Original Article

Factor V Leiden does not have a role in cryptogenic ischemic stroke among Iranian young adults

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Abstract

Background: Different risk factors have been suggested for ischemic stroke in young adults. In a group of these patients despite of extensive diagnostic work-up, the primary cause remains unknown. Coagulation tendency is accounted as a possible cause in these patients. Previous studies on factor V Leiden (FVL) as the main cause of inherited thrombophilia for clarifying the role of FVL in stroke have resulted in controversial findings. The current study investigates the role of this factor in ischemic stroke among Iranians.

Materials and Methods: This case-control study was performed between September 2007 and December 2008 in Isfahan, Iran. The case group comprised of 22 patients of which 15 were males and 7 were females with age range of ≤50 years, diagnosed as ischemic stroke without classic risk factors and the control group consisted of 54 healthy young adults. After filling consent form, venous blood samples were obtained and sent to the laboratory for genetic examination.

Results: No FVL mutation was found in the case group. There was one carrier of the mutation as heterozygous in the control group (relative frequency = 1.85%).

Conclusions: Based on our study, FVL might not be considered as an independent risk factor for ischemic stroke in Iranian individuals who are not suffering from other risk factors of ischemic stroke.

Key Words: Cryptogenic, factor V Leiden, stroke, young adults

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INTRODUCTION

Stroke is one of the most important disabling problems in the old age, but also annually occurs up to 23 young adults out of 100,000 individuals, in which most cases are ischemic.[1-4]

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Although aging, hypertension, smoking, dyslipidemia and diabetes are of the main importance in elder patients,[5] cardiogenic emboli, hypercoagulable state, dissection of extracranial arteries, drugs ingestion and premature atherosclerosis are considered as dominant risk factors of stroke in younger patients.[2,4] In 15-40% of cases in this age group, no identified etiology could be found.[2,6] Hypercoagulable states induced by antiphospholipid antibodies, hyperhomocysteinemia, elevated factor VIII, protein C deficiency and mutations such as methylenetetrahydrofolate reductase C677T, prothrombin G20210A and factor V Leiden (FVL) mutations, in spite of different opinions, are accounted as possible causes in these patients.[7-10]

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FVL is the most prevalent inherited coagulation disorder in many parts of the world. Though the role of this mutation in ischemic stroke is controversial,[11-14] but test for FVL is frequently requested in the routine diagnostic work-up of stroke patients.[15] Over the last years, some researches on cryptogenic stroke subcategory have indicated FVL's function as an independent etiological factor, particularly in young patients.[16,17] On the other hand, there are a number of studies that proposed FVL as a predisposing factor for ischemic stroke only when it is accompanied by classical risk factors of stroke.[18,19] Hence, in young stroke patients, especially those with unknown origin of the disease, screening for coagulopathies are usually suggested.[7]

As the screening for FVL is mostly indicated in a stroke of undetermined etiology, in the present study, we aimed to verify the possible contribution of FVL in cryptogenic ischemic stroke and its importance as an independent risk factor among Iranian young adults.

MATERIALS AND METHODS

Patients

In this case-control study, a total of 110 consecutive stroke patients aged ≤50 years were evaluated for the eligibility criteria. They were admitted between September 2007 and October 2008 in the Neurology ward at Alzahra University Hospital, affiliated to Isfahan University of Medical Sciences, Isfahan, Iran.

Diagnosis of ischemic stroke was based on Trial of ORG 10172 in Acute Stroke Treatment (TOAST) criteria[20] confirmed by brain computed tomography scan or magnetic resonance imaging, if necessary. To narrow our study population to cryptogenic subtype, patients with traditional risk factors and other predictors of ischemic stroke were excluded from the study: At the first stage, patients with a history of cardiac valvular and vascular diseases, hypertension, diabetes mellitus, hyperlipidemia, malignancy and chronic renal failure, heavy smokers (≥1 pack/day) or drug abusers were excluded. Electrocardiography at admission was taken to rule out cardiac arrhythmia. Trans-thoracic or trans-esophageal echocardiography — if indicated — was performed to evaluate the presence of cardiac dyskinesia, valvular disorders, prostehic heart valves and vegetations. Intra and extracranial Doppler sonography and if it was required, magnetic resonance angiography was done to further study the etiology of stroke.

