Basrah Journal ***Leading Article***

of Surgery ***Bas J Surg,September, 10, 2004***

FACTOR V LEIDEN AND THROMBOEMBOLISM

Z. Al-Barazanchi

M.Sc (Hematology), Specialist Haematologist , Laboratory Department, Basrah General Hospital**.**

AREVIATIONS

TE, Thromboembolism; ***OC***, oral contraceptives; ***FVL***, factor V leiden; ***APC***, activated protein C; ***APC-R***, activated protein C resistance; ***GP***, Glycoprotein; ***VTE***, Venous thromboembolism; ***PE***, Pulmonary embolism; ***BCS***, Budd-Chiari syndrome; ***PVT***, portal vein thrombosis, ***CAD***, coronary artery disease;***aPTT,*** activated partial thromboplastin time;***PCR***, Polymerase chain reaction;***RFLP***, Restriction fragment length polymorphism;

Introduction

Thromboembolism (TE) is a multicausal disease that includes a combination of one or more of certain genetic defects that produce hypercoagulable state with one or more of the well known acquired risk factors like inactivity, trauma, malignancy, inflammation, pregnancy, birth, oral contraceptive (OC) use or autoimmune disease. Hereditary thrombophilia is the genetic predisposition to thrombosis. Most of these thrombophilic defects act either by enhancing a pro-coagulant reaction or jeopardize an anticoagulant mechanism, leading to hypercoagulable state. Factor V leiden (FVL) mutation [**A**ctivated **P**rotein **C** **R**esistance (APC-R)], prothrombin polymorphism (G20210A) and hyperhomocysteinemia are the most common causes of hereditary thrombophilia while deficiency of protein C, protein S and anti thrombin and elevated factor VIII come next in frequency. Knowledge of FVL mutation (and other genetic defects leading to TE will affect patient management including the duration of anticoagulant treatment, the use of clotting factor replacement therapy, the need of prophylactic antithrombotic agents and counseling involving the relative risks of pregnancy and use of OC drugs or hormonal replacement. A clinical review of FVL

mutation and its effect on TE has, thus, been attempted.

Historical Review

Egeberg[1], in 1965, was the first who described hereditary thrombophilia in members of a family suffered from recurrent venous thrombosis with an autosomal dominant inheritance and reduced plasma antithrombin III. Stenflo, in 1976[2] was, then, able to purify and characterize a vitamin K-dependent anticoagulant factor that eluted in the third peak (peak C), from an anion exchange column from bovine plasma and, thus, he called it protein C. Later on, the first patients with hereditary protein C deficiency and thrombosis were described by Griffin and Colleagues[3]. In 1989, an abnormally poor response to activated protein C (APC) was described caused by the presence, in the plasma, of antibodies interfering with the expression of APC activity[4] and causing an "activated protein C resistance (APC-R)". In 1993, Dahlback and co-workers reported venous thrombosis in three unrelated families with familial APC-R and no acquired identifiable defect[5]. In May 1994, the underlying genetic defect causing familial APC-R was independently identified by three laboratories to involve a mutant form of factor V, one of them was that of Bertina and Colleagues in Leiden, thus the mutant factor was called factor V leiden[6-8] which was postulated, later, to arise in a single Caucasian founder some 21000 to 34000 years ago[9] and had expanded to Europe during the Neolithic Period from a probable Anatolian Center of origin in Turkey[10].

Genetics and molecular biology

FACTOR V: Human factor V is a high molecular weight (330000) single chain glycoprotein(GP) consisting of 2196 amino acids[11].The gene controlling it's synthesis and function is located on chromosome 1q21-25[12] and containing 25 exons[13]. Factor V is activated after several proteolytic cleavages by thrombin[14] or activated factor X (Xa)[15]. Both factors, Va and Xa form the prothrombinase complex, which, on the phospholipids (PL) membrane of platelets and in the presence of Ca^{++}, catalyzes the conversion of prothrombin to thrombin. The exclusion of factor V from the complex reduces thrombin generation by four orders[16]. Factor V is synthesized in the liver[17] and by megakaryocytes[18]. It,s plasma concentration is about 7 mg/L[19] with half life of about 12-36 hrs[20].It is inactivated by APC through limited proteolysis in the presence of protein S, Ca^{++} and either platelets or endothelial cell membrane PL[22].

