

Understanding Genetic Variations as Risk Factors for Development Venous Thrombo-Embolism (VTE)

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Rec date: Nov 16, 2015; Acc date: Feb 02, 2016; Pub date: Feb 10, 2016

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Abstract

Venous thrombosis (VT) possess a major health problem worldwide and has a high incidence in several populations across the world. Its two clinical manifestations include deep vein thrombosis (DVT) and pulmonary embolism (PE). The pathogenetic mechanism of venous thromboembolism (VTE) is still not completely elucidated, however there are clear evidences that the process occurs by complex interaction of genetic and environmental factors, wherein, genetic risk factors plays a major role. These numerous conditions that are known for predisposition of venous thromboembolism are commonly referred to as 'risk factors'. Classical risk factors for VTE include advancing age, prolonged immobilization, surgery, use of oral contraceptives or hormones, pregnancy, cancer etc. However, in the recent decades, studies have emerged that indicating a major role of novel genetic risk factors. These genetic risk factors include genes related to haemostatic system and coagulation cascade. Understanding the role of these genes as predisposing factors for VTE represent a crucial step for a better understanding of pathogenesis of thrombosis. Several genes that have been studied for their mutations playing a role in VTE are factor V Leiden (FVL), antithrombin (AT), protein C, protein S, prothrombin, fibrinogen etc. The risk associated with each genetic defect might be relatively insignificant when studied individually but simultaneous involvement of several mutations increase the risk of susceptibility. In addition to this, acquired risk factors interacting with one or more genetic variations further add to the risk of VTE. This review has been compiled to interrogate the role of several genetic and acquired risk factors across various populations as investigated in numerous studies.

Keywords: Venous thrombosis; Pulmonary embolism; Deep vein thrombosis; Genetic risk factors

Introduction

Thrombosis is a complex phenomenon that occurs as a result of blood clot formation which is due to an imbalance of procoagulant, anticoagulant and fibrinolytic factors. It may occur in arteries or in veins, arterial thrombosis being predominant as myocardial infarction (MI) and ischemic stroke (IS). Arterial thrombosis is almost invariably superimposed on vessel walls i.e. atherosclerosis. Its symptoms are acute due to blocking of vital blood flow to an organ. Although, atherosclerosis could be seen as chronic disorder related to a slowly increasing severity. In contrast to this, development of clot is relatively sudden in case of venous thrombosis [1]. Main clinical presentations of venous thrombosis include deep vein thrombosis (DVT) and pulmonary embolism (PE), representing a major health problem worldwide. Pulmonary embolism represents one of the major causes of death in developed countries with an incidence similar to that of stroke [2]. The incidence of venous thrombosis is 1-3 individuals per 1000 per year [3,4]. The deep vein thrombosis most commonly starts in leg, although it rarely also occurs in other veins such as upper extremities, liver, cerebral sinus, retina and mesenteric. A DVT can be asymptomatic, but in most cases the affected extremity is painful, swollen, red, warm and the superficial veins may be engorged. The DVT is most commonly diagnosed by blood test called D-dimer and Doppler ultrasound of affected veins. In some cases, clots are broken down using thrombolytic agents. Incidences of venous thrombosis are

almost equal in men and women, however, pregnancy, use of contraceptive pills and hormone replacement therapy are additional risk factors for female.

The coagulation system is an extremely essential homeostatic mechanism that prevents excessive bleeding from injuries. Body has to maintain a balanced coagulation system to avoid excessive bleeding as well as thrombosis. Many genetic alterations affecting the function of coagulation system have been identified in order to understand its homeostatic mechanism. The significance of the coagulation system for the development of thrombosis was observed as early as in 1874 by Virchow in the Virchow's triad [5]. Virchow suggested that thrombosis is either caused by changes in the composition of the blood affecting the coagulation system, in the vessel wall or by changes in blood flow. This broad classification is still valid. However, these classes of causes do not have same role in arterial and venous thrombosis.

Therefore, it becomes extremely important to understand the role of genetic variations in coagulation factors that could be potentially involved in venous thrombosis. Also study of genetic alterations in coagulation inhibitors such as antithrombin, protein C or protein S has gained interest in late 1980s and early 1990s. Besides mutations in the coding region of the genes, variations in the regulatory and promoter regions of the coagulation factors/inhibitor genes, have an important impact on blood coagulation system as a whole and it affects the concentration of these proteins significantly [6]. Thus identification and study of different polymorphisms and study of their roles in arterial and venous thrombosis has gained importance in last 15 years.

