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Association of MTHFR gene rs180113 A>C polymorphism with Recurrent spontaneous abortion

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Abstract

Background: DNA methylation has been linked to the development and progression of multiple disorders including recurrent spontaneous abortion (RSA) which affect 4-5% of pregnant women globally. One significant enzyme involved in DNA methylation is methylene tetrahydrofolate reductase (MTHFR). This study was designed to evaluate the association between rs1801131 polymorphism, located in the *MTHFR* gene and RSA in Iraqi population.

Materials and methods: Blood samples from 30 RSA patients diagnosed with a history of 2 or more abortions and 25 healthy individuals with no abortion history were collected and DNA was extracted. Variants were genotyped using amplification tetrarefractory mutation system-polymerase chain reaction analysis (Tetra-ARMS PCR). The data were analyzed via X^2 tests.

Results: The rs1801131 A>C polymorphism non-significantly decreased the risk of RSA in codominant heterozygous AC (OR=0.66, and P=0.541), while in homozygous increased non-significantly CC (OR=1.5, and P=0.729) models. The presence of the C allele is a potential risk factor for RSA for rs1801131 A>C (OR=1.1, and P=0.853) polymorphism.

Conclusion: The rs1801131 A>C *MTHFR* gene polymorphism increase the risk of RSA in our population. Further studies in other ethnicities are necessary to verify our findings.

Keywords: Recurrent spontaneous abortion, Homocysteine, *MTHFR* gene, Thrombophilia, rs1801131, Tetra-ARMS PCR

1. Introduction

Recurrent spontaneous abortion (RSA) is one of the major reproductive health problems (Jalilvand et al., 2022). RSA is known as miscarriage before the twentieth week (Bandeira et al., 2022). RSA abortion is defined as repeated 2 to 3 pregnancy losses or more before the 20th week of gestation (Kaur and Gupta, 2016). Affecting 4 to 5% of pregnant women with negative social and medical consequences (Abu-Asab et al., 2011). It is a multifactorial medical condition with many etiologic factors involving genetic abnormalities of the parents, anatomic and hormonal issues, hematologic and immune system abnormalities, infections, maternal thrombophilia, nutritional, and environmental factors (Al-Achkar et al., 2017). Genetic makes up 50 to 60% of RSA causes (Garrido-Gimenez and Alijotas-Reig, 2015). However, exact etiology still undetermined in up to half of RSA patients (Xu et al., 2015). Many researches have investigated the possible linking between some hereditary thrombophilia markers and RSA. Hereditary thrombophilia occur due to genetic polymorphisms and deficiencies in the production of natural anticoagulants or substances that influence coagulation (Bilibio et al., 2020). Variation in the methylenetetrahydrofolate reductase (MTHFR) gene represents

an example of hereditary thrombophilic markers (Settin et al., 2011). MTHFR is an essential enzyme in folate and homocysteine pathway (Pritchard et al., 2016). It catalyzes the reduction of 5,10-methylentetrahydrofolate into 5-methylentetrahydrofolate, the predominant circulatory form of folate which plays the main role in remethylation of homocysteine to methionine. Methionine is important for the synthesis, repair and imprinting processes of DNA (Mehta et al., 2022). MTHFR gene is located on sub band six of band six for region three of short arm of chromosome number 1 (1p36.6) with a total of 11 exons. Several single nucleotide polymorphisms (SNPs) were reported in the MTHFR gene, the A1298C (rs1801131) is one of the most commonly identified. MTHFR gene A1298C in exon number 7, a substitution of glutamate to alanine at codon 429 (E429A) (Yang et al., 2016). The rs1801131 polymorphism has shown to have a role in decreasing the enzymatic activity, and lowering the conversion of homocysteine into methionine, leading to accumulation of homocysteine in blood (Chen et al., 2016). Homocysteine has a cytotoxic effect, induces apoptosis of trophoblasts and reduces secretion of the human chorionic gonadotropin hormone (Hubacek et al., 2015). It is also associated with neural-tube defects occurrence (Yaliwal and Desai, 2012). Thrombophilia might lead to clots formation in the blood of the placental small vessels, by which in turn dropping the oxygen delivery to the fetus, and finally occurrence of fetus loss (Bigdeli et al., 2018). In current investigation, we performed an association study on MTHFR A1298C gene polymorphism with RSA patients among Iraqi women.

