The Role of Prothrombin Gene and Methylenetetrahydrofolate Reductase Gene Polymorphisms and Thrombophilia Markers as Risk Factors for Recurrent unexplained Miscarriage

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Abstract

Background: Recurrent unexplained miscarriage is still an unsolved reproductive health problem. Inherited thrombophilias have been accused as one of the causes. Secondary to an increased tendency for venous thromboembolism because of a mutation in a gene encoding a protein involved in the coagulation cascade. These include prothrombin gene (PT G20210A) and methylenetetrahydrofolate reductase (MTHFR) mutations. The study aims to evaluate the association between polymorphisms in the prothrombin gene and the MTHFR gene with recurrent miscarriage (RM). We also evaluated the association between Protein C (PC), Protein S (PS), Antithrombin III (ATIII), and homocystiene with recurrent miscarriage (RM).

Methods: We conducted a comparative study on women with a history of two or more miscarriages and healthy controls with no history of miscarriage and who had at least completed one full-term normal pregnancy. Genetic analysis of the participants was done using the 5' Nuclease Assay (TaqMan) PCR technique and various other blood tests were performed to check general health indicators and thrombophilia markers.

Results: In this study of 195 RM group (Group I) participants and 90 healthy controls (Group II), we noted significant discrepancies in health conditions. PC deficiency occurred in 7.2% of Group I, but only 1.1% of Group II. PS deficiency was found in 65.6% of Group I versus 7.8% of Group II. ATIII deficiency was observed in 9.2% of Group I and 2.2% of Group II. Hyperhomocysteinemia was noted in 10.8% of Group I, and 2.2% of Group II. For the prothrombin gene G20210A, two Group I participants were A/G, with no A/G in Group II, and no AA carriers in either group. G allele was in 99.5% of Group I and 100% of Group II, while the A allele was in 0.5% of Group I only. MTHFR C677T gene showed C/T mutation in 33.3% of Group I and 32.2% of Group II, and T/T mutation in 12.8% of Group I and 8.9% of Group II. The C allele was found in 70.5% of Group I and 75% of Group II, with the T allele in 29.5% of Group I and 25% of Group II (p=0.269).

Conclusion: Prothrombin gene G20210A and MTHFR C677T gene polymorphisms are not correlated with RM in the Egyptian population. About 70% of women in upper Egypt have at least one type of MTHFRC677T gene polymorphism. However, Egyptian women with RM are strongly associated with hyperhomocysteinemia, PC, PS, and AT deficiencies.

Keywords: Prothrombin gene; Methyltetrahydrofolate reductase; Thrombophilia markers; Recurrent miscarriage.

1. Background

The exchange of nutrients, gases, and other metabolites during pregnancy depends on the connection between the placenta and the maternal circulatory system. Recurrent miscarriages (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 a result of a mutation in a gene encoding a protein involved in the coagulation cascade. These include methylenetetrahydrofolate reductase (MTHFR) mutation, antithrombin III (ATIII) deficiency, protein C and protein S deficiency (PSD), prothrombin gene (PT G20210A) mutation, and factor V Leiden (FVL) (3). In the prothrombin G20210A variant, adenine replaces guanine at nucleotide position 20210 in the 3' untranslated region of the gene. Increases in the amount and activity of prothrombin in blood plasma are associated with the GA genotype and increased risk of thromboses (4). The MTHFR gene encodes MTHFR, 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 a 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 protein C (PC) that requires vitamin K for proper functioning. Disseminated intravascular coagulation or massive thrombosis are the two main manifestations of a PC deficiency (8). A seven-fold increase in thrombotic risk was observed in patients with

PC deficiency. While in terms of protein S (PS), a vitamin K–dependent glycoprotein, the risk ratio of thrombosis is 8.5 times more than in normal individuals (9). Women with PS deficiency are more likely to have a VTE during pregnancy or puerperium, and their risk of RM is three times higher than that of the general population (10). The vitamin K-independent glycoprotein antithrombin (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 a thrombus during pregnancy rises from 31% to 50% because of her AT deficit (12). The current study aims to evaluate the association between polymorphisms in the prothrombin gene and the MTHFR gene with RM among the Egyptian population. Additionally, we studied the prevalence of thrombophilia markers, including Homocysteine, PC, PS, and AT III, in patients with RM in Upper Egypt.

