Hemodialysis vascular access thrombosis: The role of factor V Leiden, prothrombin gene mutation and ABO blood groups

Danyelle R.A. Rios a, Ana P. Fernandes a, Maria G. Carvalho a, Roberta C. Figueiredo b, Daniela A.M. Guimarães a, Daniberg R. Reis a, Ana C. Simões e Silva c, Karina B. Gomes a, Luci M.S. Dusse a,⁎

a Department of Clinical and Toxicological Analysis, Faculty of Pharmacy, Federal University of Minas Gerais, Brazil
b Department of Public Health, Faculty of Medicine, Federal University of Minas Gerais, Brazil
c Department of Pediatrics, Division of Pediatric Nephrology, Faculty of Medicine, Federal University of Minas Gerais, Brazil

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A B S T R A C T

Background: Vascular access thrombosis increases morbidity in hemodialysis (HD) patients. The aim of this study was to investigate the association between HD vascular access thrombosis and mutations in the prothrombin and factor V Leiden (FV) genes and ABO blood system.

Methods: This cross-sectional study included 195 patients with end stage renal disease (ESRD) on HD for more than six months. HD patients were allocated into two groups according to the occurrence (cases, N=46) or not (controls, N=149) of previous vascular access thrombosis. FV and prothrombin gene mutations were investigated by polymerase chain reaction and ABO blood group phenotyping was performed by the indirect technique. Univariate analysis detected the variables with a trend to be associated with thrombosis and was followed by multivariate analysis to define independent predictors of vascular access thrombosis.

Results: FV Leiden mutation and ABO blood group were not associated with vascular access thrombosis, whereas G20210A mutation in the prothrombin gene was significantly higher in patients with vascular access thrombosis and independently associated with this complication (OR=12.0; CI 95%=1.8–83.5; p=0.012).

Conclusions: G20210A mutation emerges as an important genetic factor predisposing to vascular access thrombosis. The definition of risk factors for thrombosis will certainly enable a rational approach for HD patients.

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1. Introduction

Hemodialysis (HD) requires a well-functioning vascular access that allows sufficient blood flow to achieve adequate clearance and blood dialysis. Vascular access complications increase morbidity and contribute to 20–25% of all hospitalizations in dialysis patients, of which approximately 85% of cases is due to thrombosis [1,2].

ESRD as well as HD itself may actually increase the risk of thrombosis. Among other factors, the increasing thrombotic trend in patients under HD is due to platelets and clotting factors activation [3–5]. It is believed that almost all cases of thrombotic episodes in HD patients are associated to a reduction in vascular access blood flow due to fibromuscular and intimal hyperplasia, which may result in vascular access stenosis. The blood flow reduction causes blood stasis and favors hypercoagulability, hypotension and hypovolemia, predisposing to a prothrombotic environment [6,7]. Although thrombotic episodes are primarily related to mechanical or surgical events, the genetic background of the patient may also significantly contribute to the occurrence of vascular access thrombosis. In this context, it should be highlighted that some patients with very similar risk factors for thrombosis relative to HD procedure do not experience vascular access thrombosis, whereas others do.

Thrombophilia results from either inherited or acquired factors, which predispose to thrombosis [8] and it has been proposed as a possible cause of the vascular access thrombosis [9–11]. Among the mutations associated with thromboembolism, mutations in the factor V (FV) and prothrombin genes have been reported as the most frequent genetic determinants [12,13]. Resistance to activated protein C (APC) is regarded as an important coagulation abnormality associated with venous thrombosis. Most of the cases of APC resistance results of a missense mutation induced by the replacement of guanine by adenine at nucleotide 1691 in exon 10 of the FV gene (G1691A)-rs6025, known as FV Leiden. The resulting point mutation at nucleotide 1691 causes the replacement of arginine to glutamine at amino acid 506, located at one of the sites where FV is recognized, cleaved, and inactivated by the APC. In consequence, FVa is not enough inactivated, which in turn predisposes thrombosis [14].

