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Research Paper

Correlation Between Clinical Parameters and Genetic Markers of Thrombophilia in Ischemic Strokes

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ABSTRACT

Background and Aim: A stroke is defined as the rapid loss of brain function due to a disturbance in blood supply. Age is a major risk factor for stroke, and genetic influences may be more significant in younger stroke patients. Patients with positive results should receive appropriate counseling. This study is the first to comprehensively investigate thrombophilia genes in Iranian populations with ischemic stroke. The purpose of this study was to determine the occurrence of genetic mutations linked to thrombophilia in individuals with ischemic stroke and examine their correlation with clinical parameters and the genetic markers of thrombophilia.

Materials and Methods: This cross-sectional study was conducted on 62 patients with ischemic stroke at the Department of Stroke in Shahid Beheshti Complex Hospital affiliated with the Qom University of Medical Sciences from October 2017 to October 2018. DNA extraction was done using CVD StripAssay® kits. SPSS software, version 25, was used to analyze the data and the statistical significance level was defined as $P<0.05$.

Results: Sixty-two patients with ischemic stroke had a mean age of 67.9±3.76 years. The prevalence of risk factors included smoking at 11.1%, hypertension at 79.4%, and previous diabetes and hyperlipidemia at 44.4%. Women exhibited a lower prevalence of risk factors. The most common mutation was the polymorphism of *ACE* at 82.5%. The second and third most common mutations were the polymorphism of *FGB* at 76.2% and the polymorphism of *LTA* at 71.4%. The prevalence of polymorphisms was as follows: *ACE I/D* 82.5%, *FGB -455G>A* 76.2%, *LTA 804C>A* 71.4%, *eNOS 894G>T* 52.4%, *eNOS -786T>C* 12.7%, *Apo B R3500Q* 33.3%, *HPA1 a/b* 31.7%, and *Apo E* (*E2E2*: 54%, *E2E3*: 3.2%, *E2E4*: 9.5%, *E3E3*: 14.3%, *E3E4*: 15.9%, *E4E4*: 1.6%). The relationship between the genes and age, sex, smoking, hyperlipidemia, diabetes, and hypertension was measured, but no significant relationship was found.

Conclusion: Thrombophilia risk factors were separated into high- and low-risk factors. There was no significant association between demographic characteristics, including age, sex, smoking, preexisting comorbidities, and thrombophilia ($P>0.05$). It is suggested to conduct studies with larger sample sizes, aimed at investigating gene-environment interactions as well as gene-gene interactions.

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Introduction

Stroke is the second most common cause of death worldwide, contributing to 11.3% of all mortality cases [1]. Additionally, it is the leading cause of disability. The occurrence of stroke can be categorized into two main groups: Ischemic and hemorrhagic strokes. Both types are attributed to alterations in the brain's blood circulation. Notably, ischemic strokes account for approximately 80% of all stroke incidents [2].

Genetic factors hold significant prominence as crucial contributors to stroke development. Numerous studies have identified a prothrombotic state as a risk factor for stroke. Within the realm of genetics, two types of genetic variations play a pivotal role in thrombosis. The first involves alterations that lead to a reduction in the levels of antithrombotic proteins, while the second encompasses genetic changes that result in an elevation in prothrombotic proteins [3, 4]. A specific gene called Apo lipoprotein B (*Apo B*) *R3500Q* has rare mutations associated with severe hypercholesterolemia and an increased susceptibility to atherosclerosis [5]. Further investigation has revealed a significant correlation between the rs1042034 locus in the *ApoB* gene and plasma cholesterol levels. Moreover, the *ApoB* gene has been linked to an increased risk of ischemic stroke [6, 7]. In the Mongolian population, it has been observed that a higher ApoB/ApoA-1 ratio corresponds to an increased risk of both ischemic stroke and coronary heart disease. Additionally, elevated levels of OxPL-apoB proteins have been identified as predictive factors for recurrent stroke and initial major coronary events in patients with a history of stroke or transient ischemic attack [8, 9].