2. Materials and Methods

Study Design and Patients:

A comparative study pre-registered study (NCT03209063) recruited women from the Clinical Pathology Department, Woman's Health Hospital, Assuit University Hospital, Assuit University during the period from December 2019 through May 2022. Assuit Medical School, Ethical Review Board, had 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 and were less than 35 years old. Conversely, the control group consisted of healthy females under 35 who had no miscarriage history and 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 both study groups.

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

Data Collected and Sampling:

Eligible participants had an interview with the investigator during which the following data were collected included age, menstrual history, obstetric history (prenatal, natal, postnatal), number of previous abortions, gynecological history, contraceptive history, family history (history of abortion in the family, history of DVT), history of operations and drug history were specifically considered.

From each participant and control subject, approximately ten ml of venous blood was collected in a fully sterile environment. The collected sample was then distributed as follows: one ml was placed in an EDTA-containing tube for a complete blood count (CBC), two ml in another EDTA-containing tube for genotyping, and three ml in an anticoagulant-free tube for random blood glucose, kidney and liver function tests, and 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 (Siemens –Germany). Homocystiene was analyzed by ADVIA Centaur XPT (Siemens -Germany).

In the genetic analysis, DNA was isolated from plasma samples for the evaluation of Prothrombin gene G20210A and MTHFR C677T gene single nucleotide polymorphisms (SNP). The extraction was carried out using a real-time PCR Fast 7500 Applied Biosystems and a Genejet Whole Blood genomic DNA purification mini kit -Cat. No. (K0781) provided by Thermo Fisher Scientific, Waltham, MA, 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 involves 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, cleaves probes that have hybridized to the target sequence, separating the quencher dye from the reporter dye and leading to an increase in fluorescence. This fluorescence indicates which alleles are present in the sample. The PCR process involves 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, which are represented as Rn values, to identify the specific alleles in each sample. These results are obtained from the amplification reactions performed during the Allelic discrimination genotyping assay.

Study outcomes:

The primary outcome of this study was to examine the occurrence of prothrombin gene and MTHFR gene polymorphisms in women with recurrent miscarriages in comparison to a 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 both groups of women..

Sample size and data analysis:

Taking the percentage of patients with RM with prothrombin gene polymorphism as a primary outcome. Previous studies showed that the percentage was (10.9%) (Salim Sehirali et al., 2005). Additionally, the percentage of this genetic polymorphism in the control healthy population was (1.06%) (Arzu Ulu et al., 2006). Considering a confidence level of 95%, 80% power, and recruited 180 patients, 90 in each group ratio of 1:1, with a risk ratio of 0.09 (Epinfo 2019). 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 were analyzed using the Statistical Package for Social Science (SPSS) version 25 software for Windows. Statistics were generated for categorical data in the form of frequencies and percentages, and Shapiro–Wilk tests were used to assess the 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 value less than 0.05 was deemed statistically significant.

3. Results

We recruited 195 cases in the RM group (Group I) and 90 controls (Group II) (**Figure 1**). The median age was 26 (16-45) years among Group I and 27 (19-35) years among Group II. Nearly 52.3% and 52.2% were urban in Group I and Group II, respectively. Besides, 9.2% of Group I and 4.4% of Group II have a family history of VTE. No statistically significant difference between Group I and Group II in all previous parameters. About 44% have a history of > 2 previous abortions. Regarding the time of previous abortion, in the first abortion, 75.9% occurred in ≤ 12 weeks. In 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**).

In terms of laboratory characteristics, the median range of WBC count in Group I was 5.9 (2.47-13.60), compared to 5.79 (2.47-10.50) in Group II. The median prothrombin time level in Group I was 12.20 (10.1-15.4), and in Group II was also 12.20 (10.5-14.5). The median APTT in Group I was 31.6 (22.5-43.1) second, and in Group II was also 31.65 and ranged from 22.5-43.1. The median TT in Group I was 18.00 (12.2-35.0), and in Group II was also 17.85 (12.2-35.0). Lastly, the median Fibrinogen in Group I was 3.20 (1.7-6.2), and in Group II was also 3.20 (2.0-5.8). No statistically significant difference between Group I and Group II in all previous parameters, **Table 2**.