Large proportion of patients with venous thrombosis or PE show inherited hypercoagulable conditions. It is estimated that more than 60% of the pre-disposition to thrombosis is attributed to genetic components [7]. Over last two decades, several studies have been conducted for better understanding of the concept of inherited 'hypercoagulable states' that represent a large proportion of patients of

venous thrombosis and pulmonary embolism has been one of the major breakthrough in this area.

This review hereby describes the known and predicted genetic risk factors for VTE along with acquired risk factors. Figure 1 classifies both environmental/acquired and genetic risk factors of VTE.

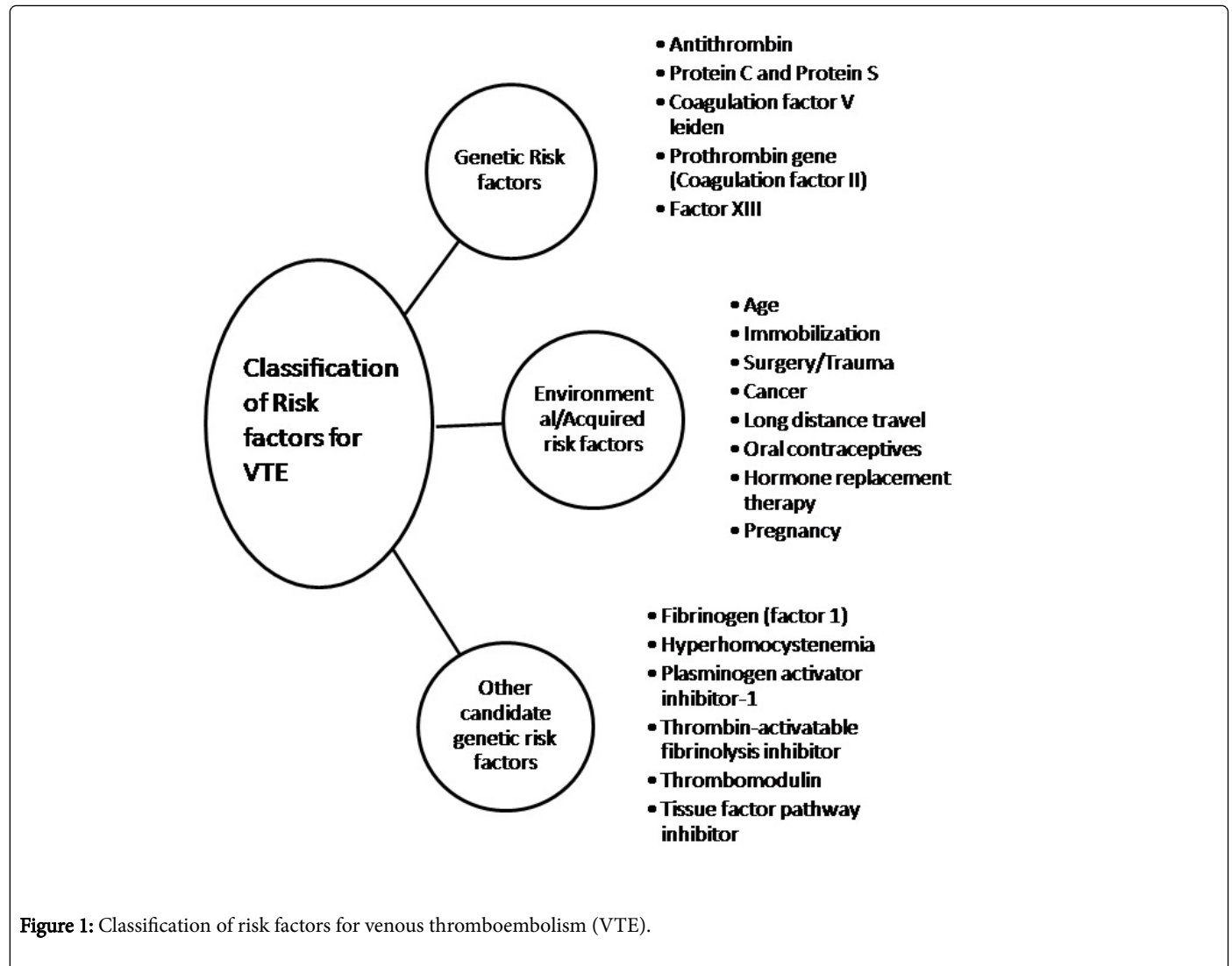


Figure 1: Classification of risk factors for venous thromboembolism (VTE).

