

Thrombophilic Genes Mutations in Preeclampsia

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ABSTRACT

Background: Preeclampsia is a multisystem disorder involving vasoconstriction and hypertension in the mother and decreased blood flow. There are inconsistent reports on whether there is an association between preeclampsia and thrombophilia. **The Aim of the Study:** The present study aimed to determine the relationship of mutations of factor V Leiden, prothrombin and methylene tetrahydrofolate reductase (MTHFR) genes in Egyptian preeclamptic patients. **Materials and Methods:** Fifty six preeclamptic women and 48 normal pregnant women were tested for detection of mutations of factor V Leiden (FVL), prothrombin and methylene tetrahydrofolate reductase genes by genotyping using multiplex allele specific PCR, restriction enzymes and agarose gel electrophoresis. **Results:** There was significant association between mutations of factor V Leiden gene in preeclamptic patients (8 positive cases out of 56) compared with control cases (No positive cases out of 48), prothrombin gene in preeclamptic patients (22 positive cases out of 56) compared with control cases (only one positive case out of 48), while, no significant association between mutations of methylene tetrahydrofolate reductase gene C677T or A1298C in preeclamptic patients when compared with control cases. **Conclusion:** This study suggests the existence of a linkage between FVL, prothrombin genes polymorphism but not MTHF reductase genes polymorphism in the pathogenesis of preeclampsia.

INTRODUCTION

Preeclampsia (PE) is a multisystem disorder involving vasoconstriction and hypertension in the mother. There are inconsistent reports on whether there is an association between preeclampsia and thrombophilia⁽¹⁾. Thrombophilia is a term used to describe a heterogenous group of coagulation abnormalities (acquired or inherited) that are generally associated with increased risk of arterial and venous thrombosis⁽²⁾.

The most common inherited thrombophilic disorders during pregnancy are mutations in factor V Leiden (FVL), prothrombin gene, and tetrahydrofolate reductase (MTHFR). During the past 2 decades, epidemiologic and case-control studies have evaluated the association between thrombophilias and adverse pregnancy outcome (APO), specifically, preeclampsia and intrauterine fetal growth restriction (IUGR)⁽³⁾.

Unfortunately, the results of previous studies are controversial, and a systematic review of the association

between maternal thrombophilia and PE has not led to definite conclusions⁽⁴⁾.

The etiology and pathogenesis of hypertensive disorders of pregnancy, including pre-eclampsia remain unknown. It is thought that genetic predisposition and immune maladaptation contribute to placental ischemia and consequent abnormalities of the maternal vascular endothelium, activation of the coagulation systems. Venous and possibly arterial diseases have resulted from many polymorphisms in various prothrombotic genes, including G1691A in FVL, prothrombin G20210A and MTHFR C677T⁽⁵⁾.

The aim of the present study is to determine the relationship of mutations of factor V Leiden, prothrombin and methylene tetrahydrofolate reductase genes in Egyptian preeclamptic patients.

SUBJECTS & METHODS

The present study included 56 preeclamptic women and 48 normal pregnant women attending the Obstetric Outpatient Clinic, Mansoura University Hospital. Five ml. venous blood samples were collected from each subject in polyethylene tubes containing EDTA as an anticoagulant for DNA extraction. The generation of DNA purification capture column kit

(Gentra systems USA) was used for DNA extraction.

FVL (G1691A) and prothrombin (G20210A) gene mutations were analyzed using a multiplex allele specific PCR amplification. The DNA extracted from all cases was also subjected to amplification of a region of factor IX gene as an internal control for assessment of the quality of the extracted DNA. Each PCR was performed with 300 ng of DNA, 200 mmol/L of each dNTP, 500 nmol/L of each primer and 2.5 units of Taq DNA polymerase (Amplitaq gold, Perkin Elmer cetus, Norwalk,conn). DNA was initially denaturated for 10 minutes at 95 °C, and then 10 cycles were performed as follows: 94 °C for 30 seconds, 60 °C for 30 seconds and 72 °C for 1 minute. Then, 25 cycles were performed as follows; 94 °C for 30 seconds, 55 °C for 30 seconds and 72 °C for 1 minute. The PCR amplification was completed by a final extension at 72 °C for 7 minutes.

