



LEIDEN FACTOR V AND G20210A IN RECURRENT SPONTANEOUS ABORTION AND STILLBIRTH

FATOR V DE LEIDEN E MUTAÇÃO G20210A EM MULHERES COM ABORTO ESPONTÂNEO RECORRENTE E ÓBITO FETAL

Renata ZACCARIA¹

Egle COUTO¹

Ricardo BARINI¹

Joyce Maria ANNICCHINO-BIZZACCHI¹

José Carlos Gama da SILVA¹

João Luiz Pinto e SILVA¹

ABSTRACT

Objective

Fetal losses and other pregnancy complications have been associated with maternal thrombosis. Inherited thrombophilia factors are possible causes for these events. To evaluate the association between inherited thrombophilia and gestational losses.

Methods

A retrospective cohort study included 42 women patients at the Hematology ambulatory, who were carriers of Leiden factor V (Group 1) and 94 women without this factor, patients at the Family Planning ambulatory (Group 2). Personal history of gestational losses and personal and familiar history of thromboembolic disease were investigated on clinical charts and by phone interviews. Descriptive analyses

¹ Departamento de Tocoginecologia, Faculdade de Ciências Médicas, Universidade Estadual de Campinas. Rua Alexander Fleming, 101, 13084-881, Campinas, SP, Brasil. Correspondência para/Correspondence to: R. ZACCARIA. E-mail: <egle@unicamp.br>.

were carried out by using frequency tables, and position and dispersion measurements. To check for associations or to compare proportions, the Chi-square or the Fisher exact tests were used.

Results

There were no statistical differences between studied groups on the occurrence of stillbirth. In Group 1, of 109 pregnancies, 26.6% ended as abortion. In Group 2, of 226 pregnancies, 9.6% ended as abortion ($p < 0.0001$). Women from Group 1 had a higher prevalence of recurrent abortion and familiar history of thromboembolic events ($p < 0.0001$). They were heterozygous Leiden factor V carriers, and three of them were also carriers of heterozygous G20210A mutation in prothrombin gene.

Conclusion

The frequency of spontaneous abortion and recurrent abortion was higher in carriers of inherited thrombophilic factors, and these women showed a higher tendency to personal thromboembolic events.

Indexing terms: spontaneous abortion, Leiden factor V, stillbirth, prothrombin, thrombophilia.

RESUMO

Objetivo

A perda fetal e outras complicações da gravidez foram associadas à trombose materna. Fatores de trombofilia hereditária são as possíveis causas destes eventos. Estudar a associação entre trombofilia hereditária e perda gestacional.

Métodos

Foi realizado um estudo de coorte retrospectiva com controle externo, que incluiu 42 mulheres portadoras do fator V de Leiden, pacientes do Ambulatório de Hematologia (Grupo 1) e 94 mulheres sem este fator, atendidas no Ambulatório de Planejamento Familiar (Grupo 2). Antecedentes pessoais de perdas gestacionais e doença tromboembólica, assim como antecedentes familiares de doença tromboembólica, foram investigados durante consultas médicas e através de entrevistas por telefone. Para a análise descritiva, utilizaram-se tabelas de frequência e medidas de posição e dispersão. Para avaliar as associações, foram utilizados os testes de Qui-quadrado e o Teste Exato de Fisher.

Resultados

Não houve diferença significativa entre os grupos estudados, quanto à ocorrência de natimortalidade. No Grupo 1, de 109 gestações, 26,6% terminaram em aborto. No Grupo 2, de 226 gestações, 9,6% terminaram em aborto ($p < 0,0001$). As mulheres do Grupo 1 apresentaram maior prevalência de aborto recorrente e história familiar de fenômenos tromboembólicos ($p < 0,0001$). Todas eram portadoras do fator V de Leiden heterozigoto; três delas também apresentavam a mutação G20210A heterozigota no gene da protrombina.

Conclusão

A frequência de aborto espontâneo e recorrente foi maior em portadoras de fatores de trombofilia hereditária, mulheres que apresentaram maior tendência aos eventos tromboembólicos.

Termos de indexação: aborto espontâneo, fator V de Leiden, natimortalidade, protrombina, trombofilia.

INTRODUCTION

The association between pregnancy loss and thrombophilia has increasingly become a subject of interest in medical research worldwide. With regard to recurrent abortion, this association has been clearly demonstrated in the literature^{1,2}.

