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ASSOCIATION BETWEEN SERUM HOMOCYSTEINE LEVELS AND METHYLENETETRAHYDROFOLATE REDUCTASE C677T AND A1298C GENE POLYMORPHISMS IN WOMEN WITH RECURRENT MISCARRIAGE

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Abstract

Recurrent miscarriage (RM) has been linked to hyperhomocysteinemia and the methylenetetrahydrofolate reductase (MTHFR) C677T and A1298C gene mutations. The study aimed to investigate the association between serum homocysteine levels and methylenetetrahydrofolate reductase C677T and A1298C gene polymorphisms in women with idiopathic recurrent miscarriage.

Material and methods: In this study, we included 30 women with idiopathic recurrent miscarriage and 28 healthy pregnant women as the control group. After genotyping, all women were divided into three groups: Control group (N = 28) (no more than one mutation in both loci of the MTHFR gene, C667T and A1298C), second group (N = 13), homozygous (both copies of either the C677T mutation or the A1298C mutation), and third group (N = 17) compound heterozygous (having one copy of the A1298C mutation and one copy of the C677T mutation).

Genotyping was performed by reversal hybridization with a CVD strip assay manufactured by Vienna Lab, Austria. A chemiluminescent immunoenzyme assay was used to determine the concentrations of homocysteine in the serum.

Results: In the control group, we found that 18% of women have elevated homocysteine levels (>15 mol/L); in the second group, 23% of women have hyperhomocysteinemia; and in the third group, 35% of women have elevated homocysteine levels.

Conclusions: Despite the high percentage of subjects with hyperhomocysteinemia in compound heterozygous, no significant differences were observed in serum homocysteine levels between the studied groups ($p > 0.05$). Serum homocysteine levels and methylenetetrahydrofolate reductase C677T and A1298C gene polymorphisms are not associated with idiopathic recurrent miscarriage.

Keywords: homocysteine, methylenetetrahydrofolate reductase, recurrent miscarriage, genotyping

Introduction

Recurrent miscarriage (RM) is the term used to describe the loss of three or more consecutive pregnancies before the 20th week of gestation. The term means a fetus or embryo weighing less than 500 g, typically at 20 to 22 weeks of gestation. 2%–5% of all women develop RM [1].

Heredity, age, antiphospholipid syndrome, uterine abnormalities, thrombosis, hormonal or metabolic problems, infection, autoimmune disease, sperm quality, habits, and mental, psychological, and environmental factors are some of the leading causes of RM. But in a substantial percentage of cases (>50%), despite intensive examination, no reason has been found, and these cases are classified as idiopathic. After two prior miscarriages, the risk increases to 35%, and it is 30% after two losses. This suggests a need for evaluation after two losses in women with no prior live births [2].

A few studies have shown that RM is related to hereditary polymorphisms of 5,10-methylenetetrahydrofolate reductase (MTHFR). With 11 exons, the MTHFR gene is found on 1p36.3. C677T and A1298C are two critical loci that cause polymorphisms within the MTHFR gene. The amino acid at base position 677 in the fourth exon shifts from alanine to valine when the CT mutation occurs. The A1298C mutation is an AC mutation at base position 1298 in the 7th exon, which changes the glutamate to alanine at the corresponding position.

Elevated homocysteine (HCY) levels are caused by mutations at these two loci, which decrease the MTHFR enzyme activity encoded by the MTHFR gene. These mutations block the metabolic conversion of 5,10-methylenetetrahydrofolate to 5-methylenetetrahydrofolate and disrupt the metabolic conversion of cysteine to methionine.

Those who support the theory have suggested that hyperhomocysteinemia may cause the formation of tiny blood clots that block nutrition flow to the placenta, essentially starving the fetus and triggering a spontaneous abortion [3,4].

This study investigated the association between serum homocysteine levels and methylenetetrahydrofolate reductase C677T and A1298C gene mutations in women with idiopathic recurrent miscarriages.

Material and Methods

Design and subjects

This retrospective study included 30 women with a history of idiopathic recurrent miscarriage and 28 women as a control group. Women who had at least one healthy pregnancy and no history of spontaneous miscarriages were considered for inclusion in the control group.

