Inherited Risk Factors in Femoropopliteal Peripheral Arterial Occlusive Disease Patients Under 55 Years

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Abstract

Purpose: We aimed in this study to examine the presence and frequency of genetic polymorphisms in individuals less than 55 years of age with significant peripheral arterial disease in the femoropopliteal arterial system and to report on their association with the disease.

Material and methods: This cross-sectional study undertaken between 2013 and 2014 included a total of 32 peripheral arterial disease patients (30 male, mean age 47.34±4.62 years) were included. Less than 55 years of age who had significant femoropopliteal stenosis/occlusion (>50%) as documented by a computed tomography arteriography and who had a complete set of thrombophilia marker and genetic risk factor analysis results.

Results: A total of 32 patients were investigated for genetic risk factors and thrombophilia markers. Of the 32 patients 7 (21.8%) had factor V Leiden mutation (7 heterozygous), 2 (6.2%) had prothrombin gene mutation, and 13 (40.6%) had methylenetetrahydrofolate reductase C677T gene mutation (3 homozygous and 10 heterozygous). Three patients had both legs involved, while 18 and 11 patients had involvement on the right and left side only, respectively.

Conclusion: Due to the presence of an association between risk factors for hereditary thrombophilia and thrombotic and occlusive events, which lead to increased failure rates in operations and interventions for peripheral arterial disease, we believe that thrombophilic markers and genetic risk factors should certainly be assessed particularly in younger patients and/or patients undergoing revascularization procedures to improve success and patency rates and to minimize thrombotic and occlusive complications.

ABBREVIATIONS

PAD: Peripheral Arterial Disease; CLI: Critical Limb Ischemia; ABI: Ankle Brachial Index; CTA: Computed Tomography Arteriography; PCR: Polymerase Chain Reaction; FVL: Factor V Leiden; MTHFR: Methylene tetrahydrofolate Reductase; PT: Prothrombin; VTE: Venous Thromboembolism.

INTRODUCTION

Atherosclerotic peripheral arterial disease (PAD), an entity with poor long-term prognosis affecting nearly >20% of the individuals over 55 years of age and reflecting the presence of systemic atherosclerosis, is a systemic condition characterized by narrowing and obstruction of the arteries, particularly those of the lower extremities, and is the result of an interplay between a multiple of risk factors [1-3]. Atherosclerosis plays an essential role in the development of PAD, which is generally an asymptomatic condition associated with increased risk of cardiovascular disease, and the most frequent clinical manifestation is represented by intermittent claudication, with the changes ankle-brachial index (ABI) being an important diagnostic parameter. The disease process generally involves the aortic bifurcation as well as femoral and popliteal arteries, and may lead to critical limb ischemia (CLI) [1]. The major risk factors for PAD include smoking, hypertension, diabetes mellitus, hyperlipidemia, impaired renal functions, advanced age, positive family history, and the role of certain inflammatory markers and hemostatic factors in the development of this condition have
been the subject of several studies [2,4]. PAD prevalence exhibits racial and ethnic differences and Afro-Americans represent the highest risk group in the US [5,6], suggesting a role for genetic factors. On the other hand, the link between atherosclerosis and the predisposition for thrombophilia has not been clearly elucidated [2], and some of the genetic polymorphisms and certain hemostatic factor disorders known to play a role in the etiology of deep venous thrombosis are also thought to play a role in arterial thrombosis and in early failures following arterial reconstructions; also, some recent publications reported an association between PAD and certain thrombophilic factors [2,5-7].

Additionally, a meta-analysis found a significant association between arterial thrombotic events and thrombophilic disorders, suggesting an important role of thrombosis in the pathogenesis of atherosclerotic complications [4]. Again, others pointed out to a potential role of genes in the occurrence of PAD despite the fact that only very few genetic variations could be associated with PAD and efforts to identify certain genetic factors had only limited success [5,7].

The objective of the present study was to examine the presence and frequency of genetic polymorphisms in individuals less than 55 years of age with significant PAD in the femoropopliteal arterial system and to report on their association with the disease.