Human immunodeficiency virus antibody and Venereal Disease Research Laboratory tests were performed in all patients. Antithrombin III, protein C and S, anti-nuclear antibody, anti-neutrophil cytoplasmic antibody, antiphospholipid antibody and anticardiolipin antibody were checked to assess the possibility of coagulopathies and vasculitises. Patients with positive test results were excluded from the study.

The patients with evidence of infective endocarditis, post-traumatic stroke or post-procedural stroke were not included in the study. There were no patients with diagnosis of cerebral autosomal dominant arteriopathy with subcortical infarcts leukoencephalopathy, mitochondrial encephalomyopathy with lactic acidosis and stroke-like episodes, sickle cell anemia, homocystinuria or Fabry disease.

After diagnostic work-ups, we included 22 patients of first-ever stroke with unknown etiology based on TOAST criteria.[20] Informed consent was obtained from all patients. Blood samples were collected into ethylenediaminetetraacetic acid (EDTA) plastic tubes and stored in –20°C until deoxyribonucleic acid (DNA) extraction.

Blood samples also obtained from 54 healthy individuals consisted of hospital staffs and volunteer medical students who did not have a history of thrombotic or ischemic disease, as the control group.

The study protocol was approved by the Research Ethics Committee of Isfahan University of Medical Sciences.

Laboratory

Venous blood samples collected into EDTA tubes were used for genomic DNA extraction with a standard DNA isolation kit (PrimePrep, Genet Bio, South Korea). Using 5'-TGCCCAGTGCTTAAAAGACCA-3' (Forward) and 5'-TGTTATCACACTGGTGCTAA-3' (Reverse) primers (Bioneer, South Korea), we could amplify a 267 bp fragment of factor V gene. After digestion by MnlI endonuclease, the normal fragment is divided to three fragments of 163 bp, 67 bp and 37 bp, but the mutated amplified fragment is divided into a 200 bp and a 67 bp fragment.[21]

The reaction setting for the DNA thermal cycler was: A 10 min denaturation period at 95°C, following by 37 cycles of denaturation for 1 min at 95°C, annealing for 2 min at 56°C, and extension for 1 min at 72°C. After 37 cycles, a final extension period for 10 min at 72°C was performed. Two units of MnlI were added to 10 μl of polymerase chain reaction products and run on a 1.5% agarose gel for electrophoresis.

Based on the finding observed in the Gel documentation system, frequency of mutated

samples was recorded and compared within the case and the control group.

RESULTS

Blood samples were analyzed for FVL in 22 ischemic stroke patients who met the inclusion criteria. The mean (±standard deviation) age of cases and controls was 39 ± 8 (15 males and 7 females) and 30.7 ± 8.6 (17 males and 37 females) years, respectively.

FVL mutation was not found in any cases of ischemic stroke. However, the mutation was found in one subject in the control group (relative frequency = 1.85%) in heterozygous form: After digestion of the amplified DNA fragments by MnlI, three bands of 163 bp, 67 bp and 37 bp were seen in all samples under gel documentation system. However, an additional band (200 bp) was also seen in the heterozygous subject [Figure 1]. No homozygote mutation was detected in the control group.

DISCUSSION

We found that FVL is not associated with cryptogenic ischemic stroke in our study population, which was consisted of young adults with ischemic stroke. We have had a highly selected case group in which classic risk factors for stroke were absent. Arranging such a selected group could increase the chance of detecting FVL in the case of being a risk for ischemic stroke.

A recent meta-analysis of 18 studies,[22] concerning the role of FVL in ischemic stroke in young adults, has reported it as a risk factor in "selected" patients that are referred with clinical susceptibility of prothrombotic state, though there was not a significant relationship in the case of consecutive neurology hospitalizations. A review of 33 studies concluded that FVL could have

