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Research Paper

GENETIC RISK FACTORS ASSOCIATED WITH THROMBOSIS IN A SAMPLE OF IRAQ POPULATION

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This study aimed to investigate the association of F5 gene Single Nucleotide Polymorphism (SNP) with the incidence of thrombosis. Blood samples were collected from 40 patients during the period from November 2014 to January 2015, from Critical Care Unit (CCU) of (Yarmook Hospital, Kadhimiya Hospital), as well as from 10 unrelated healthy control group. This study found the age group between 50 to 60 are more susceptible to thrombosis 45% and the thrombosis was more frequent in male 55% from female 45% the significant ($p<0.01$). Deoxyribonucleic acid (DNA) was extracted from whole blood samples, whereas, serum samples were analyzed using troponin test (TNT) for detection of thrombosis. Polymerase Chain Reaction (PCR) was achieved on extracted DNA using eleven specific primers for F5 gene :the first primer (Fve3) with product size (228bp), second primer (Fve4) with product size (310bp), third primer (Fve6) with product size(547bp), fourth primer (Fve7) with product size (241bp), fifth primer (Fve8) with product size (306bp), sixth primer (Fve12) with product size (286bp), seventh primer (Fve13a) with product length (260bp), eighth primer (Fve13c) with product size (317bp), ninth primer (Fve15) with product size (600bp), tenth primer (Fve16) with product size (333bp) and eleventh primer (Fve25) with product size (390bp). PCR products of F5 gene were sequenced. Result found to be change in DNA which was mostly SNP. This change was in three types: substitution 24.86%, insertion 28.57% and deletion 28.57%, a Leiden mutation was also identified among patients.

Keywords: Thrombosis, F5 gene, Mutation, Genetics risk factors, PCR

INTRODUCTION

A thrombus, or colloquially a blood clot, is the final product of the blood coagulation step in hemostasis. There are two components to a thrombus: aggregated platelets that form a platelet plug, and a mesh of cross-linked fibrin protein. The substance making up a thrombus is sometimes called cruor. A thrombus is a healthy response to injury intended to prevent bleeding, but can be harmful in thrombosis, when clots obstruct blood flow through healthy blood vessels) Saladin and Kenneth , 2012).

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Mural thrombi are thrombi that adhere to the wall of a blood vessel. They occur in large vessels such as heart and aorta, and can restrict blood flow but usually do not block it entirely. They appear grey-red with alternating light and dark lines (known as lines of Zhan) which represent bands of fibrin (lighter) with entrapped white blood cells and red blood cells (darker) (Kumar *et al.,* 2007).

Disseminated Intravascular Coagulation (DIC) involves widespread micro thrombi formation throughout the majority of the blood vessels. This is due to excessive consumption of coagulation factors and subsequent activation of fibrinolysis using all of the body's available platelets and clotting factors. The end result is hemorrhaging and ischaemic necrosis of tissue/organs. Causes are septicemia, acute leukemia, shock, snake bites, fat emboli from broken bones, or other severe traumas. DIC may also be seen in pregnant females. Treatment involves the use of fresh frozen plasma to restore the level of clotting factors in the blood, platelets and heparin to prevent further thrombi formation (Ungprasert *et al.*, 2013).

MATERIALS AND METHODS

Study Subjects

The study included 40 patients suffering from thrombosis during the period from November 2014 to January 2015 A L Kadhimiya Hospital Teaching and Yarmouk Hospital Teaching. The apparently healthy 10 person individuals from college of science/Al Nahrain University were selected to represented the control group. The maen ages of the patients were 34-76 years and the maen ages of the control were 25-40 years. Informed consents from patient as well as control were taken which included age and family history of thrombosis.

Blood Samples Collection.

Five mL of blood was collected kept in EDTA tube and preserved -20°C until be used .

DNA Extraction Mini Prep System Kit

The Reliaprep Blood genome Isa DNA MiniPrep SystemfromPromega USA, ready to use that contained the following Component:

Optimal Protocol of PCR Amplification

For PCR amplification of F5 gene (Fve3, Fve4, Fve6, Fve7, Fve8, Fve12, Fve13a, Fve13c, Fve15, Fve16, Fve25) annealing temperature in optimizing the following PCR protocols as followed.

PCR Amplification

Initial denaturation 1 cycle 94^0C for 5 min, whereas the following steps were for 35 cycles denaturation 94^0C for 1 min, extension 72^oC for 1 min, annealing show see below, and final extension 1 cycle 72^0 C for 10 min.

		Primers	Temperature –time
Annealing primers	35cycle	Fve3	58^0C for 1 minute
		Fve4	60^0C for 1 minute
		Fve6	58^0C for 1 minute
		Fve7	54^0C for 1 minute
		Fve8	54^0C for 1 minute
		Fve12	56^0C for 1 minute
		Fve13a	54^0C for 1 minute
		Fve13c	50^0C for 1 minute
		Fve15	61^0C for 1 minute
		Fve16	52^0C for 1 minute
		Fve25	52^0C for 1 minute

RESULTS AND DISCUSSION

The Distribution of the Studied Group

In this study, fourty sample have been collected from patient have thrombosis.and used to effect of difference factors in study the age and gender. Chi-square test was used to significant compare between percentage in this study (SAS, 2012).results are shown in Table (1, 2, 3, 4 and 5).

Table 1 test of trponin, Patients (40) positive and healthy (10) negative, the high significant (P<0.01).

Table 2 the ages less than 50 their percentage 20% from patients, While ages between 50 to 60 their percentage 45% from patients and the ages more than 60 their percentage 35% from patients, the high significant (P<0.01).

Table 3 the males their percentage 55% from patients, while the females their percentage 45% from patients, the significant (P<0.05).

Table 4 the ages less than 50 they numbered (8) positive from patients in the test of troponin, the ages between 50 to 60 they numbered (18) positive from patients in the test of troponin and the ages more than 60 they numbered (14) positive from patients in the test of troponin, the high significant (P<0.01).

Table 5 the males as number (22) positive from patients in the test of troponin, and the females as number (18) positive from patient in the test of troponin in Chi-square test the males were significant on the females and the P value was (P<0.01). In Azadi hospital in Kirkuk city of Iraq from 2008 to 2009, the percentage of thrombosis with gender, in the Males were more affected with thrombosis (34.2%) than females (13.4%), also with age show that the advanced age groups were more affected the disease

Table 1: Compare Between Patient and Control in Distribution of Test of Troponin

Group	Number	No. (%)	
		Ve +	Ve -
Patients	40	40 (100.0)	0 (0.00)
Healthy(Control)	10	0 (0.00)	10 (100.0)
Chi-square value (χ^2)	–	15.00 **	15.00 **

Note: ** (P<0.01).

Table 2: Distribution of Patients According to Age Group

Age group (year)	Number	Percentage (%)
Less than 50	8	20.00
50-60	18	45.00
More than 60	14	35.00
Total	40	100%
Chi-square value (χ^2)	–	9.041**

Note: ** (P<0.01).

Table 3: Distribution of Patients According to Gender

Age group (year)	Number	Percentage (%)
Male	22	55.00
Female	18	45.00
Total	40	100%
Chi-square value (χ^2)	–	4.529 *

Note: * (P<0.01).