The replacement of guanine by adenine at nucleotide 20210 in the 3′ untranslated region of the prothrombin gene (G20210A)-rs1799963,
before poli(A) tail fixation site, is another common molecular
determinant of inherited thromboembolism. This alteration increases
the messenger RNA stability, resulting in elevated prothrombin plasma
levels that leads to higher thrombin production and, consequently,
increased conversion of fibrinogen into fibrin [12].

Beyond these inherited factors, studies have also demonstrated
association between ABO blood system and venous thromboembolism
(VTE), indicating that individuals of “non O” blood groups (A, B or AB
groups) present an increased VTE risk as compared to O blood group
 carriers [15–18]. The “non O” blood group patients exhibit higher
plasma levels of factor VIII (FVIII) [19,20] and von Willebrand factor
(vWF) [19–21] than those belonging to blood group O. Moreover, it has
been hypothesized that oligosaccharides in vWF are similar to those
present in antigens that correspond to blood groups A and B. The
presence of these structures may affect the clearance of both vWF and
vWF/FVIII complex [21,22], probably by altering the proteolysis of both
vWF by the metalloprotease ADAMTS13 [23]. To our knowledge, no
previous study in the literature has evaluated the association between
ABO blood group and the risk of vascular access thrombosis. Based on
the fact that mutations may differ significantly among ethnic groups,
this is the first study in Brazilian population with the aim to investigate
the association between hemodialysis vascular access thrombosis and
the three thrombophilic disorders (mutations in the prothrombin and
FV genes and ABO blood system).

2. Subjects and methods

2.1. Study design

The present cross-sectional study included all prevalent patients
(N = 195) with ESRD undergoing HD therapy for more than six
months at two dialysis centers in Belo Horizonte/MG, Brazil from 2007
to 2009, according to the exclusion and inclusion criteria.

2.2. Ethical aspects

The Ethics Committee of our institution approved the study and
informed consent was obtained from all participants. The research
protocol did not interfere with any medical recommendations or
prescriptions.

2.3. Exclusion criteria

Patients receiving oral anticoagulation therapy or oral contra-
ceptives, with prior history of arterial or venous thrombosis, except
those with vascular access thrombosis, with acute or chronic hepatic
disease, systemic lupus erythematosus, malignant diseases, vasculitis,
acute infections, history of renal transplantation, HIV positive and
pregnant women were automatically excluded from the study.

2.4. Inclusion criteria

We allocated HD patients with arteriovenous fistula (AVF) into
two groups according to the occurrence or not of previous episode of
vascular access thrombosis. The case group consisted of 46 patients
whose functioning dialysis access had, at least, one previous episode
of thrombotic occlusion, which was defined by the absence of blood
flow and the impossibility to use the access for dialysis. The remaining
149 patients have not experienced this disturbance and were enrolled
as control group.

2.5. Study protocol

All patients required regular HD sessions for 3 to 4 h, three times a
week. Blood flow was usually 300–450 mL/min with a dialysate flow
at a constant rate of 500 mL/min. Patients were dialyzed either with
low-flux polysulphone membranes and high-flow polysulphone
membranes with bicarbonate-buffered dialysate. All patients received
regular doses of standard heparin (100 to 150 UI/kg) before hemo-
dialysis session. A detailed history, clinical variables (age, gender, BMI,
pree-dialysis blood pressure levels, etiology of ESRD, presence of
diabetes or not, type of vascular access, time on hemodialysis, inter-
dialytic weight gain, and main medications in use) and dialysis
parameters (urea reduction ratio and normal protein catabolism rate)
of each included patient were recorded in a computer specific data
bank.

After informed consent, all patients were submitted to blood
sampling for the determination of ABO group phenotype and the
evaluation of mutations in FV and prothrombin genes.

