Impact of Prothrombotic Risk Factors on the Clinical Phenotype in Haemophilia Patients

THESIS

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By
Salwa Mohamed Khedr
M.B.B.Ch.

SUPERVISED BY

Prof. Dr.

Tayssir Eyada

Professor of Clinical & chemical Pathology

Faculty of medicine

Cairo University

Prof. Dr.

Mona Salah

Professor of Clinical & chemical Pathology

Faculty of medicine

Cairo University

Dr. Amal Soliman

Lecturer of clinical & chemical pathology

Faculty of medicine

Cairo University

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Abstract

The role of genetic prothrombotic risk factors on the clinical phenotype of haemphilia namely Factor V Leiden (G1691A), prothrombin G20210A , Methylenetetrahydrofolate reductase (MTHFR) C677T and MTHFR A1298C) gene polymorphisms was studied in 101 Egyptian haemphilia patients (of which 25 patients showed mild clinical phenotype inspite of having moderate or severe laboratory factor VIII level). Our aim was to study the incidence of the most common prothrombotic risk factors among patients with hemophilia and their impact on the clinical phenotype; annual bleeding frequency and severity of hemophilic arthropathy. Patients were diagnosed by FVIII & FIX activity assays. Genotyping was carried out using real-time PCR assay based on allele-specific fluorescent oligonucleotides that contain a 3' minor groove binding (MGB) group.

Our study revealed 3%, 14%, 41.5% and 58.4% patients showing Prothrombin G20210A, factor V leiden, MTHFR (C677T) and MTHFR (A1298C) mutations respectively in the studied Egyptian haemophilia patients. Inspite of non significant statistical analysis, the presence of heterozygous cases of prothrombin G20210A or Factor V leiden was always associated with mild or moderate clinical presentation of haemphilia and never with severe presentation. This finding supports the

hypothesis of the protective effect of the prothrombotic genes (P G20210A & Factor V leiden) on the clinical presentation of haemophilia patients.

Statistical analysis revealed that the lowest bleeding frequency among the studied patients (< once/ month) was encountered among the patients with two heterozygous variants irrespective to the involved genes. In addition, our finding that the incidence of haemarthrosis was significantly higher among patients with wild genotype of prothrombin gene and factor V Leiden and that the average number of affected joints was significantly higher among patients with wild prothrombin gene than heterozygous patients, all these data collectively strongly points to the cumulative effect of these prothrombotic polymorphisms in amelioration of the severity of bleeding in hemophilic. The most prominent effect is that of prothrombin G20210A and factor V leiden. . Findings of MTHFR C677A & A1298C gene polymorphisms are less conclusive and large scale multicenter or meta-analysis studies are needed to have accurate final conclusions.

Key Words: haemophilia, Factor V leiden, prothrombin G20210A , Methylenetetrahydrofolate reductase (MTHFR) C677T , MTHFR A1298C, prothrombotic risk factors.

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List of Abbreviations

aPPT	Activated partial thromboplastin time.
APC	Activated protein C.
AT	Antithrombin III.
Bp	Base pair.
cDNA	Complementary DNA.
CJD	Creutzfeldt-Jakob disease.
CVT	Central venous thrombosis.
CT	Computed tomography.
CVS	Chrionic villous sampling.
CRM	Cross-reacting material.
CHD	Coronary heart disease.
DVT	Deep venous thrombosis.
DNA	Deoxyribonucleic acid.
Factor II	Prothrombin.
FVa	Activated Factor V.
FVIII	Factor VIII.
FVIIIa	Activated Factor VIII.
FVL	Factor V Leiden.
FIX	Factor IX.
FX	Factor X.
FXI	Factor XI.
HIV	Human Immunodeficiency Virus.
HLA B27	Human Leukocyte Antigen B27.
IgG	Immunoglobulin G.
ICH	Intracranial hemorrhage.
LDL	Low density lipoprotein.
MRI	Magnetic resonance imaging.
MTHFR	Methylenetetrahydrofolate reductase.
OC	Oral contraceptives.
PAI	Plasminogen Activator Inhibitor.
PCR	Polymerase chain reaction.
P G20210A	Prothrombin G20210A mutation.
PS	Protein S.