The inclusion criteria for the RM comprised women who had a history of two or more spontaneous abortions with a history of recurrent loss of pregnancy. Women with a history of taking teratogenic drugs during early pregnancy, those with severe digestive diseases, hepatic or renal insufficiency, and those with a diagnosis of anatomic genital anomalies confirmed by gynaecological examination, ultrasound, or hysterosalpingography were excluded, as were those with chromosomal abnormalities in couples or embryos.

Other exclusion criteria included those with coronary heart disease, diabetes, thyroid disease, malignancy, and those with a pathogenic microbial infection in the vaginal tract or hematologic or immunological disorders.

As a material, we used venous blood. For the women who were enrolled in the study before sample collection, a questionnaire about health status (alcohol use, smoking, other habits), demographic factors, pregnancy loss, and essential characteristics (height, weight) was filled out in a face-to-face interview between participants and trained hospital staff. Each participant involved in the study provided oral informed consent.

Genotyping

Venus blood samples were collected, then sample DNA was extracted using a column extraction kit. Single-nucleotide polymorphisms at the MTHFR gene's C677T and A1298C loci were found using the TaqMan-MGB method. On the two loci, a fluorescent quantitative polymerase chain reaction was performed. The TaqMan-MGB probe used in the reaction was GAAAAGCTGCGTGATGAAATCG [G/A] CTCCCGCACCTICT for MTHFRC677T and AAGAACGAAGATICAAAGACACTT [G/T] CTTCACTGGTCAGC for MTHFR A1298C. Each polymerase chain reaction equipment encounters a total volume of 10 L, containing 1 L with 20 ng/L of DNA template, 5 L of 2× TaqMan Universal Master Mix (American ABI Company), 0.5 L of 20× TaqMan-MGB probe, and 3.5 L of deionized water. Setting the reaction conditions to 95°C for 10 minutes was followed by 20 amplification cycles (92°C for 15 seconds and 60°C for 1 minute) and 30 amplification cycles (89°C for 15 seconds and 60°C for 90 seconds).

After the reactions, the end-point fluorescence in the sample well was read using an ABI 7900 fluorescence quantitative polymerase chain reaction cycler (Applied Biosystems), and the genotype of each sample was determined by converting the various fluorescence signals into a DNA sequence using the appropriate analysis software.

After genotyping, all 58 individuals were divided into three groups. First group: controls (N = 28) (no more than one mutation in both loci of the MTHFR gene, C667T and A1298C). The second group (N = 13) is homozygous (both copies of the C677T or A1298C mutation). The third group (N = 17) is compound heterozygous (one each of the A1298C mutation and the C677T mutation). Genotyping was performed by reversal hybridization with a CVD strip assay manufactured by Vienna Lab, Austria. Serum homocysteine levels were measured by chemiluminescent immunoenzyme assay

on an immunoassay system analyzer, Immulite 1000, with assay protocols referred to as Siemens, Erlangen, Germany.

Statistical analyses

Software from IBM, Inc., version 21.0 of SPSS, had been used for the statistical analysis. Quantitative data (such as body mass index, age, and serum homocysteine level) were expressed as the mean and standard deviation. We applied the Kruskal -Wallis test for quantitative data and the Chi-Square test for qualitative variables to compare the differences between the three investigation groups. When $P < 0.05$, the difference was determined to be statistically significant.

Results

The results from comparison tests of qualitative variables and quantitative data showed no statistically significant differences between the examined groups. There were no significant differences between the investigated groups in serum homocysteine levels ($p > 0.773$). The results are presented in Table 1.

Table 1. Clinical characteristics of women with RM divided according to type of mutation at the MTHFR loci.

	Heterozygous n=17	**Homozygous n=13**	**Control group n=28**	**p-value**
Age (years)	26±5.3	24±6.2	25±5.8	0.368
BMI kg/m^2	22.3±1.3	23.3±1.0	23.6±0.6	0.773
Smoking (n)	10	5	13	0.173
Alcohol (n)	2	1	1	0.77
Level of homocysteine µmol/L	13.94±7.16	13.12±6.91	12.4±7.32	0.773

The highest percentage (35%) of elevated serum homocysteine levels was found in compound heterozygous. The results are presented in Figure 1.

