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Role of Prothrombin and Methylenetetrahydrofolate Reductase Gene Polymorphisms as well as Thrombophilia Markers, as Risk Factors for Unexplained Recurrent Miscarriage: A Case-Control Study

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Abstract

Background: Unexplained recurrent miscarriage (RM) is still an unsolved reproductive health problem. Inherited thrombophilias have been one of the causes. Mutation in genes encoding coagulation proteins, including prothrombin (PT G20210A) and methylenetetrahydrofolate reductase (MTHFR) genes, increase tendency for venous thromboembolism. This study aimed to evaluate association between polymorphisms in prothrombine and MTHFR genes with RM. We also evaluated association between protein C (PC), protein S (PS), antithrombin III (ATIII), and homocystiene with RM.

Materials and Methods: We conducted a case-control study on women with history of miscarriages and healthy controls. Genetic analysis was done using (TaqMan) polymerase chain reaction (PCR) technique and the other tests were performed to check general health indications and thrombophilia markers.

Results: In this study, 195 RM group (group I) participants and 90 healthy controls (group II), PC, PS, ATIII deficiency and Hyperhomocysteinemia were in 7.2, 65.6, 9.2, 10.8% of group I respectively, but was 1.1, 7.8, 2.2, 2.2% of group II. PT *G20210A* showed two in group I were A/G, no A/G in group II, and no AA carrier in the either group. G allele was observed in 99.5% of the group I and 100% of the group II, while A allele was detected in 0.5% of group I. MTHFR *C677T* gene showed C/T mutation in 33.3% of group I and 32.2% of group II, while T/T mutation was detected in 12.8% of group I and 8.9% of the group II. C allele was found in 70.5% of group I and 75% of group II, while T allele was found in 29.5% of group I and 25% of group II (P=0.269).

Conclusion: PT *G20210A* and MTHFR *C677T* gene mutations are not correlated with RM in the Egyptian population. However, Egyptian women with RM are strongly associated with hyperhomocysteinemia, PC, PS, and ATIII deficiencies (registration number: NCT03209063).

Keywords: Antithrombin III, Protein C, Protein S, Recurrent Miscarriage

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Introduction

Exchange of nutrients, gases and other metabolites during pregnancy depends on the connection between the placenta and maternal circulatory system. Recurrent miscarriage (RM) may be caused by abnormal blood coagulation in the small blood vessels of the placenta (1). American Society for Reproductive Medicine (ASRM) defines RM as "two or more failed clinical pregnancies" (2). Inherited thrombophilia is characterized by an increased tendency for venous thromboembolism as result of mutations in the gene encoding a protein involved in the coagulation cascade. These include methylenetetrahydrofolate reductase (MTHFR) mutation, antithrombin III (ATIII) deficiency, protein C (PC) and protein S (PS) deficiency (PSD), prothrombin gene (PT *G20210A*) mutation, and factor V Leiden (FVL) (3). In the PT *G20210A* variant, adenine replaces guanine at nucleotide position of 20210 in the 3'-untranslated region of the gene. Increases of the

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amount and activity of PT in blood plasma are associated with the GA genotype and increased risk of thromboses (4). The MTHFR gene encodes MTHFR protein, the rate-limiting enzyme in the methyl cycle. *C677T* (rs1801133) and *A1298C* (rs1801131) are two of the most extensively studied single nucleotide polymorphisms (SNPs) (5).

Deficient MTHFR enzyme activity, often the result of inherited mutations, is a leading cause of hyperhomocysteinemia (6). In patients with previous abortion history, MTHFR polymorphism was shown to have a substantial effect. Hyperhomocysteinemia, leading to a hypercoagulable condition, is the leading cause of early pregnancy loss. Sperm quality and quantity, as well as reduced ovarian reserve, are affected by MTHFR isoforms (7). Liver cells produce a glycoprotein called PC that requires vitamin K for the proper functioning. Disseminated intravascular coagulation and massive thrombosis are the two main manifestations of PC deficiency (8). A seven-fold increase in thrombotic risk was observed in patients with PC deficiency. While in terms of PS, a vitamin K-dependent glycoprotein, the risk ratio of thrombosis was 8.5 times more than the normal individuals (9). Women with PS deficiency are more likely to have a VTE during pregnancy or puerperium, and their risk of RM was three times higher than that of the general population (10). The vitamin K-independent glycoprotein AT is essential in the clotting cascade (11). Approximately 60% of cases with AT deficiency occur spontaneously, and this condition is passed down in an autosomal dominant manner, making carriers more likely to have thrombosis and VTE. If a woman has a history of VTE, her risk of developing thrombus during pregnancy will rise from 31 to 50% because of her AT deficit (12). The current study aimed to evaluate association of polymorphisms in the PT and MTHFR genes with RM among the Egyptian population. Additionally, we studied the prevalence of thrombophilia markers, including homocysteine, PC, PS, and ATIII, in patients with RM in the Upper Egypt.