The *Apo E* gene possesses three alleles: *E2*, *E3*, and *E4* [10]. Studies have indicated that carriers of the *E4* allele, which constitutes 20% of the population, face an elevated risk of ischemic diseases, with a potential increase of up to 40%. Another genetic mutation of interest is the G to A mutation in the promoter of the β-fibrinogen gene (*FGB*) [11, 12]. This mutation raises plasma fibrinogen levels, leading to increased blood viscosity, fibrin formation, and platelet aggregation, ultimately culminating in ischemic stroke.

Recent research suggests that the *FGβ -148 C/T* and *-455 G/A* polymorphisms may serve as potential biomarkers for ischemic stroke in Asian populations. The *eNOS* gene plays a neuroprotective role in ischemic stroke and is primarily produced by vascular endothelial cells. The eNOS protein facilitates the production of nitric oxide (NO), which regulates the pressure within cerebrovascular vessels and plays a beneficial role in ensuring an adequate blood supply to brain tissue [13]. It has been documented that eNOS intron 4a/b polymorphisms may serve as potential serum biomarkers for the pathophysiological processes of ischemic stroke by modulating homocysteine and vitamin B12 levels [14]. The *786T>C* polymorphism in the promoter prevents the *eNOS* gene expression, but it is not associated with ischemic stroke. Changes in platelet activity and thromboembolism are linked to cerebrovascular diseases. Different forms of HPA have been identified, with HPA1-5 being relevant to clinical issues and exhibiting different geographic and racial distributions [15]. Lastly, the *894G>T* polymorphism alters the position of NOS3 on the gene and interferes with the regulatory cycle of eNOS, consequently elevating the risk of ischemic stroke [16]. The *ACE* gene polymorphism is associated with ischemic stroke and atherosclerosis. Low ACE activity and the presence of the *D* allele may increase the risk of premature death in cases of acute ischemic stroke [17, 18]. The *D* allele is associated with a high risk of atherosclerosis, especially in European populations. Inflammation is a key factor in atherosclerosis and stroke. The *LTA* gene polymorphism is associated with stroke [19-21].

This study aimed to investigate thrombophilia mutations in patients with ischemic stroke using the CVD StripAssay. The frequency of thrombophilia mutations was also examined in relation to age, sex, type 2 diabetes, hyperlipidemia, blood pressure, and smoking.

Materials and Methods

This cross-sectional study was conducted on 62 patients with a mean age of 67.9±3.76 years at the Neuroscience Research Center in Shahid Beheshti Complex Hospital affiliated with the Qom University of Medical Sciences, from October 2017 to October 2018.. All patients were over 18 years old. Along with conventional treatment, all patients underwent genetic testing to screen for thrombophilia using the CVD Panel PCR Test Kit, which included eight genes (*Apo B R3500Q*, *Apo E*, *FGB -455G>A*, *HPA1 a/b*, *ACE I/D*, *eNOS-786T>C*, *eNOS 894G>T*, *LTA 804C>A*).

Interventions and data collection

First, the patients' characteristics, including age, sex, comorbidities, habitual profile, and neurologic status were collected. Then, in addition to standard stroke management, all patients underwent genetic screening. According to the World Health Organization definition,

acute ischemic stroke is defined as the "rapid onset of focal neurological deficit due to focal disturbance of cerebral function persisting longer than 24 hours." The diagnosis was confirmed through clinical assessment and a positive computed tomography (CT) scan or magnetic resonance imaging (MRI) by the neurologist.

DNA extraction from blood samples

DNA extraction was done using CVD StripAssay® kits (A. 4-370) (Figure 1). The Nanodrop device was used to determine the quantity and quality of the extracted DNA. Most of the extracted samples had an absorption of over 200 ng/ul and were used in genetic analyses, such as PCR.

Strip assay

The CVD StripAssay was utilized to identify several mutations in different genes that are risk factors for stroke. This assay is based on the reverse hybridization of PCR products, where special probes for various variants and controls hybridize with the PCR products. It employs labeled oligonucleotides on the strip test.

Detection of genotype mutation

All reagents used in these steps were provided by CVD StripAssay® kits (A. 4-370). The CVD StripAssays® from Vienna Lab included eight genes: *Apo B R3500Q*, *Apo E*, *FGB -455G>A*, *HPA1 a/b*, *ACE I/D*, *eNOS-786T>C*, *eNOS 894G>T*, and *LTA 804C>A*.

