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Determination of Prothrombin Gene G20210A Mutation in Deep Venous Thrombosis among Sudanese Patients - Khartoum State

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Abstract:

Background: Deep vein thrombosis (DVT) is serious disorders contributing to increased morbidity and mortality worldwide. However, there is, little genetic data from Africa including Sudan. So this study was conducted to investigate the relationship between genetic mutations G20210A in the prothrombin coagulation factor and deep venous thrombosis in Sudanese patients.

Methods: This is a cross-sectional study design was carried out in Khartoum state in the period from December 2014 to May 2018. One hundred of patient group and fifty healthy one were enrolled in this study. Mutation was detected using conventional PCR depending on restriction enzyme polymorphism technique.

Results: The mutant allele (G/G) was found in 4 (4%) of patients nevertheless, there is no mutant allele was present in control group (P.value=0.152). Moreover, the mixed allele (G/A) was not detected in patients and healthy control. Exclusively, the control group showed only the wild type (A/A).

Conclusion: This study noted a few mutant alleles in patients group and only the wild type (A/A) in control one. However, carrying out a genome-wide associated study is recommended to determine relationship between prothrombin gene mutation and frequency of deep vein thrombosisin Sudanese population.

Keywords:

Throm¬bosis, Prothrombin, Mutation, Embolism, Hypercoagulable

Authors' contributions: *This work was carried out in collaboration among all authors. All authors read and approved the final manuscript*

INTRODUCTION

Efficient hemostasis is a rapid and localized response to an interruption in vascular integrity (vessel wall injury); such that clots are formed only when and where they are needed [(1)]. There are three possible contributors to formation of an abnormal clot (thrombus): decreased blood flow, vessel injury or inflammation, and changes in the intrinsic properties of the blood. Persistent physiologic changes in any of these three factors (the Virchow triad) are referred to as hypercoagulable state [(2)]. Hypercoagulable or thrombophilia state is an inherited or acquired abnormality of hemostasis that affects the delicate balance between procoagulant and anticoagulant factors, predisposing to thrombosis [(3)]. Thrombosis can be venous or arterial[(4)].Venous thromboembolism (VTE) is a disease that includes both deep vein thrombosis (DVT) and pulmonary embolism (PE). It is a common, lethal disorder that affects both hospitalized and nonhospitalized patients [(5)]. Although, DVT and PE are two clinical presentations of VTE that share the same predisposing factors [(6)]. Additionally, Deep vein thrombosis can lead to life-threatening pulmonary embolism [(7)].

Interestingly, the prothrombotic mutation found to be associated with increased prothromhin levels and a 3-fold risk of venous thrombosis [(8)]. Because, prothrombin is an inactive precursors of thrombin, which is the end product of coagulation cascade. Thus, the prothrombotic mutation occurred as results of nucleotide replacement of guanine (G) with adenine (A) at position 20210 in the sequence of the 3'-untranslated region of the gene encoding prothrombin[(8)].

Currently, there is growing need to understand the molecular mechanism that controls the relationship between inheritance diseases and many causing factors, so, this study carried out in Sudan to better understand the association betweenG20210A mutation and deep vein thrombosis in Sudanese patients.

PATIENTS AND METHODS:

One hundred and fifty unrelated participants were randomly selected. Of the 150 participants one hundred volunteers diagnosed with deep venous thrombosis (DVT), with the mean age 34.49 years. Fifty healthy volunteers were chosen in accordance to the gender and age of the study group with the mean age 27.02 years.

Blood samples

Two milliliter of whole blood were collected and poured in ethylenediamine tetra acetic acid (EDTA) container and stored at minus eighty degrees tell the time of used for the molecular examinations.

DNA extraction protocol

The genomic DNA was isolated from peripheral blood leucocytes according to Guanidine chloroforme Extraction Method.