MATERIALS AND METHODS

In this study, patients less than 55 years of age who attended and were followed-up at the Cardiovascular Surgery Unit of our hospital between 2013 and 2014 with significant stenotic or occlusive femoropopliteal PAD were examined in the light of existing literature data and with respect to history, physical examination, laboratory, and digital patient file data. The study protocol was approved by the local ethics committee (2014/83.11.6.987-134). This cross-sectional study undertaken between 2013 and 2014 included a total of 32 PAD patients less than 55 years of age who had significant femoropopliteal stenosis / occlusion (> 50%) as documented by a computed tomography angiography (CTA) and who had a complete set of thrombophilia marker and genetic risk factor analysis results. A systematic review of patient history and physical examination findings was carried out to document demographic risk factors and patient characteristics. The study only included PAD patients with significant femoropopliteal stenosis or occlusion who were less than 55 years of age, in order to better define the effect of hereditary risk factors (SS. Daskalopoulou et al) [3] and care was exercised to include patients from similar ethnic backgrounds. Patients with a past or current history of venous thromboembolism (VTE), genetic disorders, neoplasia, vasculitis, Buerger disease, or acute arterial thromboembolic disease were excluded.

Of the 68 patients with femoropopliteal PAD followed-up at our unit during the study period, 32 were eligible for inclusion. Routine work-up included a complete blood count, renal and hepatic function tests, arterial Doppler ultrasound examination of both lower extremities, and chest x-ray. Also, femoropopliteal arterial stenotic/occlusive lesions were assessed through an aorto-femoro-popliteal CTA. Blood samples obtained for thrombophilia tests were analyzed at a contracted private laboratory service provider for molecular genetics and diagnostic testing. The tests for thrombophilia included DNA isolation, gel electrophoresis, multiplex polymerase chain reaction (PCR) and point mutation analyses, as well as Protein C, S and anti-thrombin-III activity assays.

Descriptive data were expressed as the number of patients due to the fact that the total sample contained less than 100 individuals. No other statistical analyses were performed in this descriptive study.

RESULTS

Demographic characteristics of the patients are shown in (Table 1). Three patients had both legs involved, while 18 and 11 patients had involvement on the right and left side only, respectively. The distribution of each thrombophilia markers and risk factor analyses is shown in (Figure 1), and the result of genetic analyses is shown in (Figures 2).

Of the 32 patients 7 (21.8%) had factor V Leiden (FVL) mutation (7 heterozygous), 2 (6.2%) had prothrombin (PT) gene mutation, and 13 (40.6%) had methylenetetrahydrofolate reductase (MTHFR) C677T gene mutation (3 homozygous and 10 heterozygous). Of the 7 patients with FVL mutations, 2 had MTHFR C677T mutation (3 homozygous), 2 (6.2%) had prothrombin (PT) gene mutation, and 13 (40.6%) had methylenetetrahydrofolate reductase (MTHFR) C677T gene mutation (3 homozygous and 10 heterozygous). Of the 7 patients with FVL mutations, 2 had MTHFR C677T mutation as well. Thus, a total of 24 patients (75%) have been found to carry at least one hereditary risk factor and 8 (25%) had none of the genetic risk factors examined in this study.

Table 1: Patients demographics.

<table>
<thead>
<tr>
<th>Variable</th>
<th>n (%)</th>
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<tbody>
<tr>
<td>Age (year)</td>
<td>47.34±4.6</td>
</tr>
<tr>
<td>Male patients</td>
<td>30 (%93.75)</td>
</tr>
<tr>
<td>Hypertension</td>
<td>15 (%46.87)</td>
</tr>
<tr>
<td>Diabetes Mellitus</td>
<td>12 (%37.50)</td>
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<tr>
<td>Coronary Artery Disease</td>
<td>8 (%25.0)</td>
</tr>
<tr>
<td>Chronic Obstructive Lung Disease</td>
<td>6 (%18.75)</td>
</tr>
<tr>
<td>Smoking</td>
<td>19 (%59.37)</td>
</tr>
<tr>
<td>Rest pain</td>
<td>6 (%18.75)</td>
</tr>
<tr>
<td>Intermittan cludication</td>
<td>32</td>
</tr>
<tr>
<td>Average walking distance (meter)</td>
<td>150</td>
</tr>
<tr>
<td>Surgery</td>
<td>11 (%34.37)</td>
</tr>
<tr>
<td>Endovascular intervention</td>
<td>14 (%43.75)</td>
</tr>
</tbody>
</table>
Central subjects with PAD was 5.7% vs. 0.7% in controls [1,6]. Again, and more recently, an association with CLI has also been reported; associated with progression of femoral arterial atherosclerosis, similar to other studies involving PAD patients (i.e. 26.4%) [6].

