Global Proceedings Repository
American Research Foundation
ISSN 2476-017X
Available online at http://proceedings.sriweb.org
شبكة المؤتمرات العربية
http://arab.kmshare.net/

The Ninth International Scientific Academic Conference

Under the Title "Contemporary trends in social, human, and natural sciences"

المؤتمر العلمي الاكاديمي الدولي التاسع

تحت عنوان "الاتجاهات المعاصرة في العلوم الاجتماعية، الانسانية، والطبيعية"

17 - 18 يوليو – تموز 2018 - اسطنبول – تركيا

http://kmshare.net/isac2018/

Identification of factor V Leiden mutation in patients with thrombosis in Iraq

Rehab S. Ramadhan* and Muhanad N. Abdullah**

* College of Biotechnology, Al-Nahrain University, Baghdad-Iraq, email: rehabrebah2004@yahoo.com
Corresponding author
**= College of Biotechnology, Al-Nahrain University, Baghdad-Iraq, email: m_n_biotech@yahoo.com

Abstract: This study aimed to investigate the association of factor V gene (F5) single nucleotide polymorphism (SNP) with the incidence of thrombosis related to the Leiden in Iraqi patients. Blood samples were collected from one hundred patients attending (AL Yarmook Teaching Hospital and AL Kadhimiya Teaching Hospital) Baghdad / Iraq, as well as from 100 healthy subjects served as control group. Serum samples were analyzed using troponin test (TNT) for detection of thrombosis. This study found that age group 50 to 60 years old showed thrombosis 45% more than younger once, and the thrombosis was more frequent in males 55% than females 45% (P<0.01). Polymerase chain reaction showed that 28 pathogenic SNPs in DNA led to Leiden mutation identified among patients as missense and additional open reading frame in mutated sequence.

Keywords: Thrombosis, F5 gene, Leiden mutation, PCR

Global Proceedings Repository
American Research Foundation
ISSN 2476-017X
Available online at http://proceedings.sriweb.org
شبكة المؤتمرات العربية
http://arab.kmshare.net/

Introduction

A thrombus clot of blood and product of the blood coagulation step in hemostasis. The components that lead to a thrombus are aggregated platelets that form a plug, and a net work of cross-linked fibrin protein. A thrombus is a response to injury to stop bleeding, but can be harmful in thrombosis, when blocks blood flow through healthy blood vessels (Saladin and Kenneth, 2012). The factor V gene is located on chromosome (1), the gene extends 70 kb, consists of 25 exons, and the translation protein has a molecular mass of approximately 330 kDa .Patients with multiple genetic defects in the hemostaticthrombotic system may have an increased risk of thrombosis compared with patients with single defects. Several studies demonstrated a frequent association of the prothrombin G20210A mutation and the factor V Leiden mutation in patients with thrombophilia (Neil *et al*., 2000). The high allele frequency of the mutated factor V gene, homozygous carriers will not be extremely rare as in other types of hereditary thrombophilia. It has been estimated the risk of thrombosis and the clinical features of patients who were homozygous for factor V Leiden (Rosendaal *et al.*, 1995).

Purpose of the study presented in this article is to identify type and location of the genetic change that led to thrombosis in Iraqi patients and Leiden mutation in F5 gene.

Materials and Methods

Study and control population: The study included one hundred blood samples from patients suffering thrombosis during the period November 2015 to January 2016, collected from AL Kadhimiya Teaching Hospital and AL Yarmouk Teaching Hospital (Baghdad / Iraq). The average ages of patients and control group were 30-80 years.

Troponin test (TNI) was performed according to (Jahangir, 2010).

DNA Extraction: Total cellular DNA was extracted from blood samples by using the Reliaprep Blood genomic DNA MiniPrep System from Favorgene Taiwan, as instructed by the manufacture.

PCR Protocols: Extracted DNA from blood samples and healthy was used in PCR for amplification of F5 gene as shown in table (1) Initial denaturation 94^{o}C for 5 min., 35 cycle of denaturation 94^{o}C for 1 min, Annealing as given in table (1) for 1 min, extension 72^{o}C for 1 min., and final extension 72^{o}C for 10 min.