Table 4: Relationship Between Age Group and Test of Troponin in Patients

Age group (year)	Number	No. (%)		Chi-square value (χ^2)
		Ve +	Ve -	
Less than 50	8	8 (100.0)	0 (0.00)	15.00 **
50-60	18	18 (100.0)	0 (0.00)	15.00 **
More than 60	14	14 (100.0)	0 (0.00)	15.00 **

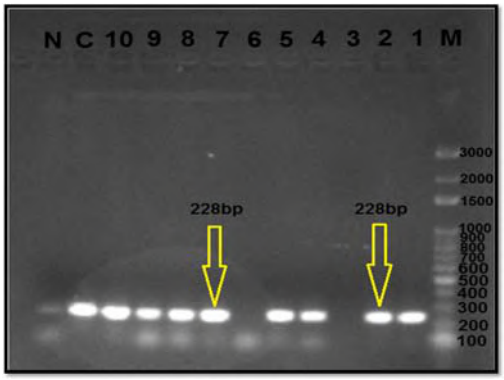
Note: * (P<0.01).

Table 5: Relationship Between Gender and Test of Troponin in Patients

Gender	Number	No. (%)		Chi-square value (χ^2)
		Ve +	Ve -	
Male	22	22 (100.0)	0 (0.00)	15.00 **
Female	18	18 (100.0)	0 (0.00)	15.00 **

Note: * (P<0.01).

Figure 1: Pcr Product For Fve3 Primer, Agarose Gel Electrophoresis (2% Agarose Gel, 10 Minutes At 100 Voltage And Then Lowered To 70 Volts, 80 Minutes). Visualized Under U.v Light After Staining Ethidium Bromide Line M: 100bp Marker, Line1-10: From Patients,Line C: From Control (Healthy) And Line N: From Negative Control



(above 65 years) (45%) than early age groups (Wafa and Lamia, 2011). Thrombosis disease are regarded as the most dangerous disease and their structure which prevent the blood supply to reach the heart that result to sudden failure in circulation, it registered Mortality of 7.6 million deaths caused by the disease in 2005, of all deaths in the world (Gregg *et al*., 2007).

Molecular Detection of Thrombosis by PCR Technique

Factor V gene

The factor V gene is located on the first chromosome (1q23). It is genomically related to the family of multicopper oxidases, and is homologous to coagulation factor VIII. The gene spans 70 kb, consists of 25 exons, and the resulting protein has a relative molecular mass of approximately 330 kDa (Huang and Koerper, 2008).

Indentification of nucleotide changes in 3, 4, 6, 7, 8, 12, 13,15, 16 and 25 exons performed in all samples with the primers used in the previously in the study.

The first primer set used in this PCR technique(Fve3) the amplifies in intron (2) of F5 gene from NCBI with product size(228bp)which is shown in the Figure 1.

Primer (Fve3) used in this study were previously designed to amplify the F5 gene, show the samples1, 2, 4, 5, 7, 8, 9 and 10 give band in PCR product (228bp) and show the sample 3 and 6 did not give any bands during PCR amplification because this primer has no attachment site on the target DNA.

The second primer set used in this PCR technique (Fve4) the amplifies in intron (3) and exon (4) of F5 gene from NCBI with product size (310bp)which is shown in the Figure 2.

Primer (Fve4) used in this study were previously designed to amplify the F5 gene, show the samples give band in PCR product (310bp).

The third primer set used in this PCR technique (Fve6) the amplifies in intron (5), exon (6) and intron (6) of F5 gene from NCBI with product size (547bp) which is shown in the Figure 3.

Primer (Fve6) used in this study were previously designed to amplify the F5 gene, show the samples give band in PCR product (547bp).

Figure 2: PCR Product For Fve4 Primer, Agarose Gel Electrophoresis (2% Agarose Gel, 10 Minutes At 100 Voltage And Then Lowered To 70 Volts, 80 Minutes). Visualized Under U.v Light After Staining Ethidium Bromide Line M: 100bp Marker,Line1-10: From Patients,Line C: From Control (Healthy) And Linen: From Negative Control

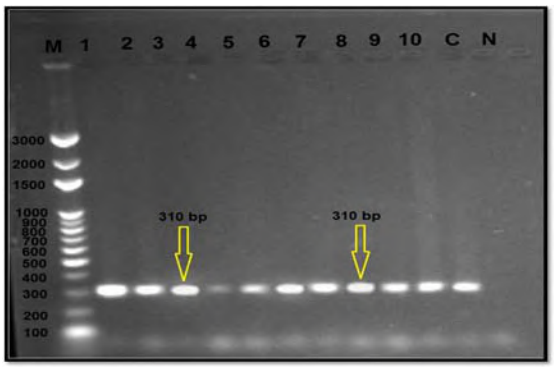
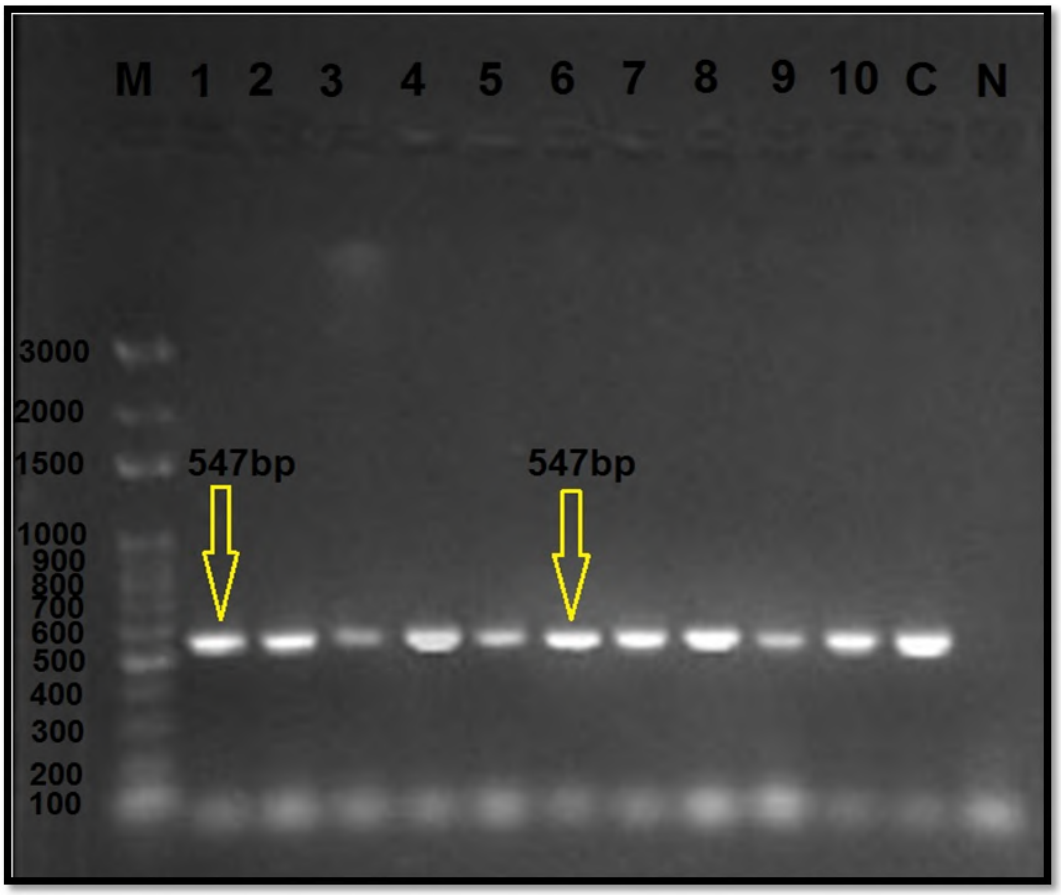