Thrombophilia is defined as a tendency to thrombosis, which may occur in young patients and at unusual sites. Such condition may be classified as either of two types: the acquired or the inherited type. Factors such as anticardiolipin antibody and lupus anticoagulant are prominent in the acquired type of thrombophilia³. Hereditary thrombophilia, a genetically determined propensity to venous or arterial thromboembolism, depends on some interactions for its clinical expression⁴. To date, our knowledge of these interactions is incomplete. In the inherited type of thrombophilia, well-known factors are the deficiencies in protein C, protein S and antithrombin III, as well as the mutations Leiden factor V, G20210A in prothrombin gene, and C677T in methylene tetrahydrofolate reductase gene⁵.

Leiden factor V is a mutation of coagulation factor V. Normally, factor V acts as a prothrombinase cofactor, increasing this enzyme's catalytic efficacy 2000-fold⁴. In 1994, Bertina *et al.*⁶ described Leiden factor V mutation, demonstrating that this genetically determined mechanism of hypercoagulation, resulted in resistance to activated protein C (APC), a natural plasma anticoagulant. Leiden factor V, results from a substitution of arginine with glutamine at position 506, one of APC's cleavage sites. Thus, in the factor V gene, replacing arginine with glutamine at position 1691, results in a partial and slower factor V proteolytic activity⁷.

Leiden factor V is transmitted as an autosomal dominant inheritance⁷. It may be found in 3 to 15% of the general population, in 5% to 15% of the Caucasian population, and in less than 1% of the Black and the Japanese populations. It has been stated that, this high prevalence in the general population suggests a positive genetic selection^{5,8}.

Leiden factor V increases the risk of venous thrombosis seven-fold and is present in 50% of

subjects coming from families carrying thrombophilia caused by unexplained factors. However, the risk for Leiden factor V may be identified early. It is suggested that there is a high tendency to thrombosis in members of families who carry hereditary thrombophilia. Clinical features are important in preventing these events⁴.

A high rate of this mutation was found in women experiencing primary abortion in the second trimester⁹. For adequate development of the maternal-fetal circulation, a balance between the procoagulant and anticoagulant systems in endothelial cells is essential. In placentas from abortions and fetal deaths, in which the hypercoagulable state predominates, it is possible to observe fibrin deposits on the decidua, arterial occlusion, and villous necrosis¹⁰.

In 1996, Poort¹¹ described the G20210A mutation in the prothrombin gene. Prothrombin is a plasma protein, which is converted to thrombin when activated. It is a key enzyme, which is involved in the processes of hemostasis and thrombosis, presenting procoagulant and anticoagulant activities. The G20210 allele is associated with elevated prothrombin levels, a risk factor for thrombosis⁵. This mutation was also found more frequently in women with preeclampsia, abruptio placentae, intrauterine growth retardation, and fetal death¹².

There is little information about hereditary thrombophilia and recurrent abortion in Brazil, so we felt the need to conduct this study. Our aim was to compare the frequency of pregnancy losses and the personal and family history of women who were carriers or non-carriers of Leiden factor V, associated or not with prothrombin gene G20210A mutation.

METHODS

A retrospective cohort study involving two groups of women was conducted to evaluate the association between Leiden factor V, correlated or not with prothrombin gene G20210A mutation, and a history of pregnancy loss.

Group 1 included 42 women attending the Hemostasis Outpatient Facility at *Universidade Estadual de Campinas* (Unicamp), from January 1999 to December 2000. All had a personal or family history of thromboembolism and carried Leiden factor V, associated or not with prothrombin gene G20210A mutation.

Group 2 included 94 women who attended the Family Planning Outpatient Facility at Unicamp and had no personal or family history of thromboembolism. All were known to be non-carriers of both mutations investigated.

Laboratory Technique

Genome DNA was extracted from peripheral blood by a standard method¹³. Polymerase chain reaction (PCR) was used to assay the Leiden factor V mutation and the prothrombin gene G20210A mutation. For Leiden factor V, amplification was performed using a mixture of 54mM Tris HCl, pH 8.8, 5.4mM MgCl₂, 5.4μM EDTA, 13.3mM (NH₄)₂SO₄, 8% DMSO, βmM β-mercaptoethanol, 0.4mg/mL BSA, 0.8mM of each nucleoside triphosphate, 400ng of each forward and reverse primer, 500ng of genome DNA and 2U Taq polymerase. Amplification was performed subjecting reaction mixtures to 36 cycles at 91°C (40 seconds), 55°C (40 seconds) and 71°C (two minutes).

