Effect of Genetic Factors on the Etiopathogenesis of Thrombosed Hemorrhoidal Disease

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Rezumat

Efectul factorilor genetici în etiopatogenia trombozei hemoroidale

Obiectiv: Scopul acestui studiu este de a investiga dacă factorii genetici care cresc riscul trombotic, joacă de asemenea un rol în etiopatogenia bolii hemoroidale trombotice (BHT).

Metode: Prezența eventualelor mutații ale factorului V Leiden, protrombinei G20210A, metilentetrahidrofolat reductazei C677T, și respectiv metilentetrahidrofolat reductazei A1298C a fost testată în materialul genomic prelevat de la pacienți cu boala hemoroidală trombozată.

Rezultate: Nu au fost depistate diferențe semnificative din punct de vedere statistic în ceea ce privește frecvența allelelor mutante la nivelul genelor menționate precedent ($p > 0.05$). Mai mult decât atât, nu au fost decelate diferențe semnificative statistic din punct de vedere al genotipului (mutații hetero- sau homozigote) factorului V Leiden, protrombinei G20210A, metilentetrahidrofolat reductazei C677T și respectiv A1298C între pacienții cu BHT și grupul de control ($p > 0.05$).

Concluzii: Studiul nostru indică faptul că mutațiile asociate cu tromboembolismul venos nu par să joace un rol în etiopatogenia BHT. Cu toate acestea, multipli factori mecanici, hemodinamici cât și cei care țin de interacțiunea cu mediul înconjurător pot contribui la etiopatogenia bolii hemoroidale.

Cuvinte cheie: hemoroizi, genetic umană, factor V Leiden, protrombină, protein umană MTHFR, mutație

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Abstract

Objective: The aim of this study is to investigate whether genetic factors known to increase thrombosis risk play a role in the etiopathogenesis of thrombosed hemorrhoidal disease.

Methods: Genomic DNA from patients with thrombosed hemorrhoidal disease was analyzed for the presence of factor V Leiden, prothrombin G20210A, methylenetetrahydrofolate reductase C677T, and methylenetetrahydrofolate reductase A1298C mutations.

Results: No significant differences were found in the allele frequencies of factor V Leiden, prothrombin G20210A, methylenetetrahydrofolate reductase C677T, and methylenetetrahydrofolate reductase A1298C mutations between patients with thrombosed hemorrhoidal disease and controls ($p>0.05$). Moreover, there were no significant differences in the genotype (heterozygous and homozygous mutations) of factor V Leiden, prothrombin G20210A, methylenetetrahydrofolate reductase C677T and A1298C mutations between patients with thrombosed hemorrhoidal disease and controls ($p>0.05$).

Conclusions: Our findings indicate that mutations associated with venous thromboembolism do not play a role in the etiopathogenesis of thrombosed hemorrhoidal disease; however, several environmental, mechanical, and hemodynamic factors may contribute to the etiopathogenesis of hemorrhoidal disease.

Key words: hemorrhoids, human genetics, factor V Leiden, prothrombin, MTHFR protein, human, mutation

Introduction

Hemorrhoids are very common disease of the anorectal region. They are defined as symptomatic growth and / or distal displacement of anal cushions (1,2). The anal cushions are formed by loose connective tissue, smooth muscle, arterial and venous vessels (3). Hemorrhoidal disease is very common in the Western world, England and the United States (4-6).

Hyperhomocysteinemia, a disorder of methionine metabolism, is suggested to represent a modifiable risk factor for myocardial infarction, peripheral arterial thrombosis, deep vein thrombosis, and pulmonary embolism (7). A large number of previous studies have linked arterial disease to an increased homocysteine level; in addition, a few controversial studies have suggested a relationship between homocysteine levels and venous thrombosis (8). It has been reported that hyperhomocysteinemia represents a risk factor for venous thrombosis. Although the link between hyperhomocysteinemia and venous thrombosis is still debated (9), several case-control studies

