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Inherited Thrombotic Risk Factors in Non-Selected Group of Egyptian Population

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ABSTRACT

In recent years, knowledge concerning inherited and acquired causes of thrombophilia has greatly increased. The association of of venous thromboembolism is reported to be different according to the genotype, being higher among the carriers of natural anticoagulant deficiencies and homozygotes for factor V Leiden.

The aim of this study was to evaluate the incidence of five inherited thrombotic risk factors (FV1691A, FV4070G, PT20210A, EPCR 23 bp insertion and ACE 300bp deletion) non-selected group of healthy individuals.

The study included one hundered and twenthy randomly selected healthy individual with age ranged from one and 62 years. This included 32 children (15 males and 17 females with age rang between 1 and 18 years with mean of 13.29 years) and 90 adults (32 males and 58 females with age range between 18.5 years and 62 years with mean of 33.52 years).

The study revealed that eighteen individuals (15%) had FV1691 Amutation with a frequency of 0.075. R2 and R3 haplotypes were found in 15 (12.5%) and 2 (1.6%) individuals respectively. Two of them carried FV1691A and R2 haplotype at the same time. Only one individual had EPCR 23bp insertion in heterozygous form (0.83%). None of the 120 individuals had PT 20210A mutation. The distribution of angiotensin converting enzyme -300bp del in homozygous state was present in 61 (50,8%) individuals. The frequency of DD allele was 0.508. Neither age nor sex was found to affect the distribution of this mutation.

Conclusion: Our preliminary data revealed that FV1691A mutation is an important risk factor for thrombosis in Egyptians and further studies on a larger scale population is needed.

Key Words: *Factor V leiden - Thrombotic risk.*

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INTRODUCTION

The term thrombophilia describes a tendency to develop thrombosis on the basis of inherited or acquired disorders of blood coagulation or fibrinolysis leading to a prothrombotic state. Familial thrombophilia was first described in 1956 on a clinical basis by Jordan and Nandroff **[1]**. Inherited thrombophilia is defined as a genetically determined tendency to thromboembolism. Dominant abnormalities or combinations of less severe defects may be clinically apparent from early age of onset, frequent recurrence or family history. Milder traits may be discovered only by laboratory investigations **[2]**. Thus venous thromboembolic disease is now viewed as a multicausal model, the thrombotic event being the result of gene-gene and gene - environment interactions **[3]**.

In recent years, two common gene polymorphisms were recognozed as important causes of hypercoagulability: Factor V leiden and prothrombin G20210A mutations. Factor V Leiden thrombophilia is the most common inherited form of thrombophilia. The mutant factor V Leiden is inactivated at an approximately tenfold slower rate than normal and persists longer in the circulation, resulting in increased thrombin generation and a mild hypercoagulable state, reflected by elevated levels of prothrombin fragment and other activated coagulation **[4]**. Prothrombin 20210 G-A change of the prothrombin gene was reported to be an important genetic risk factor for thrombosis by increasing the plasma levels of prothrombin **[5]**.

After the determination of these two coagulation factors gene defects, endothelial protein C receptor (EPCR) gene polymorphisms and angiotensin converting enzyme (ACE) gene defects were recognized. EPCR slows down protein C activation via thrombomodulin-thrombin at the endothelial surface. ACE gene defects are associated with venous thromboembolism (VTE) by affecting the vascular tone **[6,7]**. EPCR gene has a 23bp insertion into a normal DNA sequence in exon 3 that causes stop amino acid sequence into the EPCR protein which causes a truncated protein at the end **[8]**. ACE gene constitutes an insertion/deletion polymorphism of a 300bp fragment and the possible loss of function in the enzyme was suggested to be responsible for the ACE levels in circulating blood **[7]**.

The aim of this study was to investigate two common mutations of the factor V gene, (1691 G-A and 4070 A-G), prothrombin gene 20210 G-A alteration, EPCR 23bp insertion and ACE ins/del polymorphisms in a sample of Egyptian population. The Egyptian population has a mixed genetic background with an ethnic heterogeneity. The analyzed risk factors influence both the coagulation pathway and the endothelial changes in the case of vascular damage.

SUBJECTS AND METHODS

The study included 120 randomly selected healthy Egyptian individuals. Their age ranged between one and 62 years. This included 32 children (15 males and 17 females with age range between 1 and 18 years with mean of 13.29 years) and 90 adults (32 males and 58 females with age range between 18.5 years and 62 years with mean of 33.52 years).