For prothrombin, amplification was performed in a separate 50μL reaction containing 10mM Tris HCl, pH 8.3, 50mM KCl, 1.5mM MgCl₂, 0.2mM of each nucleoside triphosphate and 0.4μM of each forward and reverse primer and 2.5U of Taq polymerase. The PCR parameters were 38 cycles at 94°C (30 seconds), 54°C (30 seconds) and 72°C (30 seconds). The initial cycle was preceded by 9 minutes at 94°C to activate AmpliTaq Gold polymerase, in addition to denaturing the template. The last cycle was followed by 5 minutes at 72°C. The PCR products were digested with the appropriate restriction enzyme. After digestion, PCR products were electrophoresed on 2% agarose minigels containing ethidium bromide, at 120 V for 1 hour. For the

prothrombin gene, Hind III digestion yielded an intact 345-bp product for the normal allele, and two fragments of 322 and 23 bp for the mutant allele. For each locus, heterozygous individuals exhibited both normal and mutant digested products. The PCR assay controls included DNA from a mutation subject, a normal subject, and a water blank for each analysis.

For Leiden factor V, Mnl I digestion of the 267-bp PCR product yielded fragments of 163, 67 and 37bp for the normal allele. Digested products of the mutant allele were 200 and 67 bp.

Descriptive analyses were made by using frequency tables, and position and dispersion measurements. To check for associations or to compare proportions, the Chi-square or the Fisher exact tests were used. Statistical significance level was considered to be 5%.

RESULTS

All women in Group 1 were carriers of Leiden factor V, with three of them being also carriers of prothrombin gene G20210A mutation.

The mean age of women in Group 1 was 38.2 years (ranging from 15 to 87 years) and, in Group 2, it was 22.5 years (ranging from 18 to 40 years). The difference between these means was statistically significant ($p < 0.0001$).

While evaluating pregnancy outcome according to race and age, women with no previous pregnancies were excluded. So, in Group 1, 28 women had 109 pregnancies and, in Group 2, the women had 223 pregnancies.

Pregnancy outcome in both groups may be seen in Table 1. Of the 109 pregnancies in Group 1, 30.2% terminated in spontaneous abortion. Of the 223 pregnancies in Group 2, 10.1% terminated the same way. Such difference between the 2 groups, regarding the frequency of pregnancy termination in spontaneous abortion was statistically significant ($p = 0.0001$). No significant difference was found between both groups regarding the frequency of fetal deaths or full-term deliveries.

Table 2 shows the distribution of the number of abortions in each group. In Group 1, 13 women (46%), and in Group 2, 16 women (16%) had aborted at least once. Such difference in the number of abortions in each group, was also statistically significant.

As regards pregnancy outcome according to age, the study data showed that women younger

than 30 years experienced a higher frequency of pregnancy loss; the comparison between both groups regarding this subject, demonstrated a difference statistically significant in Group 1 (Table 3).

Table 4 shows distribution of pregnancy losses according to race in both groups. Among white women, Group 1 presented a significantly larger number of pregnancy losses than Group 2. Among

Table 1. Percentual distribution of pregnancy outcome by group.

Pregnancy outcome	Group 1 ^a (n=109)	Group 2 ^b (n=223)	p ^c
Spontaneous abortion	30.2	10.1	0.0001
Stillbirth	3.6	1.7	ns ^d
Full-term delivery	69.7	88.4	ns ^d

^a Group 1 = carriers of Leiden factor V; ^b Group 2 = non-carriers of Leiden factor V; ^c Chi-square test; ^d ns=not significant.

Table 2. Distribution of spontaneous abortions by group.

Number of abortions	Group 1 ^a (n=28)	Group 2 ^b (n=94)	p ^c
None	15	78	0.0001 ^d
One	7	13	
Two	3	1	
Three or more	3	2	

^a Group 1 = carriers of Leiden factor V; ^b Group 2 = non-carriers of Leiden factor V; ^c Chi-square test; ^d none x one or more abortions.

Table 3. Percentual distribution of pregnancy outcome by age and group.

