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ASSOCIATION ASSESSMENT OF THROMBOPHILIC GENE MUTATIONS IN KASHMIRI WOMEN WITH RECURRENT MISCARRIAGES

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ABSTRACT

Aim: To access the prevalence of factor V Leiden and prothrombin G20210A mutations in Kashmiri women with recurrent miscarriage in comparison to control group of parous women. **Method:** FVL and prothrombin G20210A mutations were analysed in 100 women with RM in comparison to 100 parous women. The mutations were analysed using ARMS- PCR approach. **Result:** All the recurrent miscarriage cases and controls were found to have FVL *GG* genotype and prothrombin 20210 *GG* genotype. There was no association of these mutations with the development of recurrent miscarriage. **Conclusion:** We conclude that FVL and prothrombin G20210A mutations do not contribute to the pathophysiology of recurrent miscarriage in Kashmiri women. Therefore, its screening is not indicated as an initial approach in Kashmiri women with RM.

KEYWORDS: Factor V Leiden; Prothrombin G20210A; Recurrent miscarriage; Kashmir.

INTRODUCTION

Recurrent miscarriage (RM) is defined as the loss of pregnancy as three early consecutive losses (<12 weeks gestation) or two late pregnancy losses (>12 weeks gestation). RM is recognised as one of the most disturbing health issues in women with a worldwide incidence of one per cent of women in the reproductive age group **(Stirrat GM, 2009; Clifford K et al, 1997; Maconochie N et al 2004)**. Several causative factors have been implicated in RM including chromosomal abnormalities of parent or fetus, anatomical malformations, autoimmune disorders, endocrine disorders, infections and other metabolic disorders

(Makino T et al 1990; Stephenson MD 1996; Rai R et al 1997; Lashen H et al 2004; Salim R et al 2003). However, the cause of 50% of RM cases still remain unexplained (**Clifford K et al, 1997; Jauniaux E et al 2006; Yang C et al, 2006).**

Thrombophilia has been indicated an the main risk factor for maternal thromboembolism thereby contributing to adverse pregnancy outcome especially recurrent pregnancy losses (RPL), pre-eclampsia, abruption placentae and intrauterine growth restriction **(Brenner B et al 1999; Visser J et al 2011; Colman-Brochu S, 2004; Abbate R et al 2003)**. A number of studies have focused on a higher prevalence of certain inherited thrombophilias, such as factor V Leiden (FVL) and prothrombin G20210A mutations in women with unexplained recurrent pregnancy losses (**Kupferminc MJ et al 1999; Rey E et al 2003; Roque H et al 2004; Jivraj S et al 2004; Robertson L et al 2006; Glueck CJ et al 2008)**. However, these reports have found conflicting results **(Coppens M et al 2007; Dilley A et al 2002; Finan RR et al 2002; Foka ZJ et al 2002)**. This heterogeneity has been also reflected in many existing meta-analyses **(Rey E et al 2003; Robertson L et al 2006; Kist WJ et al 2008; Kovalevsky G et al 2004)**. The purpose of this study was to study the prevalence of FVL and prothrombin G20210A mutation in Kashmiri women with a history of more than two miscarriages as compared to ethinicity matched healthy parous women controls.

MATERIALS AND METHODS

Participants

Our study comprised 100 consecutive women with unexplained recurrent miscarriages referred to our department by TRUST Maternity Hospital and Lal Ded Maternity Hospital, Kashmir. The inclusion criteria for the patients were those having at least two recurrent pregnancy losses and those women between 18-40 yrs of age. The women with anatomical defects of uterus, primary or secondary anti-phospholipid syndrome, previous history of thromboembolism, hormonal imbalance, hypertension, diabetes and thyroid problems, overt malignancy and abnormal self or parental karyotype were excluded from our study. The control group consisted of 100 healthy age matched parous women with at least one live child and without any history of miscarriages or gestational complications. The inclusion and exclusion criteria of patients and controls is given in **Table 1.**

Ethics

The study was approved by the ethical committee of the institute. Informed consent was obtained from both patients and controls to participate in the study.

Sample Collection and processing

About 2-3 ml of blood was collected in ethylenediamine tetraacetic acid EDTA containing vials from both patients and controls. DNA was isolated from the blood using a Zymogen (Irvine, CA, USA) DNA extraction kit. The quality of the extracted DNA was accessed by agarose gel electrophoresis. The extracted DNA was stored at 4°C.