PT	Prothrombin time.
SVT	Superficial vein thrombosis.
TPA	Tissue Plasminogen Activator.
VTE	Venous Thromboembolism.
vWF	Von Willebrand Factor.

Introduction and Aim of Work

Hemophilia is an X-linked bleeding disorder characterized by the deficiency of the coagulation factor VIII (FVIII) in hemophilia A, or the deficiency of the coagulation factor IX (FIX) in hemophilia B. Based on the level of measurable FVIII activity, hemophiliacs are classified as severe (< 1% normal FVIII activity), moderate (1–5% FVIII activity) and mild (5–25% FVIII activity) ***(Hoyer 1994).*** Patients affected by the severe form of the disease suffer from spontaneous bleedings, whereas in mild/moderate hemophilia A, bleeding occurs after minor trauma or surgery.

One of the most fascinating and debated issues in clinical hemostasis is the mechanisms underlying the variability in terms of frequency and severity of bleeding in hemophiliacs carrying the same mutation. In this context, the role of genetic risk factors for thrombosis has been the subject of a number of studies ***(Franchini et al, 2006).***

It has been observed that the clinical phenotype of severe hemophilia A, based on F VIII levels, is often variable ***(Escuriola Ettingshausen et al, 2001)***. Studies in the Caucasian populations have observed that the co-inheritance of inherited risk factors of thrombosis can ameliorate, the clinical phenotype of hemophilia patients ***(Franchini, 2004; Jayandharan, 2008)***.

While the majority of known genetic defects within the blood coagulation cascade (protein S, protein C, antithrombin III) is rare, a G>A mutation at nucleotide position 1691 in the gene for coagulation factor V (FV Leiden) is found at high frequency (20-60%) in thrombosis patients ***(Mitterer et al,1999).***

A further common point mutation in the 3´-untranslated region of the prothrombin (factor II) gene (20210: G>A) has been reported to be associated with

elevated plasma prothrombin levels and is estimated to increase the risk for venous thrombosis by 3 to 5-fold ***(Zivelin et al, 1998).***

Elevated levels of plasma homocysteine (hyperhomocysteinemia) are a well established risk factor for both arterial and venous thrombosis. Hyperhomocysteinemia may be caused by nutritional deficiencies or by defects in enzymes involved in homocysteine metabolism, such as cystathionine b-synthase (CBS) and 5, 10-methylenetetrahydrofolate reductase (MTHFR). Homozygosity for a point mutation (677: C>T) in the MTHFR gene leading to a thermolabile enzyme variant with reduced activity is very common in Caucasians (5-20% prevalence) ***(Tsai et al, 2003).***

In a multicenter cohort study of 107 children with severe hemophilia, ***Kurnik and colleagues (2007)*** observed a protective effect of thrombophilic risk factors, including FV Leiden, for annual bleeding frequency and the severity of the hemophilic arthropathy. Similar results were observed by ***Tizzano and colleagues (2002)*** in a cohort of 125 severe hemophilia A patients.

Aim of the work:

The present work aims to study the prevalence of the most common prothrombotic risk factors (namely FV G1691A, PG20210A, MTHFR C677T and MTHFR A1298C mutations polymorphisms) among patients with hemophilia and their impact on the clinical phenotype; annual bleeding frequency and severity of hemophilic arthropathy.

HEMOPHILIA

INTRODUCTION AND INCIDENCE:

The hemophilias are a group of related bleeding disorders that are usually inherited. Inherited bleeding disorders include abnormalities of coagulation factors, platelets as well as fibrinolytic system, the most common of which is Von Willebrand disease. However, when the term "hemophilia" is used, it usually refers specifically to the following two disorders:

- Factor VIII deficiency (**hemophilia A**): affects 1 in 5,000 to 10,000 males; roughly 60 percent have severe disease, with factor VIII activity less than 1 percent of normal.
- Factor IX deficiency (**hemophilia B**): affects 1 in 25,000 to 30,000 males; approximately one-half have mild to moderate disease, with factor IX activity greater than 1 percent of normal ***(Hoots & Shapiro, 2011)***.

PATHOGENESIS:

Severe factor VIII or factor IX deficiency leads to bleeding because of the role these factors play in the intrinsic pathway X-ase (ten-ase). The X-ase complex consists of activated factor IX (factor IXa) as the protease; activated