Evaluation of MTHFR Gene Polymorphisms (C677T and A1298C) as Molecular Predictors of Recurrent Pregnancy Loss

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Abstract
Miscarriage is one of the most significant problems faced by women during pregnancy, which in turn leads to numerous psychological and physical complications affecting the mother's health; however, the main cause of this condition remains unclear. The aim of this study was to investigate the relationship between polymorphisms in the methylenetetrahydrofolate reductase (MTHFR) gene and recurrent miscarriage in women in Anbar Governorate. A case-control study was conducted involving 70 women with recurrent miscarriage and 20 healthy women as a control group. Genomic DNA was extracted from peripheral blood samples, and the targeted genetic polymorphisms were analyzed using polymerase chain reaction (PCR) techniques. The frequencies of genotypes and alleles were compared between the two groups, and statistical analyses were performed to assess potential associations. The results showed differences in the distribution of certain genotypes and alleles between the patients and the control group; however, these differences did not reach statistical significance. These findings suggest that certain polymorphisms in the MTHFR gene may contribute to the risk of recurrent miscarriage, although their impact appears to be limited in the studied population. The study recommends further research using larger samples and from different ethnic groups to clarify the role of genetic variants in the MTHFR gene in causing recurrent miscarriage and to develop clinical methods for the early prediction of pregnancy loss.

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Keywords: MTHFR, Miscarriage, SNPs, PCR, DNA

Introduction

Miscarriage is the dominant negative effects suffered mother striving to get pregnant. around of 15% of gestational status from miscarriage of the world [1]. Based on to the revised recommendations of European Society of Human Reproduction and Embryology (ESHRE) and American Society for Reproductive Medicine (ASRM), recurrent pregnancy loss is described loss as two or more gestational failure before 20 weeks of pregnancy [2, 3]. Factors lead to miscarriage can be assorted namely paternal and maternal alloimmune mismatch, problems in maternal reproductive tract, endocrine disorders, genetic background and autoimmune disorders [4]. The accurate reason of miscarriage persists unclear in nearly 50% of the status [5]. Antecedent studies have observed the correlation methylenetetrahydrofolate reductase (MTHFR), vulnerability to miscarriage in adverse community [6, 7]. Methylenetetrahydrofolate reductase (MTHFR) is an enzyme contributing to changing 5,10-methylenetetrahydrofolate (5,10-methylene-THF) to 5-methyltetrahydrofolate (5-methyl-THF). MTHFR is a flavin-containing protein that streamlines NADPH-reliant reduction, harnessing FAD as a cofactor [8]. This transformation is a vital component in the folate cycle with 5-methyl-THF subsequently serving as a substrate for the methylation of homocysteine (Hcy) to methionine (Met) [Botto *et al*.,2000] The MTHFR gene is located on chromosome 1p36.22 [9]. It is transcribed alongside other enzymes involved in the folate cycle [8]. Twice of the MTHFR gene polymorphisms, C677T and A1298C, are the highly widespread and frequently studied. These two alone polymorphisms presently recognized to have a verified influence on enzyme

