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Association of factor V Leiden mutation with retinal vein thrombosis in a set of Iraqi patients

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Abstract:

BACKGROUND: Retinal vein occlusion is the most common retinal vascular disorder after diabetic retinopathy with several ocular and systemic disorders associated with retinal veins thrombosis. Factor V Leiden (FVL) mutation as part of inherited thrombophilia may associated with retinal vein thrombosis.

OBJECTIVES: Determine the presence of FVL mutation in a set of Iraqi patients with retinal vein thrombosis and evaluate its role in the etiology of thrombosis in those patients.

PATIENTS, MATERIALS AND METHODS: A case–control study conducted for 6 months, a total number of 69 patients who were diagnosed with retinal vein thrombosis while attending ophthalmology outpatient clinic in Diwania city in Iraq. Only sixty patients were eligible for the study. From each patient, venous blood was withdrawn for complete blood count, blood film, erythrocyte sedimentation rate, kaolin clotting time, anticardiolipin antibodies, antinuclear antibody, thyroid-stimulating hormone, renal function test, random blood sugar, and serum cholesterol in addition to determine the presence of FVL mutation by polymerase chain reaction (PCR)-restriction fragment length polymorphism. For 84 individuals of control group, only the presence of FVL mutation by PCR was done.

RESULTS: Sixty out of 69 patients with retinal vein thrombosis were eligible for the study. There were a total of 34 males and 26 females with a mean age of 49.1 ± 2.03 years with no significant statistical differences in mean age and sex between the patients and the control groups. The proportion of patients with FVL mutation was higher than that of control subjects, 21.7% versus 8.3% (P = 0.023). FVL mutation in patients group showing a significant risk factor to develop retinal vein thrombosis than control group (odd ratio: 3.043).

CONCLUSIONS: FVL plays a role in etiology of retinal vein thrombosis and measurement of this mutation with proper prophylaxis may be useful in prevention of venous thrombosis.

Key words:

Factor V Leiden, retinal vein occlusion, thrombophilia

A German pathologist, Rudolf Virchow at 1856, distinguishes a variety of causes for thrombosis which include stasis, injury to the vessels wall, and abnormal blood consistency and whose efforts lead to the maturity of thrombophilia as a concept.[1]

Thrombophilia refers to predispositions to thromboembolism; in practice, the term is used to describe patients who are at significantly increase long-term risk of venous and arterial thromboembolism. The predisposing factors may be genetically determined, acquired, or both.[2] At 1994, a group from Leiden city in the Netherlands recognized the most common mutation in factor V that made it to have a resistance to the action of activated protein C (APC), this was called factor V Leiden (FVL).[3]

APC is a potent inhibitor of the coagulation system. Its function is cleaving the activated forms of factors V and VIII.[4] Around 95% of APC resistance identified to cause by FVL mutation.[5] FVL is the most common known type of inherited thrombophilia, with a prevalence of 3%–8% in Caucasian population, 1.2% in African Americans, and infrequent in native African, Japanese, and Chines populations.[6]

The FVL mutation is a point mutation in the exon 10 of factor V gene in chromosome 1, single nucleotide substitution (guanine to adenine) at nucleotide 1691 in factor V gene leads to an amino acid arginine substitute by glutamine at position 506.[7] The effect of this is that more activated Factor V existing within the

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prothrombinase complex and more thrombin production that lead to hypercoagulable state (thrombophilia).[8]

Retinal vein occlusion is a common retinal vascular disease after diabetic retinopathy and it considers as multifactorial disease in its pathogenesis, many earlier studies established thrombophilia as a common pathoetiologic cause in retinal vein occlusion.[9] In this study, we try to identify the relationship between such mutation and the risk of retinal vein thrombosis.

Patients, Materials and Methods

Patients

Study design and setting

This is a case–control study among group of patients newly diagnosed with retinal vein thrombosis (central and branch retinal vein thrombosis) confirmed by suggestive clinical history and fundoscopy examination done by ophthalmologist while attending the outpatient clinic in Al Diwaniyah, Iraq. This study carried out from April 2014 to September 2014 to evaluate the role of FVL mutation as etiological factor to develop such thrombotic events.

Cases definition and ascertainment

Inclusion criteria

Patients with newly diagnosis retinal vein thrombosis with or without systemic disease were included. A detail history and clinical information collected in data sheet including patients' name, age, sex, occupation, address, history of hypertension, hyperglycemia, hypercholesterolemia, cardiovascular disease, renal failure, hypothyroidism, autoimmune diseases, local ophthalmological causes, drug history, and personal and family history of thrombosis.