Amplification of FVL and prothrombin (PT) gene resulted in 152 b.p. and 340 bp products, respectively. The internal amplification control (the region of the factor IX gene) resulted in a 250 b.p product. The amplified products were electrophoresed on 2% agarose gel, stained with ethidium bromide, and visualized under UV light. The sequence of primers used is shown in the following table⁽¹⁾.

Table 1: primer sequences used for amplification of FVL and prothrombin genes ^(6,7).

FVL common	5'-GGACTACTTGACAATTACTGTTCTCTTG-3'
FVL Wild (WT)	5'-GCAGATCCCTGGACAGACG-3'
FVL mutant (MT)	5'-GCAGATCCCTGGACAGACA-3'
PT common	5'-TCTAGAAACAGTTGCCTGGCAG-3'
PT Wild (WT)	5'-GCACTGGGAGCATTGAGGATC-3'
PT mutant (MT)	5'-GCACTGGGAGCATTGAGGATT-3'
FIX1	5'-CTCCTGCAGCATTGAGGGAGATGGACATT-3'
FIX 2	5'-CTCGAATTCGGCAAGCATACTCAATGTAT-3'

The regions containing 2 RFLPs within the MTHFR gene were amplified with Taq DNA polymerase. For evaluation of MTHFR C677T polymorphism, the polymorphic site was amplified with primers ⁽⁸⁾:

Forward (5'-TGAAGGAGAAGGTGTCTGCGGA-3')

Reverse (5'-AGGACGCTGCGGTGAGAGTG-3')

For PCR, the following procedure was used: initial denaturation for 3 minutes at 95 °C, and then 5 cycles were performed as follows: 94 °C for 1 minute, 64 °C for 1 minute and 72 °C for 30 seconds. Then, 30 cycles were performed as follows; 94 °C for 45 seconds, 62 °C for 45 seconds and 72 °C for 25 seconds. The PCR amplification was completed by a final extension at 72 °C for 7 minutes. The amplification yielded a product 198 bp. Upon cleavage with 5 units of Hinf I (Boehringer Mannheim) for 16 hours at 37 °C and then, electrophoresed on 3 % agarose and stained with ethidium bromide, and visualized under UV light. Allele T produced 2 bands at 175 b.p and 23 b.p whereas C allele remains uncut. The C677T genotypes were classified as CC, CT, or TT.

For normal individual (C677C homozygous), agarose gel electrophoresis allows visualization of a 198 bp fragment. For T677T (homozygous patient), agarose gel electrophoresis allows visualization of two bands of 175 and 23 b.p. For C677T (heterozygous patient), agarose gel electrophoresis allows visualization of three bands of 198, 175 and 23 b.p. ⁽⁹⁾.

For evaluation of MTHFR A1298C polymorphism, the polymorphic site was amplified with primers:

Forward (5'-CTTTGGGGAGGTGAAGGACTACTAC-3')

Reverse (5'-CACTTTGTGACCATTCCGGGTTG-3')

For PCR, the following procedure was used: initial denaturation for 2 minutes at 95 °C, and then 5 cycles were performed as follows: 95 °C for 1 minute, 55 °C for 2 minutes and 72 °C for 2 minutes. Then, 32 cycles were performed as follows; 95 °C for 75 seconds, 55 °C for 75 seconds and 72 °C for 90 seconds. The PCR amplification was completed by a final extension at 72 °C for 6 minutes.

The amplification yielded a product 163 b.p. Upon cleavage with

5 units of Mbo II (Boehringer Mannheim) for 16 hours at 37 C° and then, electrophoresed on 3 % agarose and stained with ethidium bromide, and visualized under UV light.

Enzymatic digestion of PCR products of A1298C polymorphism of MTHFR gene using Mbo II enzyme; Mbo II digests the 163 b.p. fragment of the homozygous mutant type CC into 84, 31, 30 and 18 b.p fragments. While the wild type AA, the 84 bp fragment is cut into 56 and 28 b.p fragments producing 5 fragments of 56, 31, 30, 28 and 18. The heterozygous mutant type CA produced 6 fragments of 84, 56, 31, 30, 28, and 18. All small fragments (31, 30, 28, and 18) have run off the gel⁽¹⁰⁾.

Statistical analysis: Statistical analysis was done by using MedCalc® program version 10.0.1⁽¹¹⁾. Student t- test was used for quantitative data (mean ± SD) to compare any variable against control group. Spearman rank correlation

coefficient was done to study the relation between variables. Odds ratio was done to calculate the ratio of the odds of the outcome in two groups. $P \leq 0.05$ is considered significant.