PROTEIN C: Protein C is a natural anticoagulant GP with a MW of 62 kDa that is synthesized in the liver. It has a plasma concentration of 4 mg/L with a half life time of 6 hrs[22]. Protein C gene, comprising nine exons and eight introns, is located on chromosome 2q14-21[23].It is converted to an active serine protease, (APC) due to a cleavage by thrombin at the Arg169-Leu170 peptide and in a Ca^{++} -dependent reaction that is accelerated by orders of magnitude by thrombomodulin[24].

PATHOPHYSIOLOGY: The genetic defect underlying FVL is a substitution of a guanine(G) with adenine(A) at nucleotide position 1691 in exon 10 of the factor V gene causing a substitution of arginine at position 506 by glutamine (Arg 506 Gln), thereby providing resistance to proteolytic cleavage by APC[6-8].Gln 506-factor Va variant is inactivated 10 times slower than the normal Arg 506-F Va[25]. It is an autosomal dominant defect[26] that contributes to >90 % of cases of hereditary APC-R[27], thus APC-R should not be used as a synonym for FVL mutation. The variant factor Va shows

only a partial resistance to APC because cleavage at Arg306 in factor Va also occurs, causing complete loss of factor Va activity. This finding, along with the incomplete penetrance of FVL helps to explain why APC-R due to Gln506-factor Va is rather mild risk factor for VTE and suggests the importance of other contributing factors, genetic (prothrombin 20210A mutation) or acquired (vascular damage, stasis, OC, etc) in the pathogenesis of thrombus formation[25,28,29].

INCIDENCE

Factor V Leiden mutation is the most common inherited risk factor for thrombosis in Caucasians[30], accounting for 3 to 12 % of population[31]. There is a significant ethnic distribution as it is rare in Asians and Chinese[32-33], while in Cyprus it shows a peak incidence[10]. However, in Arab Countries it shows a high prevalence: Syria 13.6 %, Lebanon 14.4 %, and Jordan 12.3 %[34].

CLINICAL FEATURES

Factor V Leiden is a risk factor for venous TE (VTE). Deep and superficial venous thromboses are the most common manifestations, while pulmonary embolism (PE) and thromboses in unusual sites appear to be relatively less frequent than in subjects with deficiencies in antithrombin, protein C or protein S[35]. It leads to a seven fold increased risk of VTE with a relative risk of 10.6 in heterozygous carriers[36,37], while homozygous carriers have an odds ratio for VTE of 50-100[38].The prevalence of FVL in VTE patients is around 71 % with higher prevalence among younger age groups (below 45 years)[39]. First degree relatives of symptomatic carriers of FVL develop thrombosis at a rate of 0.45 / year[40].

Factor V Leiden has been found to be associated with a higher and significant incidence of early graft perfusion defects, acute graft rejection, usually within 7 days, delayed graft function and chronic graft dysfunction after renal transplantation[40-42]. It is the most common risk factor for Budd-Chiari syndrome (BCS) and/or portal vein thrombosis(PVT)(relative risks 11,3 &1.4 respectively), where it precipitates thrombosis mostly when combined with another risk factor[43-45]. In patients with polycythemia vera, and essential thrombocythemia, carriership of FVL was associated with VTE wita prevalence of 3.6 % in symptomatic patients, 6.9 % in patients with single episode of VTE and 18.1 % in patients with recurrent VTE[46]. It is highly prevalent in patients with postthrombotic venous ulcers[47], ulcerative colitis[48] and it is attributed for 17.3 % of all thromboses in patients with central venous catheters[49]. It is reported as a risk factor for retinal vein occlusion[50]. In patients with malignant diseases, there is a significant effect of FVL on thrombosis. Most thromboses occur during the first month after tumor diagnosis[51]. Coronary artery thrombosis has been notably associated with FVL mutation in young women[52] and men[53]. The relative risk of myocardial infarction in FVL carriers from the Netherlands is 1.4 which increases to 3 to 6 folds if other risk factors such as obesity, smoking, hypertension or diabetes are present[54]. However, other studies failed to reveal a relationship between FVL mutation and CAD or acute MI development[39].