2. MATERIALS AND METHODS

2.1. Participants

This study was applied as a case-control study on two different groups. Control group consisted of 25 healthy women of reproductive age and no history of abortion. The RSA group consisted of 30 women with a history of 2 or more abortions and diagnosed with RSA.

2.2. DNA Analysis

The genetic analysis was performed at Salahaddin University-Erbil, College of Science, in Biology Department. Blood was collected from RSA women, 5 ml of peripheral blood. Blood samples were preserved, in EDTA tubes at freezing temperature. DNA extraction Kit was used to isolate genomic DNA from peripheral leukocytes. The isolation was made according to the manufacturer protocol provided in the kit. Genotyping of the single nucleotide polymorphism (SNP) rs1801131 (A1298C) MTHFR gene was implemented by Tetra amplification refractory mutation system polymerase chain reaction (TETRA-ARMS-PCR), which is a rapid and cost-effective technique for SNP detection. Four primers were used, two of them were external allele-non-specific primers (forward outer: 5'-GCAGAAGAAGTTTGCATGCTTGTGGTTG-3', reverse outer: 5'-ACTTACCCTTCT CCTTTGCCATGTC CA-3' amplifying the common band of 459bp length), while the remained two were internal allele-specific primers (forward inner 5'-GTGGGGGGAGGA GCTGACCAGTGAGGA-3' specified for amplifying the wild type allele of 231bp length , and reverse inner 5'-GGTAAAGAACGAAGACTTCAAAGACACCTG-3' specified for amplifying the mutant type allele of 281bp length). The PCR cycling program was set as the following, 5 minutes at 95°C for initial denaturation, then the next 35 cycles of three repeated steps, denaturation at 95°C for 30 seconds, annealing at 63°C for 1 minute, and extension at 72°C for 1 minute, with a final extension step set at 72°C for 10 minutes to extend all PCR fragments. Separation of the PCR products, the amplified DNA, were performed using horizontal agarose gel electrophoresis on agarose gel (2%), and then staining with DNA safe stain and banding patterns were visualized by UV transilluminator.

2.3. Statistical Analysis

Statistical analysis was done using Graph Pad Prism 6 statistical software. Genotype and allele frequencies of cases and controls were analyzed using the Chi-square (X^2) test and both genotype and allelic odds ratio (ORs) and 95% confidence interval (CI) were calculated to determine the association of *MTHFR* gene polymorphisms with RSA. A p-value of less than 5% ($p<0.005$) was set to be statistically significant.

3. Result

The genotype frequencies of MTHFR A1298C (rs1801131) polymorphism in the RSA group were wild type (AA) (73.3%), Heterozygote (AC) (20%), and CC (6.7%). The frequencies were AA (68%), AC (28%), and CC (4%) in the control group (Table 1). Genotypes expressed as AA in wild type genotype, AC in the heterozygote, while CC in mutant type genotype. In wild type genotype, two bands of 459 bp and 231 bp were produced. In heterozygote genotype, three bands of 459 bp, 231 bp indicate for A-allele, and 281 bp indicate for C-allele, and in mutant type genotype two bands of 459 bp and 281 bp were produced (Figure 1). The heterozygous genotype showed to be non-significantly protective factor for RSA with (OR=0.66, a broad spectrum 95% CI = 0.19-2.3; P-value = 0.5410) when compared with wild type genotype. But in the presence of both mutant alleles in the homozygous mutant type genotype our result showed non significantly to be a risk factor for RSA with (OR = 1.5; a broad spectrum 95% CI: 0.13-19; P-value = 0.7294). The frequency of allele C was 83.33% in the RSA group and 82% in the control group. The mutant allele showed to be non-significantly a minor risk factor for the RSA with (OR = 1.1; with a narrower spectrum 95% CI = 0.66-1.7; P-value = 0.8539).