Pregnancy outcome	< 30 years		>= 30 years	
	Group 1 ^a	Group 2 ^b	Group 1 ^a	Group 2 ^b
Pregnancy loss	45.5	5.5	32.6	21.0
Live birth	55.5	94.5	67.4	95.0
(n)	11.0	128.0	98.0	95.0
p ^c	0.00003		ns ^d	

^a Group 1 = carriers of Leiden factor V; ^b Group 2 = non-carriers of Leiden factor V; ^c Chi-square test; ^d ns=not significant.

Table 4. Percentual distribution of pregnancy outcome by race and group.

Pregnancy outcome	White		Non-white	
	Group 1 ^a	Group 2 ^b	Group 1 ^a	Group 2 ^b
Pregnancy loss	35.5	11.2	26.3	13.3
Live birth	64.5	88.8	73.7	86.7
(n)	90.0	125.0	19.0	98.0
p ^c	0.00003		ns ^d	

^a Group 1 = carriers of Leiden factor V; ^b Group 2 = non-carriers of Leiden factor V; ^c Chi-square test; ^d ns=not significant.

non-white women, there was no statistically significant difference.

No statistically significant difference was found between the studied groups regarding the number of pregnancies, although 35.7% of women from Group 1, and only 17.0% from Group 2, had 4 or more pregnancies.

A personal history of thromboembolic episode was found in approximately 74.0% of women in Group 1. The most frequently found event was deep venous thrombosis (64.2%).

DISCUSSION

The aim of this study was to compare the frequency of pregnancy losses between 2 groups of women, considering their personal and family histories: a group of women who were carriers of Leiden factor V, associated or not with prothrombin gene G20210A mutation, and another group of non-carriers of such conditions.

The age difference found between women in both groups could have acted as a confounding factor. A more thorough evaluation shows that there were no women older than 40 years of age in Group 2. This group was chosen among women who had participated as controls in another study of hereditary thrombophilia and recurrent abortion¹⁴. Group 1 consisted of women who were carriers of a thrombophilia-inducing mutation. Of these women, 10 were older than 40 years, being the cause for the statistically significant age difference found between both groups.

Nevertheless, as we can see in Table 1, a statistically significant difference in the frequency of pregnancy loss between both groups was found only in women younger than 30 years. Thus, it is unlikely that the ten women older than 40 years in Group II might have had any influence on these results.

Evaluating the studied groups according to race, there was a larger number of pregnancy losses in the white women of Group 1. It is known that Leiden factor V is more frequently found in the white

population. Our results thus suggest that the presence of this mutation may be responsible for the larger number of pregnancy losses experienced by the white women in this group. In addition, the literature reports that, among the general population, the number of spontaneous abortions in black women is larger than in white women¹⁵, a fact that, in relation to the losses experienced by the white women in our study, seems to underscore the important effects of the referred mutations on such losses.

Evaluation of the pregnancy outcomes showed a higher frequency of spontaneous abortion in women who carried the studied thrombophilic factors. This result supports our study's initial assumption and the results obtained in other series, showing a higher frequency of early pregnancy losses in women who carried some hereditary thrombophilic factor¹⁶. However, some authors disagree with such association^{10,17} and new, larger series of studies, are required to clarify this issue.

Regarding the occurrence of fetal death, our results differ from those found by Kupfermanc *et al.*¹². These authors showed a higher frequency of fetal death, not abortion, among women carrying those factors. Perhaps a systematic search for these alterations in women with fetal death might clarify the difference between results.

All women in Group 1 were carriers of Leiden factor V and had first-degree relatives with a history of one or more thromboembolic events. In recent years, there have been major advances in the study of familial thrombosis and its association with pregnancy losses. However, these mechanisms of transmission may still have unclear associations. The question is whether families who are carriers of thrombophilia, possess a positive selective factor (lower mortality from hemorrhage) outweighing the unfavorable effect (propensity to thromboembolic complications). This would eventually justify the high diagnostic rate of such genetic trait, which is maintained despite fertility being affected by pregnancy losses.

A significant increase in the incidence of thrombosis, found in first-degree relatives of families

who carry thrombophilia, supports the assumption of genetic alterations^{7,18} and justifies the implementation of efficient programs to prevent thrombotic events in such families.

This subject has still not been well-investigated from a genetic perspective. Evaluating women with hereditary thrombophilia should be thorough, and should be extended to their family members, in order to prevent deleterious clinical or obstetric consequences. Our results suggest the need to investigate hereditary thrombophilia in cases of recurrent abortions.