Primers design and preparation

Primers used were described by (Farah Parveen*et al*.2013) [(9)]; then checked by NCBI programme: Forward primer 5′TCTAGAAACAGTTGCCTGGC3′ and the reverse prime 5′GTAGTATTACTGGCTCTTCCTGAG3′

PCR protocol

Detection of prothrombin gene polymorphism was done according to method described by Farah Parveenet al[9].The amplified fragment of 345 bp was digested by 5 U/sample of Hind III enzyme at 37 ^{0}C for 18 hours. The 20210A allele generated a restriction site in the amplified fragment and was digested into two fragments of 322 and 23 bp, respectively. Wild-type allele (20210G) lacked the restriction site and therefore remained undigested (345 bp).(Farah Parveenet al).Presence of 20210GA allele was screened by PCR followed by Hind III enzyme digestion. The PCR conditions were as follow: initial denaturation at 95 ^{0}C for 5 minutes, 35 cycle of 94 ^{0}C for 30 seconds, 59.3 ^{0}C for 45 seconds, 72 ^{0}C for 1 minute followed by final extension of 72 ^{0}C for 5 minutes.

Detection of PCR products

The PCR product was checked on 2.0 % agarose gel at 120 V for 60 minutes stained with 0.5 lg/mL ethidium bromide in Tris.

RESULTS:

Prothrombin G20210A mutation was detected in 4% (4/100) of DVT patients. The prevalence of the homozygous type (GG) was 4% the heterozygous type was not detect. Both the homozygous and heterozygous were not detected in the control group (Table 3.1). The relation between DVT and this mutation was statically insignificant (*P*= 0.152).

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Table 1. Frequency and percentages of mutations in patient and control group

		Study group		Control group		P- value
		frequency	Percentage	Frequency	percentage	
prothrombin mutation G20210A	*Mutant allele*	4	4	0	0	0.152
	Mixed allele	0	0	0	0	
	Wild type	96	96	5	100	

DISCUSSION:

The results of the present study illustrate four individuals (4%) of the study group had mutant allele (A/A) for the prothrombin mutation G20210A. The remaining 96 (96%) had the wild type (G/G). results didn't show the mixed allele (G/A). The mutant allele and the mixed allele were not found in the control group. The difference between study group and control group was insignificant (*P* = 0.197) indicating that no association between this mutation and DVT. These findings are supported by a study conducted in Sudan (Yousif *et al*., 2017) [10] who didn't detect the mutation in both study and control group [10]. The absence of this mutation was also supported by similar findings in a study carried out in Saudi Arabia (Algari *et al.*, 2017) [11]; they found no association between this mutation and DVT patients (*P*-value>0.05) [11]. In France (Meyer *et.al*, 2001) found results that were consistent with our results. The mutant allele found in 8 % of their study group (DVT patients) but the association was insignificant (*P* =0.2) [12].

Nevertheless**,** our results didn't agree with a study done in turkey by Dölek and his colleague; they found 8.6 % of the study group having the mutant allele while the mutant allele represented 1.8 % of the control group. They concluded that there was association between factor II mutation and DVT **(**Dölek *et. al,* 2007*)*. Also, our results are similar to that finding noted by Martinelli and others in Italy they found the mutant allele in 9.4 % of patients and 2.5 % in the control group. They presumed that the mutation is significant if it is combined with acquired factor [(14)]. The study concluded that no association between prothrombin mutation G20210A and DVT.

CONCLUSION:

The present study noted that a few mutant alleles in patients group and only the wild type (A/A) in control one. However, carrying out a genome-wide associated study is recommended to determine relationship between prothrombin gene mutation and frequency of deep vein thrombosis in Sudanese population.

RECOMMENDATION:

We recommended that further study may be carried and large sample should be taken into account.

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ETHICAL APPROVAL AND CONSENT

The study was approved by the research committee at the faculty of medical laboratory science ,Managil University of Science & Technology. Ethical approval was achieved from the university, Informed consents were taken from each subject before enrollment in the study.

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COMPETING INTERESTS

Authors have declared that no competing interests exist.

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