Data analysis

SPSS software, version 25, was used to analyze the data and the statistical significance level was defined as $P<0.05$ (chi-square test and cross-tabulation).

Results

Sixty-two patients with a mean age of 67.9±3.76 years participated in this study. The prevalence of risk factors included smoking at 11.1%, hypertension at 79.4%, and previous diabetes and hyperlipidemia at 44.4%. Women exhibited a lower prevalence of risk factors.

The most common mutation was the polymorphism of *ACE* at 82.5%. The second and third most common mutations were the polymorphism of *FGB* at 76.2% and the polymorphism of *LTA* at 71.4%. The prevalence of polymorphisms was as follows: *ACE I/D* 82.5%, *FGB -455G>A* 76.2%, *LTA 804C>A* 71.4%, *eNOS 894G>T* 52.4%, *eNOS -786T>C* 12.7%, *Apo B R3500Q* 33.3%, *HPA1 a/b* 31.7%, and *Apo E* (*E2E2*: 54%, *E2E3*: 3.2%, *E2E4*: 9.5%, *E3E3*: 14.3%, *E3E4*: 15.9%, *E4E4*: 1.6%).

The relationship between the genes and age, sex, smoking, hyperlipidemia, diabetes, and hypertension was measured, but no significant relationships were found (Table 1).

Discussion

Stroke or cerebrovascular accident, is a highly heterogeneous disorder and is considered the second leading cause of death worldwide, accounting for 11.3% of deaths. Cryptogenic stroke accounts for 30% to 40% of acute ischemic stroke cases, with a higher prevalence of up to 50% in the youth [22]. Patients with cryptogenic stroke often undergo comprehensive diagnostic evaluations to identify its etiology. Inherited and acquired thrombophilia account for a proportion of cryptogenic strokes that may benefit from tailored treatment [23]. Several studies have suggested a possible association between various thrombophilias and acute ischemic stroke, particularly in younger adults. The factor V Leiden, prothrombin G20210A mutation, protein C deficiency, and protein S deficiency are the most frequent inherited thrombophilia associated with acute ischemic stroke [24].

The genetic contributions to ischemic stroke are important not only for explaining or predicting the minority of cases that occur in the absence of well-established risk factors, such as smoking, hypertension, and diabetes but also for accounting for the wide variability in stroke incidence among individuals who do have these common acquired risk factors. Moreover, understanding the biochemical basis of risk-associated genes can motivate novel therapeutic strategies, including pharmacogenomics.

In this study, sixty-two patients with ischemic stroke a mean age of 67.9±3.76 years participated. Among them, 11.1% were smokers, 79.4% had hypertension, and 44.4% had a history of diabetes and hyperlipidemia, which are consistent with previous studies that identified these conditions as risk factors for vascular disorders, such as stroke men had an equal or higher risk of stroke compared to women. In this study, the prevalence of risk factors, including cigarette smoking, hypertension, diabetes, and hypercholesterolemia, was less than 50% among women.

Grau et al. enrolled 5017 patients with acute ischemic stroke (42.4% women, with a mean age of 65.9±14.1 years) in a large multicenter hospital-based stroke database. The highest prevalence of hypertension, diabetes