Figure 3: PCR Product For Fve6 Primer, Agarose Gel Electrophoresis (2% Agarose Gel, 10 Minutes At 100 Voltage And Then Lowered To 70 Volts, 80 Minutes). Visualized Under U.v Light After Staining Ethidium Bromide Line M: 100bp Marker,Line1-10: From Patients,Line C: From Control (Healthy) And Linen: From Negative Control



The fourth primer set used in this PCR technique (Fve7) the amplifies in intron (6), exon (7) and intron (7) of F5 gene from NCBI with product size (241bp) which is shown in the Figure 4.

Primer (Fve7) used in this study were previously designed to amplify the F5 gene, show the samples give band in PCR product (241bp).

The fifth primer set used in this PCR technique (Fve8) the amplifies in intron (7), exon (8) and intron (8) of F5 gene from NCBI with product size (306bp) which is shown in the Figure 5.

Primer (Fve8) used in this study were previously designed to amplify the F5 gene, show the samples give band in PCR product (306bp).

The sixth primer set used in this PCR technique (Fve12) the amplifies in intron (11) and exon (12) of F5 gene from NCBI with product size (286bp) which is shown in the Figure 6.

Primer (Fve12) used in this study were previously designed to amplify the F5 gene, show the samples give band in PCR product (286bp).

The seventh primer set used in this PCR technique (Fve13a) the amplifies in intron (12)

Figure 4: PCR Product For Fve7 Primer, Agarose Gel Electrophoresis (2% Agarose Gel, 10 Minutes At 100 Voltage And Then Lowered To 70 Volts, 80 Minutes). Visualized Under U.V Light After Staining Ethidium Bromide Line M: 100bp Marker,Line1-10: From Patients, Line C: From Control (Healthy) And Linen: From Negative Control.

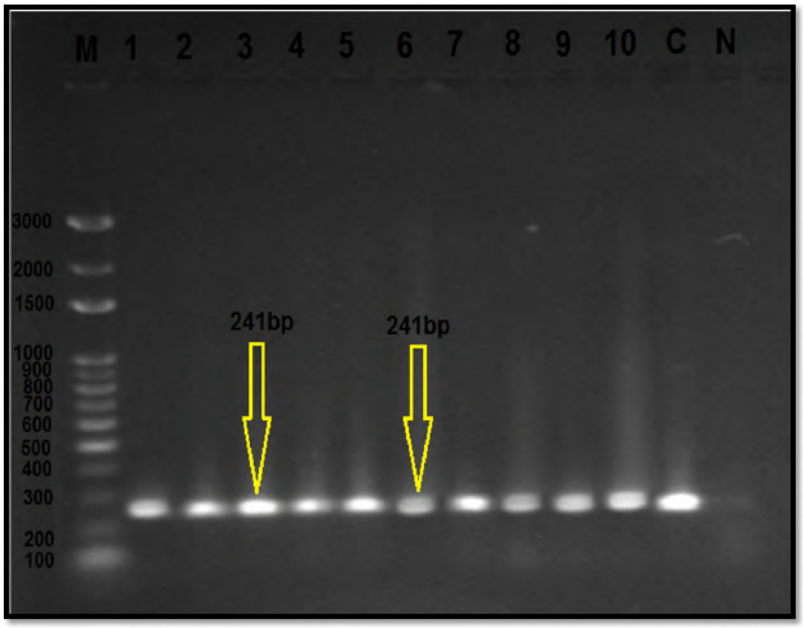


Figure 5: PCR Product For Fve8 Primer, Agarose Gel Electrophoresis (2% Agarose Gel, 10 Minutes At 100 Voltage And Then Lowered To 70 Volts, 80 Minutes). Visualized Under U.V Light After Staining Ethidium Bromide Line M: 100bp Marker,Line1-10: From Patients, Line C: From Control (Healthy) Andlinen: From Negative Control

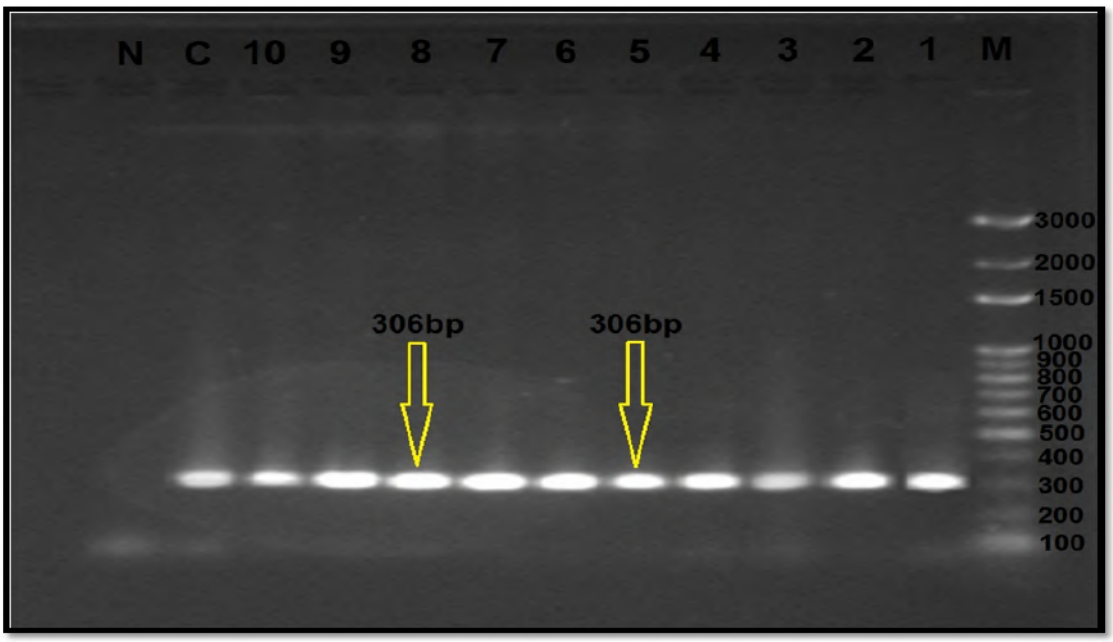
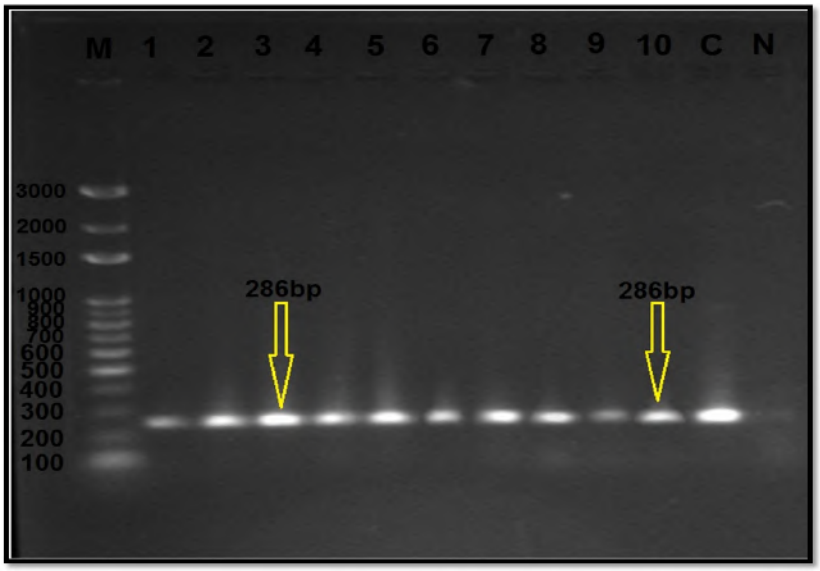