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

In terms of prothrombin gene G20210A, the wild G/G presented in 99.0 % of Group I and in 100.0 % of Group II, while Hetero-mutant A/G presented in only two patients among Group I compared to no participants among Group II. There was no AA either in Group I or Group II. Allel G presented in 99.5% of Group I and 100.0 % of Group II, while Allel A presented in 0.5% of Group I and not presented in Group II. Regarding the MTHFR C677T gene, wild C/C presented in 53.8% of Group I and in 58.9 % of Group II, Hetero-mutant C/T presented in 33.3% of Group I and in 32.2% of Group II, and Homomutant T/T presented in 12.8% among Group I and in 8.9% among Group II. Allel C presented in 70.5% of Group I and in 75 % of Group II, while Allel T presented in 29.5% of Group I and in 25 % of Group II. No statistically significant difference in the distribution of Prothrombin gene G20210A, MTHFR C677T gene and their alleles between Group I and Group II, (**Table 3**).

4. Discussion

In this comparative study, we could not find an association between prothrombin gene G20210A or MTHFR C677T gene polymorphisms and RM in the population of Upper Egypt. On the other hand, thrombophilia markers were positively correlated with RM as compared to the healthy population. Although age may affect the incidence of RM; however, our eligibility criteria were limited to less than 35 years, which nullifies the effect of age on RM. The 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 a history of two previous abortions, and 44% had a 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, the incidence of miscarriages with chromosomal abnormalities remained consistent (14).

The prevalence of PC deficiency in our RM patients was substantially greater than in the control group (7.4% vs 1.1%, respectively). Likewise, Jyotsna et al. showed a statistically significant increase in PC deficit among RM patients compared to healthy controls (33.3% vs 3.3%, respectively) (15). Hansda and Roychowdhury 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 (16). On the other hand, Osman and Abulata could not detect a statistically significant difference in PC levels in RM patients and control (8). Our results differ from those of the Osman and Abulata study, but this may be because we recruited a much larger sample size and included women who experienced both first- and second-trimester RM, whereas they only included women who had 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 in controls by both Alshammary et al. and Jyotsna et al. (15.17). Parand et al. 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 (18). A study by Matsukawa et al. on 355 Japanese women with RM and 101 parous women indicated that PS deficiency did not act as a reliable clinical predictor of RM (19). 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 (20). 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% in the control group. This finding is consistent with that of Jyotsna et al., who also reported a statistically significant correlation between the mean value of AT in the patient and control groups (15). On the other hand, Mekaj et al. did not find a significant difference in terms of AT deficiency between the RM and the control groups (20). In the present study, we found a statistically significant higher level of Homocysteine in the RM group than in controls, as hyperhomocysteinemia in cases with RM was 10.8% compared to 2.2% in the control group, and it was statistically significant ($p=0.017$), in harmony with Abd-Ellatef et al. study as the mean homocysteine level was higher in RM group than the control group (21). In the same line, Nelen et al. and Klai et al. found that elevated homocysteine level was a risk factor for recurrent early pregnancy losses (22,23). During pregnancy, levels of homocysteine typically decrease. 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 (24). There's a growing understanding of the role of increased homocysteine as an independent risk factor for both arterial and venous thrombosis. The suggested pathogenic mechanisms include 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 (25).

Regarding prothrombin gene G20210A, we could not find any significant difference between 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%) (26). Parand et al. showed that there was no homozygous case for the prothrombin G20210A polymorphism sample (18). Osman and Abulata identified the prothrombin gene G202010A mutation in 11% of case subjects and 2% of control subjects, but the difference was not statistically significant (8). Similarly, Nassour-Mokhtari and colleagues discovered the prothrombin G20210A mutation in both recurrent miscarriage (RM) and control groups, in a heterozygous form, and found no association between this mutation and RM (27). 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 Group. Results may vary if the sample size is larger. Warren et al., who investigated whether women with the G20210A mutation in prothrombin 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 prothrombin gene mutation G20210A and recurrent miscarriage (28). However, a comprehensive meta-analysis incorporating 37 case-control studies indicated an elevated risk of recurrent miscarriage linked to the G20210A prothrombin mutation, particularly noticeable in European women and those aged above 29 years (29).