Molecular Analysis

Factor V Leiden G1691A and Prothrombin G20210A mutation detection

Molecular diagnosis of FVL and prothrombin G20210A mutation was performed by using amplification refractory mutation system (ARMS). The PCR primers were designed based to amplify the sequences containing the position 1691 of the factor V gene and position 20210 of the prothrombin gene. The primers used in the PCR amplification reactions include a common primer and normal allele specific primer or a mutation specific primer. The primer sequences are given in **Table 2**. A 150bp fragment form exon 10 of Factor V gene surrounding nucleotide 1691 and 350bp fragment from PT gene surrounding nucleotide 20210 was amplified.

PCR programme

The PCR was carried out in a final volume of 30ul mixture containing 1X PCR buffer (Biotools), 0.2 mM dNTP mixture (biotools), 150 ng each primer (Sigma), 1U Taq DNA polymerase (Biotools 5U/µl) and 200 ng genomic DNA (0.2 µg/µl). Amplification was done at at 94 for 7 min, 30 cycles of 94°C for 30 s, X°C for 30 sec min, 72°C for 30sec min followed by extension at 72°C for 7 min (**Table 2**). The PCR products were visualized on a 2-3% agarose gel containing 0.5µg/ml ethidium Bromide and photographed.

Statistical analysis

Statistical analysis was performed by using Chi-square-testing and Fisher's exact test. A value of $p < 0.05$ was considered significant.

RESULTS

A total of 100 women with > 2 consecutive miscarriages were evaluated for FVL G1691A- and prothrombin G20210A mutations in an age controlled study with 100 healthy parous women who had >1 live birth without pregnancy loss. Those RM patients who were <18 or >40 yrs of age or had other gestational complications like anatomical defects of uterus, anti-

phospholipid syndrome, abnormal karyotype, malignancy, diabetes, or thyroid problem were excluded from this study.

In our study all the 100 RM cases and 100 controls were found to be GG homozygous**.** No association was found between the FVL mutation and prothrombin G20210A mutation and RM was found **(Table 3).**

Table 1: Inclusion and exclusion criteria of patients and controls.

	RM Cases	**Controls**
Inclusion criteria	> 2 consecutive miscarriages	>1 live birth without pregnancy loss
Exclusion Criteria	<18 >40 yrs of age. Anatomical defects of uterus. Anti-phospholipid syndrome. Abnormal Karyotype. Malignancy. Diabetes, Thyroid, Hypertension	Previous miscarriages or gestational complications

Table 2: Primer sequences for FVL and prothrombin gene amplification.

Mutation	**Primer Sequence**	**Annealing temperature**	**Amplicon Size**
FVL 1691G/A	(C): 5'-GGA CTA CTT GAC AAT TAC TGT TCT CTT G-3' (N): 5'-GCA GAT CCC TGG ACA GAC G-3' (M): 5'-GCA GAT CCC TGG ACA GAC A-3'	56^0	150bp
PT 20210 G/A	(C): 5'-TCTAGAAACAGTTGCCTG GCAG-3' (N): 5'-GCACTG GGA GCA TTG AGG ATC-3' (M): 5'-GCA CTG GGA GCA TTG AGG ATT-3'	60^0	340bp

Table 3: Allelic and genotypic frequency of FVL G1691A and prothrombin G20210A mutation in RM cases and healthy controls.

Group	**No**	**Factor V Leiden G1691A G > A genotype**			***P value***	**Allele Frequency**		***P value***
		GG	GA	AA		G	A	
Control	100	100	0	0	**1**	200	0	**1**
RM	100	100	0	0		200	0	
		Prothrombin G20210A						
Controls	100	100	0	0	**1**	200	0	**1**
RM	100	100	0	0		200	0	