Material and Methods

Study design and patients

A case-control pre-registered study (NCT03209063) recruited women from the Clinical Pathology Department, Woman's Health Hospital, Assuit University Hospital (Assuit, Egypt) from December 2019 to May 2022. Assuit Medical School Ethical Review Board approved the study number (17200095). We recruited women in the intervention group who were diagnosed to have RM (as defined by the ASRM) as "two or more failed clinical pregnancies" and accepted to participate in the study, while they were less than 35 years old. Conversely, the control group consisted of healthy females under 35 years old who had no miscarriage history and they had at least one problem-free full-term pregnancy. Women with unregulated diabetes mellitus, hyperthyroidism, autoimmune conditions like antiphospholipid antibody syndrome with a LA1/LA2 ratio exceeding 1.2, and those presently using oral contraceptives or anticoagulant treatments were excluded from the both study groups.

The study protocol was approved by Assiut University Ethical Review Board. We affirmed that all study procedure complied with the Declaration of Helsinki principles. All women signed the written informed consent before enrollment. We followed the STROBE guidelines by drafting this manuscript (13).

Data collection and sampling

Eligible participants had an interview with the investigator to specially collect the following data: age, menstrual history, obstetric history (prenatal, natal, postnatal), number of previous abortions, gynecological history, contraceptive history, family history of abortion, history of deep venous thrombbsis (DVT), history of operations and drug history.

From each participant and control subject, approximately 10 ml of venous blood was collected in a fully sterile environment. The collected sample was then distributed as follows: 1 ml of the blood was placed in an EDTA-containing tube for a complete blood count (CBC), 2 ml of the blood was replaced in the other EDTA-containing tube for genotyping, and 3 ml of the blood replaced in an anticoagulant-free tube for random blood glucose, kidney and liver function tests, as well as thyroid stimulating hormone (TSH) evaluation. The remaining 4 ml of blood was put into citrated tubes for the measurement of PC, PS, and ATIII. The CBC and kidney/liver function tests were performed using the CELL-DYN RUBY (Abbott, USA) and Cobas c311 (Roche, Germany). The coagulation profile was assessed using the auto analyzer Sysmex CA-1500 (Siemens, Germany), while the serum TSH was analyzed using Vidas (Biomerieux, France). The PC, PS, and ATIII were analyzed by auto-analyzer Sysmex CA-1500. Homocystiene was analyzed by ADVIA Centaur XPT (Siemens, Germany).

In terms of the genetic analysis, DNA was isolated from plasma samples for evaluation of PT gene *G20210A* and MTHFR *C677T* gene SNP. The extraction was carried out using Applied Biosystems 7500 Fast Real-Time PCR systems (Applied Biosystems, USA) and a Genejet Whole Blood genomic DNA purification mini kit (Cat. No. K0781) provided by Thermo Fisher Scientific, USA. To ensure consistency, all DNA samples were diluted with nuclease-free water to yield a concentration between 1-20 ng per well, with a recommended minimum final concentration of 0.2 ng/µl.

The study employed the 5'-Nuclease Assay (TaqMan) PCR technique. This method was composed of a specific TaqMan MGB probe annealing to its complementary sequence between the forward and reverse primer sites. The AmpliTaq Gold DNA polymerase, with its 5'-nuclease activity, cleaved probes hybridized to the target sequence, separating the quencher dye from the reporter dye and leading to an increase in fluorescence. This fluorescence indicated which alleles were present in the sample. The PCR process involved preparing

the reaction mix, DNA samples, and the reaction plate, followed by running the PCR and post-PCR analysis on a real-time PCR instrument. The software of the real-time PCR instrument analyzed fluorescence signals from each well, represented as Rn values, to identify the specific alleles in each sample. These results were obtained from the amplification reactions performed during the allelic discrimination genotyping assay.