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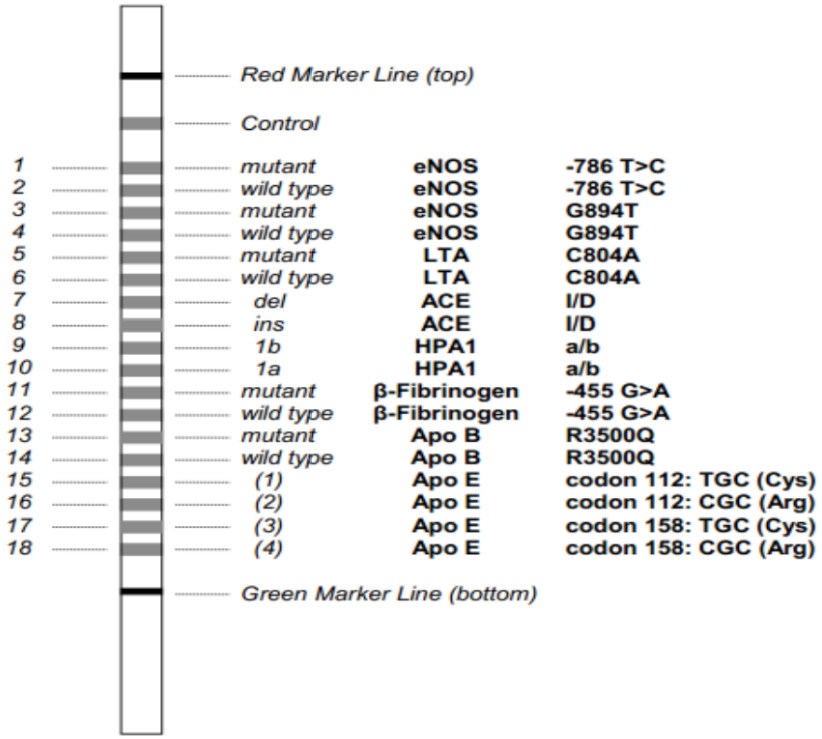

Figure 1. The CVD StripAssay® kit

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mellitus, hypercholesterolemia, and obesity was found in small-vessel disease (20.5%), which was associated with the lowest stroke severity and mortality [23]. In this study, the most common mutation was polymorphism of the angiotensin-1 converting enzyme (*ACE*) gene, with 82.5% of patients being heterozygote for the *I/D* alleles (287 bp insertion/deletion).

In a previous study, the ACE D/D genotype exhibited a significantly higher frequency in patients with small deep infarcts (40.3%; P<0.0005; OR 2.31, 95% CI, 1.49%, 3.57%) compared to the control group (22.6%) [25]. The *ACE* gene has been identified to possess two polymorphic alleles, namely *I* and *D*. Individuals with the DD genotype are more susceptible to experiencing myocardial infarction, although this finding is supported by only some studies. A comprehensive analysis of multiple studies demonstrates that the *D* allele, when acting in a recessive manner, represents a modest yet independent risk factor for the onset of ischemic stroke [26].

Previous research has demonstrated that there is a notably higher occurrence of the *D* allele of the *ACE* gene, specifically the DD genotype, in individuals with small-vessel occlusion compared to the healthy population. It appears that intricate interactions between various genes, as well as gene-environment interactions, along with alterations in protein-level functional networks, contribute to the disparities in polymorphism patterns and clinical phenotypes, resulting in differing outcomes across previous studies [27].

Although this study, consistent with previous research, indicated that no significant association between the presence of the *D* allele and an increased risk of hypertension [28], it has also been revealed that the DD genotype is linked to elevated levels of tissue-type plasminogen activator [26]. Studies have suggested that the presence of the *D* allele may be attributable to various mechanisms, including increased vessel wall thickness, increased proliferation of vascular smooth muscle cells, and vasoconstriction induced by augmented angiotensin function, which all can have detrimental effects on vascular regulatory function [29, 30]. Consequently, it can be inferred that while a causal relationship may not exist, there is a synergistic association between the presence of the *D* allele and hypertension that could elevate the risk of ischemic stroke.

In summary, it is evident that individuals with the DD genotype should be recognized as having a greater susceptibility to ischemic stroke, and the utilization of ACE inhibitors should be considered a suitable intervention to prevent ischemic stroke and endothelial dysfunction. In