Figure 6: PCR Product for Fve12 Primer, Agarose Gel Electrophoresis (2% Agarose Gel, 10 Minutes At 100 Voltage And Then Lowered To 70 Volts, 80 Minutes). Visualized Under U.V Light After Staining Ethidium Bromide Line M: 100bp Marker,Line1-10: From Patients, Line C: From Control (Healthy) Andlinen: From Negative Control

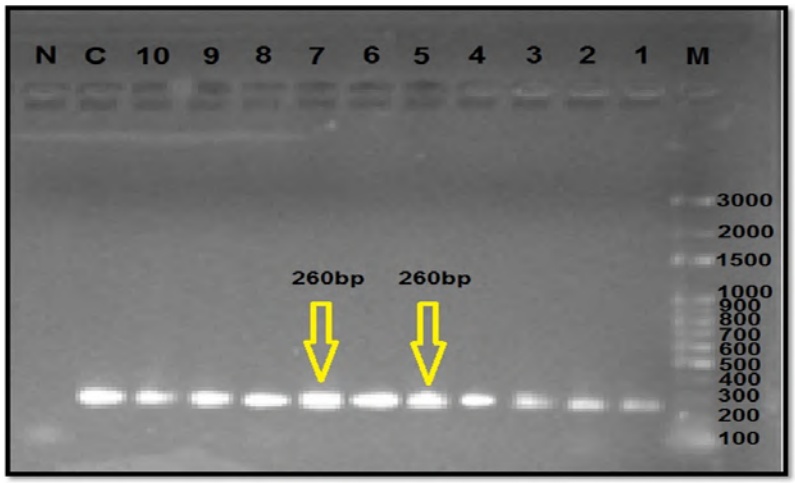


and exon (13)of F5gene from NCBI with product length (260bp) which is shown in the Figure 7.

Primer (Fve13a) used in this study were previously design to amplify the F5 gene, show the samples give the band in PCR product (260bp).

The eighth primer set used in PCR technique (Fve13c) the amplifies in exon (13) and intron (13) of F5 gene from NCBI with product size (317bp) which is shown in the Figure 8.

Figure 7: PCR Product for Fve13a Primer, Agarose Gel Electrophoresis (2% Agarose Gel, 10 Minutes At 100 Voltage And Then Lowered To 70 Volts, 80 Minutes). Visualized Under U.V Light After Staining Ethidium Bromide Line M: 100bp Marker,Line1-10: From Patients, Line C: From Control (Healthy) Andlinen: From Negative Control



Primer Fve13c used in this study were previously design to amplified the F5 gene show the samples give the band in PCR product (317bp).

The ninth primer set used in PCR technique (Fve15) the amplifies in intron (14), exon (15) and intron (15) of F5 gene from NCBI with product size (600bp) which is shown in the Figure 9.

Primer Fve15 used in this study were previously design to amplified the F5 gene show the samples give the band in PCR product (600bp).

Figure 8: PCR Product for Fve13c Primer, Agarose Gel Electrophoresis (2% Agarose Gel, 10 Minutes At 100 Voltage And Then Lowered To 70 Volts, 80 Minutes). Visualized Under U.V Light After Staining Ethidium Bromide Line M: 100bp Marker,Line1-10: From Patients, Line C: From Control (Healthy)Andlinen: From Negative Control

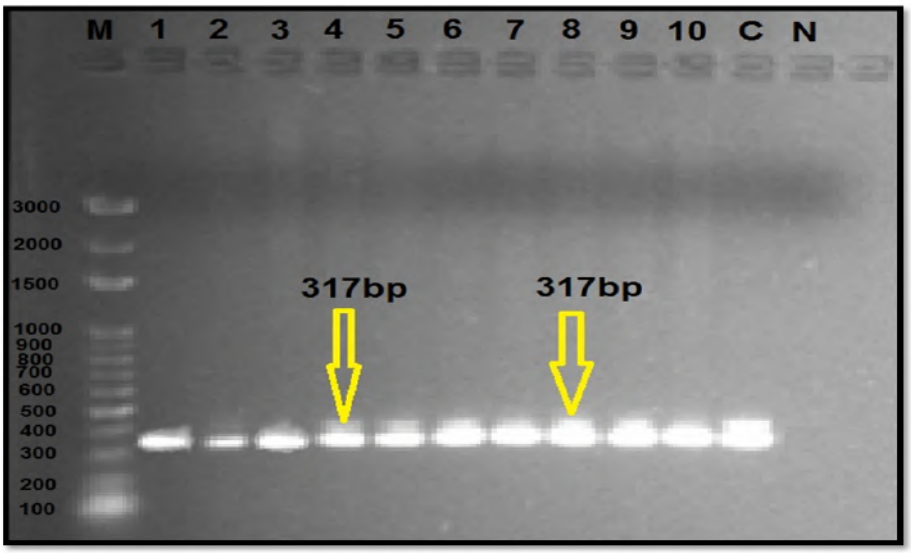
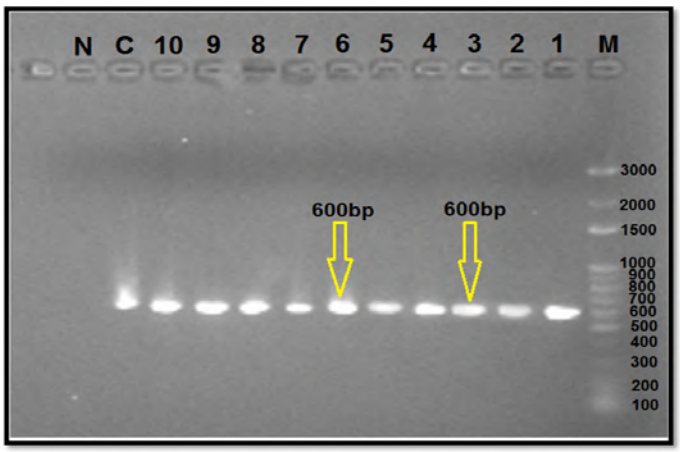


Figure 9: PCR Product for Fve15 Primer, Agarose Gel Electrophoresis (2% Agarose Gel, 10 Minutes At 100 Voltage And Then Lowered To 70 Volts, 80 Minutes). Visualized Under U.V Light After Staining Ethidium Bromide Line M: 100bp Marker,Line1-10: From Patients, Line C: From Control (Healthy)Andlinen: From Negative Control



The tenth primer set used in PCR technique (Fve16) the amplifies in intron (15), exon(16) and intron (16) of F5gene from NCBI Primer design with product size (333bp) which is shown in the Figure 10.

