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*Clinical Research*

# Risk Factors for Thrombosis Development in Mexican Patients

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**Background:** To identify inherited factors: Protein C (PC), protein S (PS), antithrombin (AT), plasminogen (Plg), the activated PC resistance (APCR), prothrombin (PT) mutation G20210 A (PTG20210 A) and methylenetetrahydrofolate reductase C677 T polymorphism (MTHFR C677 T), as well as acquired-risk factors such as: diabetes mellitus, surgeries, smoking, obesity, hypertension, trauma, alcoholism, family history; and their association, in Mexican patients with diagnostic of thrombophilia.
**Methods:** Overall, 200 patients diagnosed with thrombophilia and 100 healthy controls. Commercial kits were used for the coagulometric tests and polymerase chain reaction, restriction fragment length polymorphism for molecular alterations.
**Results:** Alterations were found with an estimated prevalence to PC 0.65%, AT 2.04% and Plg 2.5%, APCR 2%, PT 20210 2%, and MTHFR 65%. The C677 T polymorphism of the MTHFR did not associate with acquired-risk factors so we can suppose that it is an independent risk factor. For the patients that only presented acquired-risk factors (21 of 200), the association smoking—alcoholism showed to be the cause of thrombosis with high risk. The following were also associated: smoking with AT, PC, and alcoholism; obesity with Plg; smoking with alcoholism, and PS deficiency.
**Conclusions:** Risk factors for both primary and secondary and their association were present as a cause of thrombosis in the patients studied, and the possibility to suffer a recurrent thrombosis.

## INTRODUCTION

Thrombosis pathogenesis includes: (a) changes in vascular wall, (b) venous stasis, and (c) changes in blood composition.[1] Thrombophilia or hypercoagulable state involves various factors either inherited or acquired, that predispose an individual to suffer an arterial or venous occlusive event.[2,3]

There are found, among the inherited factors (or primary): prothrombin gene mutation G20210 A (PT G020210 A), which in European thrombotic population has prevalence from 3.8% to 11.5%[4,5];

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*Ann Vasc Surg 2015; ■: 1–8*
*http://dx.doi.org/10.1016/j.avsg.2015.05.035*

*Manuscript received: October 10, 2014; manuscript accepted: May 23, 2015; published online: ■■■.*

factor V Leiden mutation (FVL), which is related to activated protein C resistance (APCR),[5–7] has a very variable prevalence from 3.8%[4,8–11] to 30%.[5] Polymorphism C677 T in methylenetetrahydrofolate reductase (MTHFR) enzyme is increased in the North and South European population,[12–15] with a prevalence of 58.3% and 57.14%.[15,16] There are other alterations, such as the deficiency in the synthesis of some of the natural anticoagulants like the PC, protein S (PS), antithrombin (AT)[17] and defects in the fibrinolytic system like plasminogen (Plg) deficiency among them. Hereditary thrombophilia is an infrequent entity, and it only of explains the 5–25% of the thrombosis cases that occur in young people.[2,18,19] In some European populations, there has been reported the prevalence for the deficiency of: 3.6% of PC, 21.4% of PS, and 10.7% of AT in Greek people[20]; PC 2.56%, PS 2.55%, and 1.6% in Swiss people[4]; PC 3.19%, PS 7.27%, and AT 0.47% in Spanish people.[21] However, for Plg less than 2% has been described.[22] Among the acquired-risk factors (or secondary) described: diabetes mellitus, surgeries, smoking, obesity, hypertension, trauma, and alcoholism.[18,23–25]

Mexico shares with other countries, a similar problem related to thrombosis; however, it presents important differences, such as the lack of research programs for patients with this disease. That is why the objective of this research was to determine the prevalence of PC, PS, AT, and Plg functional deficiencies, the APCR phenotype, as well as the mutations: PT G20210 A and MTHFR C677 T and the acquired factors associated in Mexican Mestizo individuals with a presumptive diagnosis of primary thrombophilia.

## MATERIAL AND METHODS

In this transversal study conducted over a period of 3 years, 300 Mexican Mestizo participants were recruited: 200 patients with arterial or venous thrombosis (and whose origin can be of primary type), with at least, a confirmed thrombotic episode by phlebography or Doppler ultrasound. These patients were from different participating institutions: Hemostasis and Thrombosis Clinic, Hospital General de México, Medical Research Unit in thrombosis, hemostasis and atherogenesis, Instituto Mexicano del Seguro Social (IMSS), Hemostasis and Thrombosis Clinic, Centro Médico Nacional ''La Raza'' IMSS, and Instituto Nacional de Neurología y Neurocirugía. This latter institution included 50 patients with cerebral vascular event, a fact that could bias the study, regarding the anatomic location, but not to investigate the origin of thrombosis. All the patients were recruited under the following inclusion criteria (criteria described by the International Society on Thrombosis and Hemostasis-ISTH): (1) thrombosis at a younger age than 45; (2) thrombosis familiar history; (3) recurrent thrombosis without apparent precipitating factors; (4) thrombosis in unusual anatomic sites. And those with the following criteria were excluded: pregnant women, patients with systemic lupus erythematosus, and antiphospholipid antibody syndrome. Also 100 unrelated healthy individuals over 18 and less than 45 years were included and used as controls from the following blood banks: Centro Médico Nacional siglo XXI, Instituto Nacional de Rehabilitación, and Hospital General Balbuena.

### Procedure

At each participating institution, all patients underwent hemostasis and thrombosis tests to eliminate the possibility to have a secondary origin (acquired thrombophilia) such as antiphospholipid syndrome and other pathologies. During the medical consultation, information of acquired factors (or secondary) and a blood sample of each patient were taken 3 months after the thrombotic event since the function of AT and Plg decreased in the acute phase of thrombosis, and the patient was not under pharmacologic treatment with coumarin derivatives, because these reduce the activity of PC and PS. Peripheral blood was extracted in 2 tubes, 1 with sodium citrate at 3.8% and the other with EDTA.

The tubes were sent to the Hospital General de México, where the hypercoagulable markers tests were performed; to the plasma obtained from the citrate samples: AT and Plg, through the commercial kit STA®—Stachrom (Roché Diagnostics) specific for each one. PC, PS and APCR, by using the corresponding kit STA®Staclot (Roché Diagnostics). From the blood with EDTA, genomic DNA was extracted with the kit DNAzol®and through specific oligonucleotides were determined the molecular alterations: PT G20210 A and MTHFR C677 T, through the use of PCR-RFLPs.[7,26,27]

### Statistical Analysis

The Kolmogorov–Smirnov test was performed to verify that the distribution of data follows a normal distribution and to determine the use of parametric or nonparametric tests. The following tests were done: to compare these results with the healthy individuals (controls), the Mann–Whitney *U* test was done; and to compare these results with the ones obtained with other populations, the Chi-squared test was done; for the variables association and to