Exclusion criteria

Patients with establish thrombosis on anticoagulant therapy were excluded. In addition to nine cases were excluded from a total number of 69 patients by the presence of potential acquired confounders (diabetes, hypercholesterolemia, uncontrolled hypertension, autoimmune disorders, hypothyroidism, and patients with oral contraceptive pills).

- Controls: The control groups for this study were 84 persons attended for outpatient ophthalmology clinic for simple visual problem without personal or family history of thromboembolic diseases or known systemic disorders predispose to thrombosis, ages and sex matched, and representative to same geographic area of the patients.

Permission from patients and controls was taken by oral consent.

- Assessment of exposure: FVL mutation and its association with retinal vein thrombosis were assessed.

Potential confounders were adjusted in the selection of cases and controls to eliminate its bias on the result while important inherited confounders to venous thrombosis (such as protein C [PC], protein S [PS], and antithrombin [AT] III deficiencies cannot be assess due to the limitation in the cost and facilities).

Validation of the results of FVL mutation was not done due to our limitations in the cost, time, and technical facilities.

- Power calculation: Assuming that 5% of first-episode thrombosis have FVL mutation, a sample size of sixty cases, and 84 controls had a power >80% at a type 1 error rate of 5% [Flow Chart 1].

Materials and Methods

Sampling

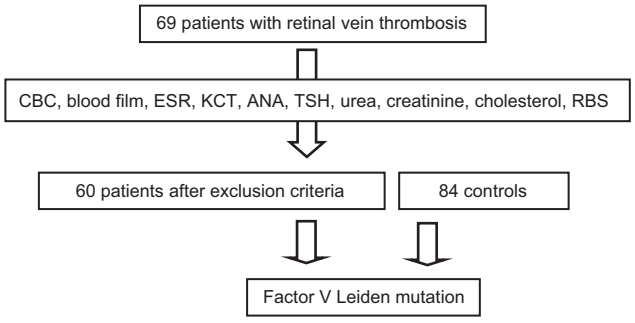
From each patient with retinal vein thrombosis, appropriate amount of venous blood was withdrawn and divided into three aliquots in proper tubes including citrated tube for coagulation studies, ethylenediaminetetraacetic acid (EDTA) tube for genetic and hematological tests, and plain tube for immunological and biochemical tests.

- Coagulation tests: These were performed on platelet poor plasma within 2 h of sampling in the same day using commercially available kits for kaolin clotting time (KCT) and KCT index estimation[10]
- Genetic and hematological tests: EDTA blood samples tested by polymerase chain reaction-restriction fragment length polymorphism (PCR-RFLP) to determine the presence of FVL mutation. Complete blood count (Hematology autoanalyzer, Swelab-Sweden), blood film, and erythrocyte sedimentation rate
- Immunological and biochemical investigation: These were performed on patient serum for estimation of aCL antibodies (IgM and IgG) and antinuclear antibody using commercially available kits (Orgentec-Diagnostika/Germany), thyroid-stimulating hormone, blood urea, serum creatinine, fasting blood sugar, and serum cholesterol.

For control group individual, only EDTA blood samples were taken tested by PCR-RFLP to determine the presence of FVL mutation.

Factor V Leiden analysis

Genomic DNA was isolated from the EDTA blood samples using "Genomic DNA extraction kit" for whole blood (Bioneer, Korea); this mutation was detected using PCR-RFLP method.[11] Briefly, the region flanking the mutation was amplified by PCR (forward primer TCAGGCAGGAACAACACCAT, reverse primer GGTTACTTCAAGGACAAAATACCTGTAAAGCT) and the PCR products digested with the restriction enzyme HindIII. The products from the digestion were separated on a 3% agarose gel, stained by ethidium bromide, and visualized on a ultraviolet transilluminator.



Flow Chart 1: Flowchart of the patients included in this study

Biostatistical analysis

The results were expressed as mean ± standard deviation. Pooled *t*-test was used for the comparison of significant difference between the patients and control groups in the measured parameters. Statistical significant was defined as a $P < 0.05$.