and meta-analyses have shown that the prevalence of homocysteinemia is higher in patients with venous thrombosis (10). Mild-to-moderate hyperhomocysteinemia may result from relative deficiencies of folic acid and vitamin B12 and homozygosity for the common polymorphism in the methylenetetrahydrofolate reductase (MTHFR) C677T gene (11). Increased homocysteine levels exert toxic effects on vascular structures (12). Therefore, hyperhomocysteinemia has been associated with numerous disorders such as cardiovascular disease, stroke, and venous thrombosis. The MTHFR C677T polymorphism is the most commonly encountered genetic anomaly related to hyperhomocysteinemia. This polymorphism is linked to reduced enzyme levels and an associated increase in homocysteine levels (13). The association of the MTHFR C677T polymorphism with folate levels is controversial (14). Li et al. demonstrated that heterozygous and homozygous MTHFR A1298C mutations were associated with higher folate levels, suggesting that the MTHFR may represent a protective factor that reduces the risk of folate deficiency (15).

Genetic factors result in a hereditary predisposition to venous thromboembolism (16). In addition, hyperhomocysteinemia represents a risk factor for thrombosis. Folic acid deficiency may lead to increased serum levels of homocysteine. A mutation in the MTHFR gene causes folic acid deficiency, which in turn leads to an increased risk of hyperhomocysteinemia and thrombosis (17). Prothrombin, a serine protease thrombin precursor, is a key enzyme in hemostasis. The heterozygous state of a single guanine for adenine substitution at nucleotide 20,210 of the prothrombin-encoding gene that is not subjected to 3' translation (20210A) (factor II: FII) is a risk factor for thrombosis (18). A mutation in the factor V Leiden gene that involves a guanine to adenine nucleotide change results in the resistance of Factor V to inactivation by Protein C, leading to a procoagulant state (19,20).

According to the widely accepted theory, If the supporting tissues of the anal cushions are damaged, they cause hemorrhoids (21). Hemorrhoids develop as a result of abnormally downward displacement of anal cushions, which causes venous dilatation. In hemorrhoid patients, the anal cushions show important pathological changes, for instance abnormal venous dilatation, vascular thrombosis, distortion and rupture of the anal subepithelial muscle. In addition to the examples, a severe inflammatory reaction involving the vascular wall and surrounding connective tissue was demonstrated in hemorrhoidal specimens (22).

Other contributors to the etiopathology of hemorrhoids include genetic factors and venous dilatation and distention (23). One or more of the above mentioned mechanisms, in combination with predisposing genetic factors, may play a role in the etiopathogenesis of the disease.

In this study, we aimed to investigate the frequency of overall mutations and the frequency of heterozygous vs. homozygous alleles independently, and to assess the role of these genetic factors in the etiopathogenesis of thrombosed hemorrhoidal disease.

Methods

Our study group included 125 patients aged between 29 and 65 years (mean age: 41.12 ± 8.12 years) who underwent colonoscopic examination in 2014 and were diagnosed with thrombosed hemorrhoidal disease (thrombosed grade IV internal hemorrhoids and thrombosed external hemorrhoids). Patients who were pregnant were excluded from the study. Controls were selected from the general population. Individuals with a history of thrombosis or vascular disorders, as well as those with family history of such disorders, were excluded from the control group. The study received approval from the local ethics committee of Haydarpaşa Numune Training and Research Hospital and conformed with the declaration of Helsinki. We declared that written informed consent was obtained from all patients who were enrolled in this study.

Blood samples were drawn from patients into EDTA-containing tubes. Genomic DNA was extracted from leukocytes using a Magrev® 24 Manual Magnetic Bead Nucleic Acid Extraction Standfor 24 Samples & PCR Setup Robot and Magrev® DNA Blood Mini Kit (AEDAA00044) (Anatolia Geneworks).

Genomic DNA was analyzed for the presence of factor V Leiden, prothrombin G20210A, MTHFR C677T, and MTHFR A1298C mutations using Bosphore Kits and Real-Time PCR (Montania 483 Anatolia, Turkey). Our control group included 197 individuals without hemorrhoidal disease, who did not have any of the above mentioned mutations.