Table 1. Patients' demographic and clinical characteristics

Variables	No.	Minimum	Maximum	Mean±SD
Age (y)	62	32.00	97.00	67.9855±13.76036
Height (cm)	24	150.00	175.00	165.3750±6.46605
Weight (kg)	24	50.00	93.00	73.0000±11.94917
PT (Sec.)	63	12.00	34.00	13.3937±2.87693
PTT (Sec.)	63	25.00	120.00	32.8778±12.23701
MRS	41	0.00	5.00	3.5610±1.68892
NIHSS	41	0.00	25.00	8.7561±7.51924
Total cholesterol (mg/dl)	63	95.00	250.00	155.2857±33.62815
TG (mg/dl)	63	36.00	378.00	122.0952±68.95784
HDL (mg/dl)	63	19.00	56.00	35.1905±7.70182
LDL (mg/dl)	63	12.00	184.00	87.3810±34.20499
FBS (mg/dl)	62	59.00	462.00	123.7903±67.79360
ALT (U/L)	45	8.00	53.00	19.8044±11.09107
AST (U/L)	46	15.00	46.00	22.8196±7.43893
Alph (U/L)	45	19.00	464.00	193.6444±75.09272
LDH (U/L)	40	144.00	801.00	382.7000±106.39072
Valid N (listwise)	12			

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Abbreviations: PT: Prothrombin time; PTT: Partial thromboplastin time; MRS: Modified rankin scale; NIHSS: National institutes of health stroke scale; TG: Triglyceride; HDL: High-density lipoprotein; LDL: Low-density lipoprotein; FBS: Fasting blood sugar; ALT: Alanine aminotransferase; AST: Aspartate aminotransferase; Alph: Alkaline phosphatase; LDH: Lactate dehydrogenase.

this particular investigation, the second mutations were characterized by the polymorphism of *FGB -455G>A*, with a prevalence of 76.2% heterozygotes.

Previous research conducted by Hu et al. revealed, for the first time, that the *A* allele of *FGB 455 G/A* was a contributing factor to cardioembolic stroke in patients with atrial fibrillation, presumably by elevating plasma fibrinogen levels The outcomes indicated that the frequency of an allele in stroke patients is approximately 0.38 [11], which exhibits a significantly higher value compared to the frequency observed in the healthy Iranian population, as reported by Kahrizi et al. (0.22) [31]. Although Lee et al. concluded that the frequency of *FGB* polymorphisms was similar in stroke patients of different races and exhibited minimal differences from the control group, our study demonstrated that the distribution of FGB genotypes in Iranian patients with lacunar stroke deviates from the reported results in these studies. Specifically, the percentage of Iranian stroke patients carrying the allele was found to be 76%, in contrast to 27% in Japanese patients, 29% in Korean patients, and 35% in Chinese and Caucasian patients [32, 33].

Kahrizi et al. suggested that the polymorphism occurring at the C148T position of the beta-fibrinogen gene may act as a risk factor for ischemic stroke, specifically for small-vessel disease. This association was observed in a North Indian population [31].

The presence of the allele has been found to elevate the concentration of plasma fibrinogen. Elevated levels of fibrinogen, considered an independent risk factor for cerebral infarction, can lead to atherosclerosis in the small vessels of the brain, consequently causing circulatory disturbances and obstruction in the small cerebral arteries [33, 34].

The findings of this study revealed that individuals with the allele exhibit a higher prevalence of diabetes and hyperlipidemia compared to other patients, although this difference was not statistically significant. Lam et al. reported that diabetes, possibly due to nephropathy, results in increased FGB levels, particularly in patients with the AA genotype, thereby increasing the prothrombotic tendency [33].

Additionally, various studies have indicated a correlation between the presence of the allele and an increased risk of hypertension and hyperlipidemia [33]. Martiskainen et al. also demonstrated that carriers of the allele are more susceptible to lacunar infarction, and the risk of ischemic stroke is heightened in hypertensive patients and smokers, as these factors are considered the most significant risk factors for stroke [35]. The differences in the results of the studies appear to be influenced by the differences in study design, as well as by the unadjusted analysis of risk factor variables.

Conclusion

Thrombophilia Thrombophilia risk groups were categorized into high-risk and low-risk groups. There was no significant association between demographic characteristics, including age, sex, smoking, preexisting comorbidities, and thrombophilia. It is suggested to conduct studies with larger sample sizes to investigate gene-environment and gene-gene interactions.

Ethical Considerations

Compliance with ethical guidelines

The study was approved by the Ethics Committee of Qom University of Medical Sciences (Code: IR.MUQ.REC.1395.122).

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Authors' contributions

All authors equally contributed to preparing this article.

Conflict of interest

The authors declared no conflict of interest.

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