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The Role of Factor X III val 34 Leu Polymorphism in Intracerebral Hemorrhagic Stroke among Jordanian Patients: Molecular Analysis

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Author's contribution

The sole author designed, analyzed and interpreted and prepared the manuscript.

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Original Research Article

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ABSTRACT

The polymorphism in the factor X III A-submit gene (F X III val 34 Leu) has been recongized as a risk factor for primary intracerebral hemorrahge (pich). In addition, F X III valu 34 leu has a significant ethnic heterogenty.

Objective: To study the possible role of a common mutation in the gene for coagulation factor X III -A (val 34 Leu) as a risk factor for primary intracerebral hemorrhage stroke (pich) among Jordanian patients.

Patients and Methods: A total thirty patients that have clinically suffered from cerebral hemorragic stroke, who are all aged between (40-62) years were entrolled in this study.

DNA was extracted from plasma samples. Genotyping of F X III val 34 Leu polymorphism was detected by a 192 bp, PCR-technique and the PCR product was digested with *DdeI* restriction enzyme.

Results: In this study two bands in fragment length (192 bp and (161 bp) were found in seven patients as positive results 25% the other patients showed one fragment of 192 bp as negative results (75%).

Conclusion: These results suggested that factor XIII val 34 Leu has a role in the pathogenesis primary intracerebral hemorrhage (pich), this may be related to impaired cross-linking of fibrin.

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1. INTRODUCTION

Blood coagulation FXlll or fibrin stabilizing factors is the plasma zymogen of transglutaminase and is last enzyme to activated in the coagulation cascade. The cascade models suggested that cloting sequences were divided into 2 pathways, intrinsic pathway and extrinsic pathway. The presence and normal functional integrity of FXlll is essential maintaining normal homeostasis [1]. Homeostatic mechanisms have envolved to protect against the threat of fatal hemorrhage. The interaction between platelets and cloting factors results in the generation of a protective homeostatic plug, whose function is to staunch the flow of blood at the site of vascular injury [2]. During clinical laboratory analysis of blood clotting, the intrinsic pathway of blood coagulation is evaluated using the activated partial thromboplastin time (PTT) and the extrinsic pathway is evaluated using the prothrombin time (PT) [2].

Blood coagulation factor Xlll plays an important role in formation and stabilization of blood clots, which require a complex interaction involving conversion of fibrinogen to fibrin and activation of plasma factor Xlll. Factor Xlll was discovered over 70 years ago by Barkan et al who observed the insolubility of fibrin clots in the presence of calcium [3].

Blood coagulation factor Xlll is a tetramere pro-transglutaminase that consist of two types of subunits (A2 B2), these subunits are synthesized by cells of bone marrow origin and by hepatocytes [4].

The genese of A and B subunits of FXlll are localised on chromosome (6) at bands p24-25 and at bands q32-32.1 respectively. Although the biological effects of different amino acid substitutions in FX lll-A have been studied widely, evidence of the consequences has been to be regulated by three different transcription factors [5]. The factor Xlll A-subunit gene has been localized to chromosome 6, contains 15 exons separated by 14 introns. The ending region is translated into a polypeptide of 731 amino acids, more than 20 mutations in the A-subunit gene of FX llll have been separated by several investigators in patients with severe FXlll deficiency which lead to complete absence of the A-subunits, 4, 5, 29 [6-7]. These points mutations or minor deletions lead to amino acid substituations, stop codons or splicing defects. Several common sequence changes in the FXlll A-subunit gene not associated with FXlll deficiency has also been described [8]. Factor Xlll is known to play an important role in three major physiologic areas, normal hemeostasis, wound healing and maintenance of viable pregnancy. Its functions to generate a stable, rigid clot that is relatively resistant to fibrinolysis due to extensive cross linking between fibrin strands [6]. Five common polymorphisms with amino acid exchange have been described in FXlll-A, one of the polymorphisms commonly occuring in a normal population is a G-T point mutation in codon 34, exon 2 of the A-subunit gene which codes a valin leucine change. This polymorphism might influence the specific acitivity of FXlll valu 34 leu [4]. The val 34 leu polymorphism was found to be more common in patients with primary intracerebral homorrhage (ICH) [7,9-11]. A hemorrhagic stroke occurs when a blood vessel in the brain leaks or ruptures, resulting in bleeding into the brain. The symptoms of hemorragic stroke depend on what part of the brain is affected and how large an area is involved and accompanied by one or more signs [12-13]. The most notable risk factor for ICH is the presnece of hypertension and it appears to be related to the severity and the duration of hypertension [14]. The multiple risk factors in the incidence of ICH has been reported [15].