The reported frequency of FVL mutations in Turkey is significantly higher compared to a normal Turkish sample and is 9 to 10% [8], as compared to 21.8% in our sample. This figure is PAD [1,6]. The reported frequency of FVL mutations in Turkey is similar to other studies reporting no link between FVL and intermittent claudication, only 1 patient was found to have stenosis at 1 month and 1 year was higher among those with FVL [11]. On the other hand, in another study examining 116 patients with intermittent claudication, only 1 patient was found to have FVL, similar to other studies reporting no link between FVL and PAD [1,6]. The reported frequency of FVL mutations in Turkey is 9 to 10% [8], as compared to 21.8% in our sample. This figure is significantly higher compared to a normal Turkish sample and is similar to other studies involving PAD patients (i.e. 26.4%) [6].

PTG polymorphisms have been previously suggested to be associated with progression of femoral arterial atherosclerosis, and more recently, an association with CLI has also been reported; the reported prevalence of PT gene polymorphisms among subjects with PAD was 5.7% vs. 0.7% in controls [1,6]. Again, in a study by Gerdes et al. PT gene mutations were reported to be linked with increased femoral intimal thickness and in the emergence of acute ischemic events in individuals with significant atherosclerotic disease [4]. However, studies specifically looking at the association between prothrombin gene mutation and PAD are scarce in number, and in an Italian study, a higher prevalence of PT gene polymorphism was detected in PAD patients than in controls, despite the absence of correlation between the stage of PAD and this increased prevalence; in another study authors suggested a limited role for the frequency of PT gene mutation in the etiology of PAD [4,6]. In our study, 6.2% of our PAD patients had PTG polymorphisms, consistent with previous reports.

Hyperhomocysteinemia is an important cardiovascular risk factor predisposing individuals' to arterial atherosclerosis that can be corrected with folic acid treatment; it has recently gained increasing attention due to fact that it can cause PAD through endothelial injury, and a strong association between MTHFR 677CT gene polymorphism and an increased risk of vascular disease in carriers of MTHFR 677CT polymorphism have been suggested [6, 12]. In a Canadian study by Pollex RL et al. involving Type 2 diabetic patients, an increased risk of PAD was found among carriers of MTHFR 677CT gene polymorphism, although there was no significant association between the stage of PAD and gene polymorphism, and as compared to other traditional risk factors, MTHFR C677T gene mutation emerged as the most significant risk factor for PAD [7]. Again in a study from Macedonia by Spiroski et al. 64.5% of PAD patients had MTHFR C667T gene polymorphism (heterozygous 51.3%, homozygous 13.2%), which was higher than the figure detected in healthy individuals, i.e. 57.8% (heterozygous 44.6%, homozygous 13.2%) [13]. In a Turkish population, 20.0% to 34.9% of the individuals had MTHFR C677T gene mutation [10]. In our study, 40.6% of the patients had this mutation (31.2% heterozygous, 9.3% homozygous), higher than that observed in normal populations consistent with previous reports.

Protein C deficiency, a known risk factor for venous thrombosis, has a less clear role in arterial diseases and in some studies examining the protein C deficiency in PAD patients reported prevalence between 2 and 15% [6]. In the current study, 4 patients (12.5%) had Protein C deficiency, consistent with the reported figures in the literature.

Protein S deficiency, a cause of venous thrombosis, is estimated to have a prevalence of 0.7% in the general population, with only limited number of small studies examining Protein S deficiency in PAD patients. Although Protein S deficiency is more common in Pad patients, its exact importance is unknown, and literature data suggests that 15 to 20% of Pad patients may have Protein S deficiency [6,14]. The observed figure in our study (21.8%) was significantly higher than that previously reported for the general population.

Limitations of our study include the small sample size, absence of a control group, and single-center cohort design. Consistent with previous reports, most of our PAD patients (75%) had at least one of the hereditary thrombophilia risk factors tested, and presence of at least one genetic risk factor seemed to increase the risk of PAD. Due to the presence of an association between risk factors for hereditary thrombophilia
and thrombotic and occlusive events, which lead to increased failure rates in operations and interventions for PAD, we believe that thrombophilic markers and genetic risk factors should certainly be assessed particularly in younger patients and/or patients undergoing revascularization procedures to improve success and patency rates and to minimize thrombotic and occlusive complications.

REFERENCES