2.6. Blood sampling

Blood samples were drawn in sodium citrate (0.129 mol/L) in 9:1
volume ratio and in EDTA from HD vascular access prior dialysis
procedure in the first dialysis session of the week. Citrated blood
samples were centrifuged at 2500 g for 20 min at 4 °C to obtain
plasma. The blood samples collected in EDTA tubes were submitted
to genomic DNA extraction using the Wizard Genomic DNA Purification
(Promega®). Samples were aliquoted and stored at −70 °C until
analysis.

2.7. FV and prothrombin genes mutations

Mutations were investigated by polymerase chain reaction (PCR-
RLFP) using oligonucleotides, restriction endonucleases and condi-
tions, as previously described [12,24]. Samples were analyzed by
silver stained polyacrylamide gel electrophoresis.

2.8. ABO group

ABO blood group phenotyping was performed by the indirect
technique using citrated plasma.

2.9. Statistical analysis

Statistical comparisons were performed using the program SIGMA
STAT (version 2.03) e STATA (version 10.0). Categorical variables
were compared using χ² or Fisher’s exact test, and continuous
variables were compared by t test or the Mann–Whitney U test, as
appropriate. It was investigated whether the mutations in the
prothrombin and FV genes and ABO blood group were associated
with vascular access thrombosis. The variables that showed a trend
toward statistical significance with vascular access thrombosis on
univariate analysis (P < 0.2) and those considered confounding factors,
such as age, gender, BMI, time on hemodialysis and diabetes were
included in the multiple logistic regression model to identify inde-
pendent predictors of vascular access thrombosis. The multivariate-
adjusted odds ratio (OR) and their 95% confidence intervals (95% CI)
were also calculated. Differences were considered significant when
P < 0.05.

3. Results

Clinical variables and hemodialysis parameters of the patients did
do not differ between cases and controls, as presented in Table 1. Age,
gender, BMI, pree-dialysis blood pressure levels, etiology of ESRD, pre-
sence of diabetes or not, type of vascular access, time on hemodialysis,
interdialytic weight gain, main medications in use and dialysis
parameters were similar in both groups (P > 0.05 for all comparisons,
Table 1).

Among the 46 patients in the case group, four (8.7%) were hetero-
zygous for G20210A mutation in the prothrombin gene, and none had
G1691A mutation in FV gene. In the control group, two (1.3%) were heterozygous for G20210A, and other two (1.3%) were heterozygous for G1691A. None was homozygous for the both mutations (Table 2).

Regarding the ABO blood group distribution, in the case group, 23 (50.0%) were group O, 13 (28.3%) group A, 7 (15.2%) group B and 3 (6.5%) group AB. In the control group, 2 (1.3%) were group O, 13 (28.3%) group A, 7 (15.2%) group B and 3 (6.5%) group AB blood groups were pooled as non-O (Table 3). Patients from the A, B and AB blood groups were pooled as different (P=0.833), as shown in Table 3.

FV Leiden mutation and ABO blood group were not associated with vascular access thrombosis in univariate analysis. On the other hand, there was a significantly higher frequency of G20210A mutation in the prothrombin gene in patients with vascular access thrombosis than in those who did not have this complication (OR: 7.0; CI 95%; 1.1–57.3; P=0.01). Despite not significantly associated with vascular access thrombosis in univariate model, patients with lower predialysis systolic pressure levels and higher interdialytic weight gain exhibited a trend to vascular access thrombosis (P=0.20). Therefore, these variables were included in the multiple logistic regression model along with the presence of G20210A mutation in the prothrombin gene and confounding factors (age, gender, BMI, diabetes and time on hemodialysis). After adjusting, only the prothrombin mutation (G20210A) had a significant and independent association with vascular access thrombosis (OR: 12.0; CI 95%; 1.8–83.5; P=0.012). Indeed, the presence of G20210A mutation in the prothrombin gene increased 12 fold the chance of vascular access thrombosis in HD patients (Table 4).