Study outcomes

The primary outcome of this study was to examine occurrence of PT and MTHFR gene polymorphisms in women with RM in comparison to the healthy control group. In addition, secondary objectives included detecting thrombophilia markers, such as PC (normal range: 70-140%), PS (normal range: 60-130%), ATIII (normal range: 79.4-112%), and homocysteine (normal range: 3.7 to 13.9 µmol/l) in the both groups of women.

Sample size and data analysis

In our study, the percentage of patients with RM with PT gene polymorphism is the primary outcome. Previous studies showed that this percentage was (10.9%) (14). Additionally, percentage of this genetic polymorphism in the control healthy population was 1.06% (15). We recruited 180 patients, 90 in each group ratio of 1:1, with a risk ratio of 0.09. However, due to the scarcity of positive cases in the interim of data analysis, we doubled the sample size of cases to perform our analysis in 195 cases and 90 controls. Data analysis was performed using statistical package for the social science (IBM-SPSS) version 25.0 software. Statistics were generated for categorical data in the form of frequencies and percentages, and Shapiro – Wilk tests were used to assess normality of numerical variables, presenting these as either median (range) for non-normal distribution or mean ± SD for normal distribution. Comparative analyses between groups were conducted using Chi-square, Fisher Exact tests, and Mann Whitney U tests for proportions and median differences, while Spearman's correlation was utilized to find relationships between continuous variables. A P<0.05 was deemed statistically significant.

Results

We recruited 195 cases in the RM group (group I) and 90 controls (group II, Fig.1). The median age was 26 (16-45) years old among the group I and 27 (19-35) years old among the group II. Nearly 52.3 and 52.2% were urban in the group I and group II, respectively. Besides, 9.2% of the group I and 4.4% of the group II had family history of VTE. No statistically significant difference was determined for the all previous parameters between the both groups. About 44% had history of >2 previous abortions. Regarding the time of previous abortion, 75.9% of the first abortion occurred in ≤12 weeks. In terms of the second abortion, 79.9% occurred in ≤12 weeks. Of more than three abortions, 77.8% occurred in ≤12 weeks and 22.2 % in >12 weeks (Table 1).

Table 1: Demographic and clinical characteristics of the included women

Variables	Group I (n=195)	Group II (n=90)	P value
Age (Y)			
<20	10 (5.1)	2 (2.2)	0.525*
20-30	118 (60.5)	56 (62.2)	
>30	67 (34.4)	32 (35.6)	
Median (range)	26.0 (16-35)	27.0 (19-35)	0.080**
Residence			
Urban	102 (52.3)	47 (52.2)	0.989*
Rural	93 (47.7)	43 (47.8)	
Consanguinity			
Yes	58 (29.7)	18 (20.0)	0.084*
No	137 (70.3)	72 (80.0)	
Family history of VTE			
Yes	18 (9.2)	4 (4.4)	0.159*
No	177 (90.8)	86 (95.6)	
Number of previous abortions			
2 abortions	109 (55.9)		
>2 abortions	86 (44.1)		
Time of abortion			
First abortion			
≤12 weeks	148 (75.9)		
>12 weeks	47 (24.1)		
Second abortion (n=194)			
≤12 weeks	155 (79.9)		
>12 weeks	39 (20.1)		
Third abortion (n=87)			
≤12 weeks	75 (86.2)		
>12 weeks	12 (13.8)		
More than 3 (n=36)			
≤12 weeks	28 (77.8)		
>12 weeks	8 (22.2)		

Data were expressed as median (range) or frequency (%). *; Chi-square/Fisher Exact tests were used to compare proportion between the groups, **; Mann-Whitney U test was used to compare the median between groups. P value is considered significant when <0.05. Group I (cases); Women having a history of two or more miscarriages, Group II (controls); Healthy controls with no history of miscarriage and at least one uncomplicated full-term pregnancy, and VTE; Venous thromboembolism.

