INTRODUCTION

Thrombophilia is a tendency to venous thromboembolism that can be acquired or determined genetically. In the last few years, the previously held concept that inherited thrombophilia is due to alterations of a single gene has been challenged. It is now accepted that in thrombophilia multiple gene defects often coexist (***Mannucci, 2005).***

Deep venous thrombosis (DVT) is the most common type of venous thromboembolism (VTE), occurring deep in veins within the muscles of the leg and pelvis. Some of the recognised factors that increase the risk of DVT include major surgery as hip or knee replacement, cancer, inherited abnormalities in the blood-borne proteins involved in coagulation, and hospitalisation for a major medical illness. Those patients are at risk of pulmonary embolism (***Cohen et al., 2007; Tapson 2008).***

Venous thromboembolism involves several genetic abnormalities including factor V Leiden, the prothrombin gene G 20210A mutation, antithrombin III deficiency, protein C deficiency, protein S

deficiency, and mutations in methylene tetrahydro folate reductase (MTHFR) (***Mannucci, 2005).***

MTHFR is an enzyme involved in homocysteine metabolism by converting folate, a cofactor for homocysteine conversion, into its major circulating form 5-methyltetrahydrofolate ***(Lewington et al., 2005).***

A common mutation in the gene encoding for methylenetetrahydrofolate reductase (MTHFR) decreases enzyme activity. MTHFR 677, is a relatively common thermolabile variant of the enzyme MTHFR in which there is replacement of cytosine by thymidine at base position 677, causes an amino acid change from alanine to valine at position 222 in the enzyme rendering the enzyme thermolabile and reduces the metabolism of homocystein leading to hyperhomocystinaemia (HHC) ***(Amplexa Genetics, 2009; Wald et al., 2002).***

Recently, Elevated homocysteine levels in patients with venous thrombosis have attracted considerable interest because it is considered an important risk factor for atherosclerotic vascular and venous thromboembolic diseases. It was found to be associated with a 2 to 3-fold elevated relative risk for deep-vein thrombosis and pulmonary embolism ***(Quéré et al., 2005)***.

AIM OF THE WORK

The aim of this work is to evaluate the role of methyltetrahydrofolate reductase (MTHFR) mutation as a genetic risk factor in patients with deep venous thrombosis.

Chapter 1

THROMBOPHILIA

Thrombophilia be used to describe the familial or acquired disorders of the haemostatic mechanism which are likely to predispose to thrombosis ***(Greer, 2003)***.

Thrombosis becomes more common as age increases and its occurrence is frequently associated with risk factors such as trauma (accidental or surgical), pregnancy, malignant disease, immobilisation or oral contraceptives. Thrombosis, however, may develop at a younger age and sometimes in the absence of an easily identifiable risk factor. Recently it has become increasingly recognised that patients who have defects or abnormalities which alter the physiological haemostatic balance in favour of fibrin formation or persistence are at increased risk of clinical thrombosis. These patients may be considered to have thrombophilia. It must, however, be realised that many patients with laboratory evidence of a thrombophilic abnormality remain clinically asymptomatic ***(Dahlbäck, 2008)***.

Mechanism of thrombosis:

At sites of vascular injury, activation of blood coagulation results in the generation of high concentrations of thrombin that activate platelets and coagulate blood (Fig. 1). The efficient coagulation system is controlled by several anticoagulant mechanisms, ensuring that the clotting process remains a local process ***(Dahlbäck, 2005; Monroe and Hoffman, 2006)***.

The initiation of the coagulation system is the result of exposure of tissue factor (TF) to blood and the subsequent binding and activation of factor VII (FVII). TF serves as a cofactor to the enzyme FVIIa, the TF-FVIIa complex efficiently activate factor IX (FIX) and factor X (FX). The ensuing reactions take place on the surface of negatively charged phospholipid membranes, exposed on activated platelets, onto which the blood coagulation proteins bind and assemble into enzymatically active complexes ***(Dahlbäck 2005; Monroe and Hoffman, 2006)***.

