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Research Article
Prevalence of Thrombophilia in Palestine and the Association of Thrombophilic Gene Polymorphisms with Recurrent Pregnancy Loss

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Abstract

Background and Objectives: Inherited thrombophilia may be caused by mutations, polymorphisms in a variety of genes mainly involved in haemostatic pathways. The aims of this study to estimate the prevalence of thrombophilia and to determine the correlation of specific inherited thrombophilias and recurrent pregnant loss (RPL) among Palestinian women. **Materials and Methods:** For this purpose, 11 thrombophilic gene polymorphisms the including Factor V Leiden and MTHFR C677T polymorphisms were evaluated. Two thousands women who were referred for thrombophilc genetic screen test between the years 2013 and 2018 were included in the study and evaluated for the presence of thrombophilic gene polymorphisms and compared to 200 healthy volunteers. **Results:** The prevalence of thrombophilia in Palestine is 11.7-27.2% among normal population in contrast to 39.90% MTHFR C677T gene polymorphism and FVL gene polymorphism 14.20%. The most common thrombophilia in our study group was MTHFR C677T mutation with 39.90% followed by MTHFR MTHFR 1298A/C with a prevalence of 31.70%, β-Fibrinogen (-455 G>A) with a prevalence of 23.60%, Factor XIII with a prevalence of 16.40%, FVL (H1299R) with a prevalence of 13.40, followed by FV Leiden 11.49% and the least common was Prothrombin (G20210A) with a prevalence of 5.30%. The MTHFR MTHFR C677T would be expected to play a major role to recurrent pregnancy loss (RPL). These findings indicate that RPL with homozygous genotype for (C677T and A1298C) either alone or compound heterozygous genotypes have a high risk of pregnancy loss in Palestine. Present study result also shows that a significantly higher frequency of factor V leiden polymorphism among the patients compared to control groups. The importance of determining at least 6 thrombophilic gene polymorphisms are most effective in increasing the risk of RPL in patients experiencing recurrent abortion losses. **Conclusion:** The study suggests the high need to determine at least 6 thrombophilic gene polymorphisms and provides evidence for a significant correlation between recurrent miscarriages and MTHFR C677T and Factor V mutation in our population.

Key words: Thrombophilia, FV leiden, MTHFR C677T, MTHFR (A1298C), Palestinians

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Competing Interest: The authors have declared that no competing interest exists.

Data Availability: All relevant data are within the paper and its supporting information files.

INTRODUCTION

Thrombophilia is the term given to abnormal blood coagulation condition leading to increased tendency towards coagulation (hypercoagulability status). Thrombophilia, commonly manifested as venous thromboembolism (VTE) is a worldwide concern but little is known on its genetic epidemiology in many parts of the globe particularly in the developing countries.

Thrombosis is a common complication of many disorders and one of the most frequent death causes. There are six abnormalities accepted as genetic risk factors for venous thrombosis. Factor V Leiden mutation (FVL) is known as the most common of them. Its presence could increase the thrombotic risk 5-10 times. Factor V mutation Leiden (FV-Leiden) is a specific point mutation, identified as a G-A substitution in nucleotide 1691 in the factor V gene that leads to Arg506-Gln conversion[1] and is associated with hypercoagulability and increased risk of venous thromboembolism. Heterozygote or homozygote mutation in factor V Leiden, protein S deficiency, protein C deficiency or anti-thrombin III were described as cause for a higher incidence of recurrent miscarriages. In a meta-analysis of 31 studies, the presence of factor V Leiden was associated with early (before gestational week 12) and late (after week 12) recurrent miscarriages. Similar results were found in women with prothrombin mutation and protein S deficiency[2]. Inherited thrombophilia is common in the Caucasian population with a prevalence[3] of up to 15%. Unsurprisingly, therefore, these abnormalities are commonly found in women with RPL but this does not prove causation. It is therefore, possible that inherited thrombophilias could play a role at this very early stage by disrupting the development of this intricate blood supply and confer a disadvantage for implantation. Thrombophilia complicates the pregnancy by interfere with the physiology of uteroplacental.