The goal of this study was to investigate a common mutation in the gene for coagulation factor Xlll-A (Val 34 leu) which is a risk factor for primary inrtracerebral hemorrhage stroke among Jordanian patients.

2. MATERIALS AND METHODS

2.1 Blood Sampel Collection

The blood samples were collected from thrity patients that have clinically sufferened from cerebral hemorrhagic stroke, who all aged between (40-62) years. The samples were collected from Prince Hamza Hospital and DNA extraction stroed at 4°C until used.

2.2 DNA Extraction

DNA was extracted from EDTA –anticoagulation blood samples by Wizard ® genomic DNA purification kit (printed in USA, revised 10/02,

part no. 9FB022). The DNA was extracted according to the manufacture's instruction.

2.3 Polymerase Chain Reaction (PCR)

Genoytping of val 34 leu gene and restriction enzyme analysis. The FxIII val 34 leu polymorphism was detected by a 192-bp polymerase chain reaction amplified fragment of exon 2/intron B in the factor XIII A –subunit gene using the TACCTTGCAGGTTGACGCCCCGGGGCACTA-3 the reverse primer. The genomic DNA that was extracted by previous method was amplified exponentially by PCR to facilitated analysis.

In a standard PCR set up, a master mix was prepared with a volume to account for the number of samples to be anlayzed, and a blank to check for contamination. The following volumes were used for single PCR reaction; (14.25 μL) of nuclease-free waters, (1 μl) of Mgcl, (5 μl) of (1x) buffer, (0.5 μl) of sense and anti-sense primers (10 μm), (0.5 μl) of dNTPs (10 mH) and (0.25 μl from (5 μ/μl) taq polymerase. Then (3 μml) of each jenomi DNA sample was added to different PCR tubes, final volume in each reaction was (25 μl). Then the samples were run in a thermal cycler (Bio-RAD USA) according to the following programm, 94°C for 30 sec, 55°C for 45sec. and 72°C for 5 min.

2.4 DNA Digestion

Factor XIII val 34 leu mutation twenty five microliter of each amplified products was digested with 0.62 μl of restriction enzyme DdeI, 2.51 of 10x enyzme buffer and 0l.25 μl BSA (10 mg/ml) a 37°C over night.

2.5 PCR Product Detection by Agarose Gel Electrophoresis

Ten Ml of the PCR product was separated electron-phoretically in 2.5% agarose gel stained with ethidium bromide (one μg/ml). Ladder was loaded in a separate well to allow the determination of molecular weight and the reaction quality, and observed under violet trans illumination using gel documentation system (Bio-RAD, USA).

3. RESULTS

The PCR product separated by using 2.5% agarose at 100V electrical fields. The F X III gene was found in all 30 samples with length 192 bp (Fig. 1). The PCR products were digested by using DdeI restriction enzyme, and separated by 2.5% agrose at 100V electrical figelds (Fig. 2).

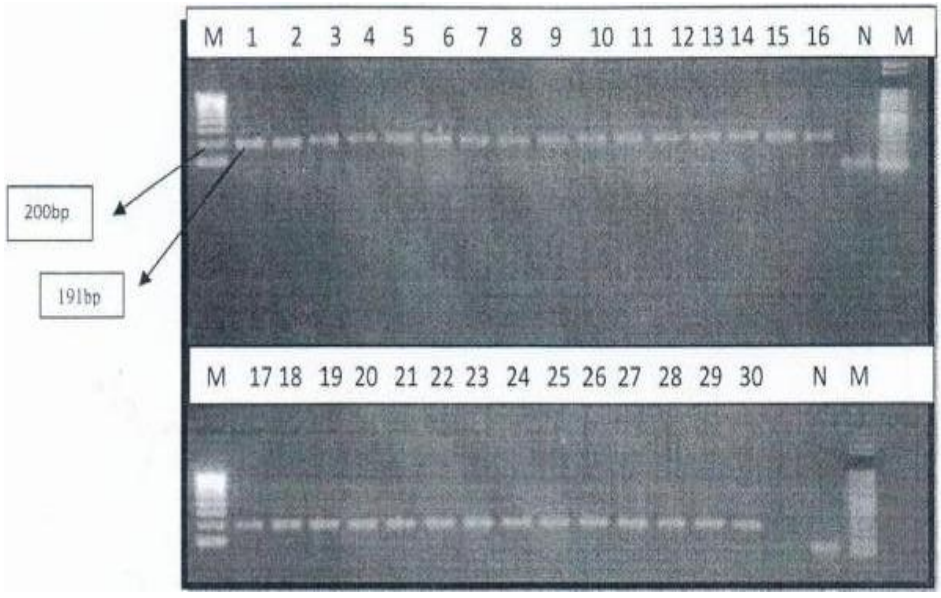

Fig. 1. Agarose gel electrophoresis of amplified PCR product of factor X111, M: ladder DNA (100 bp), N: Negative control and 1-30 represent the samples