Our research showed no significant disparity in the MTHFR C677T gene, including Wild C/C, Hetero-mutant C/T, and Homomutant T/T, between the two groups. Furthermore, there was no significant difference in the frequencies of Allele C and Allele T in 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% for cases and 21.6% for the control group, with higher homocysteine levels noted in women carrying the mutant alleles (30). As for genotype frequencies, Fard et al. noted that 30% of the recurrent miscarriage group carried the TT genotype for the MTHFR 677T gene, in contrast to 8% in the control group. Furthermore, 40% of women in the recurrent miscarriage group exhibited a CT genotype, associated with elevated homocysteine levels compared to the control group (31). However, a meta-analysis by Chen et al., including 16 articles, found that MTHFR C677T was substantially related to RM risk in the Chinese population across all genetics models (32). In previous studies, the MTHFR C677T polymorphism has been linked to an increased risk of RM. A study by Luo et al. on 136 women with a history of two or more spontaneous abortions found that women with the MTHFR 677T gene may be more likely to experience RM. According to their findings, people with a CT or TT 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 (33). 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 (34). Zhu et al. investigated MTHFR polymorphisms in 370 Chinese women with RM and found that the MTHFR C677T variant was more common in this population (35). In their analysis of 100 Iranian women, Fard et al. found that the MTHFR C677T variant was much more common among those with RM, suggesting that it could be a risk factor for miscarriage (31). In addition, Osman and Abulata reported that MTHFR was the most frequently detected gene

deficiency in both the case and control groups (63% and 41.9%, respectively) (8). However, the limited sample size and the use of an unconventional approach (FV-PTH-MTHFR strip assay) might account for these discrepancies in pregnancy outcomes. According to a study by Bigdeli et al., the homozygous frequencies of MTHFR C677T mutations were also elevated among Iranian women who had RM (36). The discrepancy across 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 the lack of adequate data to perform a subgroup analysis regarding prothrombin 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

Prothrombin gene G20210A and MTHFR C677T gene polymorphisms are not correlated with RM in the Egyptian population. About 70% of women in upper Egypt have at least one type of MTHFRC677T gene polymorphism. However, Egyptian women with RM are strongly associated with hyperhomocysteinemia, PC, PS, and AT deficiencies.

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Authors' contributions

Z.A.; contributed to protocol development, interpretation of the data, and data analysis. H.G. and O.M.S. suggested the research idea, were responsible for the study conception and design and revised the manuscript. M.I.S.; and A.A.; contributed to the study design, interpretation of the data, and data analysis. T.A.F; and G.M; Participated in data collection, data analysis, and manuscript writing. All authors performed editing and approving the final version of the manuscript for submission.

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410 **Table 1: Demographic and Clinical Characteristics of the Included Women.**

Variables	Group I (n=195)	Group II (n=90)	P-Value
Age (years)			
▪ < 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%)		

411 Data were expressed as median(range) or frequency %.
412 *Chi-square/Fisher Exact tests compare proportion between groups. **Mann-Whitney U Tests compare the median between groups
413 P value is considered significant when < 0.05.
414 **Group I (Cases)**: women having a history of two or more miscarriages.
415 **Group II (Controls)**: Healthy controls with no history of miscarriage and at least one uncomplicated full-term pregnancy.
416
417 VTE: venous thromboembolism.
418

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 (Sec)	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 were 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.

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			
▪ Wild G/G	193 (99.0%)	90 (100.0%)	1.000
▪ Heteromutant A/G	2 (1.0%)	0 (0.0%)	
Prothrombin gene G20210A *Allele*			
▪ G (Wild)	388 (99.5%)	180 (100.0%)	0.935
▪ A (Mutant)	2 (0.5%)	0 (0.0%)	
MTHFR C677T gene			
▪ Wild C/C	105 (53.8%)	53 (58.9%)	0.569
▪ Heteromutant C/T	65 (33.3%)	29 (32.2%)	
▪ Homomutant T/T	25 (12.8%)	8 (8.9%)	
MTHFR C677T gene Allele			
▪ C (Wild)	275 (70.5%)	135 (75.0%)	0.269
▪ T (Mutant)	115 (29.5%)	45 (25.0%)	

Data were expressed as frequency and %.
*Chi-square test and Fisher Exact test were used to compare proportion 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.