The prevalence of the MTHFR C677T is rather heterogeneous among different ethnic groups and varies from 2-54.5% in various populations[4]. The prevalence of the MTHFR C677T polymorphism has not been extensively studied in the Middle East or in the Arab populations and till now, no investigations were conducted on the C677T and A1298C poly morphism. It is therefore crucial to investigate the prevalence of the C677T and A1298C polymorphisms of the MTHFR gene in the healthy and unhealthy Palestinian population.

Recurrent pregnancy loss (RPL) affects 1% pregnancies and is multi-factorial in origin. By analogy, the use of low molecular weight heparin (LMWH) has become common place in women with inherited thrombophilia and also those with unexplained miscarriage to help safeguard the pregnancy. Pregnancy loss is a very significant public health issue, associated with maternal morbidity and mortality and psychological trauma. The term miscarriage is defined as the spontaneous loss of a fetus before it reaches viability and occurs in up to 15% of clinically recognized pregnancies[5].

The aims of this study were to find the prevalence of thrombophilic gene factors polymorphisms to estimate the prevalence of thrombophilia and to determine the correlation of specific inherited thrombophilias and recurrent pregnant loss (RPL) among Palestinian women. For this purpose, 11 thrombophilic gene polymorphisms the including Factor V Leiden and MTHFR C677T polymorphisms were evaluated. This also could be an early diagnostic method and preventive procedure.

MATERIALS AND METHODS

The research was carried out in two stages: the laboratory and the literature review.

Laboratory: A retrospective review 2000 women, who were referred for thrombophilc genetic screen test between the years 2013 and 2018 were included in the study and evaluated for the presence of thrombophilic gene polymorphisms and compared to 200 healthy volunteers. The study was conducted in the Euro lab medical center in which all samples were referred by different outpatient clinics for further genetic analysis during the time period between 2013 and 2018. The study samples were composed of 112 unhealthy persons (63 males, 49 females) who were send to Euro lab for further genetic investigations concerning thrombophilia.

DNA: Blood samples (5 mL) were obtained from the participants in tubes containing EDTA and genomic DNA was extracted using salting out method. The DNA was extracted from a 200 µL peripheral blood then, DNA amount and DNA purity were quantified for each DNA sample by nano-drop.

Genotypic analysis: Genotyping of polymorphisms of thrombophilic patients and controls were performed by real time polymerase chain reaction system. Polymerase chain reaction (PCR) amplification of DNA samples was performed using FV Leiden, FV 4070A/G, FV 5279 A/G, FXIII 103G/T, FXIII 614A/T, FXIII 1694C/T, BF-455G/A, PAI-1-4G/5G, ITGB3 1565T/C, MTHFR 677C/T and MTHFR 1298A/C polymorphism specific primers (7, 25, 26). The PCR

products were then confirmed by electrophoresis on 1.5% agarose gel. The PCR product variants were then digested by specific restriction enzymes and analyzed on 1.5% agarose or poly Acrylamide-Gel Electrophoresis (PAGE).

Statistical analysis: For each polymorphic position, one of three possible pat-terns may be obtained: Normal, heterozygous or homozygous mutant genotype. Genotype distributions of each polymorphism among different groups were compared by Mann-Whitney test (mean±SD). The homozygote and heterozygote groups were unified and a new group, namely, composed of those with the polymorphism was created. Results were considered significant when the p-value was less than 0.05.

RESULTS

Factor V-polymorphisms: The frequencies of FV Leiden polymorphism in the case and control groups were 21.2 and 2.4%, respectively (Table1). The proportions for heterozygous and homozygous polymorphisms in the case groups were 11.40 and 2.80%, respectively. Statistical analysis showed significant difference between the case and control groups in these polymorphisms p<0.05 (Table 1).

Prothrombin (G20210A): About 939 patients (93%) with normal polymorphism of prothrombin (G20210A) gene and 53 patients (5.30%) with its heterozygous polymorphism and 9 patient 0.90% with homozygous polymorphism were seen in the case group while the frequencies in the control group consistent of 939 women (93.9%) with normal polymorphism and 56 patients with (5.6%) heterozygous polymorphism and 3 (0.30%) with homozygous polymorphisms of the gene (Table 1).

FVL (H1299R): The frequencies of FVL (H1299R) polymorphism in the case and control groups were 86.40 and 87.90%, respectively (Table 1). The proportions for heterozygous and homozygous polymorphisms in the case groups were 13.40 and 3%, respectively.