FACTOR V LEIDEN AND OBSTETRICAL COMPLICATIONS

Factor V Leiden mutation is the most common cause of primary and recurrent VTE in pregnancy. Combined with the prothrombotic state of pregnancy, it predisposes to many pregnancy complications like recurrent pregnancy loss and still birth, severe and early onset preeclampsia (PE), placental abruptions and possibly intrauterine growth restriction[26]. The risk increases in homozygous carriers and if accompanied

by other thrombophilic abnormalities like G20210A prothrombin mutation[55,56]. Women on OC pills and FVL mutation carriage have a significantly increased risk of thrombosis[57].It increases the risk of early onset gestational hypertension and HELLP syndrome (***H***emolysis, ***E***levated ***L***iver enzymes, ***L***ow ***P***latelets)[58]. It has been shown that there is a high prevalence of FVL in mothers of growth retarded neonates (7.2 %) and in mothers of premature infants (18 %)[59].

PREDISPOSING FACTORS FOR THROMBOSIS IN SUBJECTS WITH FVL MUTATION

Despite its association with a relatively mild hypercoagulable state, FVL mutation will greatly have a magnified thrombotic phenomenon when other prothrombotic disorders also exist. These risk factors can be hereditary (protein C deficiency or prothrombin gene mutation), acquired (antiphospholipid syndrome), physical (inactivity or surgery), due to other diseases (malignancy or inflammation) or hormonal (OC or pregnancy)[60].Multiple hereditary thrombophilic defects (gene-gene interactions) are quite common and found in up to 15 % of patients presenting with VTE[61].

LABORATORY DIAGNOSIS

Coagulation assays and DNA–based assays are now available for the identification of FVL mutation. Plasma coagulation tests are often used for screening patients, followed by confirmation of positive results with the DNA assays. Only DNA tests can distinguish homozygous from hetero-zygous FVL mutation.

Plasma-based coagulation tests depend on the relative prolongation of activated partial thromboplastin time (aPTT) or other coagulation screening tests caused by the addition of purified APC. Individuals with APC-R have less prolongation of aPTT than normal. Although an aPTT assay was used, current tests use factor V-deficient plasma to make the test more suitable for screening[62]. A Pro-C Global assay has been developed by Dade Behring based on the ability of endogenous APC generated from protein C by an extract from Agkistrodon contotrix contotrix venom to prolong aPTT[30]. The STA-STACLOT APC-R test (Diagnostica Stago) is based on the specific activation of factor X by Crotalus viridis helleri snake venom The results are given as clotting time in seconds of patient's plasma in the presence of venom and activated protein C. Normal range is 136.4-174.7 sec. Clotting time < 136 sec is seen in FVL carriers with the homozygous showing time < 66 sec while the heterozygous > 80 sec[63].

Many DNA-based assays for the FVL polymorphism are now available. Genomic DNA is isolated, amplified by polymerase chain reaction (PCR), subjected to restriction fragment length polymorphism(RFLP) analysis and analyzed for G or A at nucleotide 1691[8]. PCR-independent methods for DNA analysis had been developed, one uses a homogeneous invader micro titer plate fluorescence resonance energy transfer (FRET) assay which gives results 100 % concordant with PCR-based methods as well as it's being more simple[64]. Another method developed, using single tube bi-directional allele-specific amplification and ultra thin agarose gel electro-phoresis[65].