Results

Sixty-nine patients with retinal vein thrombosis were investigated, only sixty patients eligible for the study. There were a total of 34 males (56.7%) and 26 females (43.3%) with a mean age of 49.1 ± 2.03 years. There were 44 males (52.4%) and forty females (47.6%) with a mean age of 47.8 ± 3.16 years in the control group. There were no significant statistical differences in mean age and sex between the patients and the control group. Most of patients and control group recruited from the city of Diwania and few from other areas of Middle Euphrates of Iraq. These data were illustrated in Table 1. The proportion of patients with FVL mutation was higher than that of control group, 21.7% versus 8.3% ($P = 0.023$). FVL mutation in patients group showing a significant risk factor to develop retinal vein thrombosis than control group (odd ratio: 3.043) as demonstrated in Table 2. Mean age and gender ratio of patients with FVL mutation was not significantly different from that of patients without FVL mutation as shown in Table 3.

Discussion

Several risk factors were contributed for development of retinal vein occlusion ocular or systemic; however, the exact etiology remains unclear and thought to be multifactorial in nature. Several hemostatic factors have been implicated in the pathogenesis of retinal vein occlusion such as reduced PC, PS, AT III levels, and FVL and prothrombin genes mutation.

Table 1: Characteristics of patients and control groups

Characteristics of patients	Patient (*n*=60)	Control (*n*=84)
Age/mean	49.1±2.03	47.8±3.16
Male (%)	34/60 (56.7)	44/84 (52.4)
Female (%)	26/60 (43.3)	40/84 (47.6)
Male:female ratio	1.3:1	1.1:1

Table 2: Factor V Leiden mutation in the patients and control groups

Factor V Leiden mutation	Patients, *n* (%)	Controls, *n* (%)	*P*	OR	95% CI
Presence	13 (21.7)	7 (8.3)	0.023	3.043	1.13-8.17
Absence	47 (78.3)	77 (91.7)			
Total	60 (100)	84 (100)			

OR = Odds ratio, CI = Confidence interval

Table 3: Factor V Leiden mutation in association with age and gender of the patients group

Characteristic	Factor V Leiden mutation		*P*
	Yes	No	
Age	50.2	47.51	0.486
Male (%)	6 (18)	28 (82)	0.387
Female (%)	7 (27)	19 (73)	

In this study, there is no significant statistical difference between the mean ages of patients group (49.1 years) and control group (47.8 years) that usually related to the design of study regarding age and gender statistically matched studied groups. The number of patients shown increment with increasing agers and this consist with some studies such as study of Janssen *et al*.[12] This increment may explain by association of systemic and environmental risk factors of thrombophilia in such age groups. In addition, no significant statistical difference was found in male:female ratio between patient group and control group, like in study of Rogers *et al*.,[13] which found retinal vein thrombosis prevalence did not vary according to the gender that may related to the absence of additional risk factors in female group such as oral contraceptive pills, hormone replacement therapy (HRT), immunological disease, and pregnancy association.

FVL is the most common inherited thrombophilia known, with consequence inactivated 10–20 times more slowly than native form of factor V the result is increasing the generation of thrombin.[14] In our study, the number of patients express FVL mutation was 13/60 (21.7%) while that of control group 7/84 (8.3%) and the *P* value reached to (0.023). That means, the FVL mutation is higher in patients than control group but without statistical significant regarding *P* value, while odds ratio was 3.043 with a confidence interval of (1.133–8.171), that indicate a strong association between FVL mutation and retinal vein occlusion with increased risk around three folds to develop such thrombosis. These findings were consisting with the study of Greiner *et al*. This result is highly consist with many other studies that showing FVL mutation is associated risk for retinal vein thrombosis in addition to other important inherited or acquired factors.[15-18]

The mean age of patients with FVL (50.2 years) and of patients without such mutation (47.51 years), this difference was statistically no significant that FVL mutation can occur at any age group possibly alone or in association with other risk factors, these findings are consist with study of Rehak *et al*.,[18] while not consist with study of Arsène *et al*.[17] possibly related to small sample size of our study or to the differences in the nature of mutation in different populations. Furthermore, no significant association was found between genders of patients with FVL mutation that thrombogenic risk factors for female patients were excluded such as ovarian cancer cell line panel, HRT, immunological disorders, or pregnancy association and findings mostly related to the same risk in the male groups of acquired factors.[10]

Conclusions

FVL mutation is higher in patients than control group and considers a significant risk factor for retinal vein occlusion.

Recommendations

FVL mutation and other natural anticoagulant proteins deficiencies, such as PC, PS, and AT III deficiencies, may play a role in the etiology of retinal vein thrombosis and measurement of these parameters with proper prophylaxis, especially in young patients may be useful in prevention of venous thrombosis.

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Conflicts of interest
There are no conflicts of interest.

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