performance [10]. The C677T (rs1801133) polymorphism is substitution of alanine (Ala) at position 222 with valine (Val) in the enzyme's catalytic domain (A222V) [8]. This polymorphism increases thermal instability [11] and reduces the enzyme's attraction for FAD, resulting to a 35% reduce in enzyme performance each mutated allele [8]. The occurrence of this polymorphism in community consists 30–40%, but it varies from 0- 40%, depending on regional [12]. The A1298C (rs1801131) polymorphism is a substitution of glutamate (Glu) at position 429 with alanine (Ala) (E429A) [11]. Both polymorphisms can result in decreased MTHFR activity, potentially disrupting the folate cycle and related metabolic processes. The study conducted by Nishio *et al*. suggests that homozygous individuals with the TT genotype (resulting from the C677Tpolymorphism) have 20% reduce folate value contrasted to women lacking the polymorphism, notwithstanding having the identical folate consumption [13]. This may be specifically pivotal within duration of elevated folate require, such as pregnancy [14]. Women with this homozygous polymorphism may entail not only folic acid supplementation but also active forms of folate [15] The mechanisms of HHcy widespread and influence on cellular and systemic functions, particularly in the cardiovascular and neurological systems [16] The MTHFR enzyme plays an essential role in folate metabolism, and it is essential for cell division, embryo development, and early pregnancy [17]. Reduction in MTHFR activity is associated with an increased blood level of homocysteine and cardiovascular disease [18, 19]. Associations between MTHFR gene polymorphisms (C677T and A1298C) and the risk of RM have been previously demonstrated [20, 21] The relationship between thrombophilia and recurrent pregnancy loss or poor pregnancy outcomes is relatively well known and it is possible that similar mechanisms are in play in RM [22] A study by Basha *et al*. found that the prevalence of 677C>T homozygous polymorphisms was significantly higher in women with RPL than in controls (10% vs. 2.5%). The 1298A>C polymorphism was also more common in the treatment group (13.3% vs. 5%) [23]. in a meta-analysis that included 5888 cases and 8401 controls. Interestingly, the 1298A>C polymorphism was identified as a risk factor for RPL in Caucasians but not in Asians. However, some previous studies have found the association between MTHFR polymorphisms and RPL to be insignificant[25, 26] Previous studies have produced conflicting findings, with some indicating an increased risk of RPL in women carrying specific thrombophilia mutations (MTHFR), particularly in those with homozygous or compound heterozygous mutations [27, 28]Inherited thrombophilia induces hypercoagulation that impairs the placental function with either arterial or venous thrombosis at the maternal–fetal interface. This mechanism forms the likely basis of miscarriage [29]. Conversely, other investigations have failed to demonstrate any significant association between inherited thrombophilia and recurrent miscarriage [30, 2] miscarriage are pathologies that complex diseases, determined by multiple factors, including genetic aspects. Several genes were found to be related to the pathology, and they are involved in the regulation of the endometrial receptivity [31], inflammation [32], and the vascular system. In recent years, increasing amounts of evidence have shown a relationship between the thrombophilia factor and low fertility in women. MTHFR is a key enzyme for homocysteine metabolism; mutations (homozygous and heterozygous) in the MTHFR (C677T and A 1298C) gene result in a decrease in enzyme activity and poor stability, impairing folic acid metabolism, leading to an accumulation of homocysteine, results in increase blood tendency. Previous studies have shown that miscarriage and pregnancy complications are higher among patients with either inherited or acquired thrombophilia defects [33, 34] A possible mechanism for this could be thrombosis of maternal vessels, which might reduce the perfusion of the intervillous space leading to placentation failure. Thrombosis in the placental vessels leads to hypoperfusion of the intervillous space and may cause placental dysfunction. Our data demonstrate that the alleles of MTHFR C677T and A1298C were not associated and miscarriage, which is in agreement with what was reported by [35] These discrepancies may arise from differences in study design, sample size, or the specific thrombophilia mutations investigated, and further research is needed to clarify the potential role of thrombophilia in RP

Sample collection

This study is a case -control study Collected samples 90 from AL-Ramadi Teaching Hospital for Women and Children from September 2025 into February 2026,(group A) include 70 women which suffer from pregnancy loss more than 2,(group B) include 20 women classified with viable pregnancy both groups was in the first trimester,include blood samples and Putten in EDTA tube and genomic DNA was extracted from whole blood DNA extraction kit (Qiagen,Germany) according the manufacturer's instructions. And amplification of C677T region of MTHFR gene was performed using tetra-arms by four primers (outer/inner) forward and reverse after that amplification by agarose gel electrophoresis

Agarose Gel Electrophoresis

After isolating the DNA, a technique that is optimal in separating DNA fragments was used to assessment the stability of the DNA. This method was relied on the locomotion of DNA fragments in an electric field into the positive electrode, along with the length of fragments evaluated with the agarose concentration