Table 2

<table>
<thead>
<tr>
<th>Characteristics/parameters</th>
<th>Controls (n=149)</th>
<th>Cases (n=46)</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (years)</td>
<td>52 (39–60)</td>
<td>50 (41–59)</td>
<td>0.979</td>
</tr>
<tr>
<td>Sex</td>
<td></td>
<td></td>
<td>0.100</td>
</tr>
<tr>
<td>Male [n%]</td>
<td>86 (58%)</td>
<td>20 (43%)</td>
<td></td>
</tr>
<tr>
<td>Female [n%]</td>
<td>63 (42%)</td>
<td>26 (57%)</td>
<td></td>
</tr>
<tr>
<td>BMI (kg/m²)</td>
<td>24.2 ± 4.7</td>
<td>24.5 ± 5.7</td>
<td>0.734</td>
</tr>
<tr>
<td>Cause of ESRD [n%]</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Hypertensive nephroclerosis</td>
<td>51 (34%)</td>
<td>14 (30%)</td>
<td>0.633</td>
</tr>
<tr>
<td>Glomerulopathies</td>
<td>39 (26%)</td>
<td>7 (15%)</td>
<td>0.126</td>
</tr>
<tr>
<td>Diabetic nephropathy</td>
<td>21 (14%)</td>
<td>12 (26%)</td>
<td>0.060</td>
</tr>
<tr>
<td>Polycystic kidney disease</td>
<td>7 (5%)</td>
<td>3 (7%)</td>
<td>0.624</td>
</tr>
<tr>
<td>Others or unknowns causes</td>
<td>31 (21%)</td>
<td>10 (22%)</td>
<td>0.892</td>
</tr>
<tr>
<td>Pre-dialysis arterial blood pressure</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Systolic blood pressure (mmHg)</td>
<td>130 (120–143)</td>
<td>140 (130–150)</td>
<td>0.060</td>
</tr>
<tr>
<td>Diastolic blood pressure (mmHg)</td>
<td>80 (80–90)</td>
<td>80 (80–90)</td>
<td>0.968</td>
</tr>
<tr>
<td>Time on hemodialysis (months)</td>
<td>340 (170–90.3)</td>
<td>395 (190–92.0)</td>
<td>0.226</td>
</tr>
<tr>
<td>Type of vascular access</td>
<td></td>
<td></td>
<td>0.131</td>
</tr>
<tr>
<td>Arteriovenous fistula</td>
<td>144 (97%)</td>
<td>42 (91%)</td>
<td></td>
</tr>
<tr>
<td>Arteriovenous graft</td>
<td>5 (3%)</td>
<td>4 (8%)</td>
<td></td>
</tr>
<tr>
<td>Rivaroxaban t i.v.</td>
<td>1.4 (1.3–1.6)</td>
<td>1.5 (1.3–1.6)</td>
<td>0.513</td>
</tr>
<tr>
<td>URR</td>
<td>73.6 ± 5.9</td>
<td>74.0 ± 6.4</td>
<td>0.716</td>
</tr>
<tr>
<td>nPCR (g/kg/day)</td>
<td>1.1 (1.0–1.4)</td>
<td>1.2 (1.0–1.6)</td>
<td>0.451</td>
</tr>
<tr>
<td>IWG (Kg)</td>
<td>3.2 ± 1.1</td>
<td>2.9 ± 1.4</td>
<td>0.165</td>
</tr>
<tr>
<td>Medications [n%]</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Antihypertensive drugs</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>ACE inhibitor</td>
<td>69 (46%)</td>
<td>19 (41%)</td>
<td>0.551</td>
</tr>
<tr>
<td>β-blockers</td>
<td>64 (43%)</td>
<td>21 (46%)</td>
<td>0.747</td>
</tr>
<tr>
<td>Diabetic nephropathy</td>
<td>36 (44%)</td>
<td>17 (37%)</td>
<td>0.423</td>
</tr>
<tr>
<td>Acetylsalicylic acid</td>
<td>38 (26%)</td>
<td>8 (17%)</td>
<td>0.257</td>
</tr>
<tr>
<td>Statins</td>
<td>31 (21%)</td>
<td>5 (11%)</td>
<td>0.129</td>
</tr>
<tr>
<td>Vitamin use</td>
<td>149 (100%)</td>
<td>46 (100%)</td>
<td></td>
</tr>
<tr>
<td>Insulin</td>
<td>29 (19%)</td>
<td>10 (22%)</td>
<td>0.736</td>
</tr>
<tr>
<td>Erythropoietin</td>
<td>129 (87%)</td>
<td>41 (98%)</td>
<td>0.651</td>
</tr>
<tr>
<td>Diabetes [n%]</td>
<td>40 (27%)</td>
<td>15 (33%)</td>
<td>0.448</td>
</tr>
</tbody>
</table>