Global Proceedings Repository
American Research Foundation
ISSN 2476-017X
Available online at http://proceedings.sriweb.org
شبكة المؤتمرات العربية
http://arab.kmshare.net/

Table (1): Primers (Van *et al*., 2001).

Primer name	Sequence 5′ - 3′	Annealing Temperate C^0
(T1)	**F:** ATAGTGGGCCTCAGTAAAG **R:** TTTTTTCAGCAGTAATGG	**50**
(T2)	**F:**AGCCATTTATGTTGTCATTAAAG **R:**TAATAGCCATTATCTTACTTACTG	**52**

The products from PCR amplification were sent for sequencing by Macrogen Company (Korea) and data obtained were analyzed using (Mega – 7) software and compared to their similar at the National Center for Biotechnology Information (NCBI).

Statistical Analysis: The Statistical Analysis System- SAS (2012) program was used to determine the association of thrombosis to age group and gender using chi square test.

Result and discussion

In this study, one hundred samples have been collected from patient diagnosed with thrombosis and used to determine the relation of age and gender in the manifestation of thrombosis using chi- square test. Results are shown in Table (2 and 3).

Table (2): Distribution of patients according to age group.

Age group (year)	Number of patient	Percentage (%)
Less than 50	28	20.00
50-60	38	45.00
More than 60	34	35.00
Total	100	100%
Chi-square value (χ^2)	15.00^{**}	9.041 **
** (P<0.01).		

Table (3): Distribution of patients according to gender.

Gender	Number of patient	Percentage (%)
Male	52	55.00 *
Female	48	45.00
Total	100	100%

Global Proceedings Repository
American Research Foundation
ISSN 2476-017X
Available online at http://proceedings.sriweb.org
شبكة المؤتمرات العربية
http://arab.kmshare.net/

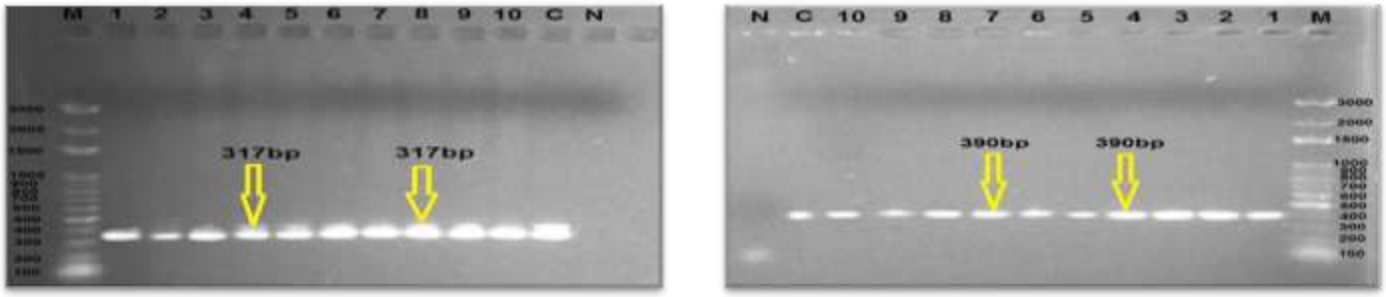
Chi-square value (χ^2)	15.00**	4.529 *
* (P<0.05). ** (P<0.01).		

Ages between 50-60 years showed higher thrombosis percentage especially in male over female in Iraqi patients. In arterial thrombosis at young age, traditional risk factors such as gender , smoking, diabetes mellitus, obesity and hypercholesterolemia might interfere the cardiovascular risk that may cause high blood pressure, arterial blood vessel block which eventually lead to thrombosis.

Molecular Detection of Thrombosis by PCR Technique

Factor V gene

Identification of nucleotide changes in, 16 and 25 exons performed in all samples and results are shown in figure 1.



(A) (B)

Figure (1): Gel electrophoresis for PCR products, A- using T1 primer, B- using T2 primer, line M: 100bp marker, line1-10: patients, line C: control (healthy) and line N: negative control.