Primer Fve16 used in this study were previously design to amplified the F5 gene show the samples give the band in PCR product (333bp).

The eleventh primer set used in PCR technique (Fve25) the amplifies in intron (24) and exon (25) of F5 gene from NCBI Primer design with product size (390bp) which is shown in the Figure 11.

Primer Fve25 used in this study were previously design to amplified the F5 gene show all samples give the band in PCR product (390bp).The F5 gene is located on the1q23 chromosome. the gene spans 70 kb, consists of

Figure 10: PCR Product for Fve16 Primer, Agarose Gel Electrophoresis (2% Agarose Gel, 10 Minutes At 100 Voltage And Then Lowered To 70 Volts, 80 Minutes). Visualized Under U.V Light After Staining Ethidium Bromide Line M: 100bp Marker,Line1-10: From Patients, Line C: From Control (Healthy)Andlinen: From Negative Control

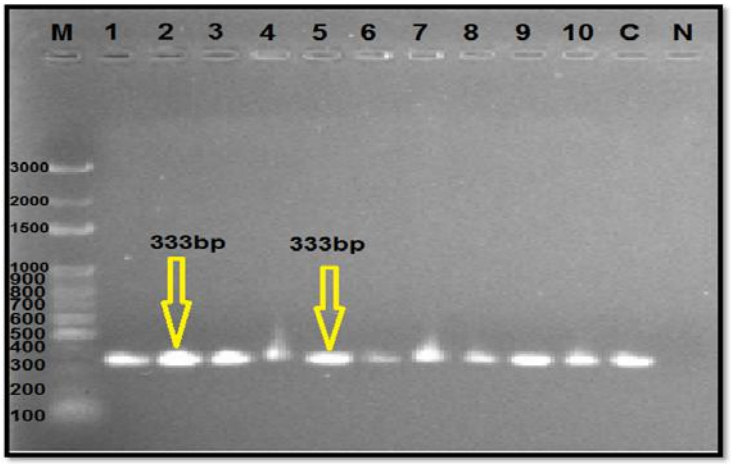
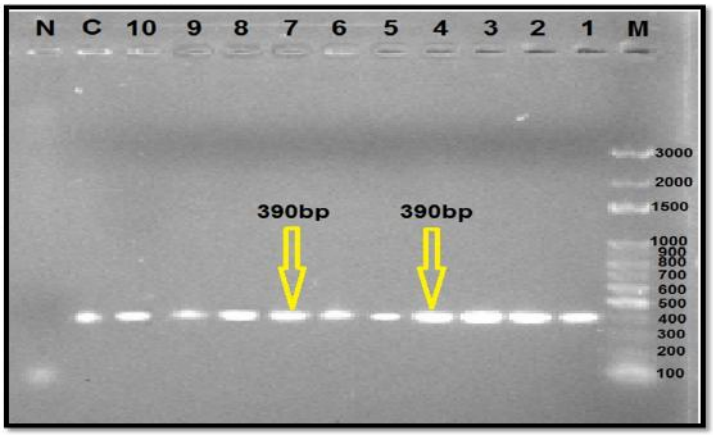


Figure 11: PCR Product for Fve25 Primer, Agarose Gel Electrophoresis (2% Agarose Gel, 10 Minutes At 100 Voltage And Then Lowered To 70 Volts, 80 Minutes). Visualized Under U.v Light After Staining Ethidium Bromide Line M: 100bp Marker,Line1-10: From Patients, Line C: From Control (Healthy)And Line N: From Negative Control



25 exons, and the resulting protein has a relative molecular mass of approximately 330 kDa , the heterozygous genotypes were identified for factor V Leiden mutation in exons of F5 gene (Press *et al*., 2002). Coagulation factor V is a critical factor of the blood coagulation cascade, this factor circulates in plasma, and is converted to the active form by the release of the activation peptide by thrombin during coagulation (Greer, 2003) according to large size of the F5 gene, many mutations have been identified in the human population, However not all of them are associated with thrombophilia (Yamakage *et al*., 2006).

Coagulation factors are a group of related proteins that make up the coagulation system, a series of chemical reactions that form blood clots. After an injury, clots seal off blood vessels to stop bleeding and trigger blood vessel repair. The F5 gene provides instructions for making a protein called coagulation factor V (Asselta *et al*., 2006).

The protein circulates in the bloodstream in an inactive form until the coagulation system is activated by an injury that damages blood vessels.when coagulation factor V is activated, it interacts with coagulation factor X. the active forms of these two coagulation factors (written as factor Va and factor Xa, respectively) form a complex that converts an important coagulation protein called prothrombin to its active form, thrombin. Thrombin then converts a protein called fibrinogen into fibrin, which is the material that forms the clot, the factor V protein is made primarily by cells in the liver (Asselta and Peyvandi, 2009).

Molecular Analysis of F5 Gene

Direct sequencing of the F5 gene from the all patients in Iraqi populations using primer Fve12, Fve13a, Fve13c, Fve16 and Fve25.

Type of Mutations

The PCR products of thrombosis patients were screened by sequencing. The result was directly compared with control (NCBI nucleotide blast) and Mega 6 program. The current utilized forwored primer for sequencing the F5 gene in 30 patients compared with control NCBI nucleotide blast.

In this study, we evaluated the presence mutations in several main exons of F5 gene in individuals which due to thrombosis.

The First DNA sequence of the F5 gene located exon 12 from patient and NCBI nucleotide blast, show the Figure (12A, B and C).

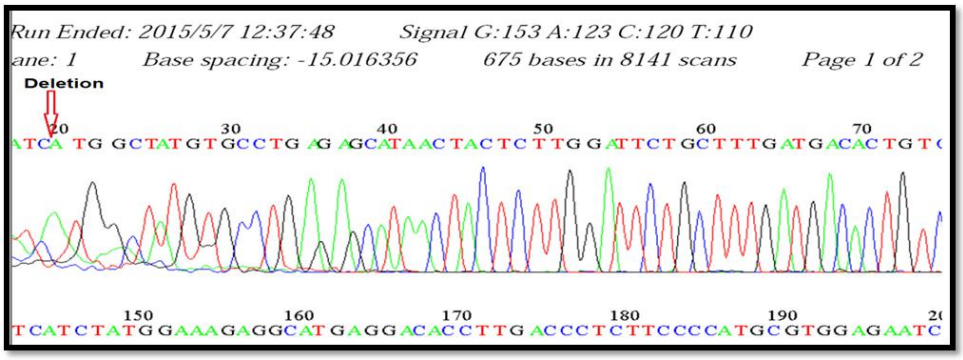
A sample of primer (Fve12) showed a deletion in nitrogen base 47028-47030/CAA , in exon (12) that caused deletion in amino acid Pro/ del (Zammiti *et al*., 2006).