Known Genetic Risk Factors for Venous Thrombosis

Thrombophilia is an inherited blood clotting disorder caused by one or more genetic risk factors or mutations that make a person susceptible to DVT/PE. These factors include deficiencies in the anticoagulation factors protein C, protein S, and antithrombin, and mutations in the factor V and prothrombin genes which result in Factor V Leiden and prothrombin G20210A respectively. Identification of various polymorphisms in different genes that are linked with an increased risk of venous thrombosis has led to the better understanding of molecular basis of thrombophilia. Some of these genes are; the genetic substrate of APCR, the factor V Arg1691 G-A mutation (factor V Leiden allele), the 677 C/T mutation in the 5, 10-methylenetetrahydrofolate reductase (MTHFR) gene which is

associated with hyperhomocystenemia and G-A mutation at nucleotide position 20110 in 3'UTR region of prothrombin gene [8-10]. The frequency occurrence of these alleles has been described in thrombophilic Caucasian patients as follows; 21% for the factor V Leiden, 10% for MTHFR 677 and 6% for prothrombin 20210 [11]. Ruiz-Arguelles et al. [12] has shown contrasting results of low prevalence of factor V Leiden mutation (10.8%) and high prevalence of the prothrombin 20210 mutation (13.5%) in Mexican mestizo patients with clinical features of primary thrombophilia. At the same time, they also showed high prevalence of the MTHFR 677 mutation gene both in normal controls (78%) and thrombophilic patients. List of genetic factors and the common genetic polymorphisms in them which are regarded as possible risk factors of VTE are enlisted in Table 1.

Genetic factors	Polymorphism	Reference
Anti-thrombin	Multiple mutations exist in this gene including gene lesions such as nonsense mutations, splice site mutations, deletions and insertions.	Lane et al. [16]
Protein C	Multiple nonsense mutations exist Ser219Gly polymorphism at position 6936 A/G exist in Endothelial protein C receptor	Reitsma et al. (1995); Galanaud et al. (2010); Chen et al. [23]
Protein S	Multiple gene defects are responsible for PS deficiency. Missense mutations are most common along with other splice site mutations, large and small deletions and insertions etc.	Gandrille et al. (2000); Makris et al. (2000)
Coagulation factor V Leiden	Single point mutation at nt 1691, R506Q, Arg/Gln amino acid change at position 506 A4070G polymorphism in exon 13 of FV gene leading to exchange of histidine by arginine at amino acid position 1299	Folsom et al. (2002)
Coagulation factor II (Prothrombin)	G/A at position 20210 in 3' untranslated region	Poort et al. [29]
Factor XIII	C/T change at nucleotide position 143, Val/Leu amino acid change at position 34, exon 2	Mikkola et al. [67]
Fibrinogen (factor I)	Bcl I polymorphism in β -chain G (-455)A polymorphism in β -chain (in complete linkage disequilibrium with C (-148) T Taq1 polymorphism in α -chain Thr312Ala polymorphism in coding region in α -chain	Humphries et al. (1992); Carter et al. (1999); Endler and Mannhalter (2003)
MTHFR (causing Hyperhomocystenemia)	MTHFR 677 C/T mutation MTHFR 1298 A/C mutation	Seligsohn and Lubetsky (2001)
Plasminogen Activator inhibitor-1	-675 4G/5G polymorphism	Arslan et al. (2011)
Thrombin activatable fibrinolysis inhibitor (TAFI)	Existence of several polymorphisms in promoter region of the gene	Franco et al. [48] Henry et al. (2001)
Thrombomodulin	Ala455Val mutation	Flem et al. [49]
Tissue factor pathway inhibitor (TFPI)	Four different polymorphisms exist Pro151Leu, Val 264Met, T384C exon 4 and C-33T intron 7	Kleesiek et al. (1998); Moatti et al. (1999)

Table 1: List of genetic factors and the common genetic polymorphisms in them, considered as possible risk factors of VTE across various studies.

Antithrombin

Antithrombin (AT) is the key inhibitor and also shows inhibitory effects on other coagulation factors such as IXa, Xa, XIa and XIIa [13]. It is a member of serine proteinase inhibitor (serpin) superfamily. The concept of AT deficiency as a risk factor for thrombophilia has been established by numerous studies [13]. Mutation studies in AT gene reveal that molecular basis of AT deficiency is highly heterogenous [14,15]. Missense mutations are most frequent genetic defects found in AT deficiency apart from other types of gene lesions such as non-sense mutations, splice site mutations, deletions and insertions [16]. Percentage of AT deficiency and its prevalence in thrombotic patients vary between different investigations ranging from 1% to 8% [17,18].