Factor V 1691 G-A and Prothrombin 20210 G-A mutations were analysed with previously described techniques and real-time PCR method using Light Cycler (Roche Diagnostics, Germany) **[9]**. For the detection of insertion/deletion polymorphisms of ACE gene and EPCR gene and HR2 haplotype of factor V gene was determined according to previously described techniques **[10,11]**.

RESULTS

The frequencies of the five thrombophilic genetic factors are shown in Table (1). Both mutations in the factor V gene were found to have a high frequency of 15 % for 1691 G-A mutation and 12.5 % for the factor V 4070 A-G. Prothrombin 20210 G-A mutation could not be detected in any of the individuals. Only one individual carried the EPCR 23bp insertion making its frequency very low. ACE 300bp ins/del polymorphism was found to be frequent like other factor V gene changes. D allele of the ACE gene polymorphism was found to be more frequent, whereas I allele was obtained in 5 individuals with its homozygous state. Of the 120 individuals we found fifteen individuals carrying R2 haplotype (12.5%) and two carrying R3 haplotype. We also found that two individuals have both the FV1691A mutation and R2 haplotype at the same time (1.66%).

Table (1): Frequency of the five risk factors in the studied group.

Genetic changes	No.	%	Allele frequency
Factor V 1691 G-A	18	15	0.075
Factor V 4070 A-G:			
R2 haplotype	15	12.5	0.062
R3 haplotype	2	1.6	0.008
Prothrombin 20210 G-A	0	0	0
EPCR 23bp insertion	1	0.83	0.004
ACE ins/del:			
I/I	5	4.1	0.041
I/D	54	45	0.225
D/D	61	50.8	0.508

DISCUSSION

Our study is the first report informing five thrombosis-related risk factors in 120 healthy Egyptians. Althogh Prothrombin 20210 G-A mutation was not found in our studied group, both factor V gene mutations were found to be very frequent. It was reported that the highest heterozygosity rate of factor V Leiden is found in Europe with a prevalence of 10-15% in southern Sweden and Greece. In the US, heterozygosity for factor V Leiden was found in 5.2% of Caucasian American, 2.2% of Hispanic Americans, 1.2% of African Americans, 0.45% of Asian Americans, and 1.25% of Native Americans **[12]**. In contrast to what was claimed before, that Factor V Leiden mutation is not found in populations with African origin, Factor V 1691 G-A and 4070 A-G mutations were found to have high frequency in our studied group of Egyptian population. Further studies on a larger

scale population is needed especially that it is known that our population has different ethnic background. The determination of the R3 haplotype of factor V gene may confirm the suggestion that the mutation is of African origin and older than factor V Leiden **[13]**.

It is worth noting that most of our understanding of inherited risk factors for thrombosis is derived from the study of largely white populations. Although some attribute the presence of factor V Leiden and prothrombin mutation in some African and Asian populations to migration or colonization, other have arrested that the presence of these mutations in small genetic isolates may argue for multiple origins for at least the factor V Leiden mutation **[14]**. Thus, although studies have begun to elucidate the basis of familial thrombophilia in white populations, a great deal remains to be learned in other populations like ours.

A specific factor V gene haplotype (HR2) is present in 8% of normal subjects and is an additional cause of resistance to activated protein C **[15]**. Its role as an independent risk factor for VTE is uncertain, yet double carriers of the HR2 haplotype and factor V Leiden have an increased plasma resistance to activated protein C and an increased risk of VTE in comparison with heterozygotes for factor V Leiden, the rare state of homozygosity for HR2 produces a 5.5 fold increase in the risk of VTE **[16]**. Lunghi et al., reported that in the exon 13 of the factor V gene there is a different restriction pattern with Rsa I digestion. They found that the haplotype have 3935 A-G (His 1254 Arg) polymorphism instead of 4070 G-A (His 1299 Arg) and referred the haplotype as R3 **[17]**. In the present study, fifteen individuals were found to carry R2 haplotype (12.5%), two of them carried FV1691A and R2 haplotype at the same time (1.66%) and R3 haplotype was present in two individuals. The former result is also higher than that reported before **[15]**.

The distribution of the 23 bp insertion polymorphism in the EPCR gene was very rare with a frequency between 0-3% in different case and control groups **[18]**. The ins/del polymorphism of the ACE gene showed a variation in the Egypt population. The influence of ACE gene defect was not clear in the issue of the levels in ACE enzyme in plasma. Recently, a quantitative trait locus was identified that is claimed to be linkage disequilibrium with APCR levels **[19]**. There is a great variation in ACE ins/del polymorphism too. Studies concerning ACE gene defects in different populations show a great variation.