DISCUSSION

In current study we analysed the frequency of FVL and prothrombin gene mutations in 100 RM cases compared to 100 parous women. Our data shows both the mutations to be absent in

both RM cases as well as healthy controls. Our results do not establish an association between recurrent miscarriage and the thrombophilia-related variants. Previously we have found FVL and prothrombin mutations to be present in arterial stroke patients from Kashmiri population **(Mahrukh et al 2017)**, but their frequency in healthy controls including parous women and those with RM is zero. It is well documented that FVL and prothrombin mutation is completely absent or extremely rare in patients of African, Japanese and South East Asian descent **(Seligsohn U et al 2001)**. Recurrent miscarriages associated with FVL and prothrombin mutations are thought to be as a result of thrombosis in utero-placental vasculature. Several studies have reported an association between inherited thrombophilias and increased pregnancy complications, such as severe preeclampsia, fetal growth restriction, stillbirth and abruptio placentae **(Kutteh WH et al 2006; Onderoglu L et al 2006, Coulam CB et al 2006; v et al 2006)**. The heparin thromboprophylaxis during pregnancy has been observed to lead to higher live birth in women with previous history of adverse pregnancy outcomes and thrombophilic defects **(Brenner et al 2000: Younis et al 2000).**

The FVL mutation (G to A substitution at position 1691 of the FV gene) prevents the inactivation of coagulation factor V by activated protein C, thereby leading to a state of hypercoagulability. FVL which is a significant increased risk factor for systemic thrombosis has also been associated with placental thrombosis **(Rai et al 1996; Dizon et al 1997)**. The placental infarction was more often seen in fetus with FVL allele as compared to normal allele **(Dizon et al 1997)**. A lower live birth rate (38%) was reported in women who had the FVL mutation compared to those who have a normal factor V genotype (69%) **(Rai *et al.*, 2002).** A histologically proven placental infarction has been reported in association with fetal carriage of FVL **(Dizon-Townson *et al.*, 1997)**. The impact of thrombophilic mutations on RM across different populations remains a controversial issue. While some studies have shown the association between recurrent pregnancy loss and FVL mutation from Brazil **(Souza SS et al 1999),** Israel **(Brenner B et al 1999)**, Sweden **(Wramsby ML et al 2000)**, Austria **(Hopmeier P et al, 2008)**, USA **(Glueck CJ et al 2008)**, UK **(Dawood F et al 2007)**, Lebanon **(Finan et al. 2002)** and Tunisia **(Mahjoub T et al 2005; Zammiti W et al 2006)** while other studies failed to demonstrate an association between Factor V leiden mutation and recurrent miscarriage **(Dizon-Townson DS, et al 1997; Pauer HU et al 1998)**, **Alfirevie et al. 2001, Altintas A et al 2007; Sotiriadis A et al 2007; Zahed LF et al 2006)**. In a study in Asian region including 85 women with RPL and 31 controls, both FVL and prothrombin gene mutation were not found to be associated with recurrent miscarriage **(Biswas A et al**

2008). This heterogeinity in the incidence of the mutation in different populations may be due to racial and ethnic differences in the gene frequency among different populations **(Burchard EG et L 2003).**

The prothrombin gene mutation (G to A substitution at position 20210 in the 3'-UTR of the prothrombin gene) is the second most common known inherited risk factor for thrombosis. The prothrombin A20210G has been identified as a risk factor for pregnancy loss in several studies and has been associated mostly to early recurrent pregnancy loss (**Martinelli I et al, 2000**). Most of the evidence that associates PT G20210A mutation to pregnancy failure originates in case– control studies **(Finan RR et al 2002; Foka ZJ et al 2002; Reznikoff-Etievan MF et al 2001).** Nevertheless, in most investigations, this relationship is consistent and, as described for FLV, is weaker with 1st trimester compared with 2nd trimester RM **(Rey E et al 2003; Robertson L et al 2002;, Kovalevsky G et al 2004)**]. Earlier in a study the prevalence of prothrombin G20210A was higher in women with both embryonic (17%) and fetal (16.9%) losses compared with controls (3%) **(Ivanov PD et al 2009).**

CONCLUSION

This is the first study conducted in Kashmiri population that has analyzed the role of thrombophilic markers on the pathogenesis of RM. The results obtained in this study are in accordance with the results of most of the previous research across the globe and indicate that the *FVL* and prothrombin G20210A mutations are not associated with recurrent miscarriage in Kashmiri women population. We suggest the thrombophilic gene screening should not be an initial approach in Kashmiri women with RM. Also large-scale studies should be performed to clarify the association between thrombophilic gene variants and RM.

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Conflict of interest: None declared.