Factor XIII polymorphisms: Frequency of FXIII polymorphism was 82.60% in the case group and 86.4% in the control group, (Table 1). On the other hand, the proportions for heterozygous and homozygous polymorphisms in the case groups were 16.40 and 1%, respectively.

β-fibrinogen (-455 G>A): The frequencies of β-Fibrinogen (-455 G>A) polymorphism in the case and control groups were 71.80 and 68%, respectively (Table 1). The proportions for heterozygous and homozygous polymorphisms in the case groups were 23.60 and 4.6%, respectively.

MTHFR polymorphisms: The proportions for homozygous and heterozygous polymorphisms of MTHFR 677C/T were 15 and 42% in the case group and 9 and 25% in the control group, respectively (Table 1). The frequencies of heterozygous and homozygous polymorphisms of MTHFR 1298A/C in the case group were 27 and 4% and in the control group 6% heterozygosity and no homozygosity were seen. There were also significant (p<0.01).

In this study the prevalence of FVL in Palestine was 14.20% however, in other middle east Mediterranean countries as Lebanon and Turkey was 9.9 and 21% however, Syria and Cyprus no studies were conducted (Table 2).

Studying the MTHFR mutation in our population sample and control cases revealed that in our group study the majority of population showed that MTHFR mutation is absent in 39.9% in contrast to 60.10% MTHFR mutation was present. Among the MTHFR mutation which was presented heterozygous mutation was in 31.7% and homozygous in a homozygous 8.20% (Table 3, 4).

Table 1: Prevalence of 11 thrombophilic polymorphism in the case and control groups

	Case			Control			
Polymorphism	Homozygote (%)	Heterozygote (%)	Normal (%)	Homozygote (%)	Heterozygote (%)	Normal (%)	p-value*
FV Leiden	2.80	11.40	85.80	2.40	21.20	74.70	<0.05
Prothrombin (G20210A)	0.90	5.30	93.00	0.30	5.60	93.90	Non-significant
FVL (H1299R)	3.00	13.40	86.30	1.60	11.70	87.90	Non-significant
Factor XIII	1.00	16.40	82.60	1.10	12.30	86.40	Non-significant
β-Fibrinogen (455 G>A)	4.60	23.60	71.80	3.60	28.20	68.00	Non-significant
MTHFR (C677T)	8.20	31.70	60.10	26.00	24.90	48.90	<0.01
MTHFR (A1298C)	8.10	47.40	87.20	23.00	26.00	46.00	<0.01

The p-value was derived from Mann-Whitney test for homozygote, heterozygote and normal genotypes

Table 2: Prevalence of FVL in patients and normal subjects in Arabs and non-Arabs living in different middle-eastern and north African countries

Country/ethnic groups	FVL patients (%)	Normal population (%)	References
Middle east Mediterranean			
Palestine	14.20	11.7-27.2	63, 97, 98
Lebanon	9.90	13.6-18.7	89, 92, 99-106
Syria	-	13.6	97, 99
Turkey	21.00	4.6-9.8	31, 107,108, 115
Cyprus	-	13.4	116
North Africa Mediterranean			
Morocco	-	0	79-80
Algeria	13.80	1.3-2	81, 82
Tunisia	20.30	3-13.6	83-92
Egypt	30.00	2.5-10.2	93-9731,
Middle east non-Mediterranean			
Jordan	23.90	10.5-27.2	97, 117-122
Iraq	-	7	123
Kuwait	15.80	2-4.5	97, 124
Saudi Arabia	-	0.2.5	89, 123, 125
Bahrain	52.00	3.1-14.7	89, 126
Oman	0.00	0	127
Yemen	-	0	63
Iran	11.4	2.0-10.6	123, 128-133

Table 3: MTHFR mutation in cases and controls (recurrent abortion)

Parameters	Cases (%)	Controls (%)	p-value
Absence of MTHFR mutation	39.90	51.1	Significant
Presence of MTHFR mutation	60.10	48.9	Significant
Heterozygous mutation	31.70	24.9	Significant
Homozygous mutation	8.20	26.0	Significant

p-value was derived from Mann-Whitney test for homozygote, heterozygote and normal genotypes

Table 4: Thrombophilia distribution among patient according to regions in Palestine

