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ORIGINAL ARTICLE

Study of some genetic predisposition in pulmonary embolism

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KEYWORDS

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Gene mutation

Abstract *Background:* There is increasing recognition of genetic deficiencies underlying pulmonary embolism in some individuals, particularly those with early onset of disease, unusual sites of venous thrombosis and recurrent disease. The aim of this work is to study the role of mutation of factor V Leiden, prothrombin (factor II) G20210A and MTHFR C677T genes in patients with pulmonary embolism.

Patients and methods: Thirty-one patients with pulmonary embolism were investigated for gene mutation. The genotyping of factor V Leiden, prothrombin G20210A, and methylenetetrahydrofolate reductase C677T was performed via real time polymerase chain reaction, using the fluorescence melting curve detection analysis.

Results: Twenty-two patients out of 31 (71% of patients) showed factor V Leiden mutation and 15 patients out of 31 (48.4% of patients) showed mutation in MTHFR C677T gene, while prothrombin 20210A gene mutation presented in 5 patients (16.1% of patients). Nine patients (29% of patients) presented with recurrent DVT and 38.7% of patients showed recurrent pulmonary embolism. Also, 17 patients (54.8% of patients) presented with other thromboses in addition to pulmonary embolism. Thirteen patients (41.9%) showed double gene mutation and only one patient presented with mutations in the three studied genes. Those patients showed a significant difference in the occurrence of other thromboses in the body and significant increase in the recurrence of pulmonary embolism.

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Conclusion: Gene mutation especially factor V Leiden mutation is very important to be considered in young patients presented with venous thrombo-embolism, patients with thrombosis in unusual sites or patients with recurrent thrombo-embolic manifestations.

Introduction

Pulmonary embolism (PE) is a common and sometimes a fatal disease. Mortality from pulmonary embolism can be reduced by prompt diagnosis and adequate treatment. Unfortunately, the clinical presentation of PE is variable and nonspecific, leading to difficulty in accurate diagnosis.

Pulmonary thromboembolic disease refers to the condition in which blood clot(s) (thrombus or multiple thrombi) migrate from the systemic circulation to the pulmonary vasculature. Most of these blood clots arise from the "deep veins" of the lower and upper extremities (deep venous thrombosis, DVT). From the clinical standpoint, DVT and pulmonary embolism can be considered a continuum of the same disease, and the two terms are often collectively referred to as venous thromboembolism (VTE) [1].

In the last decades several hereditary coagulation abnormalities, including antithrombin and protein C and protein S defects, have been identified that predispose patients to venous thromboembolism [2]. Recently, a mutation in the factor V gene and a mutation in the prothrombin gene were identified and related to an increased risk of venous thromboembolism. Combined, these two mutations can be identified in approximately 25% of patients with venous thromboembolic disorders [3].

The most common inherited or genetic risk factor for VTE is factor V Leiden (FVL) gene mutation which is a common polymorphism in FV. The first reference to FVL is in a letter to the journal Nature by Bertina and colleagues from the University of Leiden in the Netherlands [4]. A point mutation in the factor V gene that causes a missense mutation in the protein (Arg506Gln, factor V Leiden) renders the molecule less susceptible to inactivation by its naturally occurring inhibitor, activated protein C (APC). As a result, a single inactivation site of FV by APC is altered, causing thrombophilia and leading to intravascular coagulation disorders [5]. The mutation is found in 3–7% of the healthy Caucasian population [6]. It is associated with approximately a three-to 10-fold increased risk of VTE in persons who have inherited heterozygous form of the mutation, and approximately an 80-fold risk in persons who show homozygous inheritance [7].

Prothrombin (factor II) is the precursor of thrombin, the end product of the coagulation cascade. It is a vitamin K-dependent protein which is synthesized in the liver and circulates with a half-life of approximately three to five days. Vitamin K acts as a cofactor for posttranslational gamma-carboxylation of prothrombin which is required for functional activity.

G20210A mutation in the prothrombin gene (PTM) was first discovered by Poort et al. [8]. The mutation causes an increase in mRNA production due to the transitions among guanine–adenine nucleotides in 20210 position, leading to prothrombin increase and, as a result, in thromboembolic disease risk [9].

A mildly elevated homocysteine (Hcy) level is generally accepted as a risk factor for atherosclerosis & thrombotic tendency [10]. Hyperhomocysteinemia may result from inherited defects in the controlling enzymes of Hcy metabolism. Methylenetetrahydrofolate reductase (MTHFR) plays a role in the transmethylation of homocysteine to methionine. A common 677C->T transition in the MTHFR gene results in a thermolabile variant with decreased enzymatic activity. Homozygosity for the MTHFR C677T mutation has been associated with an increase in blood clotting together with plasma homocysteine increase and DVT occurrence risk [11].

The aim of this work is to study the role of mutation of factor V Leiden, prothrombin (factor II) G20210A and MTHFR C677T genes in patients with pulmonary embolism.

Patients and methods

This is a prospective study; it was carried out in the hematology department, chest department and clinical pathology department, Ain Shams University hospitals in the period between 2010 and 2013. Thirty-one patients presented with pulmonary embolism were included in the study.

The diagnosis of pulmonary embolism was based on a history of pulmonary embolism, documented clinically by a thorough clinical examination, and radiographically by CXR, C.T pulmonary–angiography. The simultaneous occurrence of DVT was tested radiologically by duplex US, in addition to careful clinical examination of the lower limbs.

The exclusion criteria included the occurrence of known conditions associated with highly increased risk of thromboembolism such as: contraceptive pills, hormonal replacement therapy, heart failure, malignancy, recent major surgery, prolonged immobilization, advanced age, etc.

Once the diagnosis of PE was established, FVL (G 1691A), prothrombin 20210 A, and MTHFR (C677T) were tested in peripheral venous blood. EDTA-anticoagulated peripheral blood samples were obtained from patients. Human plasma was obtained by centrifugation of EDTA samples at 3000*g* at 4 °C for 15 min.

In testing for factor V Leiden, prothrombin, and methylenetetrahydrofolate reductase mutations, DNA was isolated using a Magna Pure Automatic Isolation System (Roche Diagnostics, Indianapolis, IN). The genotyping of factor V Leiden G1691A, prothrombin G20210A, and methylenetetrahydrofolate reductase C677T was performed via real time polymerase chain reaction, using the fluorescence melting curve detection analysis according to a Light Cycler System (Roche Diagnostics, Manheim, Germany). Primers were obtained from TIB MOLBION, Berlin, Germany.