REFERENCES

1. Abbate R, Lenti M, Fatini C, Gazzini A, Lapini I, Fedi S. L'ipercoagulabilita gravidica e puerperale. Haematologica, 2003; 88: 1–2.

2. Alfirevic Z, Roberts D, Martlew V. How strong is the association between maternal thrombophilia and adverse pregnancy outcome? A systematic review. Eur J Obstet Gynecol Reprod Biol., 2002; 101(1): 6–14 [Feb 10].
3. Altintas A, Pasa S, Akdeniz N, Cil T, Yurt M, Ayyildiz O, et al. Factor V Leiden and G20210A prothrombin mutations in patients with recurrent pregnancy loss: data from the southeast of Turkey. Ann Hematol, 2007; 86(10): 727–31.
4. Biswas A, Choudhry P, Mittal A *et al*. Recurrent abortions in Asian Indians: No role of factor V Leiden Hong Kong/ Cambridge mutation and MTHFR polymorphism. *Clin Appl Thromb Hemost*, 2008; 14: 102–104.
5. Brenner B, Sarig G, Weiner Z, Younis J, Blumenfeld Z, Lanir N. Thrombophilic polymorphisms are common in women with fetal loss without apparent cause. Thromb Haemost, 1999; 82: 6–9.
6. Brenner B, Hoffman R, Blumenfeld Z, Weiner Z, Younis JS. Gestational outcome in thrombophilic women with recurrent pregnancy loss treated by enoxaparin. BJOG. 2000 Mar; 107(3): 415-9.
7. Burchard EG, Ziv E, Coyle N, Gomez SL, tang H, Karter AJ, et al. The importance of race and ethnic background in biomedical research andclinical practice. N Eng J med, 2003; 348.
8. Clifford K, Rai R, Regan L (1997) Future pregnancy outcome in unexplained recurrent first trimester miscarriage. Hum Reprod, 12: 387–389.
9. Colman-Brochu S. Deep vein thrombosis in pregnancy. MCN Am J Matern Child Nurs, 2004; 29: 186–92.
10. Coppens M, Folkeringa N, Teune MJ, Hamulya´k K, van der Meer J, Prins MH, et al (2007) Outcome of the subsequent pregnancy after a first loss in women with the factor V Leiden or prothrombin 20210A mutations. J Thromb Haemost, 5(7): 1444–1448.
11. Coulam CB, Jeyendran RS, Fishel LA, Roussev R. Multiple thrombophilic gene mutations rather than specific gene mutations are risk factors for recurrent miscarriage. *Am J Reprod Immunol*, 2006; 55: 360–368.
12. Dawood F, Mountford R, Faruquharson R, Quenby S. Genetic polymorphisms on the factor V gene in women with recurrent miscarriage and acquired APCR. Hum Reprod, 2007; 22(9): 2546–53.
13. Dilley A, Benito C, Hooper WC, Austin H, Miller C, El-Jamil M, et al (2002) Mutations in the factor V, prothrombin and MTHFR genes are not risk factors for recurrent fetal loss. J Matern Fetal Neonatal Med, 11(3): 176–182.

