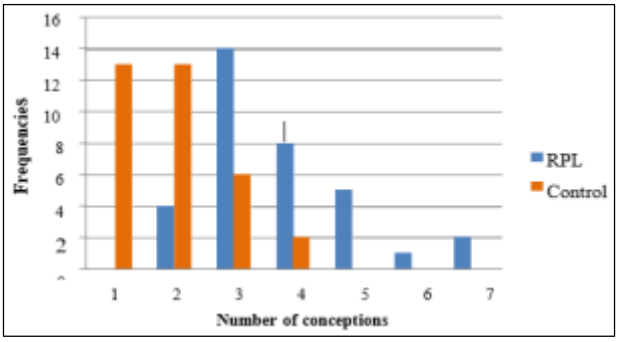

Figure 1: Bar chart shows the number of conceptions of two groups (n=68).

Figure 2 showed that 20(59%) of the RPL cases had 3 pregnancy losses and 5(14%) had 2 pregnancy losses.

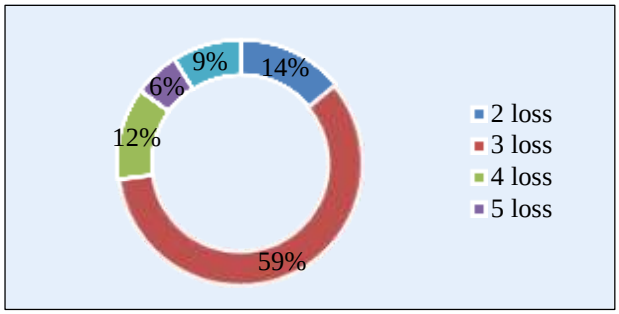

Figure 2: Ring chart showed the number of pregnancy losses among RPL group (n=34).

Figure 3 illustrates that in the RPL group, 23 patients had no previous children, indicating they were primary RPL cases, while the remaining 11 were secondary RPL cases. Among the primary RPL cases, 10 patients had 1 child, and only one had two children. In contrast, all participants in the control group had at least one child.

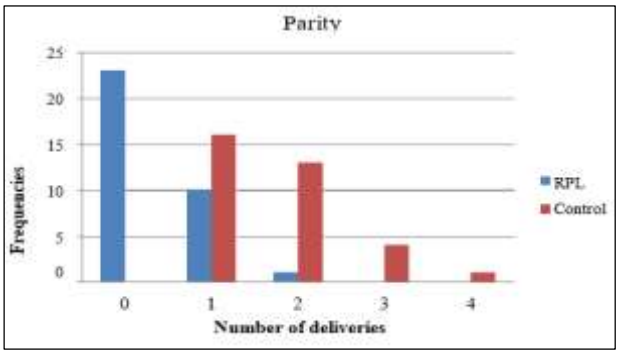

Figure 3: Parity of two groups of the sample population (n=68).

Figure 4 showed that among the RPL group, 68% were in the primary RPL and 32% were in the secondary RPL category.

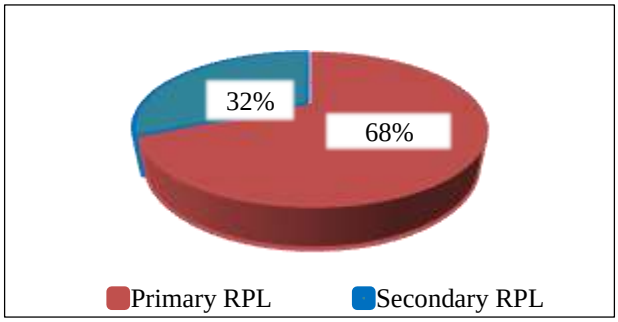

Figure 4: Pie chart showed RPL category distribution among case patients (n=34).

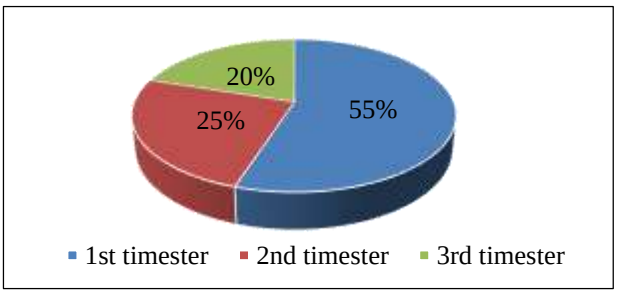

Figure 5: Pie chart showing pregnancy loss according to trimester (n=34).

Figure 5 illustrates those 31 (55%) patients in the RPL group experienced pregnancy loss in the first trimester, which includes both first-trimester losses and combined first and second-trimester losses. Additionally, 14 (25%) patients had pregnancy loss in the second trimester, comprising both second-trimester losses and combined first and second-trimester losses. 11 (20%) patients

experienced pregnancy losses in both the first and second trimesters.