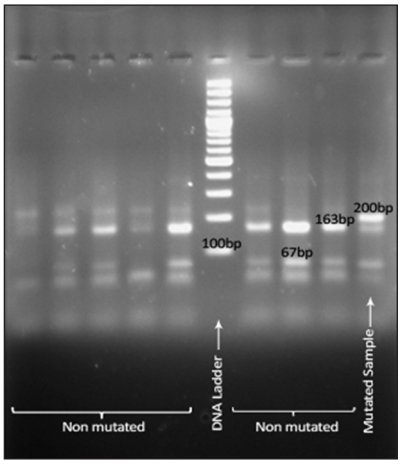

Figure 1: Electrophoresis gel after dyeing with ethidium bromide in nine samples of control group

a modest effect in vascular ischemic events in patients < 55-years-old.[9] Similarly an additional meta-analysis of 26 studies in population of European decent, noted a positive association between ischemic stroke and FVL, though with a smaller odd ratio than cerebral vein thrombosis patients.[23] Another available meta-analysis of eight studies[24] failed to find a relationship between ischemic stroke and FVL in adults. Albeit still exist controversies, the result of most studies, as well as our study, are on the contrary with FVL's role in ischemic stroke.

In a similar study designed by Szolnoki *et al.*,[16] the role of FVL and seven other genetic coagulation abnormalities were evaluated in a small group of 17-57-year-old risk free stroke patients. They found FVL as heterozygous in all of their five patients and concluded that it would be a risk for stroke in cryptogenic subgroup. We performed our study with a larger case group. However this mutation was not detected in any of our patients. Although this discrepancy could be a result of different prevalence of this mutation among these two studied populations, our data directed us to a negative conclusion toward the role of FVL in cryptogenic ischemic stroke in Iran.

Since the association between venous thrombosis and FVL was reported before, several studies investigated the prevalence and importance of this mutation in different regions of the world and showed that it has a wide range of diversity: A high prevalence in European and Middle-Eastern countries, with a decreasing trend when it goes to the east.[25,26] The highest prevalence rates have been reported in Mediterranean countries of the Middle-East.[27,28] The occurrence of FVL in a normal population in Turkey, which extended from Mediterranean area to the western border of Iran at the central Middle-East, varies from 2.1% to 10.4%.[29,30] Available data from Iraq-the other western neighbor of Iran- and Pakistan in the eastern borders of Iran-shows the frequency of 3% and 1.3%, respectively.[31,32] Eventually, a range of 0-0.2% has been observed in eastern Asian countries.[33-35] This low prevalent distribution pattern extremely dilutes the effect of FVL in thrombotic events and even practically eliminates its role in thrombotic events in that region. Iran is geographically located at a transition area between high prevalence countries at the west and low prevalence countries at the east. The frequency of FVL mutation in our control group (1.85%) is in accordance with this decreasing trend, but is lower than some earlier studies in the country. The prevalence of FVL mutation was estimated to be between 2% and 2.97% in Kurdish ethnic group[36,37] in the western Iran, and up to 5.5% in Tehran,[38] the

capital, with diverse ethnicity. Our result is more comparable to the data collected in Tehran by Zeinali *et al*.,[38] since the Kurdish ethnicity is limited to parts of Iran in the west. While Zeinali *et al*. asserted that they chose their samples from all ethnicities living in Iran, but according to other studies and also the results of our study, this factor does not seem to have such a high prevalence in Iran. The method of samples' inclusion and presence of some certain ethnic groups in the study by Zeinali *et al*. can be the cause of this reported higher prevalence. Besides, in smaller sample size, two other studies observed no FVL mutation in Iranian general population.[39,40]

In the present study, we could investigate the presence of FVL in a small group of 22 patients, which could be the main limitation of our study. Since we intended to evaluate the role of FVL as an independent risk factor in stroke with unknown origin and usefulness of FVL testing in these patients, we had to exclude a large number of patients and it brought us a small sample size. A larger sample size could provide a more precise data about the prevalence of FVL in this locality. Besides, cases and controls should be matched for gender, because the prevalence of FVL mutation might be different two genders. However, in our study we could not provide this condition.

Regarding to our findings, FVL has no significant role in cryptogenic ischemic stroke. Accordingly, screening for FVL in these patients may not have an advantage and is not suggested. In addition, the scarcity of FVL attenuates its role in thrombogenesis among Iranian population. Further studies on the scope of examining the sequence of factor V at the activated protein C cleavage sites of factor V are recommended to investigate the possibility of presence of other mutations of factor V in this society and elucidate their possible thrombophillic role.

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