RESULTS

In the present study 56 patients with preeclampsia were used and 48 healthy pregnant females were also used as a control group. As shown in table (2), there is significant association between prothrombin gene mutations, $p = < 0.0001$ (8 out of 56 preeclamptic pregnant patients 14.28%) compared with pregnant control groups (zero out of 48 subjects 0 %). Also, significant association was founded between FVL gene mutations $p = 0.0185$ (22 out of 56 pregnant patients with preeclampsia 39.22%) compared with pregnant control groups (1 out of 48 pregnant control groups 2.08%).

Table (2): The frequency of FVL and prothrombin genes mutations in different groups with their statistical analysis:

Thrombophilic defect	Patients with PE(n=56)	Controls (n=48)	Odds ratio	P value
FVL	8/56(14.28%)	0/48 (0 %)	7.8333	0.0185
Prothrombin II	22/56(39.22%)	1/48(2.08%)	30.4118	< 0.0001

Table (3): Frequency of MTHR C677T genotypes among preeclamptic patients and control with their statistical analysis:

MTHR C677T	Control group (48)	Patients with PE (n=56)	Odds ratio	P value
CC	20/48(41.67%)	19/56(33.93%)		0.5421
CT	28/48(58.33%)	37/56(66.07%)		0.5421
TT	0/48(0%)	0/56(0%)	
C allele	68/96(70.83%)	75/112(66.96%)		0.6525
T allele	28/96(29.17%)	37/112(33.04%)	1.1981	0.6525

$P \leq 0.05$ is considered significant.

As shown in table (3), there is no significant association between MTHR T allele, $p = 0.6525$ (37 out of 112 preeclamptic pregnant patients 33.04%) compared with pregnant control groups (28 out of 96 subjects 29.17%).

The relative risk of preeclampsia with patients carrying T allele as compared with control subjects were 1.1981 (OR=1.1981, P=0.6525).

Table (4): Frequency of MTHR A1298C genotypes among pre eclamptic patients and control with their statistical analysis:

	Control group (48)	Patients with PE (n=56)	Odds ratio	P value
AA	13/48(27.09%)	14/56(25%)		0.9856
AC	27/48(56.25%)	30/56(53.57%)		0.9393
CC	8/48(16.66%)	12/56(21.43%)		0.7145
A allele	53/96(55.21%)	58/112(51.78%)		0.7237
C allele	43/96(44.79%)	44/112(58.22%)	0.9350	0.7237

$P \leq 0.05$ is considered significant.

As shown in table (4), there is no significant association between MTHR C allele, $p = 0.7237$ (44 out of 112 preeclamptic pregnant patients 58.22%) compared with pregnant control groups (43 out of 96 subjects 44.80%).

The relative risk of preeclampsia with patients carrying C allele as compared with control subjects were 0.9350 (OR= 0.9350, P= 0.7237).

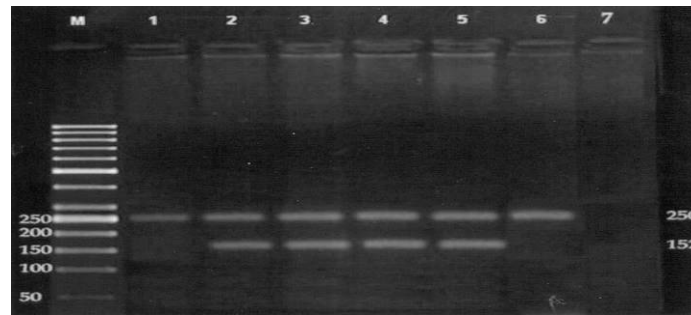


Figure (1): amplification of FVL (G1691A): using primers for normal allele (lanes 1,3,5) and mutant allele (lanes 2,4,6) showing positive bands (152 bp) in lane 2,3,4, 5 with negative bands in lanes 1,6. Case 1 (lanes 1, 2) shows normal negative and mutant positive indicating carrier. Case 2 (lanes 3, 4) shows normal positive and mutant positive indicating heterozygous. Case 3 (lanes 5, 6) shows normal positive and mutant negative indicating normal. Lane M indicates molecular marker, bands of size 250 bps belongs to FIX used as internal controls. Lane 7: negative control.