\*P<0.05. The normally distributed data were expressed as mean±SD (t test). The non-Gaussian data were presented as median (range). (Mann–Whitney test). Frequencies (%) was evaluated by \( \chi^2 \) test. BMI: body mass index; HD: hemodialysis; URR: urea reduction ratio; nPCR: normal protein catabolism rate; IWG: interdialytic weight gain; ACE: angiotensin-converting enzyme.

Table 3

<table>
<thead>
<tr>
<th>Blood group</th>
<th>Controls (n=149)</th>
<th>Cases (n=46)</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>O</td>
<td>75 (50.3%)</td>
<td>23 (50.0%)</td>
<td>0.833</td>
</tr>
<tr>
<td>A</td>
<td>47 (31.6%)</td>
<td>13 (28.3%)</td>
<td>0.726</td>
</tr>
<tr>
<td>B</td>
<td>21 (14.1%)</td>
<td>7 (15.2%)</td>
<td>0.611</td>
</tr>
<tr>
<td>AB</td>
<td>6 (4.0%)</td>
<td>3 (6.5%)</td>
<td>0.381</td>
</tr>
</tbody>
</table>

\*P<0.05. BMI: body mass index; IWG: interdialytic weight gain.

Table 4

<table>
<thead>
<tr>
<th>Variables</th>
<th>Odds ratio</th>
<th>CI 95%</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age</td>
<td>0.99</td>
<td>0.97–1.10</td>
<td>0.986</td>
</tr>
<tr>
<td>Gender</td>
<td>0.50</td>
<td>0.99–1.00</td>
<td>0.083</td>
</tr>
<tr>
<td>BMI</td>
<td>0.97</td>
<td>0.89–1.10</td>
<td>0.501</td>
</tr>
<tr>
<td>Time on hemodialysis (months)</td>
<td>1.00</td>
<td>0.99–1.00</td>
<td>0.702</td>
</tr>
<tr>
<td>IWG</td>
<td>0.99</td>
<td>0.99–1.00</td>
<td>0.467</td>
</tr>
<tr>
<td>Systolic blood pressure</td>
<td>1.02</td>
<td>0.99–1.14</td>
<td>0.080</td>
</tr>
<tr>
<td>Diabetes</td>
<td>1.08</td>
<td>0.43–2.72</td>
<td>0.874</td>
</tr>
<tr>
<td>G20210A mutation</td>
<td>12.0</td>
<td>3.81–83.5</td>
<td>0.012*</td>
</tr>
</tbody>
</table>

\*P<0.05. BMI: body mass index; IWG: interdialytic weight gain.

4. Discussion

The present study was proposed to investigate the possible role of G20210A mutation, FV Leiden and ABO phenotype in predisposing vascular access thrombosis. After adjusting for relevant clinical variables (age, gender, BMI, time on hemodialysis and diabetes) statistical analysis has shown that only the G20210A mutation in the prothrombin gene was independently associated to an increased risk for vascular access thrombosis (OR: 12.0; CI 95%; 1.8–83.5; P=0.012). This mutation has been identified as one of the main risk factors for thrombosis development, especially deep venous thrombosis. In this study, four patients (8.7%) in the case group and two (1.3%) in the control group were detected as heterozygous for G20210A. The reduced number of G20210A carriers found in our study may reflect the low frequency of this mutation in Brazilian population. In a previous study, our group detected a frequency of 1.3% among Brazilian subjects with no previous history of thrombosis [18].