CONCLUSION

The frequency of spontaneous abortion and recurrent abortion was higher in carriers of inherited thrombophilic factors.

REFERENCES

- Rai R, Reagan L, Hadley E, Dave M, Cohen H. Second-trimester pregnancy loss is associated with activated protein C resistance. *Br J Haematol.* 1996; 92(2): 489-90.
- Forastiero R, Martinuzzo M, Adamczuk Y, Iglesias Varela ML, Pombo G, Carreras LO. The combination of thrombophilic genotypes is associated with definite antiphospholipid syndromes. *Haematologica.* 2001; 86(7):735-41.
- Brenner B, Mandel H, Lanir N, Yonis J, Rothbart H, Ohel G, Blumenfeld Z. Activated protein C resistance can be associated with recurrent fetal loss. *Br J Haematol.* 1997; 97(3):551-4.
- Lane DA, Manucci PM, Bauer KA, Bertina RM, Bochkov NP, Boulyjenkov V, et al. Inherited thrombophilia: part 1. *Thromb Haemost.* 1996; 76(5):651-62.
- Arruda VR, Figueiredo MS. *Biologia molecular para o hematologista clínico.* Temas Hematol Clin. 1997; 187-99. Autor: indicar o volume e o fascículo
- Bertina RM, Koeleman BP, Koster T, Rosendaal FR, Dirven RJ, Ronde H. et al. Mutation in blood coagulation factor V associated with resistance to activated protein C. *Nature.* 1994; 369(6475):64-7.
- Dalbach B. Inherited thrombophilia: Resistance to activated protein C as a pathogenic factor of venous thromboembolism. *Blood.* 1995; 85(3):607-14.
- Kujovich J, Goodnigt S. *Factor V de Leiden Thrombophilia: Hereditary resistance to activated protein C; factor V R506Q.* Seattle: University of Washington; 1999.
- Foka ZJ, Lambropoulos AF, Saravelos H, Karas GB, Karavida A, Agorastos T, et al. Factor V Leiden and prothrombin G20210A mutations, but not methylene tetrahydrofolate reductase C677T, are associated with recurrent miscarriages. *Hum Reprod.* 2000; 15(2): 458-62.
- Dizon-Townson DS, Meline L, Nelson LM, Varner M, Ward K. Fetal carriers of the factor V Leiden mutation are prone to miscarriage and placental infarction. *Am J Obstet Gynecol.* 1997; 177(2):402-5.
- Poort FE, Rosendaal FR, Reitsma PH, Bertina RM. A common genetic variation in the 3'-untranslated region of the prothrombin gene is associated with elevated plasma prothrombin levels and an increase in venous thrombosis. *Blood.* 1996; 88:3698-703.
- Kupfermanc MJ, Eldor A, Steinman N, Many A, Bar-Am A, Jaffa A, et al. Increased frequency of genetic thrombophilia in women with complications of pregnancy. *N Engl J Med.* 1999; 340(1):9-13.
- Sambrook J, Fritsch EF, Maniatis T. *Molecular cloning: A laboratory manual.* 2nd ed. New York: Cold Spring Harbor; 1989.
- Couto E, Barini R, Annichino-Bizzacchi JM, Negrão P, Oliveira G. Comparative study of the frequency of thrombogenic factors in spontaneous aborting and fertile women. *J Autoimmun.* 2000; 15(2):A66.
- Rooney B. Racism, poverty, abortion, and other reproductive outcomes. *Epidemiology.* 2000; 11(6):740-2.
- Stirling SS, Woolf L, North WR, Seghatchian MJ, Meade TW. Homeostasis in normal pregnancy. *Thromb Haemost.* 1984; 52(2):176-82.
- Dulíček P, Chrobák L, Kalousek I, Pesavová L, Pecka M, Stránský P. Is factor V Leiden a risk factor for fetal loss? *Acta Med.* 1999; 42(3):93-6.
- Koster T, Rosendaal FR, Ronde HD, Briet E, Vandenbroucke JP, Bertina RM. Venous thrombosis due to poor anticoagulant response to activated protein C: Leiden Thrombophilia Study. *Lancet.* 1993; 342(8886-8887):1503-6.

Recibido para publicação em 25 de maio e aceito em outubro de 2004.