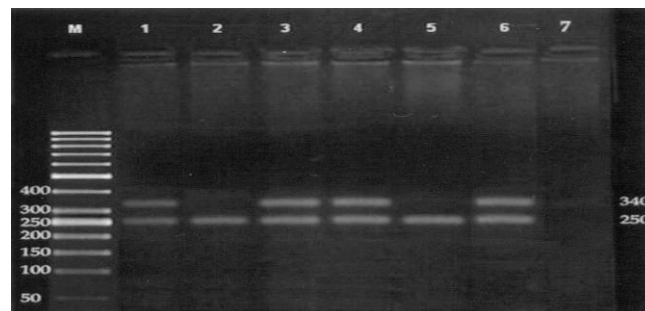


Figure (2): amplification of FII (G20210A) using primers for normal allele (lanes 1, 3, 5) and mutant allele (lanes 2, 4, 6) showing positive bands (340 bp) in lane 1,3,4, 6 with negative bands in lanes 2,5. Case 1 (lanes 1, 2) shows normal positive and mutant negative indicating normal. Case 2 (lanes 3, 4) shows normal positive and mutant positive indicating heterozygous. Case 3 (lanes 5, 6) shows normal negative and mutant positive indicating carrier. Lane M indicates molecular marker, bands of size 250 bps belongs to FIX used as internal controls. Lane 7: negative control.

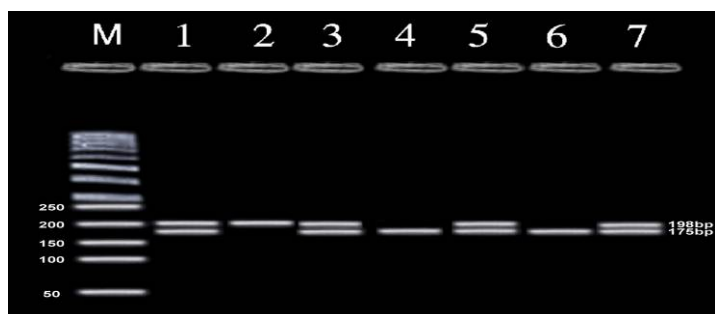


Figure (3): Enzymatic digestion of PCR product of C667T polymorphism of MTHFR gene using *Hinf*I enzyme. *Hinf*I enzyme digests the 198 b.p fragment into 175 and 23 bp fragments; the small 23 bp fragment has run off the gel. DNA marker (50 b.p), lane 2 (wild type CC is found which appears at 198 b.p), lane 1, 3, 5 And 7 (heterozygous mutated genotype CT which has 198, 175, 23 b.p fragments) and lane 4 and 6 (homozygous mutated genotype TT is found which has 175, 23 b.p fragments).



Figure (4): Enzymatic digestion of PCR product of A1298C polymorphism of MTHFR gene using *Mob* II enzyme. *Mob* II enzyme digests the 163 b.p fragment of the mutated type CC into 84, 31, 30 and 18 b.p fragments, while the wild type AA yields five fragments 56, 31, 30, 28 and 18; the small fragments (18, 28, 30, 31 b.p) have run off the gel. Lane M: DNA marker (50 b.p), lanes 2, 4 (wild type AA), lanes 1, 3, 5 and 7 (heterozygous mutated genotype AC which has 84, 56, 31, 30, 28 and 18 b.p fragments) and lane 6 (homozygous mutated genotype CC)

DISCUSSION

Thromboembolic complications, including pulmonary embolism, that occur during pregnancy and the puerperium are a major cause of maternal morbidity. During normal pregnancy, there are changes in the plasma concentrations and activities of several proteins involved in blood coagulation and fibrinolysis. These changes may promote coagulation, decrease anticoagulation, and inhibit fibrinolysis and thus may increase the risk of thromboembolic events, especially among pregnant women who have acquired or genetic risk factors for thrombosis⁽¹²⁾.

Point mutations in the genes encoding coagulation factor V (a change from guanine to adenine at nucleotide 1691 and prothrombin (a change from guanine to adenine at nucleotide 20210) have been found to be associated with thrombophilia⁽¹³⁾.