14. Dizon-Townson DS, Meline L, Nelson LM, Varner M, Ward K. Fetal carriers of the factor V Leiden mutation are prone to miscarriage and placental infarction". Am J Obstet Gynecol, 1997; 177: 402–5.
15. Dizon-Townson DS1, Kinney S, Branch DW, Ward K. The factor V Leiden mutation is not a common cause of recurrent miscarriage. J Reprod Immunol. 1997 Oct; 34(3): 217-23.
16. Finan RR, Tamim H, Ameen G, Sharida HE, Rashid M, Almawi WY (2002) Prevalence of factor V G1691A (factor V Leiden) and prothrombin G20210A gene mutations in a recurrent miscarriage population. Am J Hematol, 71: 300–305.
17. Foka ZJ, Lambropoulos AF, Saravelos H, Karas GB, Karavida A, Agorastos T, et al (2000) Factor V Leiden and prothrombin G20210A mutations, but not methylenetetrahydrofolate reductase C677T, are associated with recurrent miscarriages. Hum Reprod, 15: 458–462.
18. Glueck CJ, Gogenini S, Munjal J, Tracy T, Pranikoff J, Wang P (2008) Factor V Leiden mutation: a treatable etiology for sporadic and recurrent pregnancy loss. Fertil Steril, 89: 410–416.
19. Hopmeier P, Puehringer H, van Trotsenburg M, Atamaniuk J, Oberkanins C, Dossenbach-Glaninger A. Association of endothelial protein C receptor haplotypes, factor V Leiden and recurrent first trimester pregnancy loss. Clin Biochem, 2008; 41(12): 1022–4.
20. Ivanov PD, Komsa-Penkova RS, Konova EI, Kovacheva KS, Simeonova MN, Popov JD (2009) Association of inherited thrombophilia with embryonic and postembryonic recurrent pregnancy loss. Blood Coagul Fibrinolysis, 20(2): 134–140.
21. Jauniaux E, Farquharson RG, Christiansen OB, Exalto N (2006) Evidence-based guidelines for the investigation and medical treatment of recurrent miscarriage. Hum Reprod, 21: 2216–2222.
22. Jivraj S, Rai R, Underwood J, Regan (2006) Genetic thrombophilic mutations among couples with recurrent miscarriage. Hum Reprod, 21(5): 1161–1165.
23. Kist WJ, Janssen NG, Kalk JJ, Hague WM, Dekker GA, de Vries JI (2008) Thrombophilas and adverse pregnancy outcome: a confounded problem. Thromb Haemost, 99(1): 77–85.
24. Kovalevsky G, Gracia CR, Berlin JA, Sammel MD, Barnhart KT (2004) Evaluation of the association between hereditary thrombophilias and recurrent pregnancy loss: a meta-analysis. Arch Intern Med, 164(5): 558–563.

25. Kupferminc MJ, Eldor A, Steinman N, Many A, Bar-Am A, Jaffa A et al (1999) Increased frequency of genetic thrombophilia in women with complications of pregnancy. N Engl J Med, 340: 9–13.
26. Kutteh WH, Triplett DA. Thrombophilia and recurrent pregnancy loss. *Semin Reprod Med*, 2006; 24: 54–66.
27. Lashen H, Fear K, Sturdee DW (2004) Obesity is associated with increased risk of first trimester and recurrent miscarriage: matched case–control study. Hum Reprod, 19: 1644–1646.
28. Maconochie N, Doyle P, Prior S (2004) The national women's health study: assembly and description of a population-base reproductive cohort. BMC Public Health, 4: 35.
29. Mahjoub T, Mtiraoui N, Tamim H, et al. Adverse pregnancy outcomes and maternal factor V (Leiden) and Prothrombin G20210 genotype in women with a history of recurrent idiopathic miscarriages. Am J Hematol, 2005; 80: 12–9.
30. Mahrukh H Zargar, Syed Shafia, Qurteeba Mahajan, Rehana Ahmad, Nabeela Khan. Factor V Leiden and prothrombin gene mutations in patients with arterial thrombotic diseases from Kashmiri population. *International journal of scientific research.* Volume 6;Issue-10;October-2017.
31. Makino T, Tabuchi T, Nakada K, Iwasaki K, Tamura S, Iizuka R (1990) Chromosomal analysis in Japanese couples with repeated spontaneous abortions. Int J Fertil, 35: 266–270.
32. Martinelli I, Taioli E, Cetin I, et al. Mutations in coagulation factors in women with unexplained late fetal loss. *N Engl J Med*, 2000; 343: 1015-1018.
33. Onderoglu L, Baykal C, Al RA, Demirtas E, Deren O, Gurgey A. High frequency of thrombophilia disorders in women with recurrent fetal miscarriage. *Clin Exp Obstet Gynecol*, 2006; 33: 50–54.
34. Pauer HU, Neesen J, Hinney B. Factor V Leiden and its relevance in patients with recurrent abortions. Am J Obstet Gynecol. 1998 Mar; 178(3): 629.
35. Rai R, Backos M, Elgaddal S, Shlebak A and Regan L (2002) Factor V Leiden and recurrent miscarriage—prospective outcome of untreated pregnancies. Hum Reprod, 17: 442–445.
36. Rai R, Regan L (1997) Antiphospholipid antibodies, infertility and recurrent miscarriage. Curr Opin Obstet Gynecol, 9: 279–282.

