

Thrombophilia: Factor V Leiden Mutation as a Genetic Background in Patients with Retinal Vein Occlusion

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Abstract

Aim: This study was designed to detect the association between factor V Leiden mutation and retinal vein occlusion. **Methods:** A total 60 adult newly diagnosed retinal vein occlusion patients (branch or central types) with or without systemic disorders were included in this study in addition to 84 apparently healthy adult control persons. Factor V Leiden mutation by PCR- RFLP technique was done for all cases of patients and controls groups. **Results:** The number of patients express FVL mutation was 13/60 (21%), while that of control subjects 7/84 (8.3%) and the P- value reach to (0.023). The Odd 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. At the same time the etiologic fraction (EF) was 0.436 which mean that FVL mutation remain as a risk factor not a cause for retinal vein occlusion. **Conclusions:** FVL mutation is significantly higher in patients than control group, and consider a strong risk factor for retinal vein occlusion.

Keywords: Factor V Leiden, Thrombophilia, Retinal vein occlusion.

Introduction:

A German pathologist, Rudolf Virchow at 1856, distinguish 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 Netherlands, recognized the most common mutation in factor V that made it to have a resistance to the action of activated protein C, this was called factor V Leiden[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 caused by factor V Leiden mutation[5]. FVL is the most common known type of inherited thrombophilia, with a prevalence of 3% to 8% in Caucasian population, 1.2% in African Americans and infrequent in native African, Japanese and Chinese 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 lead to an amino acid arginine substitute by glutamine at position 506 [7]. The effect of this is that more activated FV existing within the 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 consider as multifactorial disease in its pathogenesis, many earlier studies established thrombophilia as a common pathologic cause in retinal vein occlusion[9].

Patients, materials and methods:

The present study is an observational case control study. A total 60 adult newly diagnosed retinal vein occlusion patients in addition to 84 apparently healthy adult control persons was included in this study. All patients were collected from Al-Diwanyia Teaching Hospital/units of ophthalmology, the diagnosis of retinal vein occlusion (branch or central types) was made from specialist of ophthalmology by suggestive clinical history and Fundoscopic examination, all of them have normal (CBC, Blood film, and ESR Levels). The following tests were done for all including random blood, blood urea, serum cholesterol, thyroid stimulating hormone, antinuclear antibody, Kaolin clotting time, anti-cardiolipin antibodies IgG and IgM in order to exclude the common acquired causes of thrombophilia. All patients and controls blood samples tested by PCR-RFLP in order to determine the presence of factor V Leiden mutation.

Factor V Leiden analysis: genomic DNA was isolated from the EDTA blood samples by using "Genomic DNA extraction kit" for Whole Blood (Bioneer. Korea), this mutation was detected by using PCR-RFLP method [10]. 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 UV Transilluminator.

Statistical analysis: Achieved by Chi-square test which used to study association between nominal variables. Odd ratio was used to calculate risk with 95% confidence interval, in addition to Student t-test. P-value was considered significant when it was less than or equal to 0.05.

Results: The proportion of patients with factor V Leiden mutation were significantly higher than that of control subjects, **21 %** versus **8.33 % (P=0.023)**, as shown in **table 1**. the odd ratio was **3.043** with a confidence interval of **(1.133 – 8.171)**. The etiologic fraction (EF) was **0.436**. These results suggested a strong association between factor V Leiden mutation and retinal vein occlusion.

Table 1: Proportion of patients and control subjects expressing factor V Leiden mutation

Factor V L	Patients		Control		P-value	Odd ratio	95 % CI		EF
	No.	%	No.	%			Lower	Upper	
Yes	13	21.67	7	8.33	0.023	3.043	1.133	8.171	0.436
No	47	78.33	77	91.67					
Total	60	100.00	84	100.00					

Mean age of patients with factor V Leiden mutation was not significantly different from that of patients without factor V Leiden mutation as shown in **table 2**.

Table 2: Comparison of mean age between patients with factor V Leiden mutation and patients without

Parameter	Factor V L (Yes)		Factor V L (No)		P-value
	Mean	SE	Mean	SE	
Age	50.00	3.14	47.51	1.65	0.486

No significant association was found between gender of patients and factor V mutation (P= **0.387**), as shown in **table 3**.

Table 3: Association between gender of patients and factor V Leiden mutation

Factor VL	Male		Female		Total	
	No.	%	No.	%	No.	%
Yes	6	17.65	7	26.92	13	21.67
No	28	82.35	19	73.08	47	78.33
Total	34	100.00	26	100.00	60	100.00

P= 0.387

Discussion

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

In this study there is no significant statistical difference between the mean ages of patients group (48years) and control group (46 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 is more frequency in age more than 50 years, this consist with some studies like study of Janssen MC et al.[11]. 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 S. Roger, R. L. Mcintosh, N. Cheung et al.[12], that found RVO prevalence did not vary according to the gender, that may related to the absence of additional risk factors in female group like oral contraceptive pills, hormone replacement therapy, 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 [13]. In our study the number of patients express FVL mutation was 13/60 (21%), while that of control subjects 7/84 (8.3%) and the P- value reach to (0.023). That mean the FVL mutation is significantly higher in patients than control group. Odd 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. These findings were consist with the study of Greiner K, Peetz D, Winkgen A, et al.[14]. At the same time the etiologic fraction (EF) was 0.436, which mean that FVL mutation remain as a risk factor not a cause for RVO, and this result is highly consist with many other studies that showing FVL mutation is associated risk for RVO in addition to other important inherited or acquired factors [15, 16, 17].

The mean age of patients with FVL (50 years) was statistically not significant difference from patients without such mutation (47.51 years), 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 M, Rehak J, Muller M. et al. [17], while not consist with study of Arsène S, Delahousse B, Regina S, et al.[16] possibly related to small sample size of our study or to the differences in the nature of mutation in different populations. Also no significant association was found between genders of patients with FVL mutation, that thrombogenic risk factors for female patients were excluded like OCCP, HRT, immunological disorders or pregnancy association and findings mostly related to the same risk in the male groups of acquired factors.

In this study no significant statistical difference in the mean of most biochemical and immunological parameters in patient with FVL mutation and those without such mutation, that FVL mutation may play a sole role in pathogenesis of RVO separated from other acquired risk factors which already excluded in most of our patients. So the inherited causes of thrombosis play a major role in the pathogenesis and subsequently prevention of RVO even in the absence of associated acquired systemic factors.

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