Placental insufficiency resulting in fetal loss has been recognized in women with thrombophilic conditions clearly related to venous thromboembolism⁽¹⁴⁾ and deficiencies of antithrombin, protein C, and protein S⁽¹⁵⁾.

A guanine-to-adenine mutation in the prothrombin gene, G20210A, associated with elevated plasma prothrombin concentrations and an increased risk of venous thrombosis, has been identified⁽¹⁶⁾. In addition, homozygosity for a common cytosine-to-thymidine mutation in the gene encoding 5, 10-methylenetetrahydrofolate reductase (MTHFR), C677T, is associated with high plasma homocysteine

concentrations and venous thrombosis⁽¹⁷⁾.

The results of the present study suggest the existence of a linkage between FVL, prothrombin but not MTHF reductase genes polymorphism in the pathogenesis of preeclampsia. This result is in agreement with **Martinelli et al.**⁽¹⁸⁾ who, demonstrated that women who are carriers of factor V or prothrombin mutations are at higher risk for late fetal loss than noncarriers.

Dirula et al.⁽¹⁹⁾ had found higher prevalence of FVL mutation in women with preeclampsia compared with control subjects. **Grandone et al.**⁽²⁰⁾ reported that the prothrombin gene mutation was significantly more frequent in preeclamptic patients than in controls.

The presence of either of these mutations was associated with no significant increased risk for RFL in first trimester. Genetic thrombophilic defects are common in women with RFL and are associated with late fetal loss. This association is manifest by FVL rather than total number of defects involved⁽²¹⁾.

The findings of **Kahn et al.**⁽²²⁾ do not indicate an increased risk of preeclampsia among women with any of the three studied inherited thrombophilias. The increase in placental underperfusion among women with preeclampsia seems to be associated with low plasma folate levels but not with the three studied thrombophilias examined. Also, **Morrison et al.**⁽²³⁾ concluded that these prothrombotic genotypes are not associated with the development of PE or GH in their population

Also, studies of the factor V mutation have revealed an association between that mutation and first- or second-trimester fetal loss⁽²⁴⁾. Carriers of that mutation also seem to be at a higher risk for third-trimester fetal loss than noncarriers, although this is not a consistent finding⁽²⁵⁾. However, the prothrombin-gene mutation is associated with fetal death is not known⁽¹⁵⁾.

Resistance to activated protein C caused by an adenine-to-guanine mutation at nucleotide 506 in the factor V gene has been linked with an increased risk of venous thromboembolism⁽²⁶⁾. Homozygosity for the mutation of cytosine to thymine at nucleotide 677 in the gene encoding MTHFR results in decreased synthesis of 5-MTHF that results in increase in plasma homocysteine concentrations producing a risk factor for venous and arterial thrombosis⁽²⁷⁾. Women who carried this mutation are at higher risk for various obstetrical complications, such as preeclampsia, abruptio placentas, intrauterine growth retardation, and late fetal loss⁽²⁸⁾.

A point mutation at nucleotide 1691 in exon 10 of the Factor V gene makes factor Va resistant to proteolytic inactivation by activated protein C⁽²⁹⁾ leading to physiological hypercoagulation in pregnancy and this may contribute to increased thrombus formation in the placenta and thus may be a hereditary risk factor for preeclampsia⁽³⁰⁾.

On the other hand, there was no difference in thrombophilic mutations between preeclamptic patients and normal pregnant women. Therefore, it would be suggested that preeclamptic

patients should not be tested for thrombophilia⁽³¹⁾.

Kupferminc et al.⁽²⁸⁾ stated that, there was no difference in the prevalence of genetic risk factors for thrombosis in women with preeclampsia compared with control subjects. Also, **Lindqvist et al.**⁽³²⁾ found no difference in prevalence for this mutation in preeclampsia or IUGR patients. This finding is supported by the findings of **Livingston et al.**⁽³³⁾, who found no association of maternal or fetal genetic polymorphisms (Factor V Leiden, Factor II, MTHFR) and severe preeclampsia.

In the population analyzed, the presence of the genotype risk factors alone does not seem to be associated with the development of preeclampsia even in the severe presentation form. However, the interactions between more than one thrombophilic gene (MTHFR, F II, FVL and PAI-1) polymorphisms were involved in the development of preeclampsia⁽³⁴⁾.

There was important association between FVL mutation only not all genetic thrombophilias and preeclampsia in the population studied by **Driula et al.**⁽¹⁹⁾.