Variables	Number	Percentage
Center 1	19	1.9
North 1	230	23.0
North 2	400	40.0
North 3	69	6.9
Center 2	60	6.0
Center 3	41	4.1
Center 4	22	2.2
North 5	23	2.3
South 1	20	2.0
Center 5	10	1.0
Center 6	6	0.6
South 2	41	4.1
Others	33	3.3

DISCUSSION

In the present study, the result showed that a significantly higher frequency of factor V leiden polymorphism compared to control groups. Among these samples the prevalence of FV Leiden was 21.2% in the control group and 11.40% in the population samples. The high prevalence rate of FVL among healthy subjects from Palestine was interestingly matched with similarly high rates from neighboring Jordan[6] and Lebanon[7]. This suggested that FVL mutation must have originated as a single mutational event outside of Europe, then spreading by migration of mutation-carrying individuals[8]. The rate of FVL in our study is much more elevated than those reported in Iran (2.5%), India (4.7%), a USA hospital (20%), (7.9%) in Turkey. Data could not be retrieved from the literature on prevalence of FVL in Libya, Syria, Morocco, Iraq and Yemen.

It further evaluated the impact of MTHFR C677T and A1298C polymorphisms on the risk of RPL in a Palestinian population. Homozygous TT genotypes frequency was found to be significantly higher among RPL than the other two groups, this would indicated that MTHFR C677T polymorphism would be expected to play a major role to bring about RPL. Results from several meta-analyses that have examined the possibility of an association between MTHFR polymorphisms and the risk of RPL are variable[9-17]. Some studies reported significantly increased prevalence of MTHFR C677T among cases[18-21]. In contrast to that some other studies which reported that an insignificant association between the MTHFR C677T and RPL[22-24]. The differences in ethnicity may be one major reason for the controversy.

Molecular and epidemiological studies provided evidences that FVL and MTHFR C677T polymorphism would be expected to play a major role to bring about RPL in the Mediterranean region and has highest prevalence of FVL in the world. Screening high risk patients for this polymorphism and the use of specific thromboprophylaxis to prevent recurrent thrombotic disease.

CONCLUSION

This study suggests the high need to determine at least 6 thrombophilic gene polymorphisms and provides evidence for a significant correlation between recurrent miscarriages and MTHFR C677T and Factor V mutation in our population.

The prevalence of thrombophilia (i.e., MTHFR (A1298C)) was higher in our study group than in the general population in Palestine. The present data showed that FVL, MTHFR polymorphisms have a strong association with RPL.