MANAGEMENT & COUNSELING

Patients with FVL mutation who develop a DVT or PE are initially treated with heparin or LMW heparin for the acute illness and warfarin for the long term protection. Warfarin dose should be adjusted to give an INR range of 2-3 with an optimal duration of 6 months following a thrombotic event[66]. Prophy-lactic oral anticoagulant therapy is usually not warranted in subjects with FVL mutation discovered on routine

family testing or for another reason and who have not yet developed a thrombotic event as the risk of hemorrhage due to warfarin (about 1.3 % per year) out weights the risk of thrombosis (about 0.4 % per year in asymptomatic carriers). In contrast, long term antithrombotic treatment is needed for all with recurrent thrombosis and having especially more than one hereditary or acquired hypercoagulable states. An alternative approach, if oral anticoagulant therapy is not used, is to use intensive antithrombotic prophylaxis (eg LMWH), for events with a high risk of thrombosis such as surgery, infections (eg pneumonia), or inflammatory diseases (eg inflammatory bowel disease) or prolonged periods of inactivity. This should reduce the risk of thrombo-embolism by half since about 50 % of thromboses in patients with hereditary hypercoagulable states can be attributed to a known provoking factor[67]. Screening for the Leiden mutation is advisable in women with previous pregnancy complications and carriers of such a mutation should be given the appropriate counselling[68]. VTE that occurs during pregnancy requires therapeutic doses of heparin for the remainder of the pregnancy, followed by postpartum anticoagulants for at least 4 weeks[69]. All women attended to be put on estrogen replacement or OC therapy should be screened for FVL mutation which is a relative or absolute contraindication to give ERT or OC because of the increase risk of VTE[70,71]**.** In October 1997, an elective liver transplantation was performed and it has led to the disappearance of APR-R (hereditary, caused by FVL mutation or acquired, since IgG anticardiolipin antibodies were found to be negative since then). It proved especially effective if Budd–Chiari syndrome is the outcome since liver transplantation not only treats the chronic disease but also it cures the state of thrombophilia since factor V is mainly synthesized in the liver[72].

RFERENCES

1. Egeberg O: Inherited antithrombin deficiency causing thrombophilia. Thromb Diath Haemorrh, 1963, 13: 516

2. Stenflo JA: A new vitamin K-dependent protein: purification from bovine plasma and preliminary characterization. J Biol Chem, 1976, 251: 355

3. Griffin JH, Evatt B, Zimmerman TS, et al: Deficiency of protein C in congenital thrombotic disease. J Clin Invest, 1981, 68: 1370

4. Marciniak E, Romond EH: Impaired catalytic function of activated protein C: a new in vitro manifestation of lupus anticoagulant. Blood, 1989, 74: 2426

5. Dahlback B, Carlsson M, and Svenson PJ: Familial thrombophilia due to a previously unrecognized mechanism characterized by poor anticoagulant response to activated protein C: Proc Natl Acad Sci, 1993, 90: 1004

6. Bertina RM, Koeleman BPC, Koster T, et al: Mutation in blood coagulation factor V associated with resistance to activated protein C. Nature, 1994, 369: 64

7. Greengard JS, Sun X, Xu X, et al: Activated protein C resistance caused by Arg506Gln mutation in factor Va. Lancet, 1994, 343: 1361

8. Voorberg J; Roelse J; Koopman R, et al: Association of idiopathic venous thromboembolism with single point-mutation at Arg506 of factor V. Lancet, 1994, 343: 1535

9. Zivelin A, Griffin JH, Xu X, et al: A single genetic originfor a common Caucasian risk factor for venous thrombosis. Blood.1997, 89: 397

10. Lucotte G, Mercier G: Population genetics of factor V Leiden in Europe. Blood Cells Mol Dis, 2001, Mar-Apr, 27(2):362-7

11. Kone WH, Davie EW: Blood coagulation factors V & VIII: Structural and functional similarities and their relationship to hemorrhagic and thrombotic disorders. Blood, 1988, 71:539

12. Wang H, Riddell DC, Guinto ER, et al: Location of the gene encoding human factor V to chromosome 1q21-25.Genomics, 1988, 2: 324

13. Cripe LD, Moore KD, Kane WH: Structure of the gene for human coagulation factor V. Biochemistry, 1992, 31:3777

14. Suzuki K, Dahlback B, Stenflo J: Thrombin catalyzed activation of human coagulation factor V. J Biol Chem, 1982, 257: 6556