The coagulation factors represent a group of similar proteins that construct the protective coagulation system, multiple chemical reactions that form blood clots. When injury, clots seal off blood vessels to prevent bleeding and start blood vessel repair. The F5 gene controls a protein called coagulation factor V (Asselta *et al.*, 2006). The protein is inactive in the blood stream till the coagulation system is active by an injury to the blood vessels. When coagulation factor V is active, it combines with factor X, and the active forms of these two coagulation factors make a complex that affect coagulation protein called prothrombin to its active form, thrombin. Thrombin affects the fibrinogen to become fibrin, which is the substance that forms the clot (Asselta and Peyvandi, 2009).

Molecular Analysis of F5 Gene

Global Proceedings Repository
American Research Foundation
ISSN 2476-017X
Available online at http://proceedings.sriweb.org
شبكة المؤتمرات العربية
http://arab.kmshare.net/

DNA sequencing of the F5 gene product from the all Iraqi patients using T1, and T2, primers were compared with control group and NCBI. Types of mutations in F5 gene are shown in table 4.

Global Proceedings Repository
American Research Foundation
ISSN 2476-017X
Available online at http://proceedings.sriweb.org
شبكة المؤتمرات العربية
http://arab.kmshare.net/

Table (4): Single nucleotide changes dected in Patients DNA.

Variation*Location*		**Gene(s)**	**Condition(s)**	**Clinical significance (Last reviewed)**
	NM_000130.4(F5):c.5037dup (p.Ser1680Ilefs) *GRCh37:*Chr1:169500195 *GRCh38:* Chr1:169530957	F5	not provided	Pathogenic (Oct 23, 2015)
	NM_000130.4(F5):c.1601G= (p.Arg534=) *GRCh37:* Chr1:169519049 *GRCh38:* Chr1:169549811	F5	not provided, hormonal contraceptives for systemic use response - Toxicity/ADR	drug response
	NM_000130.4(F5):c.5668G>A (p.Glu1890Lys) *GRCh37:* Chr1:169495187 *GRCh38:* Chr1:169525949	F5	not provided	Pathogenic
	NM_000130.4(F5):c.5392G>A	F5	not provided	Pathogenic

Global Proceedings Repository
American Research Foundation
ISSN 2476-017X
Available online at http://proceedings.sriweb.org
شبكة المؤتمرات العربية
http://arab.kmshare.net/

Variation*Location*	**Gene(s)**	**Condition(s)**	**Clinical significance (Last reviewed)**
(p.Glu1798Lys) *GRCh37:* Chr1:169498873 *GRCh38:* Chr1:169529635			
NM_000130.4(F5):c.3187dupA (p.Arg1063Lysfs) *GRCh37:* Chr1:169511142 *GRCh38:* Chr1:169541904	F5	not provided	Pathogenic
NM_000130.4(F5):c.1160T>C (p.Ile387Thr) *GRCh37:* Chr1:169521931 *GRCh38:* Chr1:169552693	F5	Thrombophilia due to activated protein C resistance	Pathogenic (May 1, 2004)
NM_000130.4(F5):c.6304C>T (p.Arg2102Cys)	F5	Factor V deficiency	Pathogenic (Jan 1, 2003)

Global Proceedings Repository
American Research Foundation
ISSN 2476-017X
Available online at http://proceedings.sriweb.org
شبكة المؤتمرات العربية
http://arab.kmshare.net/

Variation*Location*	**Gene(s)**	**Condition(s)**	**Clinical significance (Last reviewed)**
GRCh37: Chr1:169487691 *GRCh38:* Chr1:169518453			
NM_000130.4(F5):c.3481C>T (p.Arg1161Ter) *GRCh37:* Chr1:169510847 *GRCh38:* Chr1:169541609	F5	Factor V deficiency	Pathogenic (Sep 1, 2001)
NM_000130.4(F5):c.2401C>T (p.Gln801Ter) *GRCh37:* Chr1:169511927 *GRCh38:* Chr1:169542689	F5	Factor V deficiency	Pathogenic (Jul 15, 2001)
NM_000130.4(F5):c.5189A>G (p.Tyr1730Cys) *GRCh37:* Chr1:169500043	F5	Factor V deficiency	Pathogenic (Jul 15, 2001)