Polymerase Chain Reaction (PCR)

The PCR reaction entails three stages: denaturation, annealing, and extension and four essential constituents, First, the four basic nucleotides—alanine, thymine, cytosine, and guanine—are primers, and their presence is essential for amplifying the DNA output. Second, DNA polymerase is the primary enzyme that connected the separate nucleotides to generate the PCR output

Statical Analyses of MTHFR Genotyping

Allele and genotype frequencies were determined by direct counting, and their associations with disease susceptibility were analyzed using the Chi-square test and ORs with 95% CIs. The Hardy–Weinberg equilibrium (HWE) for genotype distributions was assessed using the Chi-square goodness-of-fit test. A two-tailed p-value < 0.05 was considered statistically significant throughout the study

Figures and Tables

Table 1: Sequence of Primers Used in The Present Study for rs (180133&RS180131)

primers	sequences	Tm©	Product size(bp)
rs1801133 C677T	IF33 AAGAAAAGCTGCGTGATGATGAAATAGG	28	432
	IR33 GAGAAGGTGTCTGCGGGCGT	20	
	OF33 GCTGTGCAAGTTCTGGACCTGAGAG	25	
rs1801131 A1298C	OR33 AGCAGAGGACTCTCTCTGCCCAGTC	25	
	IF31 GGTAAAGAACGAAGACTTCAAAGACACCTT	30	499
	IR31 GGAGGAGCTGACCAGTGAGGC	21	
	OF31 TTTGGCACCCTGAGTCCCTCT	21	
	OR31 CAGAATTTACAGGAATGGCCTCCTG	25	

Result

Table 2: Showed the distribution of allele frequency and genotype form for (rs1801133, and rs1801131) among study groups.

SNPs	Alleles	All Subjects (n=90)	Controls Group(n=20)	patients Group (n=70)	O. R	CI (95%)	p-value
rs1801133	G	173	37	136	2.76	0.38 – 16.97	0.185
	A	7	3	4	0.36	0.06 – 2.60	0.155
rs1801131	T	156	35	121	0.91	0.25 – 2.77	0.549
	G	24	5	19	1.10	0.36 – 4.04	0.559

This table demonstrated the relationship between allele frequency and distribution in the study group,the G allele was in the patients group (136)and this was elevated than controls group was (37),although two of each group that confirms widespread occurrence of G allele and establishes the G allele was an essential allele among groups of study, although elevated an frequence of G allele but didn't illustrated a significant statically, in the SNPs rs1801133.the SNPs rs1801131,the allele T frequency was in patients 121but in controls (35) although an elevated of frequency but O.R was 0.19 and p-value (0. 549) and the CL% was include 1 than evidence of non-relationship between this SNPs

Table 3: Genotype form of (rs1801133, and rs21801131) of all subjects, Control and patients' group

SNPs	Genotype	All subjects (n=90)	C (Group) (n=20)	Patients (Group) (n=70)	O. R	CI (95%)	p-value
rs1801133	GG	85	18	67	2.48	0.19 – 23.14	0.307
	GA	3	1	2	0.56	0.03 – 34.69	0.534
	AA	2	1	1	0.28	0.001 – 22.74	0.397
rs1801131	TT	72	16	56	1.00	0.21 – 3.82	0.611
	TG	12	3	9	0.84	0.18 – 5.34	0.528
	GG	6	1	5	1.46	0.15 – 72.72	0.599

The GG genotype in the SNP(rs1801133) was more prevalence in patient group than control group but although from prevalence but O.R was 2.48 and CL was 0.19-23.14 and p-value (0.307) however,this an indicator of non - significantly statically,in SNP(rs1801131)was genotype TT more prevalence than other genotype and in patient group than controls although from prevalence but O.R(1.00), CL(0.21-3.82) and P-value(0.611) but this confirm a non-significant statically.