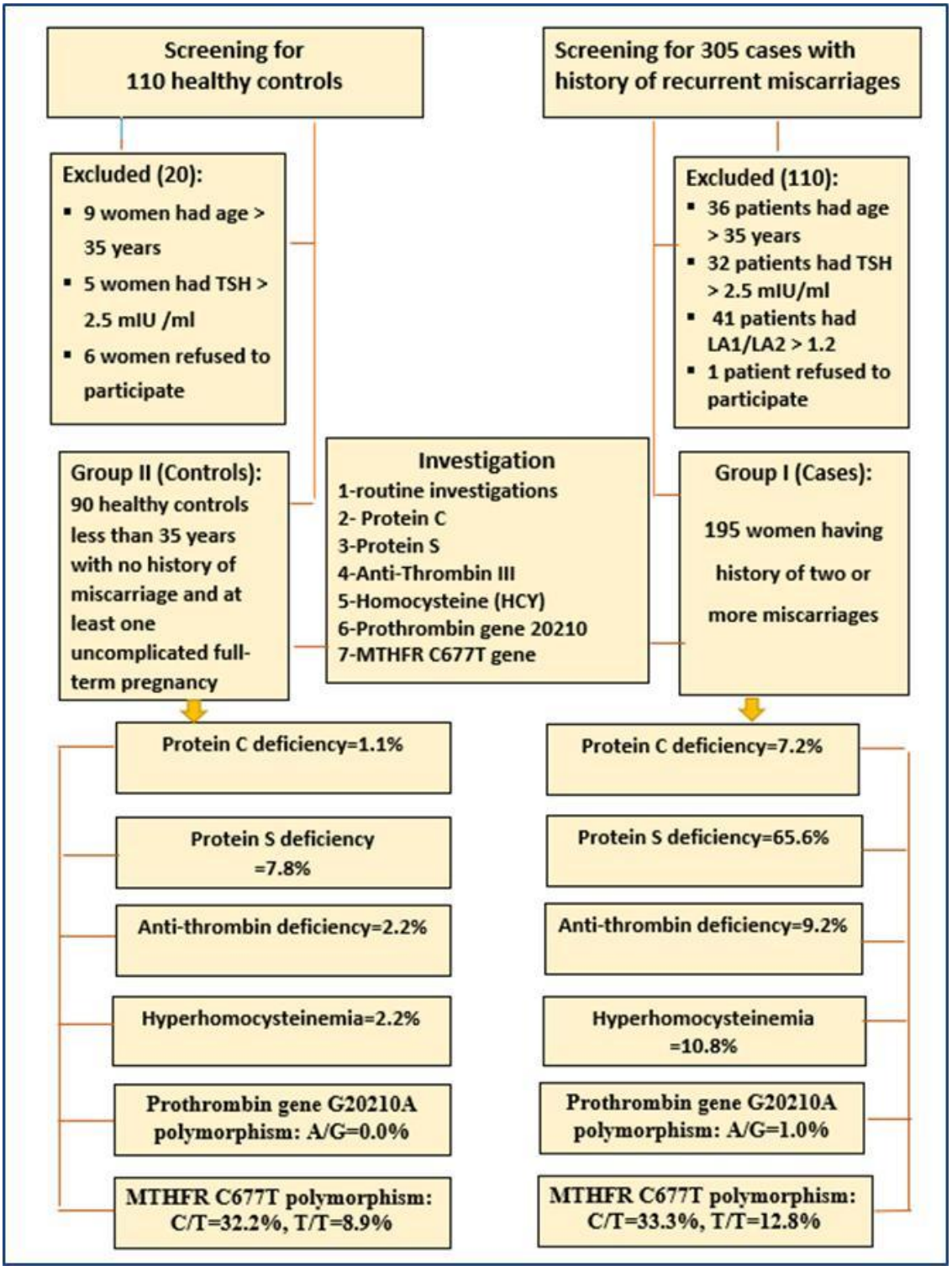

Fig. (1): Study flow chart.