Only Factor II: protein C activity levels, uterine arterial Doppler and a history of familial hypertension are useful in predicting poor pregnancy outcome in gestational hypertension⁽³⁵⁾.

Mello et al.⁽³⁶⁾ demonstrate a significant association between maternal thrombophilia and severe preeclampsia in white women. Thrombophilia also augments the risk of life-threatening maternal

complications and adverse perinatal outcomes in preeclamptic patients.

Factor V Leiden mutation is present in the Asian population and may very well serve as one of the genetic factors responsible for preeclampsia and other adverse pregnancy outcomes⁽³⁷⁾.

In conclusion, in the population analyzed in our region, the presence of the genotype risk factors seems to be associated with the development of preeclampsia. However, not all thrombophilic genes polymorphisms on the development of the preeclampsia were indicated.

REFERENCES

1. **Brown MA, Hague WM, Higgins J, Lowe S, Mc-Cowan L, Oats J, Peek MJ, Rowan JA, Walters BN (2000):** The detection, investigation and management of hypertension in pregnancy, Aust NZ. J. Obstet. Gynaecol., 40:139-155.
2. **Paidas MJ, Ku DH, Arkel YS (2004):** Screening and management of inherited thrombophilias in the setting of adverse pregnancy outcome. Clin. Perinatol., 31: 783–805
3. **Lin J and August P (2005):** Genetic thrombophilias and preeclampsia. A meta-analysis. Obstet. Gynecol., 105: 182–192
4. **Alfirevic Z, Roberts D, Martlew V (2002):** How strong is the association between maternal thrombophilia and adverse pregnancy outcome? A systematic review Eur. J. Obstet. Gynecol. Reprod. Biol., 101: 6–14.
5. **Schlembach D, Beinder E, Zingsem J, Wunsiedler U, Beckmann MW and Fischer T (2003):** Association of maternal and/or fetal factor V Leiden and G20210A prothrombin mutation with HELLP syndrome and intrauterine growth restriction. Clin. Sci.; 105, 279–285
6. **Ripoll L, Paulin D, Thomas S and Drouet LO (1997):** Multiplex PCR mediated site directed mutagenesis for one step determination of factor V Leiden and G20210A transition of the prothrombin gene. Thromb. Haemost., 78(2):960-961,
7. **Ranguelov RD, Rosenthal N, Bromley C and Vasef MA (2002):** Detection of factor V Leiden and prothrombin gene mutations in patients who died with thrombotic events. Arch. Pathol. Lab. Med., 126(10): 1193-1196.
8. **Siemianowicz K, Gminski J, Garczorz W, Slabiak N, Goss M, Machaski M, and Magiera-Molendowska H (2003):** Methylene tetrahydrofolate reductase gene C667T and A1298C polymorphisms in patients with small cell and non small cell lung cancer. Oncol. Rep., 10: 1341- 1344
9. **Mtiraoui N, Ezzidi I, Chaieb M, Marmouche H, Aouni Z, Chaieb A, Mahjoub T, Vaxillaire M, Almawi WY (2007):** MTHFR C677T and A1298C gene polymorphisms and hyperhomocysteinemia as risk factors of diabetic nephropathy in type 2 diabetes patients. Diabetes Res. Clin. Pract., 75(1):99-106.