In agreement with the present study, Ataç et al. [9] detected 4 (9%) heterozygous for G20210A among 46 patients under HD with vascular access thrombosis, whereas the mutation was not identified in 44 patients without this complication, suggesting that the G20210A mutation may in fact contribute to vascular access thrombosis. In contrast, other studies did not find a significant association between the presence of G20210A and vascular access thrombosis [10,11,25–27].

FVL constitutes the genetic alteration more frequently associated to venous thrombosis in Caucasian populations by increasing 6.6 times the risk for this event. Although, the association between FV Leiden and arterial thrombosis is still controversial [13] in Brazilian populations.
young patients this association has been reported [28]. In the present study, only two patients were heterozygous for FV Leiden in the control group and none in the case group, suggesting that this mutation is not associated to thrombotic complications in the vascular access. This finding is consonant with other studies that did not also observed association between FV Leiden and vascular access thrombosis [25, 27, 29, 30]. On the other hand, the association between FV Leiden mutation and vascular access thrombosis was previously reported for patients undergoing HD [9–11]. Therefore, larger series of patients are still required to confirm this association, considering the limited sample size and the reduced number of participants with this mutation in this study.

The conflicting results obtained in different studies on the association between both G20210A and FVL and vascular access thrombosis may be explained, at least in part, by ethnic differences of the populations, the inadequate control of other risk factors for thrombosis, as well as the limited sample size assayed in the different studies. The ethnic origin of participants was not considered in the present study, since the Brazilian population is one of the most heterogeneous around the world. This aspect certainly impairs the definition of ethnic origin of the participants of this study based on phenotypic characteristics only [31].

In general, the blood groups distribution for the participants of this study was similar to that obtained for blood donors from the same region, with a higher frequency for group O, followed by groups A, B and AB [32]. The classification of controls and cases into groups O and “non O” (groups A, B and AB included) showed a very similar frequency for both (Table 3). The literature suggests that “non O” subjects have shown an increased predisposition to thrombotic events [15–18]. However, in the present study, this finding was not confirmed, indicating that this factor seems to be not important in predisposing vascular access thrombosis in HD patients.

In spite of the limited number of participants enrolled in this study, our preliminary results suggest that G20210A mutation may be an important genetic factor predisposing to vascular access thrombosis [9]. Hemodialysis per se leads to a number of hemostatic alterations that contribute to a hypercoagulability state in HD patients. Furthermore, fistula surface promotes platelet and fibrinogen adhesion [33], leading to a prothrombotic microenvironment that favors clot formation. Knowing that hyperprothrombinemia is the phenotype associated to G20210A mutation, it is reasonable to suppose that, in patients with an already compromised hemostatic system, the increased prothrombin plasma levels and consequent thrombin generation may culminate with thrombosis at vascular access.

Although the frequency of G20210A mutation in Brazilian general population is lower than that observed for the majority of the European countries [34], in Brazilian patients with venous and arterial thrombosis this mutation has been associated to increased risk for these pathologies, reaching frequencies near 5% in some studies [35]. Our group has observed that G20210A mutation is associated to increased risk of venous thrombosis in young Brazilian patients (OR: 3.36; CI 95%: 1.15–9.84; P = 0.027) [18]. Therefore, is reasonable to expect that presence of G20210A may additionally contribute to trigger the blood clot especially on the fistula surface, since multiple factors interact leading to thrombembolic events and that a contributing for a better understanding of risk factors enrolled in this condition. The definition of risk factors for thrombosis will certainly enable a rational approach of patients under HD regarding the prescription of oral anticoagulants and/or platelets antiagregants.

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References