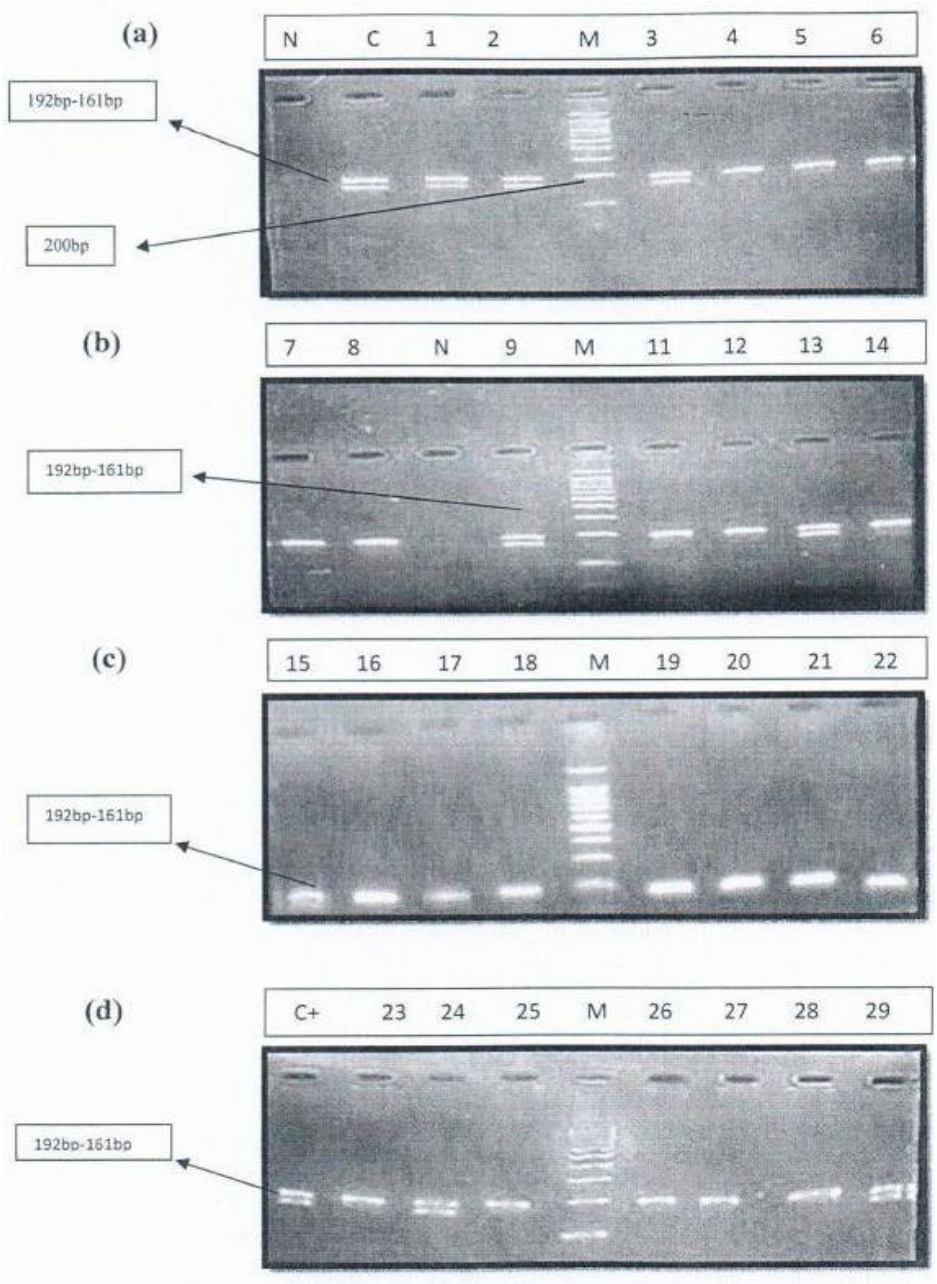

Fig. 2. Agarose gel electrophoresis of amplified PCR product after digested with Ddel restriction enzyme, N: Negative control, C: positive control (192 bp and 161bp fragments DNA), M: ladder DNA (100 bp). In Fig. 2a. The lanes 1-6 represent sample no.1 to sample no.6.), and Fig. 2b. The lanes 7-14 represent sample no.7 to sample no 14, and Fig. 2c. The lanes 15-22 represent sample no.15 to sample no 22, also the Fig. 2d. The lanes 23-29 represent sample no. 23 to sample no. 29