10. **Moczulski D, Fojcik H, Zukowska-Szczechowska E, Szydłowska I, Grzeszczak W (2003):** Effects of the C677T and A1298C gene polymorphisms of the MTHFR gene on the genetic predisposition for diabetic nephropathy. *Nephrol. Dial. Transplant.*, 18(8):1535-1540.
11. **Schoonjans F, Zalata A, Depyudt CE, and Comhaire FH (1995):** MedCalc: a new computer program for medical statistics. *Computer Methods and Programs in Biomedicine* 48: 257-262.
12. **Hellgren M, Svensson PJ, Dahlback B. (1995):** Resistance to activated protein C as a basis for venous thromboembolism associated with pregnancy and oral contraceptives. *Am. J. Obstet. Gynecol.*, 173:210-213.
13. **Lensing AWA, Prandoni P, Prins MH, Büller HR (1999):** Deep-vein thrombosis. *Lancet* 353:479-485.
14. **Rand JH, Wu X-X, Andree H, et al. (1997):** Pregnancy loss in the antiphospholipid antibody syndrome, a possible thrombogenic mechanism. *N. Engl. J. Med.*, 337:154-160.
15. **Gris JC, Quéré I, Monpeyrroux F (1999):** Case-control study of the frequency of thrombophilic disorders in couples with late foetal loss and no thrombotic antecedent the Nimes Obstetricians and Hematologists Study5 (NOHA5). *Thromb. Haemost.*, 81:891-899.
16. **Poort SR, Rosendaal FR, Reitsma PH, Bertina RM (1996):** A common genetic variation in the 3'-untranslated region of the prothrombin gene is associated with elevated plasma prothrombin levels and an increase in venous thrombosis. *Blood* 88:3698-3703.
17. **Margaglione M, D'Andrea G, d'Addeda M, et al. (1998):** The methylenetetrahydrofolate reductase TT677 genotype is associated with venous thrombosis independently of the coexistence of the FV Leiden and the prothrombin A20210 mutation. *Thromb. Haemost.*, 79:907-911.
18. **Martinelli I, Taioli E, Cetin I, Marinoni A, Gerosa S, Villa MV, Bozzo M, Mannucci PM (2000):** Mutations in coagulation factors in women with unexplained late fetal loss. *N. Engl. J. Med.*, 343:1015-1018.
19. **Driula L, Damanteb G, D'Elia A, Iannia A, Springoloa F and Marchesoni D (2005):** Genetic thrombophilias and uterine artery Doppler velocimetry and preeclampsia. *International Journal of Gynecology & Obstetrics*; 88(3): 265-270.
20. **Grandone E, Margaglione M, Colaizzo D, Cappucci G, Scianname N, Montanaro S, Paladini D, Martinelli P, Di Minno G (1999):** Prothrombotic genetic risk factors and the occurrence of gestational hypertension with or without proteinuria. *Thromb. Haemost.*, 81:349-352.
21. **Kovacheva K, Ivanov P, Konova E, Simeonova M, Komsa-Penkova R. (2007):** Genetic thrombophilic defects

- (Factor V Leiden, prothrombin G20210A, MTHFR C677T) in women with recurrent fetal loss. *Akush. Ginekol.*, 46(7):10-16.
22. **Kahn SR, Platt R, McNamara H, et al. (2009):** Inherited Thrombophilia and Preeclampsia within a Multicenter Cohort: The Montreal Preeclampsia Study. *Obstetrical & Gynecological Survey* 64(6): 366-368.
 23. **Morrison ER, Miedzybrodzka ZH, Campbell DM, Haites NE, Wilson BJ, Watson MS, Greaves M, Vickers MA (2002):** Prothrombotic genotypes are not associated with pre-eclampsia and gestational hypertension: results from a large population-based study and systematic review. *Thromb. Haemost.*, 87(5):779-785.
 24. **Tormene D, Simioni P, Prandoni P, et al. (1999):** The risk of fetal loss in family members of probands with factor V Leiden mutation. *Thromb. Haemost.*, 82:1237-1239.
 25. **Meinardi JR, Middeldorp S, de Kam PH, et al. (1999):** Increased risk for fetal loss in carriers of factor V Leiden mutation. *Ann. Intern. Med.*, 130:736-739.
 26. **Dahlback B (1994):** Inherited resistance to activated protein C, a major cause of venous thrombosis, is due to a mutation in the factor V gene. *Hemostasis* 24:139-151.
 27. **Kluijtmans LAJ, van den Heuvel LPWJ, Boers GHJ, et al. (1996):** Molecular genetic analysis in mild hyperhomocysteinemia: a common mutation in the methylenetetrahydrofolate reductase gene is a genetic risk factor for cardiovascular disease. *Am. J. Hum. Genet.*, 58:35-41.
 28. **Kupferminc MJ, Fait G, Many A, Gordon D, Eldor A, Lessing JB (2000):** Severe preeclampsia and high frequency of genetic thrombophilic mutations. *Obstet. Gynecol.*, 96: 45-49.
 29. **Bertina RM, Koeleman BP, Koster T (1994):** Mutation in blood coagulation factor V associated with resistance to activated protein C. *Nature* 369:64-67.
 30. **Hefler L, Jirecek S, Heim K, Grimm C, Antensteiner G, Zeillinger R, Husslein P, Tempfer C (2004):** Genetic polymorphisms associated with thrombophilia and vascular disease in women with unexplained late intrauterine fetal death: a multicenter study. *J. Soc. Gynecol. Investig.*, 11:42-44.
 31. **Yalinkaya A, Erdemoglu M, Akdeniz N, Kale A, Kale E (2006):** The relationship between thrombophilic mutations and preeclampsia: a prospective case-control study. *Ann. Saudi. Med.*, 26(2):105-109
 32. **Lindqvist PG, Svensson PJ, Dahlback B, Marsal K (1998):** Factor V Q506 mutation (activated protein C resistance) associated with reduced intrapartum blood loss a possible evolutionary selection mechanism. *Thromb. Haemost.*, 79: 69-73.
 33. **Livingston JC, Barton JR, Park V, Haddad B, Phillips O, Sibai BM (2001):** Maternal and fetal