Table 4: Number and percentage frequencies of (SNP rs1801133) genotypes and their Hardy-Weinberg equilibrium (HWE) in Control group and patients' group

Groups	Genotype form						Allele frequency		p-value
	GG	GA			AA		G	A	
	Obs.	Exp.	Obs.	Exp.	Obs.	Exp.			
All subjects	85	83	3	7	2	0	173	7	0.0001
Controls	18	17	1	3	1	0	37	3	0.0042
Patients	67	66	2	4	1	0	136	4	0.0001

1degree of freedom (d.f.) for Chi-squared distribution. Obs.: observed, Exp: expected.
This table demonstrated the genotype of this SNPs and observed, Expected rely on Hardy-Weinberg Equilibrium, that showed difference between in control and patient group that an indicator of deviation regarding HWE.

Table 5: Number and percentage frequencies of (SNP rs1801131) genotypes and their Hardy-Weinberg equilibrium (HWE) in Control group and patients' group

Groups	Genotype form						Allele frequency		p-value
	TT		TG		GG		T	G	
	Obs.	Exp.	Obs.	Exp.	Obs.	Exp.			
All subjects	72	68	12	21	6	1	156	24	0.0001
Controls	16	15	3	4	1	1	35	5	0.1599
Patients	56	52	9	16	5	2	21	19	0.0002

1degree of freedom (d.f.) for Chi-squared distribution. Obs.: observed, Exp: expected

This table demonstrated the genotype of this SNPs and observed, Expected rely on Hardy-Weinberg Equilibrium, but this SNP in constrict of another SNP, in patient group p-value was 0.002 and this value a highly significant differences was showed

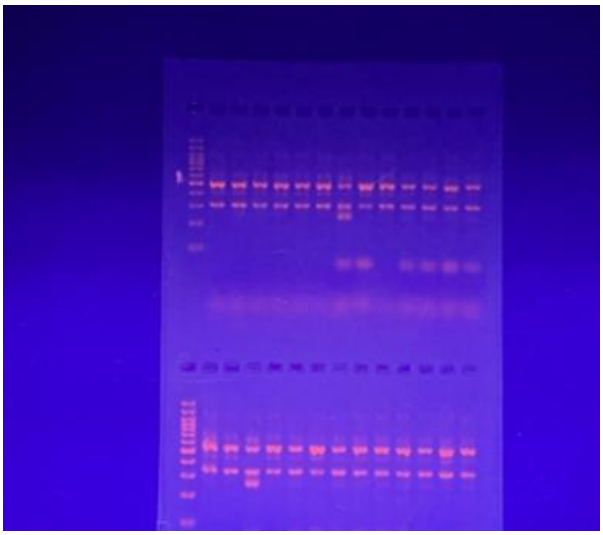
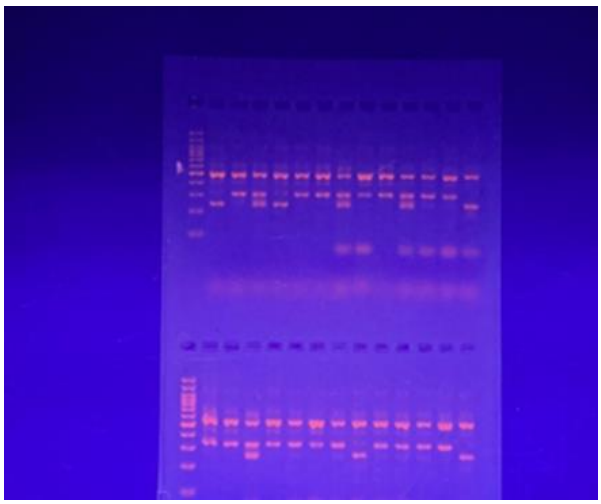


Fig 1: These graphs demonstrate the results of rs180131 SNPS in the PCR machine.



