Methylenetetrahydrofolate Reductase Gene Mutation as a Predisposing Factor for Primary Deep Venous Thrombosis

Thesis

Submitted in Partial Fulfillment of MD in General Surgery

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LIST OF ABBREVIATIONS

DVT	Deep vein thrombosis
PE	Pulmonary embolism
MTHFR	Methyl Tetra-Hydro-Folate Reductase
C	Cytosine
T	Thymine
VTE	Venous thromboembolism
IVC	Inferior Vena Cava
LITE	The Longitudinal Investigation of Thromboembolism Etiology study
HRT	Hormonal Replacement Therapy
IPG	Impedance plethysmography
CT	Computed tomography
CTV	Computed tomography venography
MRI	Magnetic resonance imaging
LMWH	Low Molecular Weight Heparin
PT	The prothrombin time
INR	International normalized ratio
ATIII	Antithrombin III
Xa	Activated factor X
aPTT	Activated partial thromboplastin time
DTIs	Direct thrombin inhibitors
TFPI	Tissue Factor Pathway Inhibitor
vWF	Von Willebrand factor
ADP	Adenosine diphosphate
PGI2	Prostacyclin
NO	Nitric Oxide
HMWK	High-molecular-weight kininogen
EPI	Extrinsic Pathway Inhibitor
t-PA	Type Plasminogen Activator
u-PA	Urokinase-type Plasminogen Activator
PK	Prekallikrein
AHG	Antihemophilic globulin
FVL	Factor V Leiden
Type II RS,	Reactive site
Type II HBS	Heparin-binding site
Type II PE	Pleiotropic effect
HCII	Heparin cofactor II
tHcy	Total Homocysteine
CAD	Coronary Cartery disease
PTT	Partial Thromboplastin Time
TT	Thrombin Time
HRP	Horse radish peroxide
TF	Tissue factor

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ABSTRACT

Key words:
Deep venous thrombosis (DVT)
Methyl Tetra-Hydro-Folate Reductase (MTHFR)
Hyperhomocystenemia
Pulmonary embolism (PE)

Primary DVT is a serious, potentially fatal disease that may affect genetically predisposed individuals. MTHFR gene mutation has been identified as risk factor for venous thrombosis in several studies due to the associated mild Homocystenemia. The mutation is due to a C→ T substitution at position 677 in the MTHFR gene.

The study underhand has revealed that there is a statistically significant relationship between MTHFR gene mutation and incidence of DVT with a relative risk of 1.5. The results also revealed a higher incidence of Proximal DVT in patients with MTHFR gene mutation as opposed to distal DVT.

ملخص تجريدي

التخثر الدموى الوريدي الاولي هو مرض خطير و احيانا قاتلا قد يؤثر على الافراد المؤهلين جينيا. التحول الجيني للميثايل تتراهيدروفوليت قد تم التعرف علية كعامل خطر للتخثر الوريدى الدموي العميق فى عدة ابحاث و ذلك بسبب استبدال الحمض الاميني الالانين بفالين عند الموقع[677]جين التتراهيدروفوليت ردكتيز و ما يترتب علية من خلل جيني. كشفت دراستنا ان هنالك انتشارا ذا دلالة احصاءية للتحول الجيني لميثايل تتراهيدروفوليت وارتفاع عدد حالات التخثر االوريدي العميق. كما كشفت النتائج فى مجموعة مرضانا عن زيادة التخثر الدموى العميق الدانى فى مقابل القاصي.

Introduction

INTRODUCTION

Deep vein thrombosis (DVT) and its potentially lethal complication: pulmonary embolism (PE), are leading causes of morbidity and mortality**.** *(White, 2003*). While the majority of cases of DVT have been documented to be caused by prolonged immobilization, major surgery, trauma, cancer; thrombophilias due to genetic abnormalities, including those leading to elevated plasma Homocysteine levels have been strongly and clearly implicated. *(Den Heijer et al., 2005).*

Elevated plasma total homocysteine concentration has been considered a risk factor for venous thrombosis. It reflects an abnormal amino acid metabolism. Elevated homocysteine levels, in patients with venous thrombosis is a potentially reversible thrombophilic marker, and hence has attracted multiple research interest.*(Que et al, 2005)*

Initially, retrospective epidemiological case-control studies suggested evidence of an association between elevated plasma homocysteine levels and venous thrombosis (plasma homocysteine measurements was collected after onset of thrombotic events). *(Bienvenn et al., 1991) (Brattstrom et al., 1991); (Falcon et al., 1994); (Den Heijer et al. 1995); (Den Heijer et al., 1998)*

It was then through subsequent prospective studies (in which plasma was collected for homocysteine level before the onset of the thrombotic events and patients followed till thrombotic event) appeared to confirm these findings. *(Ridker et al., 1997); (Eichinger et al., 1998); (Tsai et al., 2003).*

Homocysteine lies at the branch point of Methionine metabolism, between the remethylation and trans-sulfation pathways, producing Methionine or