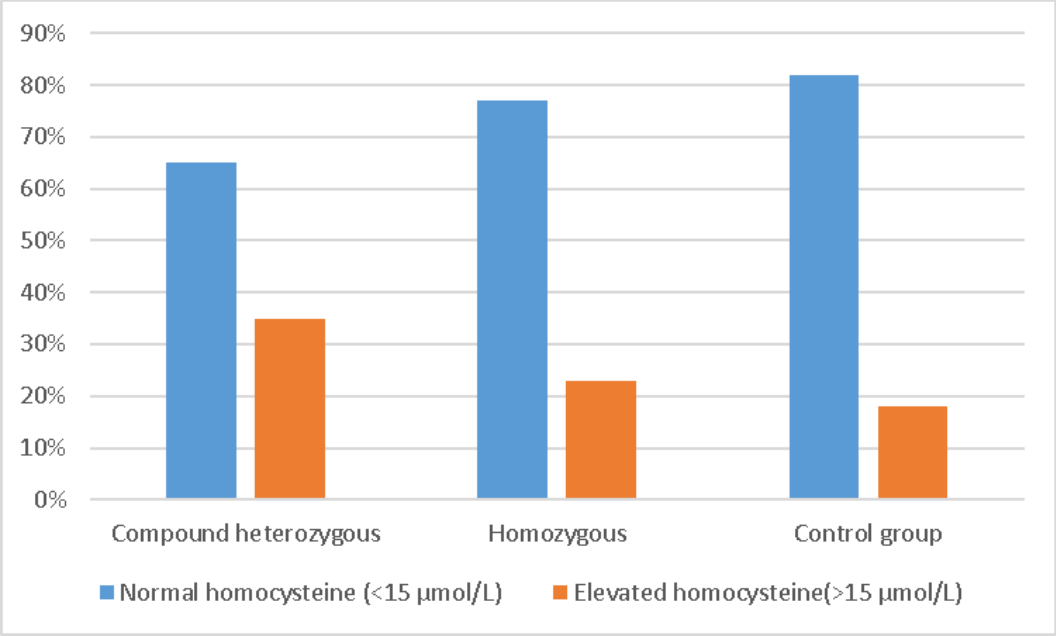

Figure 1. Level of homocysteine in groups of women with RM divided according to type of mutation at the MTHFR loci.

Discussion

The cause of recurrent miscarriage is due to many factors. Data has indicated that mothers who are older, overweight, and have poor lifestyle habits (smoking and alcohol abuse) are high-risk factors for spontaneous abortion.

In recent years, many studies have examined the relationship between spontaneous abortion risk and MTHFR gene polymorphisms. Some of them reported an association between these polymorphisms and spontaneous abortion, while others did not find a correlation [5].

More recent studies have shown that the occurrence of recurrent miscarriages of unknown cause is statistically significantly associated with polymorphisms at the MTHFR loci C677T and A1298C. The heterozygosity of the MTHFR C667T and A1298C mutations is associated with decreased enzyme activity and increased blood homocysteine [3, 6].

Several researchers have recently proposed that the mutation may be responsible for certain women's recurring miscarriages. The theory emphasizes the potential impact genetics may play in otherwise unexplained pregnancy loss, even though it has not yet been proven [4].

Our study did not find statistically significant differences between homozygous, compound heterozygous, and healthy women and the serum homocysteine level. However, a high percentage (35%) of compound heterozygous women had elevated serum homocysteine levels. In the study of Zammiti et al., it was concluded that there is no association between total serum homocysteine levels and the risk of RM. Creus et al. also found no association between the risk of RM and serum homocysteine levels and/or the MTHFR gene mutation [7, 8].

However, in a few recent studies, it was found that there was an association between hyperhomocysteinemia and RM. Juan et al.'s study found that patients who experienced RM had higher serum homocysteine levels than the controls, which was statistically significant. This study concluded that high serum homocysteine levels may be an essential risk factor for RM, indicating that homocysteine may be useful as a non-invasive marker for diagnosing RM [9].

In one prospective study, it was also found that hyperhomocysteinemia is associated with RM [10]. A cross-sectional case-control study conducted in Saudi Arabia found hyperhomocysteinemia to be a potential risk factor for RM [11].

The limitation of our study is the fairly small sample size of the study population. Consequently, further studies on more extensive series are needed to understand better the association between serum homocysteine level, mutations on the MTHFR gene, and recurrent miscarriage.

Conclusion

Serum homocysteine levels and methylenetetrahydrofolate reductase C677T and A1298C gene polymorphisms are not associated with idiopathic recurrent miscarriage.

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