Protein C and protein S

Protein C is the key component of the natural anticoagulant pathway [19]. It performs its anticoagulant function when it is converted to a serine protease by the thrombin-thrombomodulin complex or thrombin alone [20]. Protein C and Protein S deficiencies result in defects in the activated PC anticoagulant system. PC gets activated upon by binding of thrombin to its endothelial receptor (thrombomodulin). Activated PC cleaves and inactivates factor Va and VIIIa, thereby inhibiting clot formation wherein PS acts as non-

enzymatic cofactor for activated PC optimizing the efficiency of these reactions [21].

Genetic mutations in protein C gene results in its deficiency and individuals homozygous or heterozygous for protein C deficiency have been found to be at increased risk for severe thrombotic disorders [22]. Altinisik et al. [22] found a higher prevalence of mutated protein C allele in the pulmonary emboli group (42.8%). Also, protein C mutation incidence was higher in the pulmonary emboli group than in DVT (8.33%) and cerebral vein thrombosis (16.1%). Recently, Chen et al. [23] found 6936A/G polymorphism of endothelial cell protein C receptor gene to be associated with increased plasma levels of sEPCR and subjects carrying 6936AG were predicted to be associated with an increased risk of thrombosis.

Coagulation factor V Leiden mutation: This mutation is a G-A transition at nucleotide position 1691 leading to substitution of arginine (R) by glutamine (Q) at amino acid position 506, which is a cleavage site for activated PC in factor V molecule [24,25]. The mutant factor V is resistant to activated PC-mediated neutralization, yielding to hypercoagulable state and significantly increases the susceptibility to VTE). This resistance to APCR in patients of venous thrombosis was first identified by Dahlback et al. by modified activated partial thromboplastin time (APTT) assay [26]. FVL is highly prevalent in

Caucasians with carrier frequencies in population ranging from 1% to 15% [27,28]. Approximately 5% of Caucasian population has FV R506Q mutation, which represents one of the most important risk factors of inherited thrombophilia and venous thrombosis. Coagulation factor V (FV) acts as a cofactor of FXa and plays an important role in coagulation process. It can be activated by a limited proteolysis of several peptide bonds by thrombin and FXa [29]. Several independent case studies have revealed a high prevalence of FV506Q allele in patients of MI without signs of coronary atheromatosis.

Prothrombin gene mutation: Prothrombin is a glycoprotein which is a proenzyme of thrombin circulating in human plasma. Factor IIa converts fibrinogen to fibrin and is activated by Factor Xa (in presence of FVa and phospholipids). Genetic defects in this gene can lead to inherited prothrombin deficiencies which are associated with an increased bleeding tendency [30]. Patients bearing factor II G20210A polymorphism have shown an increased plasma factor II level. In 1996, a novel genetic factor involved in etiology of VTE was described i.e. G-A transition at nucleotide position 20,210 in 3'UTR region of coagulation factor II gene [31]. This mutation is found in 1-3% of subjects in general population and in 6-18% of patients with VTE [30,31].

Factor XIII mutation

A point mutation G/T occurring in exon 2 of factor XIII gene results in substitution of valine with leucine at amino acid position 34 (Val34Leu). This SNP has been reported to be protective against the occurrence of VTE [32]. Mutant allele (Leu34) influences the transglutaminase activity of factor XIII whereas homozygosity for this mutation is associated with increased enzyme activity. For heterozygotes enzyme activity is intermediate when compared with non-carriers. Previous studies [33,34] had confirmed these findings however other studies by Corral et al. [35] and Balogh et al. [36] did not find any statistically significant association between this polymorphism and VTE.

Predicted Genetic Risk Factors for Venous Thrombosis

Apart from established genetic risk factors for VTE, recent studies have suggested number of new possible candidate genetic risk factors which include mutations in fibrinogen, methylene hydrofolate reductase (MTHFR), plasminogen activator inhibitor-1 (PAI-1), thrombin-activatable fibrinolysis inhibitor (TAFI) etc genes.