Figure 12A: The Automated Sequencing of F5 Gene Display A Deletion In(47029/ A) Base Pair In Exon(12) Of The F5 Gene When Comparing In The Ncbi Nucleotide Blast

Figure 12B: Comparing Between Control (NCBI nucleotideblast) and Thrombosis Patient in the MEGA 6 Program

Figure 12C: A Chromatogram for Sample Display a Sequence and the Deletion Region

TC-ATGGCTATGTGCCTGAGAGCATAACTACTCTTGGATTCTGCTTTGAT
GACACTGT



Deletion of a number of pairs that is not evenly divisible by three will lead to a frameshift mutation, causing all of the codons occurring after the deletion tobe read incorrectly during translation, producing severely altered and potentially nonfunctional protein. In contrast, a deletion that is evenly divisible by three is called an in-frame deletion (Ren, 2005).

The Second DNA sequence of the F5 gene located exon 13 from patient and NCBI nucleotide blast, show the Figure (13A, B and C).

A sample of prime (Fve13a) showed a deletion in nitrogen base 48418-48420/CAA, in exon (13) that caused deletion in amino acid Ser/del. DNA sequence analysis revealed causative mutations in heterozygous form base deletion in exon (13) (Montefusco *et al.*, 2000). A mutation in which a part of a chromosome or a sequence of DNA is lost during DNA replication. Any number of nucleotides can be deleted, from a single base to an entire piece of chromosome (Lewis, 2004).

The Thrird DNA sequence of the F5 gene located exon 13 from patient and NCBI nucleotide blast, show the Figure (14A, B and C).

In the sample (I) of primer (Fve13c) was show a SNP as substitution A/C in nitrogen base 51148-51150/CAA, in exon (13) that caused a Missense

Figure 13A: The Automated Sequencing of F5 Gene Display A Deletion In (48419/ A) Base Pair in Exon (13) Of The F5 Gene When Comparing in the NCBI Nucleotide Blast

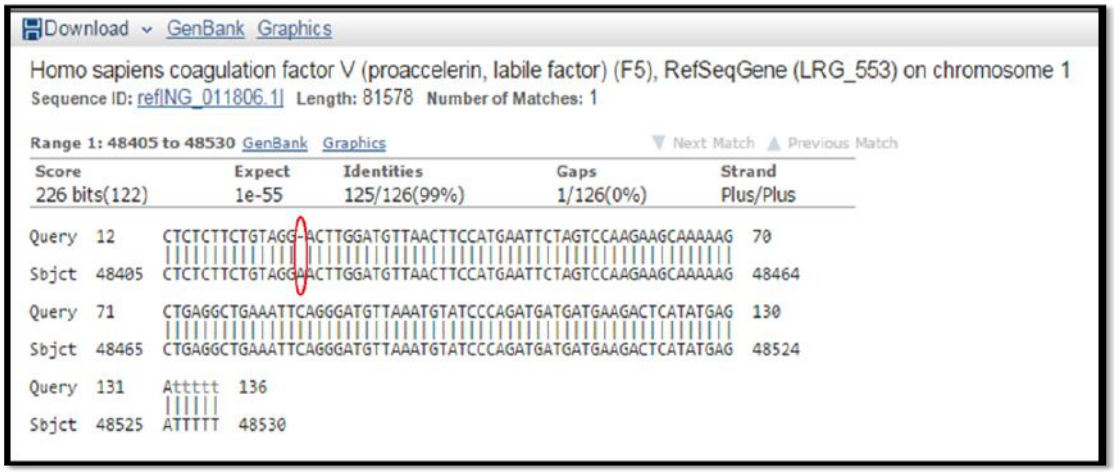


Figure 13B: Comparing Between Control (NCBI nucleotideblast) and Thrombosis Patient in the MEGA 6 Program

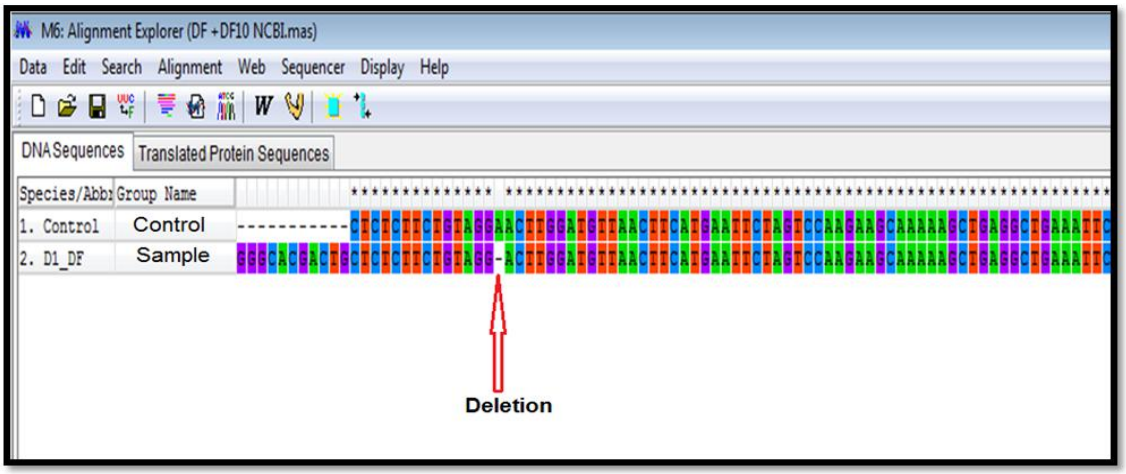


Figure 13C: A Chromatogram For Sample Thrombosis Patient Display a Sequence and the Deletion Region

GG-ACTTGGATGTTAACTTCCATG

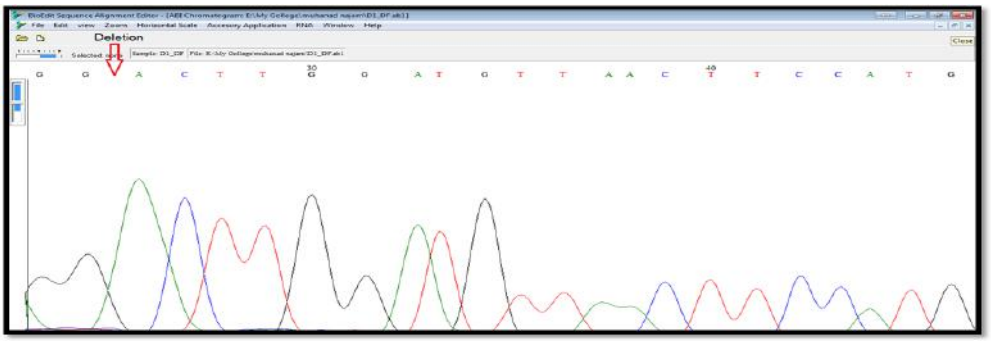


Figure 14A: The Automated Sequencing Of F5 Gene Display A Substitution in (51168/C/A), Isertion in (51176/T) and Substitution in(51196/G/C) Base Pair in Exon (13) of the F5 Gene When Comparing In The Ncbi Nucleotide Blast