Thus, FIXa binds to its cofactor FVIIIa forming the tenase (FIXa-FVIIIa) complex that activates additional FX, whereas FXa together with FVa form the prothrombinase (FXa-FVa) complex that efficiently

converts prothrombin (PT) to thrombin. In these membrane-bound complexes, FVIIIa and FVa serve as important cofactors to the enzymes FIXa and FXa, respectively ***(Dahlbäck, 2008)***.

Indeed, without the cofactors and the negatively charged phospholipid, the efficiency of the 2 enzymes FIXa and FXa is negligible, further ensuring that the enzymatic reactions remain localized. The generated thrombin positively feedback-activates the coagulation system by converting circulating precursors FVIII and FV into their active forms. The whole system is designed to provide massive amplification of an initiating stimulus and if not appropriately controlled, it would rapidly convert circulating blood into a clot. Natural anticoagulants e.g. antithrombin and the Protein C/Protein S (PC/PS) system oppose this generation of thrombin ***(Dahlbäck, 2008)***.

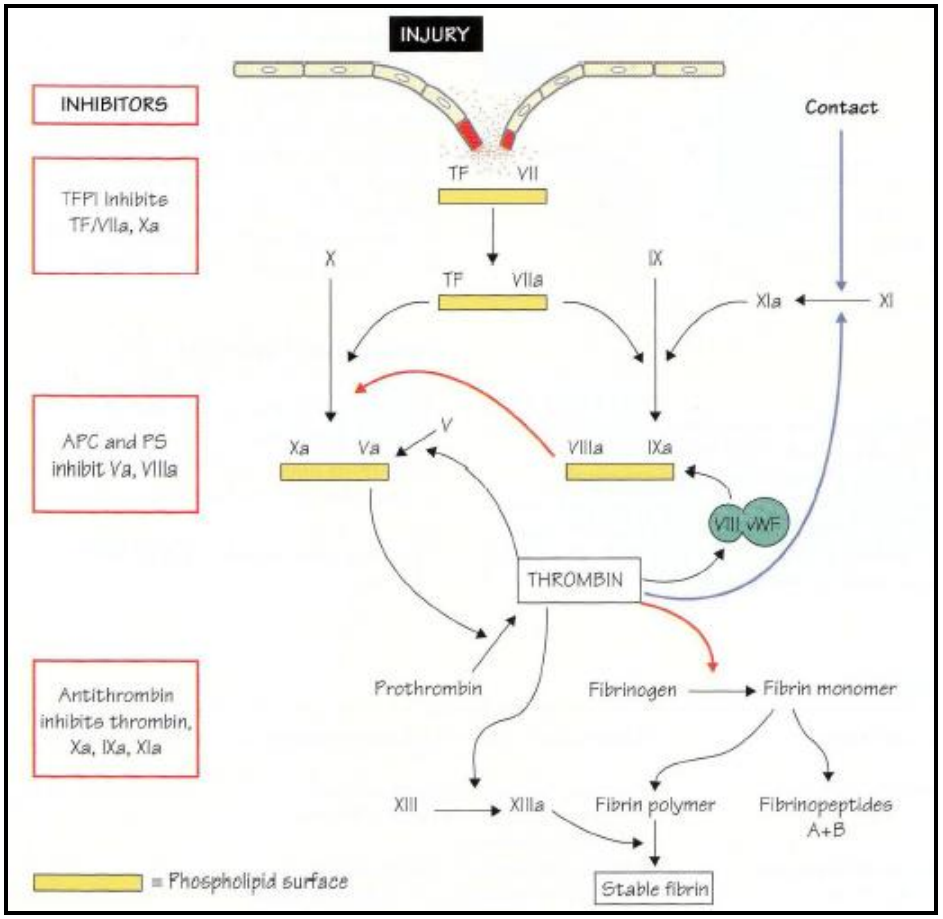

Fig. (1): The coagulation pathway ***(Mehta and Hoffbrand, 2000).***

History of thrombophilia:

Egeberg in 1965 was the first to describe a thrombophilia caused by a hereditary deficiency of antithrombin. Members of the family described in the report suffered from recurrent venous thrombosis, and the disorder was inherited in an autosomal dominant pattern. The deficiency of this naturally occurring

anticoagulant protein remained the only identified cause of inherited thrombophilia for many years ***(De Stefano, 1996; Greer, 2003)*.**

Since the early 1980s there has been an explosion of new knowledge, with the identification of protein C (PC) deficiency, and three years later, protein S (PS) deficiency was described as additional causes of inherited thrombophilia. However, altogether these three defects only account for less than 15% of selected cases of juvenile and/or recurrent venous thrombosis and for less than 10% of unselected cases, and this was disappointing at that time.