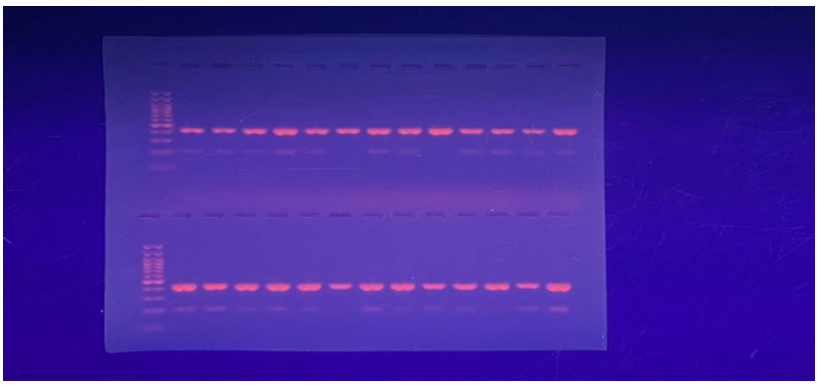


Fig 2: This graph demonstrates the rs180133 SNPS in the PCR machine.

Discussion

Miscarriage is one of the most common problems of gestation and for this status more of side effect for psychological health for the partner. But, although from vulnerate but mechanism of occurrence yet non-described. Single nucleotide polymorphisms (SNPs) A1298T and C677T of MTHFR gene has potential to be serving as a diagnostic probe in an inherited mutation of miscarriage is hypothesized to be connected with heredity thrombophilia's [36]

MTHR A1298C variation fails significantly impede enzymatic- activity function nevertheless a corresponds to insufficiency in folate [37] The MTHFR C677T, situated inside the enzyme-functional site, causes both a thermolabile protein and elevated in Hcy via its effect on Hcy value, MTHFR C677T has been contributing to a predisposing in the miscarriage, The frequency of homozygous TT genotypes was discovered significantly superior between the pregnant with recurrent miscarriage. Some studies illustrated significantly elevated distribution of MTHFR C677T between affected individuals [38] in fetus development during gestation, the fetus enlarges and strengthen by promote its own blood furnish through angiogenesis. A good substitute among embryo and mother is needed to guarantee typical physiological embryo development for this reason, defective placental villous structures vascularization that give rise to miscarriage [39] MTHFR C677TT genotype related with miscarriage, autonomously of elevated homocysteine level, was attributed to impeding with red blood cell folate metabolic activity, as previously stated [40] But no significant correlation among MTHFR A1298C polymorphism and unexplained miscarriage was demonstrated in a number of studies [33, 41, 42] More recent studies have shown that the occurrence of recurrent pregnancy loss of unreported cause is statistically significantly related with polymorphisms at the MTHFR C677T and A1298C The heterozygosity of the MTHFR C667T and A1298C mutations is associated with reduce enzyme activity and elevated blood homocysteine, [43] Multiple researchers have lately postulated that the mutation may be responsible for certain women's recurring pregnancy loss. The hypothesis accentuation the potential possible effect genetics might contribute in differently unknown gestation loss, furthermore via it has not yet been confirmed [44]

In the study of Zammit it was determined that there is no correlation among homocysteine levels and the danger of miscarriage. also, study found no correlation among the danger of miscarriage and homocysteine levels and the MTHFR gene mutation [45, 46] In contrast to[42] result that indicated no correlation among the A1298C polymorphism and miscarriage but [34] observed each of two maternal and paternal MTHFR gene C677T and A1298C polymorphisms are linked with undesirable effects of pregnancy.

Conclusions

This study suggests that MTHFR gene polymorphisms may be associated with miscarriage in Iraqi women. Although no statistically significant associations were found for the genetic variants studied, this is due to several reasons, including sample size and ethnic diversity. The study recommends further research using larger samples and from diverse population groups to verify these findings and clarify the underlying factors contributing to recurrent miscarriage

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Conflict of Interest

we declare that they have no conflict of interest.

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