37. Rai RS1, Regan L, Chitolie A, Donald JG, Cohen H. Placental thrombosis and second trimester miscarriage in association with activated protein C resistance. Br J Obstet Gynaecol. 1996 Aug; 103(8): 842-4.
38. Rey E, Kahn SR, David M, Shrier I (2003) Thrombophilic disorders and fetal loss: a meta-analysis. Lancet, 361(9361): 901–908.
39. Reznikoff-Etievan MF, Cayol V, Carbonne B, Robert A, Coulet F, Millez J (2001) Factor V Leiden and G20210A prothrombin mutations are risk factors for very early recurrent miscarriage. BJOG, 108: 1251–1254.
40. Robertson L, Wu O, Langhorne P *et al.* Thrombophilia in pregnancy: A systematic review. *Br J Haenmatol*, 2006; 132: 171–196.
41. Robertson L, Wu O, Langhorne P, Twaddle S, Clark P, Lowe GD, Walker ID, Greaves M, Brenkel I, Regan L, Greer IA (2006) Thrombophilia in pregnancy: a systematic review. Thrombosis: risk and economic assessment of thrombophilia screening (TREATS) Study. Br J Haematol, 132(2): 171–196.
42. Roque H, Paidas MJ, Funai EF, Kuczynski E, Lockwood CJ (2004) Maternal thrombophilias are not associated with early pregnancy loss. Thromb Haemost, 91: 290–295.
43. Salim R, Regan L, Woelfer B, Backos M, Jurkovic D (2003) A comparative study of the morphology of congenital uterine anomalies in women with and without a history of recurrent first trimester miscarriage. Hum Reprod, 28: 162–166.
44. Seligsohn U, Lubetsky A (2001) Genetic susceptibility to venous Thrombosis. New Eng J Med, 344: 1222–1231.
45. Sotiriadis A, Vartholomatos G, Pavlou M, Kolaitis N, Dova L, Stefos T, et al. Combined thrombophilic mutations in women with unexplained recurrent miscarriage. Am J Reprod Immunol, 2007; 57(2): 133–41.
46. Souza SS, Ferriani RA, Pontes AG, Zago MA, Franco RF. Factor V Leiden and factor II G20210A mutations in patients with recurrent abortion". Hum Reprod, 1999; 14: 2448–50.
47. Stephenson MD (1996) Frequency of factors associated with habitual abortion in 197 couples. Fertil Steril, 66(1): 24–29.
48. Stirrat GM (2009) Recurrent miscarriage: definition and epidemiology. Lancet, 336: 673–675.
49. Visser J, Ulander VM, Helmerhorst FM, Lampinen K, Morin-Papunen L, Bloemenkamp KWM, et al. Thromboprophylaxis for recurrent miscarriage in women with or without

thrombophilia HABENOX*: A randomized multicentre trial. Thromb Haemost, 2011; 105: 295–301.

50. Wramsby ML, Sten-Linder M, Bremme K. Primary habitual abortions are associated with high frequency of factor V Leiden mutation". Fertil Steril, 2000; 74: 987–91.
51. Yang C, Stone P, Stewart AW (2006) The epidemiology of recurrent miscarriage: a descriptive study of 1214 prepregnant women with recurrent miscarriage. Aust NZ J Obstet Gynaecol, 46: 316–322.
52. Younis JS1, Ohel G, Brenner B, Haddad S, Lanir N, Ben-Ami M. The effect of thrombophylaxis on pregnancy outcome in patients with recurrent pregnancy loss associated with factor V Leiden mutation. Thromb Haemost. 2000 May; 83(5): 693-7.
53. Zahed LF, Rayes RF, Mahfouz RA, Taher AT, Maarouf HH, Nassar AH. Prevalence of factor V Leiden, prothrobmin and methylenetetrahydrofolate reductase mutations in women with adverse pregnancy outcomes in Lebanon. Am J Obstet Gynecol, 2006; 195(4): 1114–8.
54. Zammiti W, Mtiraoui N, Mercier E, Abboud N, Saidi S, Mahjoup T, et al. Association of factor F gene polymorphisms (Leiden; Cambridge; Hong Kong and HR2 haplotype) with recurrent idiopathic pregnancy loss in Tunisia. A case control study. Thromb Haemost, 2006; 95(4): 612–7.