This situation changed dramatically in 1993 when Dahlback and coworkers reported that venous thrombosis often is associated with hereditary resistance to activated protein C (APC) ***(Svensson, and Dahlbäck,1994)***; the protease generated by the thrombomodulin-PC anticoagulant pathway to inactivate activated factor V and VIII (Va and VIIIa).

De Stefano et al. (1996); Greer, (2003) noted that APC resistance is associated with factor V leiden which is the most frequent cause of inherited thrombophilia, accounting for 20% to 50% of cases.

Mild hyperhomocysteinemia was found in 19% of patients with juvenile venous thrombosis and family studies showed that in most cases the abnormality was inherited ***(Oger et al., 2007).*** While the genetic lesions for deficiencies of Antithrombin III deficiency, Protein C deficiency, Protein S deficiency, and activated protein C resistance can be found in single genes encoding the defective proteins, inherited hyperhomocysteinemia may be caused by defects in several genes encoding different enzymes involved in the metabolism of the amino acid ***(Oger et al., 2007)***.

Venous thrombosis:

The annual incidence of venous thrombosis is 1 to 3 individuals per 1000 per year ***(Rosendal et al., 1997; White, 2003)***. It is a major sources of morbidity and mortality affecting people annually ***(Hizem et al., 2008)***. Its major manifestations are deep vein thrombosis (DVT) of the leg and pulmonary embolism. Thrombosis may rarely occur in other veins (upper extremities, liver, cerebral sinus, retina and mesentery). Major complications of venous thrombosis are disabling postthrombotic syndrome, occurring in up to 20% of patients, and acute death from pulmonary embolism, occurring in 1-2 % of patients. The incidence of thrombosis is age dependent; it is

extremely uncommon (l in 100,000 per year) in childhood, and rises to nearly 1% per year in old age ***(Ageno et al., 2006).***

Deep vein thrombosis and pulmonary embolism are typical multifactorial disease, involving both genetic and circumstantial risk factors. Risk factors for lower extremity acute venous occlusion include prolonged immobilization, hypercoagulability syndromes, trauma, and malignancy ***(Hizem et al., 2008).***

Causes of venous thromboembolism:

Thrombosis develops as a consequence of predisposing factors that may be genetically determined, acquired, or combined. Acquired and genetic causes frequently interacts which makes it difficult to decide which patients should be tested for inherited thrombophilia ***(Brouwer et al., 2006).***

1- Acquired causes of venous thromboembolism

The acquired causes of venous thrombosis are:

- Immobility
- Surgery and trauma
- Advanced age
- Pregnancy and puerperium
- Hormonal replacement therapy
- Oral contraceptives
- Malignancy
- Antiphospholipid antibodies

(Hunt and Greaves, 2005)

- Immobility

Thrombosis may occur in many circumstances that are associated with immobilization, such as paralysis, bed rest, plaster casts, and prolonged travel. All of these situations have in common the fact that they interfere with the function of the calf musculature in pumping the blood upstream through the veins ***(Rosendaal, 2005).***

- Surgery and Trauma

Without thromboprophylaxis, surgery will lead to thrombosis in up to 50% of the patients, dependent on the type of surgery. Major trauma is also an

important risk factor for thrombosis, which occurs in 50-60% of patients with head trauma, spinal injury, pelvic fracture, femoral fracture, and tibial fracture. Nowadays, these high risks are no longer observed because of the use of anticoagulant prophylaxis ***(Brouwer et al., 2006)*.**