15. Foster WB, Nesheim ME, Mann KG: The factor Xa-catalyzed activation of factor V.J Biol Chem, 1983, 258:13970

16. Tracy PB, Mann KG: Abnormal function of prothrombinase complex: Factor V deficiency and related disorders. Hum Pathol.1987, 18:162

17. Wilson DB, Salem HH, Mruk JS, et al: Biosynthesis of coagulation factor V by human hepatocellular carcinoma cell line. J Clin Invest, 1983, 73:654

18. Gerwitz AM, Keefer M, Doshi K, et al: Biology of human megakaryocyte factor V. Blood, 1986, 67, 1639

19. Tracy PB, Eide LL, Bowie EJ, et al: Radioimmunoassay of factor V in human plasma and platelets. Blood, 1982, 60: 59

20. Seeler RA: Parahemophilia: Factor V deficiency. Med Clin North Am, 1972, 56: 119

21. Suzuki K, Stenflo J, Dahlback B, et al: Inactivation of human coagulation factor V by activated protein C. J Biol Chem, 1983, 358: 1914

22. Heeb MJ; Schwarz HP; White T; et al: Immunoblotting studies of the molecular forms of protein C in plasma. Thormb Res; 1988; 52: 33

23. Foster DC; Yoshitake S; Davie EW: The nucleotide sequence of the gene for human protein C. Proc Natl Acad Sci USA, 1985; 82: 4673

24. Owen WG; Esmon CT: Functional properties of an endothelial cell cofactor for thrombin-catalyzed activation of protein C. J Biol Chem; 1981; 256:5532

25. Heeb MJ, Kojima Y, Greengard JS, et al: Activated protein C resistance: Molecular mechanisms based on studies using purified Gln506 factor V. Blood, 1995, 85: 3405

26. Bloomenthal,-Dena; Delisle,-Marie-France; Tessier,-Francine, et al: Obstetric implications of factor V Leiden mutation: a review. Am J Perinatol, 2002, Jan; 19(1):37-47

27. Gherman RB; Goodwin TM: Obstetric implications of activated protein C resistance and factor V Leiden mutation. Obstet Gynecol Surv, 2000, Feb; 55(2): 117-22

28. Eitzman,-Daniel T; Westrick,-Randal J; Bi,-Xiaoming; et al: Lethal perinatal thrombosis in mice resulting from the interaction of tissue factor pathway inhibitor deficiency and factor V Leiden. Circulation, 2002, May, 7105(18):2139-42

29. Becattini,-Cecilia; Angelli,-Giancarlo: Pathogenesis of venous thromboembolism. Curr Opin Pul Med, 2002, Sep; 8(5):360-4

30. Quincampoix, JC; Legarff M; Rittling C; et al: Modification of the Pro C global assay using dilution of patient plasma in factor V-depleted plasma as a screening assay for factor V Leiden mutation. Blood Coagul Fibrinolysis, 2001, Oct, 12(7): 569-76

31. Gregg JP; Yamane AJ; Grody WW: Prevalence of the factor V Leiden mutation in four distinct American ethnic populations. Am J Med Genet, 1997, 73: 334

32. Angehaisuksiri P; Piagsuthiwong S; Aryuchai K, et al: Prevalence of the G1691A mutation in the factor V gene (Factor V Leiden) and the G20210A prothrombin gene mutation in Thai population. Am J Hematol, 2000, Oct, 65(2); 119-22

33. Ho CH; Chau WK; Hsu HC, et al: Prevalence of factor V Leiden in the Chinese population. Zhonhua-Yi-Xue-Za-Zhi (Taipei); 1999, Dec, 62(12): 875-8

34. Irani-Hakime N, Tamim H; Kreidy R; et al: The prevalence of factor V 506Q mutation-Leiden among apparently healthy Lebanese. Am J Hematol, 2000, Sep; 65(1): 45-9

35. Dahlback B: Resistance to activated protein C as a risk factor for thrombosis: molecular mechanisms, laboratory investigation and clinical management. Sem Hematol, 1997, 34: 217

36. Lesen R; Rosendaal F; Vandenbroucke J; Bertina R: Factor V Leiden: the venous thrombotic risk in thrombophilic families. Br J Haematol, 2000, Sep; 110(4): 939-45