We used the following commercial kits: Bosphore Prothrombin Detection Kit v1, Bosphore FVL Detection Kit v1, Bosphore MTHFR C677T Detection Kit v1 and Bosphore MTHFR A1298C Detection Kit v1 (Anatolia Geneworks, Turkey); these kits employ Real-Time PCR method based on dual labeled hydrolysis probes to achieve allelic discrimination.

FAM and HEX fluorescent dyes were used for detection of the mutant and wild type alleles respectively, using the Montania 4896

Real-Time PCR Instrument (Anatolia Geneworks, Turkey). The thermal protocols were applied according to the instructions of the kit manufacturer. Thermal protocol was the same for all the kits except Bosphore MTHFR A1298C Detection Kit v1: an initial denaturation of 95°C for 14:30 minutes, followed by 35 cycles of: denaturation at 95°C for 30 seconds, annealing and synthesis/ fluorescent data collection step at 64°C for 30 seconds, followed by a final hold at 22°C for 5 minutes. For the MTHFR A1298C Detection Kit v1, the thermal protocol was follows: an initial denaturation of 95°C for 14:30 minutes, followed by 35 cycles of: denaturation at 95°C for 30 seconds, annealing and synthesis/ fluorescent data collection step at 60°C for 30 seconds, followed by a final hold at 22°C for 5 minutes.

Factor V Leiden mutations were analyzed by Real-Time PCR using the Bosphore® FVL Detection Kit v1 (FVLHA000229) (Anatolia Geneworks®), which detects the G1691A mutation in the F5 gene by using dual-labeled fluorescent hydrolysis probes.

The prothrombin G20210A mutation was detected in the FII gene by Real-Time PCR using the Bosphore® Prothrombin Detection Kit v1 (PROHA000201) (Anatolia Geneworks®) with dual-labeled fluorescent hydrolysis probes.

The MTHFR C677T mutation was detected by Real-Time PCR using the Bosphore® MTHFR C677T Detection Kit v1 (677HA000248) (Anatolia Geneworks®) with dual-labeled fluorescent hydrolysis probes.

The MTHFR A1298C mutation was detected by Real-Time PCR using the Bosphore® MTHFR A1298C Detection Kit v1 (129HA000179)

(Anatolia Geneworks®) with dual-labeled fluorescent hydrolysis probes.

Montania® 4896 (Anatolia Geneworks®) was used as the Real-Time PCR instrument.

Statistical analyses were performed using the SHEsis (24) software package. The heterozygous and homozygous genotype frequencies were determined for the factor V Leiden, prothrombin G20210A (FII), MTHFR C677T, and MTHFR A1298C mutations. Chi-Square test was used for inter-group comparisons. A *p* value of less than 0.05 was considered to represent statistical significance.

Results

The distribution of allele frequencies for the factor V Leiden, prothrombin G20210A (FII), MTHFR C677T, and MTHFR A1298C mutations in patients with thrombosed hemorrhoidal disease is shown in *Table 1*. There were no significant differences in the allele frequencies of factor V Leiden, prothrombin G20210A (FII), MTHFR C677T, and MTHFR A1298C mutations between patients with thrombosed hemorrhoidal disease and controls (*p*>0.05).

Table 2 shows the distribution of patients with thrombosed hemorrhoidal disease that were heterozygous or homozygous for factor V Leiden, prothrombin G20210A (FII), MTHFR C677T, and MTHFR A1298C mutations. No significant difference was found between patients with thrombosed hemorrhoidal disease and controls with respect to factor V Leiden, prothrombin G20210A (FII), and MTHFR C677T heterozygous and homozygous mutations (*p*>0.05).

Table 1. Distribution of allele frequencies for factor V Leiden (FVL), prothrombin G20210A (FII), MTHFR C677T, and MTHFR A1298C mutations in patients with thrombosed hemorrhoidal disease

Allele	Patient		Control		p value
	Normal	Mutant	Normal	Mutant	
FVL	241 (96%)	9 (4%)	388 (98%)	6 (2%)	0.088597
FII	250 (100%)	0 (0%)	391 (99%)	3 (1%)	0.166752
MTHFR C677T	189 (76%)	61 (24%)	313 (79%)	81 (21%)	0.251855
MTHFR A1298C	155 (62%)	95 (38%)	263 (67%)	131 (33%)	0.218306

p < 0.05 was considered to represent statistical significance

Table 2. Distribution of the genotype of patients with thrombosed hemorrhoidal disease who were heterozygous or homozygous for factor V Leiden (FVL), prothrombin G20210A (FII), MTHFR C677T, and MTHFR A1298C mutations