inherited thrombophilia are not related to the development of severe preeclampsia. *Am. J. Obstet. Gynecol.*, 185: 153-157.

34. **Dalmáz CA, Santos KG, Botton MR, Tedoldi CL, Roisenberg I (2006):** Relationship between polymorphisms in thrombophilic genes and preeclampsia in a Brazilian population. *Blood Cells Mol. Dis.*, 37(2):107-110.
35. **D'Aniello G, Florio P, Sabatini L, Severi FM, Fineschi D, Cito G, Guidoni CG, Petraglia F (2003):** The search for thrombophilic gene mutations in women with gestational hypertension does not help in

predicting poor pregnancy outcome. *J. Hypertens.*, 21(10):1915-1920.

36. **Mello G, Parretti E, Marozio L, Pizzi C, Lojacono A, Frusca T, Facchinetti F, Benedetto C (2005):** Thrombophilia is significantly associated with severe preeclampsia results of a large-scale, case-controlled study *Hypertension*;46:1270..
37. **Siti Z. O, Rajes Q, Si LK, Sekaran M and Sunil B (2008):** Thrombophilic mutations in pre-eclampsia and pregnancy-induced hypertension. *J. Obstet. Gynaecol. Res.*, 34(2):174 – 178.

الطفرات المتعلقة بالجلطات فى مرضى ما قبل تسمم الحمل

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 كلية الطب-جامعة المنصورة

هدف البحث: يهدف هذا البحث إلى دراسة العلاقة بين بعض أنواع التباينات المختلفه فى جينات البروثرومبين (عامل التجلط الثانى) و معامل التجلط الخامس (لايدن) و انزيم الميثيلين تتراهيدروفوليت ريدكتيز فى حدوث مرض ما قبل تسمم الحمل.

وقد اشتملت هذه الدراسة على ١٠٤ سيدة حامل منهم ٤٨ سيدة ذات ضغط طبيعى اثناء الحمل ويمثلون المجموعه الضابطه للبحث ، فى حين اشتملت مجموعه المرضى على ٥٦ سيدة حامل يعانون من ما قبل تسمم الحمل من المرضى المترددى على العيادة الخارجيه لأمراض النساء والتوليد فى مستشفى المنصورة الجامعى .

تم أخذ ٥ سم^٣ دم على مادة مانعة للتجلط و تم حفظه فى درجة حرارة (-٣٠) درجة مئوية حتى تم استخلاص الحامض النووي (دي إن إيه) واستخدامه فى تحديد النمط الجينى للبروثرومبين (أدنين ٢٠٢١٠ جوانين) و عامل التجلط الخامس لايدن (أدنين ١٦٩١ جوانين) و جين الميثيلين تتراهيدروفوليت ريدكتيز بنوعيه (سيتوزين ٦٧٧ ثايمين) و (أدنين ١٢٩٨ سيتوزين) بواسطة تفاعل البلمرة المتكرر ثم التحليل باستخدام الإنزيمات الحصرية.

وقد أظهرت هذه الدراسه وجود علاقته ارتباطية ايجابية بين جينات البروثرومبين (أدنين ٢٠٢١٠ جوانين) و عامل التجلط الخامس لايدن (أدنين ١٦٩١ جوانين) النوع الطافرى بينما لا توجد علاقة نو دلالة احصائية فى جين الميثيلين تتراهيدروفوليت ريدكتيز بنوعيه فى حدوث مرض ما قبل تسمم الحمل.