Table 3 indicates that in terms of the frequency of MTHFR C677T genotypes, the wild-type CC and heterozygous mutant CT genotypes were observed in 61.8% and 38.2% of cases, respectively. No homozygous mutant TT genotype was identified in cases. In contrast, controls had frequencies of 94.1% (CC) and 5.9% (CT) genotypes respectively, with no occurrence of the mutant TT genotype. The heterozygous mutant CT genotype was more prevalent in cases than in controls (38.2% vs. 5.9%). Additionally, the mutant T allele was more frequent in cases compared to controls (19.1% vs. 2.9%). Both differences were statistically significant (P=0.001 and 0.003, respectively).

Table 3: Distribution of MTHFR C677T genotypes (n=68).

MTHFR	Genotype frequency (%)						Allele (%)			
C677T	**CC**	**%**	**CT**	**%**	**TT**	**%**	**C**	**%**	**T**	**%**
Cases	21	61.8	13	38.2	0	0	55	80.9	13	19.1
Controls	32	94.1	2	5.9	0	0	66	97.1	2	2.9
p-value	0.001[s]						0.003[s]			

Table 4: Distribution of MTHFR A1298C genotypes (n=68).

MTHFR	Genotype frequency (%)						Allele (%)			
A1298C	**AA**	**%**	**AC**	**%**	**CC**	**%**	**A**	**%**	**C**	**%**
Cases	14	41.2	19	55.9	1	2.9	47	69.1	21	30.9
Controls	30	88.2	4	11.8	0	0	64	94.1	4	5.9
p-value	0.000*						0.000*			

Table 4 reveals that in terms of the frequency of MTHFR A1298C genotypes, the wild-type AA and heterozygous AC genotypes were observed in 41.2% and 55.9% of cases, respectively. One mutant homozygous CC genotype was found among RPL cases. In contrast, controls exhibited frequencies of 88.2% (AA) and 11.4% (AT), with no occurrence of the mutant CC genotype. The heterozygous mutant AC genotype was more prevalent in cases than in controls (55.9% vs. 11.8%). Additionally, the mutant C allele was more frequent in cases compared to controls (30.9% vs. 5.9%). Both differences were statistically significant (P=0.000 and 0.000, respectively).

Table 5 indicates that heterozygous mutant genotypes CC/AC and CT/AA were significantly more prevalent in RPL patients, with odds ratios of 4.091 and 2.759 (95% CI, 1.162-14.397 and 0.496-15.330), respectively. The compound heterozygous mutant genotype CT/AC was present in 20.6% of RPL patients, while no such mutation was identified in the control group.

Table 5: Combination of MTHFR C677T and A1298C genotypes among patients and controls (n=68).

No. of mutations	Genotype combination	Genotype frequency n (%)		OR	95% CI	P-value
		RPL	Controls			
0	CC/AA	9 (26.5)	28 (82.3)	0.077	0.024-0.247	0.000*
1	CC/AC	12 (35)	4 (11.8)	4.091	1.162-14.397	0.022
	CT/AA	5 (14.7)	2 (5.9)	2.759	0.496-15.33	0.231
2	CC/CC	0	0	N/A	N/A	N/A
	CT/AC	7 (20.6)	0	N/A	N/A	N/A
	TT/AA	0	0	N/A	N/A	N/A
3	CT/CC	1 (2.9)	0	N/A	N/A	N/ A
	TT/AC	0	0	N/A	N/A	N/A
4	TT/CC	0	0	N/A	N/A	N/A

DISCUSSION

Recurrent pregnancy loss has devastating consequences on the happiness of the couple and on the maintenance of the marital harmony. It is frustrating for both patients and Obstetricians because a causative etiology cannot be identified in about 50% of cases even after extensive workup. At present, one of the possible causes increasingly