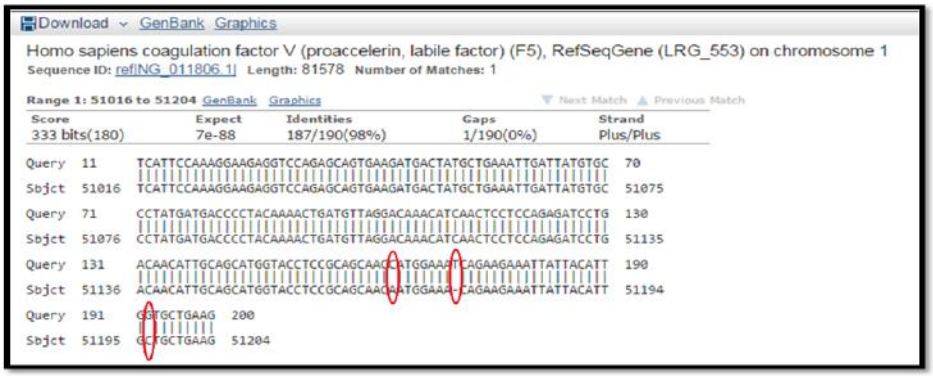


Figure 14B: Comparing Between Control (NCBI Nucleotideblast) and Thrombosis Patient in the Mega 6 Program

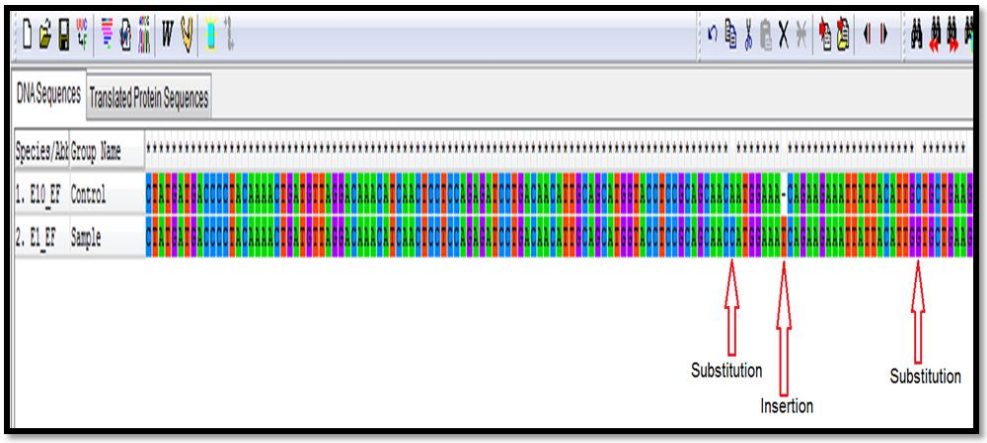
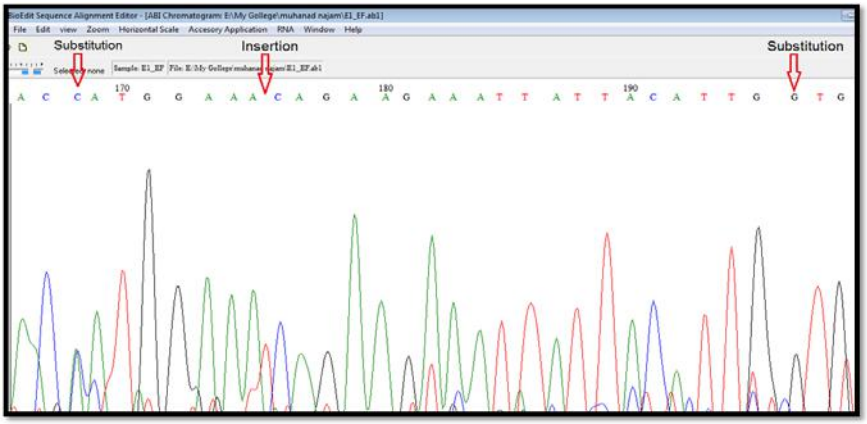


Figure 14C: A Chromatogram for Sample Thrombosis Patient Display a Sequence and the Substitution and Insertion Regions

ACCATGGAAATCAGAAGAAATTATTACATTGGTG



mutation because its coded for amino acid Gln/ Pro (Castoldi *et al*., 2000).

A sample (II) of prime (Fve13c) showed insertion (T) in nitrogen base 51157 that caused insertion in amino acid His /Ser (Kling *et al*., 2006),and sample (III) of prime (Fve13c) was show a SNP as substitution C/Gin nitrogen base 51195-51197/GCT, in exon (13) that caused a Missense mutation because its coded for amino acid Ala/Gly (Huang *et al*., 2010).

The Fourth DNA sequence of the F5 gene in located exon16 and intron16 from patient and NCBI nucleotide blast, show the Figure (1-15A, B and C).

Figure 15A: The Automated Sequencing of F5 Gene Display a Substitution in (61795/G/A) Base Pair in Exon (16) and Substitution In (61936/G/A) Base Pair in Intron (16) of the F5 Gene When Comparing In The Ncbi Nucleotide Blast

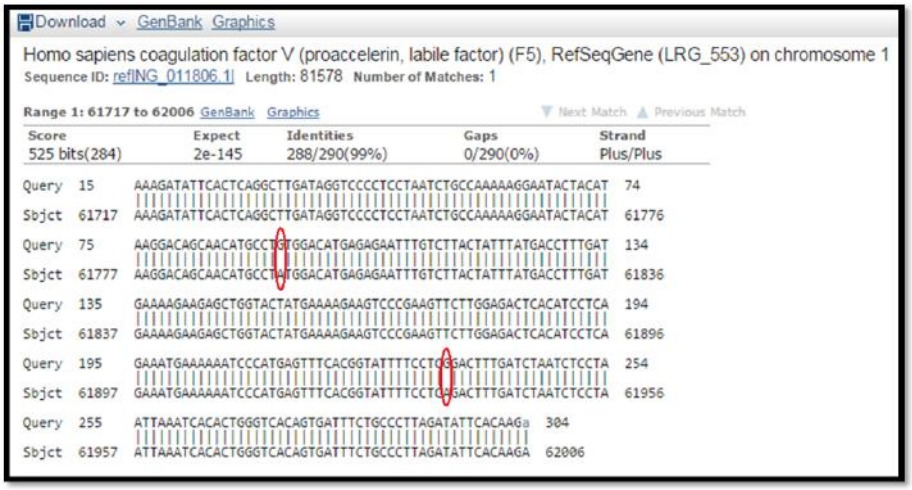
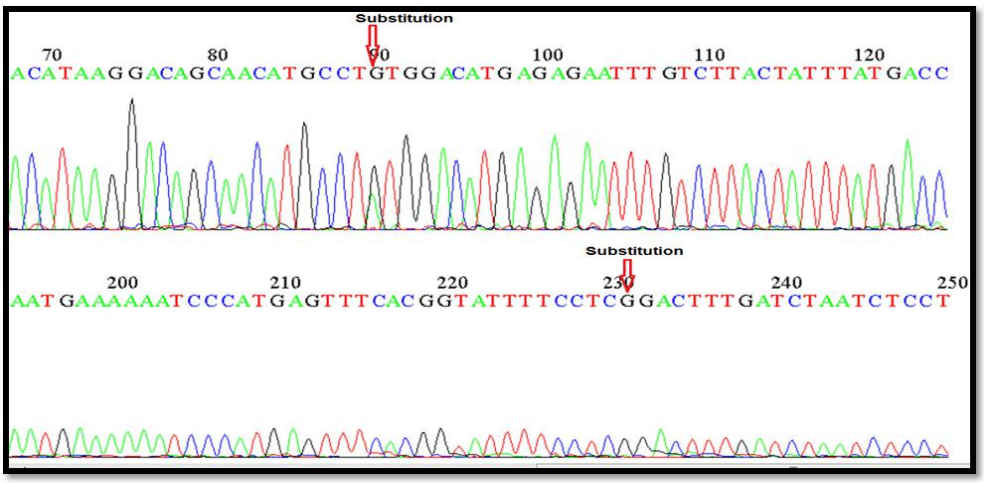