Genotype	Patient			Control			p value
	Normal	Heterozygous	Homozygous	Normal	Heterozygous	Homozygous	
FVL	116 (93%)	9 (7%)	0 (0%)	191 (97%)	6 (3%)	0 (0%)	0.084796
FII	125 (100%)	0 (0%)	0 (0%)	194 (99%)	3 (1%)	0 (0%)	0.165758
MTHFR C677T	68 (54%)	53 (43%)	4 (3%)	121 (61%)	71 (36%)	5 (3%)	0.457241
MTHFR A1298C	42 (34%)	71 (57%)	12 (9%)	73 (37%)	117 (59%)	7 (4%)	0.078875

p < 0.05 was considered to represent statistical significance

Discussion

The roles of factor V Leiden, FII, and MTHFR gene mutations in deep vein thrombosis, pulmonary embolism, cerebrovascular events, and similar thromboembolisms are well established. However, to our knowledge, there are no studies supporting the role of thrombosis and embolism in the pathogenesis of hemorrhoidal disease. The present study was performed to examine whether genetic risk factors for thrombophilia are linked with the etiopathology of hemorrhoidal disease.

In our study, the difference in allele frequencies of MTHFR C677T and MTHFR A1298C mutations between patients and controls were not statistically significant ($p > 0.05$). We hypothesized that, despite the lack of statistically significant differences in terms of allele frequency, differences between patients and controls may be observed based on their genotype; therefore, we additionally compared the two groups based on homozygosity and heterozygosity. No significant differences between the patients and healthy controls were observed with regard to heterozygous and homozygous MTHFR C677T mutations ($p > 0.05$). Our data indicate that the MTHFR C677T mutation, which is associated with an increased risk of venous thrombosis, was not linked with the development of thrombosed hemorrhoidal disease in the population studied. Factor V Leiden and prothrombin gene heterozygosity, which result in systemic hypercoagulability, represent major risk factors for venous thrombosis. Factor V Leiden mutation is associated with resistance of factor V to inactivation by protein C, leading to a procoagulant

state (19,20); 5% of the Caucasian race harbor are heterozygous for the Factor V Leiden mutation (25). We found that the allele frequency of this mutation did not differ significantly between the patient and control groups ($p > 0.05$). In addition, no significant differences were observed between the two groups in terms of heterozygosity or homozygosity for Factor V Leiden mutations ($p > 0.05$). Our data indicate that the Factor V Leiden mutation, which is associated with an increased risk of venous thrombosis, was not linked with the development of thrombosed hemorrhoidal disease in the population studied. The prothrombin gene mutation involves a guanine to adenine change in the 20,210th nucleotide of the FII gene, resulting in a procoagulant state that is associated with higher plasma prothrombin levels (26). The prothrombin gene mutation is similar to the Factor V Leiden mutation in that it is frequently observed in the Caucasian race (prevalence range: 1-4%) (27) and associated with thromboembolism (28-30). In the present study, there was no significant difference between the patient population and the healthy control population with respect to the frequency of this allele ($p > 0.05$). Furthermore, there were no significant differences between the patients and healthy controls with regard to heterozygosity and homozygosity for this mutation ($p > 0.05$). Our data indicate that the prothrombin gene mutation was not linked with the development of thrombosed hemorrhoidal disease in the population studied.

Taken together, our findings indicate that the mutations in the four genes studied are not implicated in the etiopathogenesis of throm-

bosed hemorrhoidal disease. We suggest that other environmental, mechanical, and hemodynamic factors, such as nutritional and defecation habits, working environment, and other behaviors, may contribute to the etiopathogenesis of hemorrhoidal disease. As hemorrhoidal disease is a vascular disorder, thromboembolism may additionally contribute to the abovementioned factors.

Conflicts of Interest: None declared.

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