37. Martinelli Ida; De-Stefano-Valerio; Taioli-Emanuela; et al: Inherited thrombophilia and first venous thromboembolism during pregnancy and puerperium. Thromb Haemost; 2002; May; 87(5): 791-5

38. Rosendaal FR; Koster T; Vandenbroucke JP; et al: High risk of thrombosis in patients homozygous for factor V Leiden (Activated protein C resistance). Blood, 1995; 85: 1504

39. Irani-Hakime N, Tamim H; Elias G; et al: Factor V R506Q mutation-Leiden: an independent risk factor for venous thrombosis but not coronary artery disease. J Thromb Thrombolysis, 2001, Apr; 11(2):111-6

40. Middeldorp S; Henkens CMA; Koopman MMW; et al: The incidence of venous thromboembolism in family members of patients with factor V Leiden mutation and venous thrombosis. Ann Intern Med; 1998; 128: 15

41. Wuthrich RP; Cicvara-Muzar S; Booy C; et al: Heterozygosity for the factor V Leiden (G1691A) mutation predisposes renal transplant recipients to thrombotic complications and graft loss. Transplantation, 2001; Aug; 15; 72(3): 549-50

42. Hocher,-Berthold; Slowinski,-Torsten; Hauser,-Ingeborg; et al: Association of factor V Leiden mutation with delayed graft function, acute rejection episodes and long term graft dysfunction in kidney transplant recipients. Thromb Haemost; 2002; Feb; 87(2): 194-8

43. Jansen HL; Meinardi JR; Vleggaar FP; et al: Factor V Leiden mutation, prothrombin gene mutation and deficiencies in coagulation inhibitors associated with Budd-Chiari syndrome and portal vein thrombosis: results of a case-control study. Blood; 2000; Oct 1; 96(7): 2364-8

44. Mohanty D; Shetty S; Ghosh K; et al: Hereditary thrombophilia as a cause of Budd-Chiari syndrome: a study from Western India. Hepatology; 2001; Oct; 34(4pt1):666-70

45. Deltenre P; Denninger MH; Hillaire S; et al: Factor V Leiden related Budd-Chiari syndrome. Gut; 2001; Feb; 48(2): 264-8

46. Ruggeri,-Marco; Gisslenger,-Heinz; Tosetto,-Alberto; et al: Factor V Leiden mutation carrier ship and venous thromboembolism in polycythemia vera and essential thrombocythemia. Am J Hematol; 2002; Sep; 71(1):1-6

47. Hanfer J; Kuhne A; Schar B; et al: Factor V Leiden mutation in post-thrombotic and non-post-thrombotic venous ulcers. Arch Dermatol 2001; May; 137(5): 599-603

48. Haslam N; Standen GR; Probert CS: An investigation of the association of the prothrombin G20210A gene

mutation and inflammatory bowel disease: factor II and IBD. Inflamm Bowel Dis 2001; May; 7(2): 133-5

49. Fijnheer,-Rob; Paijmans,-Birgit; Verdonck,-Leo F; et al: Factor V Leiden in central venous catheter-associated thrombosis. Br J Haematol; 2002; Jul; 118(1): 267-70

50. Scott JA; Arnold JJ; Currie JM; et al: No excess of factor V: Q506 genotype but high prevalence of anti-cardiolipin antibodies without anti-endothelial cell antibodies in retinal vein occlusion in young patients. Ophthalmologica, 2001; May-Jun; 215(3): 17-21

51. Pihusch,-Rodolf; Danzl,-Gudrun; Scholz,-Michael; et al: Impact of thrombophilic gene mutations on thrombosis risk in patients with gastrointestinal carcinoma. Cancer; 2002; Jun; 15; 94(12): 3120-6

52. Rosendaal FR; Siscovick DS; Schwartz SM; et al: Factor V Leiden (Resistance to activated protein C) increases the risk of myocardial infarction in young women. Blood; 1997; 89:2817

53. Inbal A; Freimark D; Modan B; et al: Synergistic effects of prothrombotic polymorphisms and atherogenetic factors on the risk of myocardial infarction in young males. Blood; 1999; 93: 2186