- Advanced age:

The risk factor with the greatest gradient of risk is undoubtedly age, with a 1000-fold difference in risk of thrombosis between the very young (<2yrs) and very old (>70 yrs) ***(Rosendaal, 2005)*.**

- Pregnancy and puerperium

About 1 in 2000 women will develop thrombosis during pregnancy. This risk is about 10-fold that of the risk for non pregnant women of the same age. The risk is also increased in puerperium, with most studies finding a higher risk than during pregnancy. Anticoagulant prophylaxis is, therefore more frequently prescribed postpartum than during pregnancy. In a large study of over 72,000 deliveries in Scotland, DVT and pulmonary embolism was 0.86 per 1000 deliveries. Thrombophilic abnormalities affect the risk of thrombosis during pregnancy, particularly antithrombin deficiency ***(De Stefano et al., 2002)*.**

- Hormonal replacement therapy

Postmenopausal hormone replacement therapy has been prescribed for the treatment of symptoms of menopause, to reduce the progression of osteoporosis and to prevent ischemic heart disease ***(Folkeringa et al., 2007).*** Prolonged use of estrogens reduces the progression of osteoporosis. Several studies have demonstrated that hormone replacement therapy increases the risk of thrombosis 2- to 4-folds. Oral administration was associated with an increased risk of venous thrombosis, and transdermal administration was not. It has been postulated that the absence of a first-pass effect through the liver with transdermal administration might lead to less risk, but an increased risk has been shown for patches too ***(Scarabin and Oger, 2003).***

- Oral contraceptives

Some women have a higher risk of venous thrombosis when using oral contraceptives than others do. These are older, obese women, and women with prothrombotic abnormalities. Obesity itself mildly affects the risk of thrombosis, with a doubling of the risk for those with a body mass index (BMI) over 30. Overweight (BMI >25kg/m^2) and obese (BMI

>30kg/m^2) women have a 10-fold increased risk of thrombosis when they use oral contraceptives ***(Abdollahi et al., 2003)***.

Oral contraceptives also greatly increase the risk of thrombosis in familial thrombophilia caused by deficiencies of protein C, protein S or antithrombin. Heterozygous FV Leiden carriers or prothrombin G20210A carriers have a 15- to 30-fold increased risk of thrombosis when they use oral contraceptives. High levels of several procoagulant factors (FII, FVIII, FIX, FX, and FXI) confer a 2- to 3-fold increased risk when levels exceed the 90th percentile of the distribution in the population ***(van Hylckama et al., 2003)***.

- Malignancy

The thrombogenic effect of cancer may be the result of several factors, which probably all play a role to some extent. *First,* tumor cells can activate the coagulation pathway by tissue factor expression that activates FVII or by cystein containing proteases that are capable of activating factor X. Resistance to activated protein C, that is unrelated to the mutant FV Leiden and perhaps due to elevated level of FVIII or fibrinogen, may contribute to thrombosis through mechanical (venous obstruction), and general (acute

phase reactions) effects. *Second,* cancer may indirectly promote thrombus formation due to consequences of being ill e.g., reduced mobility, reduced dietary intake of vitamins, such as folate. And *third,* there may be effects of treatment e.g., surgical/radiologic scarring or effects of chemotherapeutic treatment e.g. tamoxifen in breast cancer. The prevalence of cancer among patients with venous thrombosis varies between 3% and 18% in different studies ***(Unal et al., 2005).***

- Antiphospholipid antibodies

Primary antiphospholipid antibody syndrome comprises a cluster of clinical and laboratory features in patients with systemic lupus erythematosus (SLE) or independent of SLE, arterial occlusions, fetal wastage, and thrombocytopenia. It is accompanied by a positive assay for antibody against one or another of phopholipids-associated targets of antibodies ***(Harris and Pierangeli, 2000).***

Various mechanisms have been proposed to explain the increased risk of thrombosis, including inhibition of endothelial activation or production of prostacyclin. The risk of thrombosis is increased in patients with antiphospholipid antibodies, both among those with SLE (half of whom have these antibodies)