In terms of laboratory characteristics, the median range of white blood cell (WBC) count in the group I was 5.9 (2.47-13.60), compared to 5.79 (2.47-10.50) in the group II. The median PT time level in the group I was 12.2 seconds (10.1-15.4), and in the group II was also 12.2 (10.5-14.5). The median APTT in the group I was 31.6 (22.5-43.1) seconds, and in group II was also 31.65 seconds ranging from 22.5-43.1. The median TT in the group I was 18.00 (12.2-35.0), and it was 17.85 (12.2-35.0) in the group II. Lastly, the median Fibrinogen in the group I was 3.20 (1.7-6.2), and it was also 3.20 (2.0-5.8) in the group II. No statistically significant difference of the all previous parameters was detected between the group I and group II (Table 2).

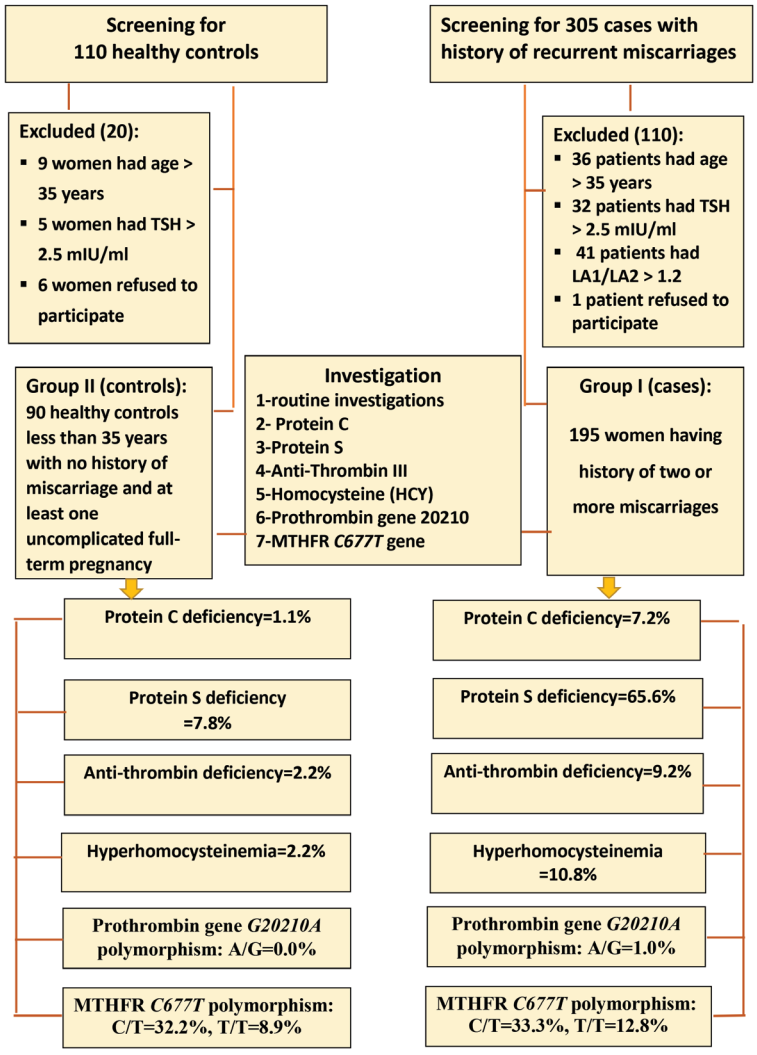

Fig.1: Study flow-chart. TSH; Thyroid stimulating hormone.

Table 2: Laboratory characteristics of the included women

Median (range)	Group I (n=195)	Group II (n=90)	P value*
WBC	5.9 (2.47-13.60)	5.79 (2.47-10.50)	0.096
HB (g/dl)	12 (8.5-14.6)	12.2 (8.5-13.9)	0.227
Platelets ($\times 10^3/\mu l$)	282.70 (161.10-430.20)	290.00 (163.10-430.00)	0.275
Prothrombin time	12.20 (10.1-15.4)	12.20 (10.5-14.5)	0.810
Prothrombin concentration (%)	103.00 (79.0-174.7)	102.15 (84.0-143.0)	0.794
INR	1.00 (0.83-1.19)	1.00 (0.86-1.15)	0.433
APTT (Seconds)	31.60 (22.5-43.1)	31.65 (22.5-43.1)	0.996
TT	18.00 (12.2-35.0)	17.85 (12.2-35.0)	0.290
Fibrinogen	3.20 (1.7-6.2)	3.20 (2.0-5.8)	0.648

Data are expressed as frequency and %. *; Mann Whitney U test was used to compare the median difference between the two groups. P value is considered significant when <0.05. Group I (cases); Women having a history of two or more miscarriages, Group II (controls); Healthy controls with no history of miscarriage and at least one uncomplicated full-term pregnancy, WBC; White blood cells, INR; International normalized ratio, APTT; Activated partial thromboplastin time, and TT; Thrombin time.