Global Proceedings Repository
American Research Foundation
ISSN 2476-017X
Available online at http://proceedings.sriweb.org
شبكة المؤتمرات العربية
http://arab.kmshare.net/

Variation*Location*	**Gene(s)**	**Condition(s)**	**Clinical significance (Last reviewed)**
GRCh38: Chr1:169530805			
NM_000130.4(F5):c.439G>T (p.Glu147Ter) *GRCh37:* Chr1:169529939 *GRCh38:* Chr1:169560701	F5	Thrombophilia due to activated protein C resistance	Pathogenic (Nov 1, 2003)
NM_000130.4(F5):c.1001G>C (p.Arg334Thr) *GRCh37:* Chr1:169524537 *GRCh38:* Chr1:169555299	F5	Thrombophilia due to activated protein C resistance	Pathogenic (Feb 15, 1998)
NM_000130.4(F5):c.1000A>G (p.Arg334Gly) *GRCh37:* Chr1:169524538 *GRCh38:* Chr1:169555300	F5	Factor V Hong Kong	Pathogenic (Oct 1, 1998)

Global Proceedings Repository
American Research Foundation
ISSN 2476-017X
Available online at http://proceedings.sriweb.org
شبكة المؤتمرات العربية
http://arab.kmshare.net/

Variation*Location*		Gene(s)	Condition(s)	Clinical significance (Last reviewed)
	NM_000130.4(F5):c.1601G>A (p.Arg534Gln) *GRCh37:* Chr1:169519049 *GRCh38:* Chr1:169549811	F5	Factor V deficiency, Thrombophilia due to activated protein C resistance, Thrombophilia due to factor V Leiden, Ischemic stroke, susceptibility to, Budd-Chiari syndrome, susceptibility to, Recurrent abortion, not specified	Conflicting interpretations of pathogenicity, risk factor (Feb 27, 2018)

Gene(s)	Condition(s)	Gene(s)	Conditions Review status	
NM_000130.4(F5):c.5037dup (p.Ser1680Ilefs) *GRCh37:*Chr1:169500195 *GRCh38:* Chr1:169530957		F5	not provided	Pathogenic (Oct 23, 2015)
NM_000130.4(F5):c.1601G=		F5	not provided, hormonal contraceptives for systemic use response - Toxicity/ADR	drug response

Global Proceedings Repository
American Research Foundation
ISSN 2476-017X
Available online at http://proceedings.sriweb.org
شبكة المؤتمرات العربية
http://arab.kmshare.net/

Variation*Location*	**Gene(s)**	**Condition(s)**	**Clinical significance (Last reviewed)**
(p.Arg534=) *GRCh37:*Chr1:169519049 *GRCh38:* Chr1:169549811			
NM_000130.4(F5):c.5668G>A (p.Glu1890Lys) *GRCh37:*Chr1:169495187 *GRCh38:* Chr1:169525949	F5	not provided	Pathogenic
NM_000130.4(F5):c.5392G>A (p.Glu1798Lys) *GRCh37:*Chr1:169498873 *GRCh38:* Chr1:169529635	F5	not provided	Pathogenic
NM_000130.4(F5):c.3187dupA (p.Arg1063Lysfs) *GRCh37:*Chr1:169511142 *GRCh38:* Chr1:169541904	F5	not provided	Pathogenic

Global Proceedings Repository
American Research Foundation
ISSN 2476-017X
Available online at http://proceedings.sriweb.org
شبكة المؤتمرات العربية
http://arab.kmshare.net/