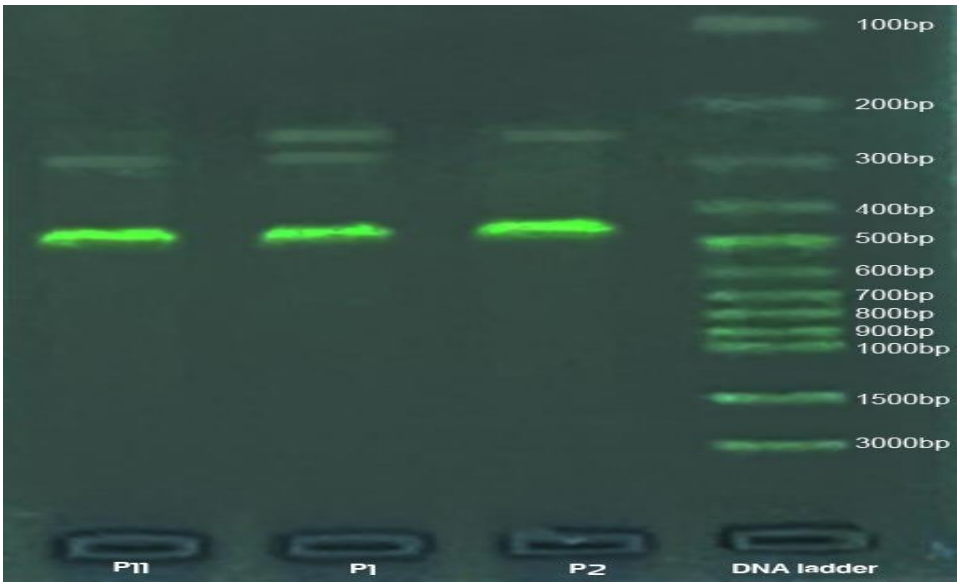

Figure 1. Agarose gel electrophoresis showing results of Tetra Amplification Refractory Mutation System-Polymerase Chain Reaction of three RSA patients (P2, P1, P11) illustrating an amplified 459-bp fragment; common band of MTHFR gene as a control, fragment of 281-bp; specific band for C-Allele, and 231-bp; specific band for A-Allele. Lane DNA ladder, lane P2; AA-Genotype, lane P1; AC-Genotype, and lane P11; CC-Genotype.

Table 1. Distribution of the MTHFR A1298C Genotypes in the RSA and Control Groups.

Polymorphism	RSA (n=30)		Control (n=25)		OR	95% CI	p value
	No.	%	No.	%			
AA	22	73.3	17	68.0	1.0	-	-
AC	6	20.0	7	28.0	0.66	0.19 to 2.3	0.541
CC	2	6.7	1	4.0	1.5	0.13 to 19	0.729
A-Allele	50	83.33	41	82.0	1.1	0.66 to 1.7	0.853
C-Allele	10	16.66	9	18.0			

Abbreviations: RSA, Recurrent spontaneous abortion; OR, odds ratio; CI, confidence interval.

4. Discussion

In the current study, we found the association between the MTHFR gene polymorphism (A1298C) and the risk for RSA in Iraqi women.

A1298C is an important polymorphism for *MTHFR* gene that might affect the activity of MTHFR enzyme in blood. Polymorphisms in MTHFR reduce the activity and enhanced the removability of the enzyme and subsequent elevation in homocysteine levels. Clinically, hyperhomocysteinemia caused by polymorphisms has been associated with coronary artery disease, thrombophilia, neural-tube defects, and recurrent miscarriage (Saraswathy et al., 2012). Thus, it was important to study MTHFR in women diagnosed with RSA due to the association placental infarcts with RSA (Mtiraoui et al., 2006). Many researches have investigated the association between MTHFR gene A1298C SNP and the risk of RSA, but with conflicting results (Khaleghparast et al., 2011). Several researches found a significant association between patients with MTHFR A1298C polymorphism and an increased RSA risk. The C-allele and CC and AC genotypes are suggested to be risk factors for RSA (Ramadan et al., 2020). In contrast, numerous studies found no significant contribution of the MTHFR A1298C SNP with RSA (Poursadegh Zonouzi et al., 2012).

In the present study, the association between the prevalence of homozygous genotype CC in the RSA group (6.7%) and the control group (4%) was observed, and this difference was not statistically significant (OR = 1.5; 95% CI: 0.13-19; P-value = 0.7294). However, the frequency of heterozygous genotype AC has no significant difference in both groups as well (P-value = 0.5410).

5. Conclusion

We conclude that the *MTHFR* gene rs1801131 A>C polymorphism might be genetic biomarkers for RSA in Iraqi population sample. However, the authors suggest further investigation on a larger sample and other ethnicities to verify these results.

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