The 192 bp amplified fragment from a normal factor XIII gene has no cutting site for the restriction enzyme *Ddel,* but the G to T point mutation in codon 34, exon 2 of the factor XIII A-subunit gene create a recognition site for restruction enzyme D del resuting in fragments 161bp and 31 bp. So, the expected results for the patients samples after enzyme digested are one band (192 bp) for the normal alleles (Wild type) and three bands (192 bp, 161 bp and

31 bp) a rising from nromal alleles and mutant allelels (heterozygous) and two bands (161 bp and 31 bp) a rising from mutant alleles (homozygous), the homozygous are not found in this results. The fragment 31 bp cannot be seen in a garose gel electrophoresis due to its short length.

The results, showed seven positive sample has two bands (192 bp and 161 bp) arising from normal alleles and mutant alleles (heterozygous).

In Fig. 2a the samples no. 1,2 and 3 were positive in Fig. 2b the samples no 9 and 13 produced two bands for each. In Fig. 2c the sampel no. 15 was positive and in Fig. 2d the samples no. 24 and 29 were positive.

4. DISCUSSION

Blood coagulation factor XIII plays an important role in clot stabilization by cross linking fibrin chains in the final stages of blood coagulation, to increasing the resistance of the clot to degradation by plasmin.

There is a wide range of plasma FX III-A transglutaminase activity in the normal population that is not directly dependent on FX III levels. Recently, distinct genetic polymorphisms of the FX III-A gene have been correlated with FX III A specific activity, that polymorphisms associated with high FX III A citivity could factor the formation of weaker fibrin structures, thereby increasing the risk of developing bleeding disorders. Nowadays, one of these common polymorphisms val 34 Leu has been the focus of attention for two reasons, first, the val 34 Leu changes is located three amino acids aways from the thrombin cleavage site (Arg 37-Gly 38) and could therefore affect the acitivation process or the activity of the resulting enzyme. Second, this polymorphism has been found to be associated with the risk of primary intracerebral haemorrhage [16,17]. Association studies of this polymorphism with ICH have generated conflicting results [7,9,11,18].

Therefore, this study was designed to investigage the mutation in the factor XIII A-subunit gene and is establish its frequenices in the Jordanian population. Ag eneral screening method for mutation identification is essential for detection of mutation in populations previously not studied.

The results of this study indicate an association between possession of a G-to T, point mutaiton coding for factor X III val 34 Leu and Pich.

It is now well demonstrated that the FXIII val34 Leu polymorphism associates with FX III specific with FX III specific transglutaminase activity, due to thrombin activity.

The role of common polymorphism of FX in val 34 Leu has been recently recongized as a protective genetic factor against arterial and venous thrombosis. The less frequent Leu 34 allele has been described as risk factor for ICH.

The FX III val 34 Leu has been recently reported to confer protection against arterial venous thrombosis [9-10],7,16], our result agree with these studies.

However, [19] reported that FX111-A val 34 Leu has protective effect against thrombosis) [20-22].

To my knowledge, there has been no information on the prevalence of FX III val 34 Leu polymorphism in Jordanian population until now. My results are in line with those of previous studies that showed FX111-A in Val34Leu was low prevalence among Japanese. Moreover, this polymorphism has been sugested to be the first one to increase the risk of cerebral hemorrhage in caucasian patients.

A common G to T polymorphism in codon 34 of exon 2 of the Fx111-A subunit gene results in the substitution of amino acid valine (GTG) to leucine (TTG), three amino acid valine away from the thrombine cleavage site. The amino acid change at this location might influence the process of proteolyses activation and FX111 activity. Association studies have investigated the relationship between FX111 Val 34 Leu polymorphism and the risk of primary intracerebral hemorrhagic stroke. Other five common mutation in polymorphisms have been identified in the FX111-A deficiency [23-24].

5. CONCLUSION

In this report, the expected result from the patient samples according to the DNA digested by restriction enzyme for FX111 Val 34 Leu polymorphism mutation are one band (191 bp) for wild type DNA, and tow bands (191 bp, 161 bp) for heterozygous found in samples (no. 1,2,3,9,13, 15, 24 and 29) and no samples were found for homozygous. The fragment (31 bp) can

not be seen in the gel due to its short length the incidence of the Val34Leu polymorphism is about 25% in heterozygous state and 3% in the homozygous form.

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COMPETING INTERESTS

Author has declared that no competing interests exist.

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