54. Doggen CJM; Cats VM; Bertina RM; et al: Interaction of coagulation defects and cardiovascular risk factors-increased risk of myocardial infarction associated with factor V Leiden or prothrombin 20210A. Circulation; 1998; 97: 1037

55. Reznikoff-Etievan MF; Cayol V; Carbonne B; et al: Factor V Leiden and G20210A prothrombin mutations are risk factors for very early miscarriage. BJOG; 2001; Dec; 108(12): 1251-4

56. Tormene D; Simioni P; Prandoni P; et al: Factor V Leiden mutation and the risk of venous thromboembolism in pregnant women. Haem; 2001; Dec; 86(12): 1305-9

57. Sass-Amy E; Neufeld-Ellis J: Risk factors for thromboembolism in teens: When should I test? Curr Opin Pediatr; 2002; Aug; 14(4): 370-8

58. Bloomenthal-Dena; von-Dadelszen-Peter; Liston-Robert; et al: The effect of factor V Leiden carriage on maternal and fetal health. CMAJ; 2002; Jul 9; 167(1): 48-54

59. Erhardt E; Stancovics J; Molnar D; et al: High prevalence of factor V Leiden mutation in mothers of premature neonates. Biol-Neonate; 2000; 78(2): 145-6

60.Rosendaal FR: Venous thrombosis: a multicausal disease. Lancet; 1999; 353:1167

61. Salomon O; Steinberg DM; Zivelin A; et al: Single and combined prothrombotic factors in patients with idiopathic thromboembolism-Prevalence and risk assessment. Arterioscler Thromb Vasc Biol. 1999; 19:511

62. de-Ronde H; Bertina RM: Careful selection of sample dilution and factor V-deficient plasma makes the modified activated protein C resistance test highly specific for the factor V leiden mutation. Blood Coagul Fibrinolysis; 1999; Jan; 10(1):7-17

63. Quenhenberger P; Hardler S; Mannhalter C; et al: Evaluation of a highly specific functional test for the detection of factor V Leiden. Int J Clin Lab Res; 2000; 30(3): 113-7

64. Ledford M; Friedman KD; Hessner MJ; et al: A multi-site study for the detection of the factor V (Leiden) mutation from genomic DNA using a homogeneous invader microtiter plate fluorescence resonance energy transfer (FRET) assay. J Mol Diagn; 2000 May; 2(2): 97-104

65. Sasvari-Szekely M; Gerstner A; Ronai Z; et al: Rapid genotyping of factor V Leiden mutation using single-tube bidirectional allele-specific amplification and automated ultrathin-layer agarose gel electrophoresis. Electrophoresis; 2000; Mar; 21(4):816-21

66. Hirsh J; Kearson C; Ginsberg J: Duration of anticoagulant therapy after first episode of venous thrombosis in patients with inherited thrombophilia. Arch Intern Med; 1997; 157:2174

67. Lensing AWA; Prandoni P; Prins MH; et al: Deep vein thrombosis. Lancet; 1999; 353: 479

68. Spina V; Aleandri V; Morini F: The impact of the factor V Leiden mutation on pregnancy. Hum Report Update; 2000; May-Jun; 6(3): 301-6

69. Toglia MR; Weg JG: Venous thromboembolism during pregnancy. N Engl J M; 1996; 335: 108

70. Glueck CJ; Wang P; Fontaine RN; et al: Effect of exogenous estrogen on atherothrombotic vascular disease risk related to the presence or absence of the factor V Leiden mutation (resistance to activated protein

C) Am J Cardiol; 1999; Sep1: 8(5): 549-54

71. Undas A; Sanak M; Jankowski M; et al: Factor V Leiden and venous thromboembolism in a woman taking second generation oral contraceptives: a case report. Ginekol Pol; 1999; Feb; 70(2): 93-7

72. Avenhaus W; Ullerich H; Menzel J; et al: Budd-Chiari syndrome in a patient with factor V leiden-successful treatment by TIPSS placement followed by liver transplantation. Z Gastroenterol; 1999; Apr; 37(4): 277-81