Factor V Leiden Mutation in Iraqi Patients with Deep Venous Thrombosis.

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Summary:

Background: Factor V Leiden is considered the most common inherited risk factor for venous thrombosis in Caucasian populations, including those in the Eastern Mediterranean region. While several studies have addressed Factor V Leiden prevalence in patients with venous thrombosis in the Eastern Mediterranean countries, none have been reported from Iraq.

Objective: To study the prevalence of Factor V Leiden in an unselected group of Iraqi patients with Deep Venous thrombosis.

Materials and Methods: A total of 50 unselected patients with deep venous thrombosis referred to the Medical City Teaching Hospital in Baghdad, Iraq, as well as 40 age and sex matched controls, were enrolled. The evaluation included in addition to detailed history, Factor V Leiden by polymerase Chain reaction and reverse hybridization.

Results: Factor V Leiden mutation was documented in 8 patients (16%), compared to 1 control (2.5%) (Odds Ratio 7.4; p= 0.0397). The mutation was more frequent among younger patients, those with family history of thrombosis and those with recurrent thrombosis, but only the latter was of significance.

Conclusions: The study suggests that Factor V Leiden is frequently encountered in Iraqi patients with Deep venous thrombosis from Baghdad, but less so than in some surrounding Eastern Mediterranean countries. Although further larger studies maybe warranted, the current study favors screening for Factor V Leiden in the workup of newly diagnosed venous thrombosis cases in this city.

Keywords: Factor V leiden, thrombophilia, Venous thrombosis, APC resistance, Iraq.

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Introduction:

Factor V Leiden (FVL) mutation is a conserved single point mutation at nucleotide 1691 (G to A) in exon 10 of the factor V gene, that leads to the substitution of arginine at position 506 by glutamine. Position 506 is one of the key cleavage sites of Activated Protein C (APC), and loss of this cleavage site makes the resultant factor V less susceptible to inactivation by APC (1) leading to the phenomenon of APC resistance (APCR) and thus increased venous thrombotic tendency. Compared to non-carriers, the increased venous thrombotic risk varies from 2.7-16 folds in FVL heterozygotes and up to about 80 folds in homozygotes in different studies. (2) Factor V Leiden (FVL) is highly prevalent in Caucasians, and is considered to be the most common cause of inherited thrombophilia in these populations. (3) Some of the highest FVL frequencies were reported from Eastern Mediterranean countries like Lebanon, Syria and Turkey. (4, 5) Despite being at the heart of the Eastern Mediterranean region, a recent study from Iraq revealed a lower FVL frequency of 3% among healthy individuals. (6) However, no data is available on the prevalence of FVL in Iraqi patients with venous thrombosis. The current study aimed at addressing the latter issue by studying a group of unselected Iraqi patients with Doppler-confirmed deep venous thrombosis (DVT).

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Materials and Methods:

Fifty unselected Iraqi patients diagnosed as Deep Venous thrombosis by color Doppler and referred to the Hematology department at the Medical City Hospital, Baghdad, Iraq, in the period between 21st September 2002 and the 31st December 2003, were enrolled. The Medical City Hospital is the largest and the main referral teaching hospital in the country. In addition to relevant history, all included patients had their DNA extracted using a phenol-chloroform method[7]. The DNA was thereafter amplified using Primus 25 thermocycler (MWG-Germany) with specific primers (ViennaLab-Austria), and a cycling program consisting of pre-polymerase chain reaction (PCR) at 94°C for 2 minutes, followed by 30 cycles at 94° C for 15 sec; 58° C for 30 sec; 72° C for 30 sec, and a final extension of 3 minutes at 72°C. Factor V Leiden mutation status was thereafter detected in the amplified products using reverse hybridization to

specific wild and mutant oligonucleotide probes by a colorimetric microwell plate method, according to the instructions of the manufacturer (ViennaLab-Austria). The study also included a concomitant similar evaluation of forty age and sex matched healthy controls who had no history of arterial or venous thrombosis, were not suffering from any illness of significance and were not taking any medications at the time of sampling. The study was approved by the ethical committee of the college of Medicine, University of Baghdad, Iraq, and informed consent was obtained from all participants. Statistical analysis included wherever appropriate, the Mann Whitney U and Chi Squared tests (with Fisher's exact test when a cell value was less than 5) and $p < 0.05$ was considered significant.