PC deficiency in the group I was 7.2% compared to 1.1% in the group II. PS deficiency in the group I was 65.6% compared to 7.8% in the group II. ATIII deficiency in the group I was 9.2% compared to 2.2% in the group II. Hyperhomocysteinemia in the group I was 10.8% compared to 2.2% in the group II. There was a statistically significant difference between the group I and group II for the all above parameters (Fig.2).

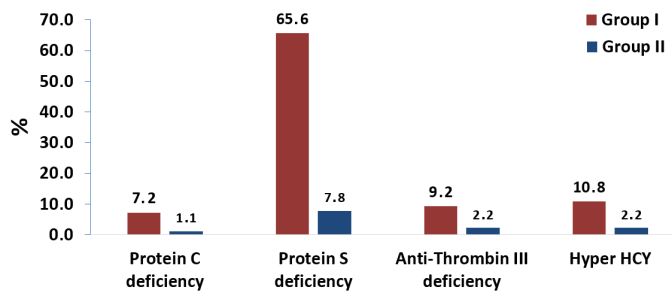

Fig.2: Comparison of Thrombophilia markers (protein C, protein S, antithrombin III and homocysteine) between the group I and group II. HCY; Homocysteine.

In terms of PT gene *G20210A*, the wildtype genotype (G/G) was present in 99.0% of the group I and 100% of the group II, while heterozygote alleles (G/A) was present in only two patients among the group I compared to no participants among the group II. There was no mutante genotype (AA) either in the group I or group II. Allele "G" was present in 99.5% of the group I and 100% of the group II, while Allele "A" was present in 0.5% of the group I and not present in the group II. Regarding the MTHFR *C677T* gene, wildtype genotype (C/C) was detected in 53.8% of the group I and 58.9% of the group II. Heterozygote alleles (C/T) was present in 33.3% of the group I and 32.2% of the group II. Homozygote mutation (T/T) presented in 12.8% of the group I and 8.9% of the group II. Allele "C" was present in 70.5% of the group I and 75 % of the group II, while Allele "T" was present in 29.5% of the group I and in 25% of the group II. No statistically significant difference in distribution of PT gene G20210A, MTHFR *C677T* gene and their alleles between the group I and group II was detected (Table 3).

Table 3: Comparison of prothrombin gene *G20210A* and MTHFR *C677T* between group I and group II

Variables	Group I (n=195)	Group II (n=90)	P value*
Prothrombin gene *G20210A*			
Wildtype G/G	193 (99.0)	90 (100.0)	1.000
Heterozygote A/G	2 (1.0)	0 (0.0)	
Prothrombin gene *G20210A* allele			
G (wildtype)	388 (99.5)	180 (100.0)	0.935
A (mutant)	2 (0.5)	0 (0.0)	
MTHFR *C677T* gene			
Wildtype C/C	105 (53.8)	53 (58.9)	0.569
Heterozygote C/T	65 (33.3)	29 (32.2)	
Homozygote mutant T/T	25 (12.8)	8 (8.9)	
MTHFR *C677T* gene allele			
C (wildtype)	275 (70.5)	135 (75.0)	0.269
T (mutant)	115 (29.5)	45 (25.0)	

Data are expressed as frequency and %. *; Chi-square test and Fisher Exact test were used to compare proportion between the groups. P value is considered significant when <0.05. Group I (cases); Women having a history of two or more miscarriages and Group II (controls); Healthy controls with no history of miscarriage and at least one uncomplicated full-term pregnancy.

Discussion

In this comparative study, we could not find an association of PT gene *G20210A* or MTHFR *C677T* gene polymorphisms with RM in the population of Upper Egypt. On the other hand, thrombophilia markers were positively correlated with RM, compared to the healthy population. Although age may affect incidence of RM, our eligibility criteria were limited to less than 35 years old, which nullified the effect of age on RM. Rate of family history of VTE ranged from 4.4 and 9.2%, with no statistically significant difference between cases and control groups. In the presented study, about 56% of the cases had history of two previous abortions, and 44% had history of >2 previous abortions. Research conducted by Ogasawara and his team noted a rise in the rate of miscarriages from 25 to 80%, as the number of previous miscarriages increased from 2 to 7 or more, predominantly due to a growing occurrence of miscarriages with a standard karyotype. On the other hand, incidence of miscarriages with chromosomal abnormalities remained consistent (16).