Variation*Location*	**Gene(s)**	**Condition(s)**	**Clinical significance (Last reviewed)**
NM_000130.4(F5):c.1160T>C (p.Ile387Thr) *GRCh37:*Chr1:169521931 *GRCh38:* Chr1:169552693	F5	Thrombophilia due to activated protein C resistance	Pathogenic (May 1, 2004)
NM_000130.4(F5):c.6304C>T (p.Arg2102Cys) *GRCh37:*Chr1:169487691 *GRCh38:* Chr1:169518453	F5	Factor V deficiency	Pathogenic (Jan 1, 2003)
NM_000130.4(F5):c.3481C>T (p.Arg1161Ter) *GRCh37:*Chr1:169510847 *GRCh38:* Chr1:169541609	F5	Factor V deficiency	Pathogenic (Sep 1, 2001)
NM_000130.4(F5):c.2401C>T (p.Gln801Ter) *GRCh37:*Chr1:169511927 *GRCh38:*	F5	Factor V deficiency	Pathogenic (Jul 15, 2001)

Global Proceedings Repository
American Research Foundation
ISSN 2476-017X
Available online at http://proceedings.sriweb.org
شبكة المؤتمرات العربية
http://arab.kmshare.net/

Variation*Location*	**Gene(s)**	**Condition(s)**	**Clinical significance (Last reviewed)**
Chr1:169542689			
NM_000130.4(F5):c.5189A>G (p.Tyr1730Cys) *GRCh37:* Chr1:169500043 *GRCh38:* Chr1:169530805	F5	Factor V deficiency	Pathogenic (Jul 15, 2001)
NM_000130.4(F5):c.439G>T (p.Glu147Ter) *GRCh37:*Chr1:169529939 *GRCh38:* Chr1:169560701	F5	Thrombophilia due to activated protein C resistance	Pathogenic (Nov 1, 2003)
NM_000130.4(F5):c.1001G>C (p.Arg334Thr) *GRCh37:*Chr1:169524537 *GRCh38:* Chr1:169555299	F5	Thrombophilia due to activated protein C resistance	Pathogenic (Feb 15, 1998)
NM_000130.4(F5):c.1000A>G	F5	Factor V Hong Kong	Pathogenic (Oct 1, 1998)

Global Proceedings Repository
American Research Foundation
ISSN 2476-017X
Available online at http://proceedings.sriweb.org
شبكة المؤتمرات العربية
http://arab.kmshare.net/

Variation*Location*	**Gene(s)**	**Condition(s)**	**Clinical significance (Last reviewed)**
(p.Arg334Gly) *GRCh37:*Chr1:169524538 *GRCh38:* Chr1:169555300			
NM_000130.4(F5):c.1601G>A (p.Arg534Gln) *GRCh37:*Chr1:169519049 *GRCh38:* Chr1:169549811	F5	Factor V deficiency, Thrombophilia due to activated protein C resistance, Thrombophilia due to factor V Leiden, Ischemic stroke, susceptibility to, Budd-Chiari syndrome, susceptibility to, Recurrent abortion, not specified	Conflicting interpretations of pathogenicity, risk factor (Feb 27, 2018)

Discussion

Thrombosis is a dangerous disease that may cause lethal clinical cases if not diagnosed earlier. About 50000 deaths per year may result from this disease (Slusher, 2010). Factor V Leiden thrombophilia is diagnosed by a poor anticoagulant response to activated protein C (APC) and and high risk for venous thromboembolism (VTE). Deep vein thrombosis (DVT) is the most common VTE, with the legs being the most common site. The molecular basis of Factor V Leiden is a missense mutation in the factor V (FV) gene at G1691A, resulting in R506 being changed to glutamine (R506Q) (Van Cott *et al*., 2015). Gender specific mutation of Factor V Leiden may be associated with evolutionary state. Women who carry this mutation may lose less blood during menstruation (Dunné *et al*., 2006).

However, we found an increased risk of Factor V Leiden with age. Most of patients showed the symptoms of thrombosis are elderly ages. Most of them suffered from high blood pressure, high blood

Global Proceedings Repository
American Research Foundation
ISSN 2476-017X
Available online at http://proceedings.sriweb.org
شبكة المؤتمرات العربية
http://arab.kmshare.net/

viscosity, high LDL, and less movement due to age which consequently drove the DVT to be diagnosed in most of them.