Figure 15B: Comparing Between Control (NCBI nucleotide blast) and Thrombosis Patient in the MEGA 6 Program

Figure 15C: A Chromatogram for Sample Thrombosis Patient Display a Sequence and the Substitution Regions

ACATAAGGACAGCAACATGCCTGTGGACATGAGAGAATTTGTCTTAC
TATTTATGACC
AATGAAAAAATCCCATGAGTTTCACGGTATTTTCCTCGGACTTTGATCTAATCTCCT



In the sample (I) of primer (Fve16) was show a SNP as substitution A / G in nitrogen base61794-61796/TAT, in exon (16) that caused a Missense mutation because its coded for amino acid Tyr / Cys (Lunghi *et al* ., 2008).

Factor V Leiden mutation is a point mutation in factor V gene which is obtained by the substitution of adenine for guanine at nucleotide position 1691, were homozygous for 48571 A>G missense mutation in 16 exon (Ali Nazemi *et al*., 2013). Interest in the genetic basis of thrombosis was accelerated with the discovery of the Factor V Leiden (FVL) mutation, which is considered the most common genetic risk factor (Rosendaal, 1999).

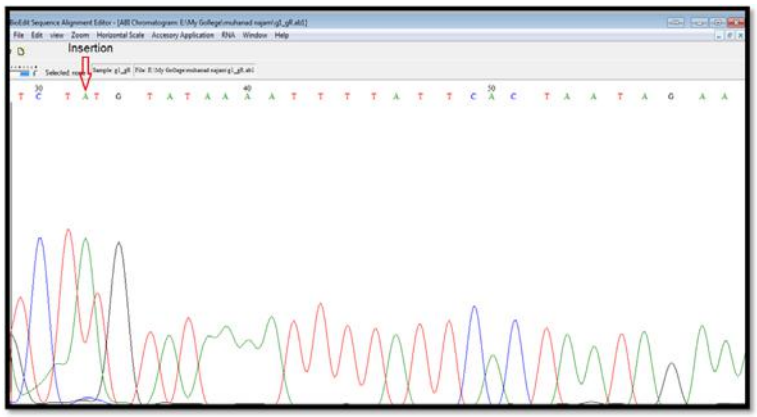
The Fifth DNA sequence of the F5 gene inlocated exon25 from patient and NCBI nucleotide blast, show the Figure (16A, B and C).

Figure 16A: The Automated Sequencing Of F5 Gene Display Insertion In (77362 / A) Base Pair in Exon 25 Of The F5 Gene When Comparing In The Ncbi Nucleotide Blast

Figure 16B: Comparing Between Control (Ncbi Nucleotideblast) and Thrombosis Patient In The MEGA 6 Program

Figure 16C: A Chromatogram for Sample Thrombosis Patient Display a Sequence and the Insertion Region

TCTATGTATAAAATTTTATTCACTAATAGAA



A sample (I) of prime (Fve 25) showed insertion (A) in nitrogen base 77353 that caused insertion in amino acid Cys /Met (Shinozawa *et al*., 2007).

Different mutation of one or more than located gene region. However point mutation, substitution, insertion and deletion affected the F5 gene in Iraqi patients as show in Table 6.

Different genetic variants within a species are referred to as alleles, and so a new mutation is said to create a new allele. Each allele is characterized by a selection coefficient, which measures the expected change in an allele's frequency over time (Wielgoss *et al*., 2011).

The direct sequencing of the of F5 gene located in exon 12, exon 13 ,exon 16, intron 16 and exon 25 change the position in 21, 26, 163, 171, 192, 89 and 44 these different type mutation caused the factor V gene. This result agree with those of (Montefusco *et al*., 2000; Shinozawa *et al*., 2007 and Ali Nazemi *et al*., 2013).

Table 6: Point Mutations Detect in Patient Samples						
Name of Primers	Wild type	Mutant type	Change in Amino Acids	Site of NA	Type of Mutation	Effect on Translation
(Fve12)	CAA	-AT	Pro_Deletion	21	Deletion	Frame shift
(Fve13a)	GAA	-AC	Ser_Deletion	26	Deletion	Frame shift
(Fve13C)	CAA	CCA	Gln- Pro	163	Substitution	Missense
	C AC	TCA	His- Ser	171	Insertion	Frame shift
	GCT	GGT	Ala- Gly	192	Substitution	Missense
(Fve16)	TAT	TGT	Tyr- Cys	89	Substitution	Missense
(Fve25)	TGT	ATG	Cys- Met	44	Insertion	Frame shift

Percentage of Mutations

The rate at which various types of mutations occur over time. mutation rates are typically given for a specific class of mutation, for instance point mutations, small or large scale insertions or deletions. The rate of substitutions can be further subdivided into a mutation spectrum which describes the influence of genetic context on the mutation rate (Ossowski *et al*., 2010).

Analysis of F5 gene by sequencing for Iraqi patients exhibited the existence of many genetic variation. three types of mutations namely deletion , insertion and substitution were present. percentage of mutation types that showed 25% for deletion , 37.5% for substitution and 25% for insertion. as show in Table 7.

Table 7: Percentage of Mutation Type	
Type of Mutation	Percentage%
Substitution	42.86%
Insertion	28.57%
Deletion	28.57%

Effect of Mutations

Mutation can result in several different types of change in sequences of F5 gene.point mutations typically refer to alterations of single base pairs of DNA or to a small number of adjacent base pairs. in this section, we shall consider the effect of such changes at the phenotypic level. Point mutations are classified in molecular, which shows the main types of DNA changes and their functional effects at the protein level (Freeman and Company, 2000).

Table 8 shows that the substitution mutation was a missense (42.86%) causing impact on phenotype that leads to replacement in amino acid, the deletion and insertion mutation lead to frame shift which represented 57.14% usually introduces premature stop codons in addition to lots of amino acid changes in this study. These mutations result in a completely different translation in F5 gene.

Table 8: Percentage of Effect of Mutation	
Effect of Mutation	Percentage%
Frame shift	57.14%
Missense	42.86%

If a mutation changes a protein produced by a gene, the result is likely to be harmful, with an estimated 70% of amino acid polymorphisms that

have damaging effects, and the remainder being either neutral or weakly beneficial (Sawyer *et al*., 2007).

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