Results:

The fifty DVT patients had ages ranging between 17 and 65 years (median 38 years) and included 20 males and 30 females. In 22 (44%) of the enrolled patients an acquired thrombotic risk factor was identified, while in the remaining 28 (56%) patients no such risk factor was detected. The risk factors were pregnancy-related in 36.7% of females enrolled, while they were related to surgery/trauma or immobilization in 8% and 6% respectively. In 11 patients (22%) a history of one or more previous venous thrombotic event(s) could be elicited (i.e. had recurrent venous thrombosis). Five patients (10%) gave a family history of venous thrombosis. DNA studies revealed that 8 patients (16%) were carriers of FVL mutation (six heterozygous and two homozygous), while one (2.5%) of the controls was heterozygous for the mutation (Figure 1). FVL carrier rate was significantly higher in patients, compared to the controls (OR=7.4, p=0.0397). FVL carriers had their first thrombotic episode at a median age of 30 years compared to 38.5 years for the non-carriers, however this was not significant (p=0.412). No significant difference was found between the frequency of FVL in those with an associated acquired risk factors (18.2%) compared to those with no such association (14.3%)(p= 0.5016842). Three out of the 11 patients (27.3%) with recurrent DVT were FVL carriers compared to 5/39 (12.8%) with single episodes and 1/40 (2.5%) of the healthy controls, a finding which was significant (p=0.03894531). It was found that the Odds Ratio for having FVL in recurrent thrombosis was about 2.5x higher than those with single episode with ratios of 14.6 versus 5.7 (figure 2). On the other hand, a family history of venous thrombosis was found in 2/8 (25%) of FVL carriers compared to 3/42 (7.1%) of the non-carriers, however this was not significant (p=0.1758821).

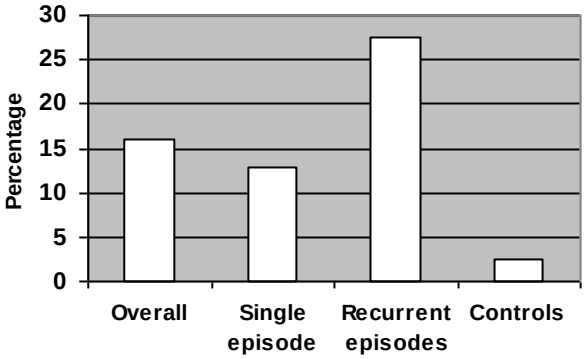

Figure 1: The frequency of FVL in those with DVT and controls in the current study.

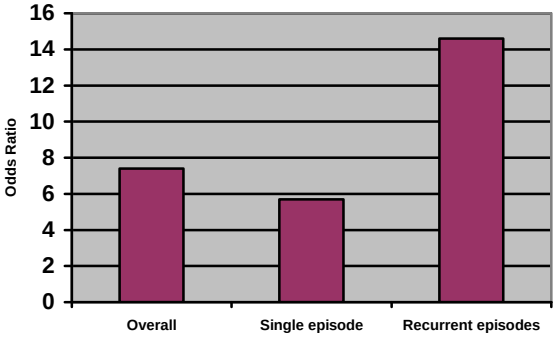

Figure 2: The Odds Ratio for being FVL carriers overall and in those with single or recurrent deep venous thrombotic episode(s) in the current study.

Discussion:

The overall prevalence of FVL mutation of 16% in the unselected Iraqi DVT patients in the current case-control study is comparable with results reported by several major studies from other Caucasian populations in Europe and North America[8,9].This frequency is however much lower than those reported in DVT patients from some other neighboring Eastern Mediterranean populations like the Lebanese and the Turks where rates of 70.5% and 34.9% were reported respectively(10,11), while it is much higher than those reported from Chinese and those of African origin where rates of 0% and 2.8% were reported respectively. (9,12) This variation in prevalence of FVL among venous thrombosis patients could be best explained by respective variation in the background prevalence of mutation in the above populations. (3,4,5) The higher frequency of FVL among those with recurrent thrombosis compared to those with single episodes and controls, is consistent with the conclusions of a recent meta-analysis of prospective studies, which demonstrated that heterozygous carriers who experience one episode of venous thromboembolism have on average a 40% increased risk of recurrence over non-carriers, and this increase

is statistically significant. (13) In conclusion, it was demonstrated and for the first time from Baghdad-Iraq, that FVL is frequently and significantly associated with deep venous thrombosis, although less so than in some of the neighboring Eastern Mediterranean countries. This still warrants in our opinion considering FVL testing for all DVT patients in this city, since it may alter treatment decisions in particular clinical settings[14]. Further studies are recommended in the future, including larger numbers of patients and covering other less common inherited risk factors, as well as other parts of the country.

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