Cystathionine respectively. Several enzymes balance and regulate this pathway (*Pathare et al., 2004)*. The amino acid Methionine is a critical component of protein biosynthesis. *(Arruda et al., 1997).* Methionine formation is dependent on the enzyme Methionine Synthetase, which requires vitamin B12 as a cofactor. It acts on a substrate synthesized by MTHFR and requires vitamin B6 as a cofactor. In steady state, normal levels of homocysteine are remethylated to methionine. Reduction in the levels of any of these enzymes, because of an inherited defect or an environmental interaction, will effectively raise the potentially cytotoxic homocysteine level. *(Thare et al., 2004*)

Elevated Homocysteine levels can be caused by a number of reasons. These include: Genetic polymorphism, dietary deficincies of both of B vitamins and Folate, kidney disease, low levels of thyroid hormones, Psoriasis, and with certain medications (such as antiepileptic drugs and methotrexate). *(Malinow, et al., 1999)*

Genetic polymorphisms of the genes encoding the enzymes, regulating the metabolism of Homocysteine, affect plasma total homocysteine concentrations. More than 21 polymorphisms have been described in 10 genes involved in Folate or Homocysteine metabolism, of which the C677T polymorphism for Methyl Tetra-Hydro-Folate Reductase (MTHFR) has been the most widely studied. *(Lievers et al., 2003).*

The MTHFR nucleotide at position 677 at the gene responsible for coding the enzyme has two possibilities: C (cytosine) or T (thymine). C at position 677 (leading to an Alanine at amino acid 222) is the normal allele. The 677T allele (leading to a Valine substitution at amino acid 222) encodes a thermolabile enzyme with reduced activity**.** The decreased activity is due to the enzymes thermolability. *(Cortese and Motti, 2001)*

The C677T genotype and its resulting thermolabile MTHR enzyme lead to a state of hyperhomocysteinemia which is believed to be a risk factors for thrombosis, ischemic stoke and cardiovascular disease. *(Vinnkonda, 2008).*

Literature Review

1. DEEP VEIN THROMBOSIS (DVT)

Overview

Deep vein thrombosis (DVT) refers to the formation of thrombi in the deep veins- most commonly of the extremities or pelvis due to a disordered balance between the thrombotic and fibrinolytic systems.

DVT is a significant and costly health-care and social problem. *(Malone and Agutter, 2006).* It is one of the most prevalent medical problems today, with a reported cumulative annual incidence ranging anywhere from 70 to 113 cases per 100,000 persons/year, depending on the literature reviewed, study population age and study specific methodologies.*(Silverstein et al., 2006)*

The most serious and life threatening complication of DVT is Pulmonary embolism. P.E (whether symptomatic or asymptomatic) is estimated to occur in anywhere from 30-60% of patients with proximal DVT and in around 25% of all cases of DVT. Approximately 10% of all PEs are fatal within the first hour of presentation and the mortality rate for untreated PE is around 26%. *(Moser et al., 1994); (Monreal et al., 1989);(Kistner et al., 1972); (Plate et al., 1985); (Huisman et al., 1989)*

Around 200,000 patients die annually from DVT in the USA alone**.** *(Line, 2001)*

Untreated lower extremity DVT has a 3% PE-related mortality rate while the mortality rate in DVT patients treated with anticoagulants is less than 1% *(Mismetti et al., 2008).*

The most common sources of pulmonary embolism are proximal leg deep venous thrombosis (DVTs) and pelvic vein thromboses. This pathological continuum is termed: venous thromboembolism (VTE). *(Goldhaber, 2005).* In

simpler terms: DVT and PE are manifestations of a single disease entity, namely, VTE. *(Patel and Brenner, 2011).*

The understanding of this definition and continuum is paramount to understanding and analysing modern literature on the subject due to the ubiquitous relationship between both conditions. Some authors have used VTE as a surrogate/or indicator when studying DVT and or PE.

Furthermore, 23% to 60% of patients with lower limb DVT will go on to develop significant post thrombotic limb syndrome over a 20 year cumulative incidence rate period. This is a potentially debilitating condition with major adverse: medical, social, and economic consequences. *(Herrara, 2011)*

The Post-thrombotic syndrome in the USA alone is thought to cost around 200 million dollars annually and is responsible for the loss of approximately 2 million work days. *(Ashrani, 2009*)

The economic burden of deep vein thrombosis (DVT) is estimated at 3 to 4 billion dollars annually in the USA alone. *(De Lissovoy et al., 2001).*

A Historical Facts

A review of the history of venous thrombosis, portrays in miniature the developmental history of modern medicine itself.

Peripheral venous disease was first chronicled in the Ebers papyrus, describing bleeding from lower extremity varicose veins. This account dated back to 1550 BC. The first described case of thrombophlebitis could be found in the ancient Hindu medicine writings of Susruta circa 800 BC. *(Meetoo, 2010).*