REFERENCES

1. Irani-Hakime, N., H. Tamim, G. Elias, R.R. Finan, J.L. Daccache and W.Y. Almawi, 2000. High prevalence of factor V mutation (Leiden) in the Eastern Mediterranean. Clin. Chem., 46: 134-136.
2. Rey, E., S.R. Kahn, M. David and I. Shrier, 2003. Thrombophilic disorders and fetal loss: A meta-analysis. Lancet, 361: 901-908.
3. Greer, I.A., 2003. Thrombophilia: Implications for pregnancy outcome. Thromb. Res., 109: 73-81.
4. Pepe, G., O.C. Vanegas, B. Giusti, T. Brunelli and R. Marcucci *et al*., 1998. Heterogeneity in world distribution of the thermolabile C677T mutation in 5,10-methylenetetrahydrofolate reductase. Am. J. Hum. Genet., 63: 917-920.
5. Warburton, D. and F.C. Fraser, 1964. Spontaneous abortion risks in man: Data from reproductive histories collected in a medical genetics unit. Am. J. Hum. Genet., 16: 1-25.
6. Eid, S.S. and G. Rihani, 2004. Prevalence of factor V Leiden, prothrombin G20210A and MTHFR C677T mutations in 200 healthy Jordanians. Clin. Lab. Sci., 17: 200-202.
7. Almawi, W.Y., S.H. Keleshian, L. Borgi, N.A. Fawaz, N. Abboud, N. Mtiraoui and T. Mahjoub, 2005. Varied prevalence of factor V G1691A (Leiden) and prothrombin G20210A single nucleotide polymorphisms among Arabs. J. Thromb. Thrombol., 20: 163-168.
8. Zivelin, A., J.H. Griffin, X. Xu, I. Pabinger and M. Samama *et al*., 1997. A single genetic origin for a common Caucasian risk factor for venous thrombosis. Blood, 89: 397-402.
9. Wiwanitkit, V., 2005. Roles of methylenetetrahydrofolate reductase C677T polymorphism in repeated pregnancy loss. Clin. Applied Thromb./Hemost., 11: 343-345.
10. Ren, A. and J. Wang, 2006. Methylenetetrahydrofolate reductase C677T polymorphism and the risk of unexplained recurrent pregnancy loss: A meta-analysis. Fertil. Steril., 86: 1716-1722.
11. Wu, X., L. Zhao, H. Zhu, D. He, W. Tang and Y. Luo, 2012. Association between the *MTHFR* C677T polymorphism and recurrent pregnancy loss: A meta-analysis. Genet. Testing Mol. Biomarkers, 16: 806-811.
12. Cao, Y., J. Xu, Z. Zhang, X. Huang and A. Zhang *et al*., 2013. Association study between methylenetetrahydrofolate reductase polymorphisms and unexplained recurrent pregnancy loss: A meta-analysis. Gene, 514: 105-111.
13. Nair, R.R., A. Khanna, R. Singh and K. Singh, 2013. Association of maternal and fetal *MTHFR* A1298C polymorphism with the risk of pregnancy loss: A study of an Indian population and a meta-analysis. Fertil. Steril., 99: 1311-1318.
14. Rai, V., 2014. Methylenetetrahydrofolate reductase gene A1298C polymorphism and susceptibility to recurrent pregnancy loss: A meta-analysis. Cell Mol. Biol., 60: 27-34.
15. Yang, Y., Y. Luo, J. Yuan, Y. Tang and L. Xiong *et al*., 2016. Association between maternal, fetal and paternal MTHFR gene C677T and A1298C polymorphisms and risk of recurrent pregnancy loss: A comprehensive evaluation. Arch. Gynecol. Obstet., 293: 1197-1211.
16. Chen, H., X. Yang and M. Lu, 2016. Methylenetetrahydrofolate reductase gene polymorphisms and recurrent pregnancy loss in China: A systematic review and meta-analysis. Arch. Gynecol. Obstet., 293: 283-290.
17. Rai, V., 2016. Methylenetetrahydrofolate reductase C677T polymorphism and recurrent pregnancy loss risk in Asian population: A meta-analysis. Indian J. Clin. Biochem., 31: 402-413.
18. Nelen, W.L., E.A. Steegers, T.K. Eskes and H.J. Blom, 1997. Genetic risk factor for unexplained recurrent early pregnancy loss. Lancet, 350: 861-861.
19. Lissak, A., A. Sharon, O. Fruchter, A. Kassel, J. Sanderovitz and H. Abramovici, 1999. Polymorphism for mutation of cytosine to thymine at location 677 in the methylenetetrahydrofolate reductase gene is associated with recurrent early fetal loss. Am. J. Obstet. Gynecol., 181: 126-130.
20. Couto, E., R. Barini, R. Zaccaria, J.M. Annicchino-Bizzacchi and R. Passini Jr. *et al*., 2005. Association of anticardiolipin antibody and C677T in methylenetetrahydrofolate reductase mutation in women with recurrent spontaneous abortions: A new path to thrombophilia? Sao Paulo Med. J., 123: 15-20.
21. Altomare, I., A. Adler and L.M. Aledort, 2007. The 5, 10 methylenetetrahydrofolate reductase C677T mutation and risk of fetal loss: A case series and review of the literature. Thromb. J., Vol. 5. 10.1186/1477-9560-5-17.
22. Kobashi, G., E.H. Kato, M. Morikawa, S. Shimada and K. Ohta *et al*., 2005. *MTHFR* C677T Polymorphism and factor V Leiden mutation are not associated with recurrent spontaneous abortion of unexplained etiology in Japanese women. Semin. Thromb. Hemost., 31: 266-271.
23. Mukhopadhyay, R., K.N. Saraswathy and P.K. Ghosh, 2009. *MTHFR C677T* and *Factor V Leiden* in recurrent pregnancy loss: A study among an endogamous group in North India. Genet. Testing Mol. Biomarkers, 13: 861-865.
24. Settin, A., R. Elshazli, A. Salama and R. ElBaz, 2011. Methylenetetrahydrofolate reductase gene polymorphisms in Egyptian women with unexplained recurrent pregnancy loss. Genet. Testing Mol. Biomarkers, 15: 887-892.