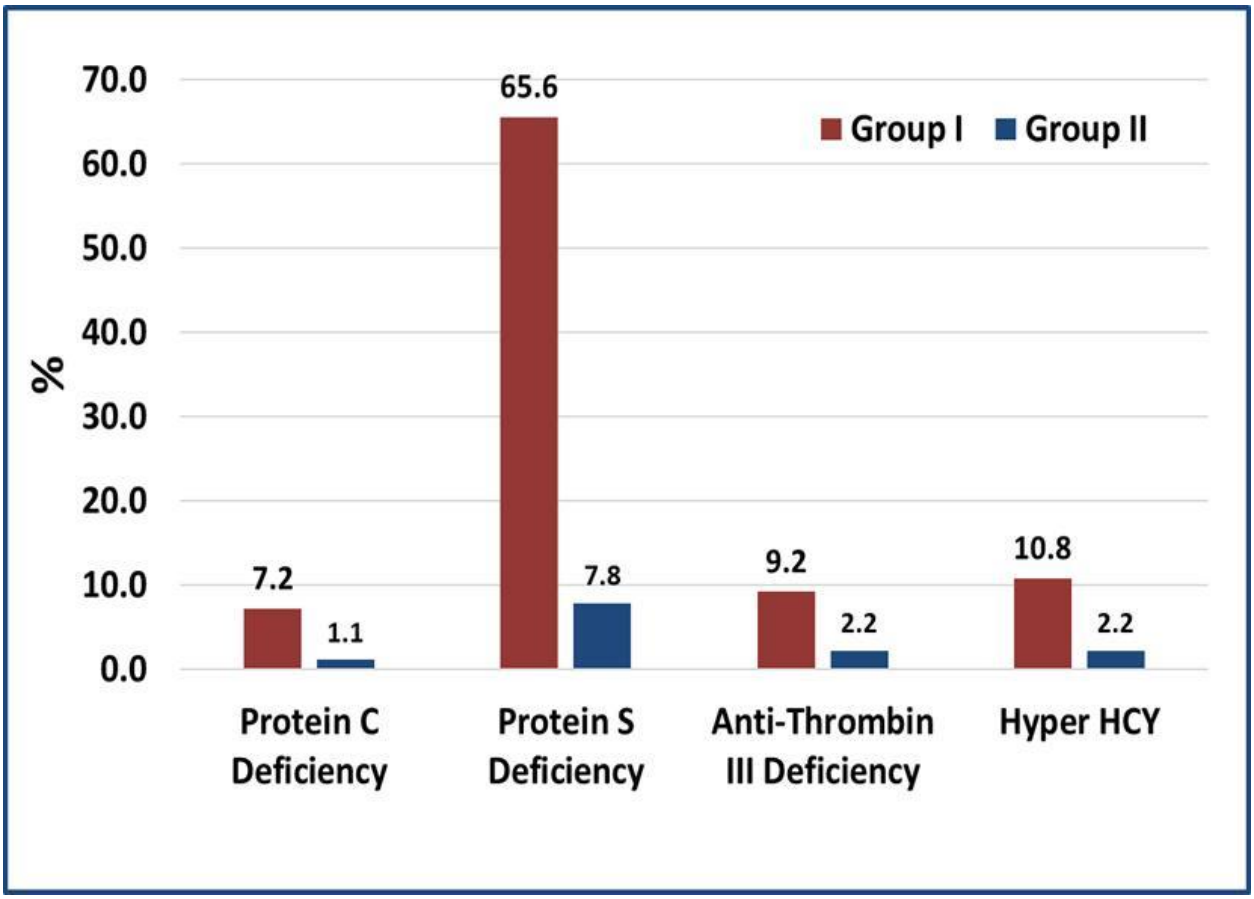

Fig. (2): Comparison of Thrombophilia markers (Protein C, Protein S, and Antithrombin III and Homocysteine) between Group I and Group II.