From this study we revealed the genetic tendency and the frequency of the five thrombotic risk factors in a group of Egyptian population. Further studies are needed in Egyptian patients with thrombosis, as testing may be used for better understanding of the origin of these mutations and better evidence based management of these patients.

REFERENCES

1- Jordan FLJ, Nandorff A. The familial tendency in thromboembolic disease. Acta Med Scand. 1956, 156: 267-75.

2- Lane DA, Mannucci PM, Bauer KA, Bertina RM, Bochkov NP et al. Inherited thrombophilia: Part 1. thromb Haem. 1996, 76: 651-62.

3- Rosendaal FR. Venous thrombosis: A multicausal disease. Lancet. 1999, 353: 1167-73.

4- Zoller B, Holm J, Svensson P, Dahlback B. Elevated levels of prothrombin activation fragment 1 + 2 in plasma from patients with Arg 506 to Gln in the factor V (APC-resistance) and/or inherited S deficiency. Thromb Haemost. 1996, 75: 270-4.

5- Poort SR, Rosendaal FR, Reitsma PH, Bertina RM. A common genetic variation in the 3'-untranslated region of the prothrombin gene is associated with elevated plasma prothrombin levels and an increase in venous thrombosis. Blood. 1996, 88: 3698-703.

6- Esmon CT. The Endothelial Protein C Receptor. Thromb Haemost. 2000, 83: 639-43.

7- Okumus G, Arseven O. Insertion/Deletion polymorphism of the angiotensin-converting enzyme gene: really a risk factor for venous thromboembolism?. Thromb Haemost. 2003, 90: 766-7.

8- Zecchina G, Bosio S, Brusa E, Rege-Cambrin G, Camaschella C. EPCR 23bp Insertion an a Patient with Arterial Disease: A dominant loss of function mutant in conditions of increased APC request. B J Haematol. 2002, 119: 874-84.

9- Akar N. Site specific mutagenesis: Usage for the mutation detection in thrombosis. Turk J Haematol. 1998, 15 (3): 123-24.

10- Akar N, Akar E, Yılmaz E. Factor V (His 1299 Arg) in Turkish patients with venous thromboembolism. Am J Hematol. 2000, 63 (2): 102-3.

11- Akar N, Aras Ö, Ömürlü K, Cin S. Deletion polymorphism at the angiotensinogen converting enzyme gene in Turkish patients with coronary artery disease and controls. Scand J Clin Lab Invest. 1998, 58: 491-95.

12- Ridker PM, Miletich JP, Hennekens CH, Buring JE.

Ethnic distribution of factor V Leiden in 4047 men and women. Implication for venous thromboembolism screening. JAMA. 1997, 277: 1305-7.

13- Lunghi B, Iacoviello L, Gemmati D, Dilasio M, Castoldi E et al. Detection of new polymorphic markers in the factor V gene: Association with factor V levels in plasma. Thromb Haemost. 1996, 75: 45-48.

14- Pawar AR, Shetty S, Ghosh k, Mohanty D. How old is the factor V Leiden mutation? Thromb Haemost. 2001, 86: 1591-92.

15- Bernardi F, Faioni EM, Castoldi E, Lunghi B, Castaman G, et al. A factor V genetic component differing from factor VR506Q contributes to the activated protein resistance phenotype. Blood. 1997, 90: 1552-57.

16- Folsom AR, Cushman M, Tsai MY, Aleksic N, Heckbert SR, et al. A prospective study of venous thromboembolism to factor V Leiden and related factors. Blood. 2002, 99: 2720-25.

17- Lunghi B, Castoldi E, Mingozzi F, Bernardi F. A New Factor V Gene Polymorphism (His 1254 Arg) Present in Subjects of African Origin Mimics the R2 Polymorphism (His 1299 Arg). Blood. 1998, 91 (1): 364-5.

18- Akar N, Gökdemir R, Özel D, Akar E. Endothelial Cell Protein C Receptor (EPCR) Gene Exon III 23bp Insertion Mutation in the Turkish Pediatric Thrombotic Patients. Thromb Haemost. 2002, 88: 1068-69.

19- Soria JM, Almasy L, Souto JC, Buil A, Martinez-Sanchez E, Mateo J, Borrell M, Stone WH, Lathrop M, Fontcuberta J, Blangero J. A new locus on chromosome 18 that influences normal variation in activated protein C resistance phenotype and factor VIII activity and its relation to thrombosis susceptibility. Blood. 2003, 101 (1): 163-7.