Mutation led to Factor V Leiden in Iraqi patients did not differ from that found around the world, but interestingly Hong Kong V mutation was detected in some patients subjected to this study.

Moreover, analysis of open reading frame in the sequence studied showed a significant difference, since the normal sequence appeared with one ORF, where as the affected sequence appeared with 2 ORFs suggesting complete disruption response to activated C protein.

Figure (2). ORF schematic of Factor V Leiden showing the presence of 2 ORFs from which transcription begins. Each ORF is directed oppositely from the other one.

Table (5). ORFs start and stop codon and no. of amino acids transcribed from each one detected in mutated sequence.

Label	Strand	Frame	Start	Stop	Length (nt | aa)
ORF2	**-**	**3**	**165**	**>1**	**165 | 54**
ORF1	+	2	44	>199	156 | 51

Figure (3). Normal ORF analyzed from normal sequence where no Factor V Leiden is found.

Global Proceedings Repository
American Research Foundation
ISSN 2476-017X
Available online at http://proceedings.sriweb.org
شبكة المؤتمرات العربية
http://arab.kmshare.net/

Table (6).ORF start and stop codon and no. of amino acids transcribed from this position detected in normal sequence.

Label	Strand	Frame	Start	Stop	Length (nt | aa)
ORF1	**+**	**1**	**34**	**>249**	**216 | 71**

Ethics approval and consent to participate: this study did not include any human subjects and was performed on animals postmortem.

Consent for publication: this work did not include any personal, written information, pictures and videos to any person.

Competing interests: this work was conducted without conflict of interest among authors or any other research group in others institutes.

Funding: this study was performed without any funding from any institute or sponsorship agency.

Authors' contributions: all authors contributed to this work according to their major in designing, following, interpreting and data analysis.

References

Asselta, R. and Peyvandi, F. (2009), Factor V deficiency SeminThrombHemost ., 35(4): 382-389.

Asselta, R.; Tenchini, M. and Duga, S. (2006). Inherited defects of coagulation factor V: the hemorrhagic side, J.Thromb. Haemost. , 4(1): 26-34.

Dunné, A.J.M.; Craen, B.T.; Heijmans, F.M.and Helmerhorst, R.G.J. (2006). Gender-specific association of the factor V Leiden mutation with fertility and fecundity in a historic cohort. The Leiden 85-Plus Study. Human Reproduction, 21, 4, 967–971,https://doi.org/10.1093/humrep/dei422

Jahangir, H. (2012). Fundamental pharmacology for pharmacy technicians. Cengage Learning p. 218. ISBN., 978(1):4180-5357.

Neil, R.; Anthony, A.; Nahida, A.; Daniel, N. and Alvin, H. (2000). Association of the Prothrombin G20210A Mutation With Factor V Leiden in a Midwestern American Population. Am. J. Clin. Pathol. 114:272-175.

Rosendaal, F.; Koster, J.; Vandenbroucke and Reitsma,P.(1995). High Risk of Thrombosis in Patients Homozygous for Factor V Leiden. Blood. 85(6): 1504-1508.

Global Proceedings Repository
American Research Foundation
ISSN 2476-017X
Available online at http://proceedings.sriweb.org
شبكة المؤتمرات العربية
http://arab.kmshare.net/

Saladin, A. and Kenneth, S. (2012). Anatomy and Physiology: The Unity of Form and Function, 6 ed., McGraw-Hill., p. 710.

Slusher, KB. (2010). Factor V Leiden: a case study and review.

Van Cott, EM.; Bernard, K. and James L. Zehnder, (2015). Factor VLeiden. Hematology. 91 (1): 46-49.

Van, R. ; Montefusco, M. and Duga, S. (2001). Coexistence of a novel homozygous nonsense mutation in exon 13 of the factor V gene with the homozygous Leiden mutation in two unrelated patients with severe factor V deficiency. Br. J.Haematol . 114:871.