Fibrinogen (Factor I)

Fibrinogen is a 340 KD a glycoprotein consisting of three non-identical polypeptide chains (α , β and γ) linked by disulphide bridges. It has been consistently associated with the development of arterial thrombosis. Also, levels of fibrinogen are associated with other cardiovascular risk factors including hypertension, diabetes, smoking and peripheral artery disease [37,38]. Elevated levels of plasma fibrinogen has been established as an indicator of coronary artery disease, stroke and peripheral vascular disease in whites [39] and factors influencing circulating levels of fibrinogen in these subjects include age, gender, smoking and genetic factors. Behague et al. [40] suggested a relationship between β Arg448 Lys, β -455G/A and A α Thr312Ala polymorphisms and fibrinogen levels. However findings of Kain and co-workers (2002) suggest that increased fibrinogen levels among South Asians versus Whites are not due to differences in prevalence of genetic polymorphisms that encode for fibrinogen. Levels of fibrinogen are subject to biological variation and genetic

factors contribute to ~50% of total variability of fibrinogen plasma levels [41].

Hyperhomocystenemia

A variant in the gene coding for methylene hydrofolate reductase (MTHFR), plays an important role in homocysteine metabolism and has been found to be associated with mildly elevated levels of homocysteine [42]. Homozygous individuals for C677T variant show hyperhomocystenemia, which is the term referred to abnormal elevation of plasma concentration of amino acid homocysteine. It is an established risk factor for VTE [43]. Nutritional deficiency of vitamin B12, vitamin B6 and folate, advanced age, renal failure and use of anti-folic drugs are some of the acquired causes of hyper-homocystenemia.

Plasminogen activator inhibitor-1 (PAI-1)

This gene has a major role in the regulation of the fibrinolytic process as it functions as primary inhibitor of both tissue type plasminogen activator (t-PA) and urokinase type plasminogen activator [44]. Data from various epidemiological investigations suggest an association of plasma concentrations of PAI-1 and risk of arterial thrombosis [45]. Environmental factors influence plasma levels of PAI-1 but circulating PAI-1 is under genetic control. An insertion (5G)/deletion (4G) polymorphism at position-675 of PAI-1 promoter has been extensively studied (Kohler et al. 2000). This polymorphism has been shown to play a role in the risk of MI in presence of underlying insulin resistance [46].

Thrombin activatable fibrinolysis inhibitor (TAFI)

It is a plasma zymogen that plays an important role in haemostasis as a potent fibrinolysis inhibitor and is also referred to as plasma carboxypeptidase B, procarboxypeptidase U and procarboxypeptidase R [47]. Tilburg and co-workers (2000) demonstrated increased risk for DVT (1.7 folds) with elevated plasma levels of TAF-1. Promoter region of TAF-1 gene has several polymorphisms which regulate its plasma levels. These polymorphisms have been shown to modify the risk of venous thrombosis in young patients [48].

Thrombomodulin: Endothelial cells and monocytes contain thrombomodulin as an integral membrane protein. Thrombin loses its procoagulant activity when it binds to thrombomodulin but becomes capable of activating protein C. Since activated PC inhibits clot formation, thrombomodulin plays a role in development of thrombotic disease. Commonly occurring polymorphism Ala455Val in this gene has not been established as a risk factor for venous thrombosis but may predispose to varicose vein formation [49].

Tissue factor pathway inhibitor (TFPI): Tissue factor is the major initiator of blood coagulation cascade. TFPI is a so-called Kunitz-type inhibitor that plays a major role in the inhibition of extrinsic coagulation pathway (Bronze 1995). This property makes TFPI a candidate risk factor for thrombotic disease.

Acquired Risk Factors for Venous Thrombosis

Pathogenesis mechanism of VTE is influenced by complex interactions of genetic and environmental factors, although the mechanism is not fully elucidated. The risk of VTE is increased during, stress, trauma, surgery, bed rest, malignancy etc. [50]. Hence, it becomes crucial to characterize the acquired risk factors for VTE for better understanding of etiology.

Age

It has been investigated that the incidences and risk of thrombosis is a thousand fold higher in older people compared to young [29,51]. There is no definite cause for this; however decreased motility and muscular tone with advancing age could be the possible explanations. Aging of veins and their valves are considered as one of the strongest risk factors for thrombosis.

Immobilization

Immobilization interferes with function of calf musculature in pumping the blood upstream through the veins. There is an increased risk of venous thrombosis during all circumstances that are associated with immobilization of body extremities such as bed rest, paralysis, plaster casts etc [52].