investigated in the literature is thrombophilic status which may alter the placental circulation (Creus et al, 2013). The MTHFR gene mutation is a part of the thrombotic risk factors and a number of studies have been investigated its potential association with RPL with inconclusive and controversial results. In this study, regarding the genotype distribution of MTHFR C677T and A1298C, it was observed that there was no homozygous mutation of MTHFR C677T either in the case or control group. Only one homozygous mutation of MTHFR A1298C was identified in the case group. This suggests that homozygous mutation of MTHFR is likely very rare in our country, as there is already wide variation in the prevalence of MTHFR polymorphism. Creus et al (2013) state that the frequency of the homozygous MTHFR C677T genotype among the general population in Europe varies according to the geographical area studied, ranging from 6% to 10% in the Nordic countries and 13% to 18% in the Mediterranean area.[12] The frequency of the heterozygous mutant CT of the MTHFR C677T genotype in this study was 38.2% for the case group and 5.9% for the control group. The mutant T allele was also found to be more frequent in cases compared to controls (19.1% vs. 2.9%). Both differences were statistically significant (p=0.001 and 0.003, respectively). In contrast, Puri et al (2013) found in their study that MTHFR C677T genotype distribution among cases and controls showed no significant difference (p=0.409).[15] Xu et al (2019) showed that the frequency of the T allele of the MTHFR C677T locus was statistically significantly higher in the unexplained RPL group than in the control group (p=0.039), and no significant difference was found in the frequencies of the TT+CT genotypes compared with the control group (p=0.305).[5] In Farahmand et al, (2015), heterozygosity for MTHFR C677T mutation was found in 34.55% of patients and in 24.29% of controls, which was significant.[1] Homozygosity for this mutation was observed in 10.9% and 10% in the case and control groups, respectively, and the difference was not significant. The frequency of the mutant T allele among cases and controls was 28.18% and 22.14%, respectively, which was also not significant. Fard et al found a significant increase in the prevalence of TT and CT mutant genotypes of MTHFR C677T among women with RPL (30% vs. 8% and 40% vs. 30%).[16] The frequency of the T allele was also found to be significantly higher in the case group than in the control (50% vs. 23%, P=0.001). In Creus et al (2013), there were no significant differences in the prevalence of homozygous and heterozygous MTHFR C677T gene mutations between the two groups studied.[12] Hwang et al (2017) found that the genotype distributions of MTHFR C677T in the RPL group did not differ from those of the control (p=0.142 and p=0.142, respectively).[17] In Nair et al (2012), the genotype frequencies of C677T and the mutant T allele differ significantly between these two groups (p=0.016 and 0.003, respectively).[18] This study indicates that homozygosity and heterozygosity for the MTHFR C677T polymorphism confer a 6.3009 and 1.9591-fold increased risk of idiopathic RPL, respectively. A meta-analysis conducted by Rai included 25 articles that concluded that there is a strong association between the MTHFR C677T mutation and RPL in the Asian population.[19] Regarding MTHFR A1298C genotypes, this study found that the heterozygous mutant AC genotype was more frequent among the case group when compared to the control (55.9% vs 11.8% respectively). The mutant C allele was also found to be more frequent in cases compared to the controls (30.9% vs. 5.9%). Both differences were statistically significant (p=0.000 and 0.000, respectively). Xu et al (2019) showed that the frequency of the C allele of the MTHFR A1298C locus was statistically significantly higher in the unexplained RPL group than in the control group (p=0.021).[5] In Farahmand et al (2015), the MTHFR 1298AC genotype was diagnosed in 46.07% of patients and 5.71% of controls, which was significant.[1] The homozygous genotype 1298CC was seen in 13.33% of cases and 0.29% in controls. The mutant 1298C allele occurred with a frequency of 36.36% and 3.14% among the patients and controls, respectively. All were statistically significant. In Hwang et al (2017), it was found that the genotype distributions of MTHFR A1298C in the RPL group did not differ from those of the control (p=0.142).[17] Nair et al showed that the presence of allele ‘‘C’’ and heterozygous and homozygous genotypes of MTHFR A1298C significantly increased the risk of RPL.[18] Cao et al, in a case-control study, also revealed a significant association between MTHFR A1298C polymorphism and idiopathic RPL (AC/AA, OR=2.58, 95% CI 1.40-4.74, p=0.002; AC+CC/AA, OR=2.53, 95% CI 1.40-4.57, p=0.002; C/A, OR=2.13, 95% CI 1.27-3.57, p=0.004, respectively).[20] Xu et al (2019) state in their study that the frequency of carrying the compound heterozygous genotype MTHFR 677CT/1298AC was statistically significantly higher in the unexplained RPL group (18.8%) than in the control group (10.6%).[5] Farahmand et al (2015) also found that the prevalence of combined mutations (more than one mutation) was significantly higher in the RPL women (18.79%) than in the control group (2.86%).[1] In Cao et al (2014), it was also found that the MTHFR 677T-MTHFR 1298C allele combination was associated with RPL (P=0.001).[20] This study also shows in Table V that the compound heterozygous mutant genotype CT/AC was much more prevalent in RPL patients with an odds ratio of 9.5 (95% CI, 2.739-32.949).

Limitations

This study has some limitations. This was single-centered nature and the relatively small sample size. Additionally, the study was conducted over a brief period, raising the possibility that the findings may not accurately represent the overall situation in the entire country. Caution is advised when generalizing the results, and further research with larger, more diverse samples and a more extended study duration would be valuable for a comprehensive understanding of the subject.

CONCLUSION

According to the results of this present study, the existence of MTHFR C677T and A1298C mutations is associated with an increased risk of unexplained recurrent pregnancy loss (RPL). Individuals with a history of recurrent pregnancy loss who have been identified with MTHFR mutations may find value in tailored management strategies. Healthcare providers might consider treatment with low dose aspirin and low molecular weight heparin to mitigate potential risks and improve the chances of successful pregnancies in this particular population. However, it's important to interpret these findings cautiously and in the context of other research, and personalized medical advice should be sought based on individual health circumstances.

Funding: No funding sources
Conflict of interest: None declared
Ethical approval: The study was approved by the Institutional Ethics Committee

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Cite this article as: Sultana M, Banu FA, Chowdhury S, Bul-Bul S, Parveen T, Begum F. Association of methylene tetrahydrofolate reductase gene mutation in unexplained recurrent pregnancy loss. Int J Reprod Contracept Obstet Gynecol 2024;13:564-9.