Prevalence of PC deficiency in our RM patients was substantially greater than the control group (7.4 vs. 1.1%, respectively). Likewise, Jyotsna et al. (17) showed a statistically significant increase in PC deficit among RM patients compared to the healthy controls (33.3 vs 3.3%, respectively). Hansda and Roychowdhury (18) conducted another Indian investigation on 53 RM cases and 47 healthy age-matched controls, and they found that 15.09% of the RM patients had a deficit PC. On the other hand, Osman and Abulata (8) could not detect statistically significant difference in PC levels of the RM patients and control. Our results differ from those of the Osman and Abulata study. This may be since we recruited larger sample size and included women who experienced both first- and second-trimester RM, whereas they only included women who experienced RM in the first trimester.

Sixty-five percent of our recruited patients in the RM group were shown to have a significantly lower level of PS compared to 7.8% in the control group. Similarly, PS deficiency was observed to be more common in RM patients than the controls (17, 19). Parand et al. (20) found a significant association of RM with PS deficiency in a sample of 90 patients who had experienced three or more consecutive miscarriages with the same partner at less than 20 weeks of gestation. A study by Matsukawa et al. (21) on 355 Japanese women with RM and 101 parous women indicated that PS deficiency did not act as a reliable clinical predictor of RM. The discrepancy between their findings and ours might be attributed to the fact that lupus anticoagulant was not taken into account in their research. In addition, Mekaj et al. observed no statistically significant difference between 104 cases of RM in the first trimester and 110 controls, in their research conducted in Kosovo (22). Disagreements with our findings may be attributable to differences in study population age and ethnicity.

Deficiency in AT was observed in 9.2% of the RM group vs. 2.2% of the control group. This finding is consistent with that of Jyotsna et al. (17), who also reported a statistically significant correlation between the mean value of AT in the patient and control groups. On the other hand, Mekaj et al. (22) did not find a significant difference in terms of AT deficiency between the RM and the control groups. In the present study, we found a statistically significantly higher level of homocysteine in the RM group than the control group, as hyperhomocysteinemia in cases with RM was 10.8% compared to 2.2% of the control group. It was statistically significant in harmony with Abd-Ellatef et al. (23) study as the mean homocysteine level was higher in the RM group than the control group. In

the same line, Nelen et al. (24) and Klai et al. (25) found that elevated homocysteine level was a risk factor for recurrent early pregnancy losses. During pregnancy, level of homocysteine was typically decreased. High concentrations of homocysteine might be linked with fetal abnormalities and potential issues with the blood vessels in the placenta, which could lead to abruption (26). There is a growing understanding of the role of increased homocysteine, as an independent risk factor for the both arterial and venous thrombosis. The suggested pathogenic mechanisms included elevated levels of asymmetric dimethylarginine, impaired methylation, oxidative damage to the endothelium due to suppression of the vasodilator nitric oxide, promoting platelet activation and aggregation, vascular smooth muscle proliferation, and disruption of the usual balance between procoagulants and anticoagulants, favoring thrombosis (27).

Regarding the PT gene *G20210A*, we could not find any significant difference between the both groups. Similarly, Ashour and Sharif demonstrated that the "A" allele was more prevalent in RM patients (2.25%), as compared to controls (0.75%) (28). Parand et al. (20) showed that there was no homozygote case for the PT *G20210A* polymorphism sample. Osman and Abulata (8) identified the PT gene *G202010A* mutation in 11% of the case subjects and 2% of the control subjects, but the difference was not statistically significant. Similarly, Nassour-Mokhtari and colleagues discovered the PT *G20210A* mutation in the both of RM and control groups, in a heterozygous form, and found no association between this mutation and RM (29). The difficulty in identifying a link between this polymorphism and RM may be due to the low frequency of the minor "A" allele in our groups. Results may vary if the sample size is larger. Warren et al. who investigated whether women with the *G20210A* mutation in PT were at increased risk of RM, came to the same conclusion. For this study, investigators enrolled 5188 pregnant women and reviewed the results of 4167 blood samples collected during the first trimester to screen for the *G20210A* gene mutation. The conclusion of their study revealed no association between the PT gene mutation *G20210A* and RM (30). However, a comprehensive meta-analysis incorporating 37 case-control studies indicated an elevated risk of RM linked to the *G20210A* PT mutation, particularly noticeable in European women and those aged above 29 years (31).