Surgery/Trauma

Surgical interventions result in increased risk of thrombosis. The risk of thrombosis rises to 30-50% in case of hip and knee surgery [53,54]. The highest risks for VTE are conferred by orthopedic surgery and neurosurgery. In patients with multiple traumas such as head or spinal injury, pelvic fractures, femoral and tibial fractures, the incidence of venous thrombosis may rise to 50-60% [55].

Cancer

The relationship between cancer and venous thrombosis was observed several decades ago. However, several factors seem to be involved in this complex mechanism. Large tumors may cause thrombosis due to mechanical effects and venous obstruction [56,57].

Air travel

Over the years, many cases of thrombosis have been reported as a result of long hours of air travel. This phenomenon is more commonly known as 'economy class syndrome'. A detailed study was published in 1986 in which sudden deaths occurring over several years at Heathrow Airport in London had been collected and categorized. It was observed that more deaths from PE took place in arrival area compared to departure hall [58]. Similar studies have been performed at various other places but none of them gave any idea about the magnitude of the risk involved. Studies have indicated the direct correlation between long air travel and VTE, however it still remains unknown whether this is due to the effects of prolonged sitting, immobilization or there is some other specific factor is playing a role.

Oral contraceptives

One of the first case of PE in relation to the use of oral contraceptives was observed in a nurse in 1961 [59]. This was followed by cases of MI and ischemic stroke in oral contraceptive users [60]. The absolute risk of VTE in women of reproductive age is less than 1 per 10,000 per year which increases to 2-3 per 10,000 per year with use of oral contraceptives. However, with increasing use of these drugs, it remains the most common cause of venous thrombosis in young women. Levels of estrogen and progesterone content in the drugs affects the magnitude of risk as levels of procoagulant factors rise and anticoagulant factors decrease with estrogen levels.

Hormone replacement therapy

Postmenopausal hormone replacement therapy is done for treatment of symptoms of menopause. Estrogens are important components of hormone replacement therapy but its use can increase risk of endometrial cancer, hence it is combined with progestin. Several studies have demonstrated that hormone replacement therapy can increase risk of thrombosis by 2 to 4 folds [61-63]. The risk is even

more increased in case of older and overweight women or those with factor V Leiden or high factor IX levels [64,65].

Pregnancy

Pregnancy increases risk of venous thrombosis. In an elaborated study of over 72,000 deliveries in Scotland, 62 cases of venous thrombosis occurred. It is estimated that risk of venous thrombosis is at least 10 fold increased during delivery time compared to non-pregnant women [35].

Future Prospective

Venous Thrombosis events often occur when multiple risk factors, including genetic and environmental (acquired risk factors), are present at the same time. Most important acquired risk factors include age, post-operative state, venous stasis from immobility, malignancy, oral contraceptive use, estrogen therapy, obesity, diabetes mellitus, trauma, lupus anticoagulant etc. In addition to well-established risk factors several lines of evidences indicate the role of inherited risk factors, mainly related to the haemostatic system also influencing the thrombotic risk. Variations in the genes responsible for blood coagulation factors, fibrinolytic factors and platelet membrane receptors could be responsible for developing thrombo-embolism as well. These include mutations in the genes that encode factor V Leiden, anti-thrombin, protein C and protein S, factor II G20210 A, MTHFR etc [66,67]. Coexisting inherited thrombophilic disorders like haemophilia, antiphospholipid antibodies, hyperhomocysteinemia, protein C, protein S deficiencies etc have an additive effect on overall thrombotic risk. Current view on genetic predisposition towards venous thrombosis suggests that single gene defect confers increased risk that may not necessarily lead to thrombosis. However, the interaction of genetic, plasma and environmental risk factors could significantly increase the chances of thrombotic events.

Other confounding factors like blood hydration levels, viscosity and other haemorheological variables responsible for blood stasis along with dietary intake are equally essential to understand thrombo-embolic disorders at HA. Genetic factors that influence the risk of a TED continue to modulate the risk of subsequent venous embolism. Much larger prospective studies will be required to narrow the confidence intervals on the risk conferred by individual genotypes and will need to account for treatment, which can strongly modify conferred risks. This will make meta-analyses across studies difficult except in cases where very similar treatment regimens are applied. In the future, an index of genetic risk may be calculated from a number of genotyping studies of multiple genes, which could identify patients at higher risk who may possibly benefit from more aggressive treatment. Such genotyping assays may be helpful in formulating therapies tailored for particular genotypes, if clear and reproducible genotype-specific treatment effects emerge from multiple studies.

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