Our research showed no significant disparity in the MTHFR *C677T* gene, including Wildtype genotype (C/C), heterozygote (C/T), and homozygote-mutant (T/T), between the two groups. Furthermore, there was no significant difference in the frequencies of Alleles “C” and “T” in the both groups. A separate study involving 133 women with a history of three or more consecutive miscarriages before the 20th week of pregnancy revealed allele frequencies for “T” at 34.6% of the cases and 21.6% of the control group, with higher homocysteine levels noted in women carrying the mutant allele (32). As for genotype frequencies, Zarfeshan Fard et al. (33) noted that 30% of the RM group carried “T/T” genotype for the MTHFR 677T gene, in contrast to 8% in the control group. Furthermore, 40% of women in the RM group exhibited “C/T” genotype, associated with elevated homocysteine levels compared to the control group. However, a meta-analysis performed by Chen et al. (34), including 16 articles, found that MTHFR *C677T* was substantially related to RM risk in the Chinese population across the all genetics models. In the previous studies, MTHFR *C677T* polymorphism has been linked to an increased risk of RM. A study performed by Luo et al. (35) on 136 women with a history of two or more spontaneous abortions found that women with MTHFR *C677T* gene may be more likely to experience RM. According to their findings, people with “C/T” or “T/T” genotype should increase their consumption of folic acid supplements to avoid miscarriage, and MTHFR *C677T* might serve as an early genetic screening signal for RM. It is possible that folic acid supplementation during pregnancy, particularly in the first trimester, had something to do with this. Homozygotes for the MTHFR gene have a much lower homocysteine threshold, and this is in large part due to their elevated folate levels. This led researchers to hypothesize that women with two MTHFR mutation variants would benefit from taking folic acid to mitigate the negative consequences of this mutation and lower their risk of RM (36). Zhu et al. (37) investigated MTHFR polymorphisms in 370 Chinese women with RM and found that the MTHFR *C677T* variant was more common in this population. In their analysis of 100 Iranian women, Zarfeshan Fard et al. (33) found that the MTHFR *C677T* variant was much more common among those with RM, suggesting that it could be a risk factor for miscarriage. In addition, Osman and Abulata (8) reported that MTHFR was the most frequently detected gene deficiency in both of the case and control groups (63 and 41.9%, respectively). However, the limited sample size and use of an unconventional approach (FV-PTH-MTHFR strip assay) might account for these discrepancies in pregnancy outcomes. According to a study performed by Bigdeli et al. (38), homozygous frequencies of MTHFR *C677T* mutations were also elevated among Iranian women who had RM. The discrepancy across the studies might be due to factors like the diversity of the studied populations, sample sizes, and research designs or methods.

We acknowledge that our study has some limitations, including lack of adequate data to perform a subgroup analysis regarding PT gene *G20210A* polymorphism. Further, we could not perform more extensive studies of MTHFR variants and other genetic mutations that may be associated with RM, due to the limited financial resources. Additionally, we did not measure vitamin B12 or folic acid serum levels in patients with hyperhomocysteinemia. Multicenter trials with larger sample sizes and different ethnic groups are required.

Conclusion

PT gene *G20210A* and MTHFR *C677T* gene

polymorphisms are not correlated with RM in the Egyptian population. About 70% of women in the Upper Egypt have at least one type of MTHFR *C677T* gene polymorphism. However, Egyptian women with RM are strongly associated with hyperhomocysteinemia, PC, PS, and ATIII deficiencies.

Acknowledgments

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Authors' Contributions

Z.A.E.; Contributed to protocol development, Interpretation of the data, and Data analysis. H.G.A.E., O.M.S.; Suggested the research idea, Responsible for the study conception, Design, and Revised the manuscript. M.I.S., A.A.; Contributed to the study design, Interpretation of the data, and Data analysis. T.F., Gh.M.; Participated in data collection, Data analysis, and Manuscript writing. All authors performed editing and approved final version of the manuscript for submission.

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