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Analysis of 18 bp Ins/Del at -2549 polymorphism of the vascular endothelial growth factor gene in patients with systemic lupus erythematosus

Análise do polimorfismo -2549 18 pb Ins/Del do gene do fator de crescimento vascular endotelial em indivíduos com lúpus eritematoso sistêmico

Évelin M. Cassemiro¹; Daiani Cristina C. Alves²; Daniel O. Freire³; Lusitano B. Ferreira³; Carlos Eduardo C. Lins⁴; Izabel Cristina R. Silva¹; Rodrigo Haddad¹

1. Universidade de Brasília (UnB), Brasília, Distrito Federal, Brazil. 2. Centro Universitário Euro-americano (UNIEURO), Brasília, Distrito Federal, Brazil. 3. Faculdade LS, Brasília, Distrito Federal, Brazil. 4. Hospital de Base de Brasília, Brasília, Distrito Federal, Brazil.

ABSTRACT

Systemic lupus erythematosus (SLE) is considered an autoimmune disease characterized by the action of autoantibodies, which cause chronic inflammation in various tissues of the body. Considering the vascular endothelial growth factor (VEGF) participation in the development of inflammation, this study aimed to evaluate the frequency of the polymorphism at the -2549 position (Ins/Del 18pb, rs35569394) in patients with SLE and in healthy individuals. No statistical differences were found when comparing the allele and genotype frequencies between patients and controls, suggesting that there is no association between the studied polymorphism and the development of SLE.

Key words: vascular endothelial growth factor; genetic polymorphism; systemic lupus erythematosus.

INTRODUCTION

Systemic lupus erythematosus (SLE) is a chronic autoimmune disease characterized by an exacerbated autoimmune response directed against autoantigens of any organ or tissue in the human body⁽¹⁾. Systemic inflammation and tissue damage, characteristic of this disease, contribute to the clinical presentation of SLE and may cause severe sequelae that result in disability or death⁽²⁾. In Brazil, there are no accurate studies to evaluate the real prevalence of SLE. It is estimated that the incidence is 0.098%; in the world, prevalence ranges from 14.6 to 122 casos/100,000 inhabitants⁽³⁾.

The pathophysiology of SLE is complex and multifactorial. It is known that most patients are genetically predisposed to the development of SLE⁽⁴⁾ and, therefore, to the identification of the genetic factors associated with the susceptibility or the development of antibodies specific or related to the clinical characteristics and under intense investigation⁽¹⁾. In this context, the investigation of the vascular endothelial growth factor (VEGF) gene becomes

relevant, since this growth factor is of great importance in the angiogenesis process and could be related to the SLE development process. This possibility is reinforced by the observation that patients with SLE present an increased risk of vascular and coronary artery disease with premature atherosclerosis and arterial stiffness⁽⁵⁾. It has also been shown that patients with SLE have higher VEGF levels when compared to individuals without the disease^(6, 7). Recently, an in silico analysis demonstrated the participation of the VEGF pathway in the development of SLE-related manifestations⁽⁸⁾. Therefore, the study of genetic polymorphisms that may influence the production of VEGF, such as the insertion/deletion 18 base pair (bp) polymorphism at position -2549 of the VEGF gene (Del/Ins 18pb, rs35569394), is important in order to prove, along with the already established literature, the relations between this polymorphism and the development of this pathology. The presence of homozygous deletion (Del Del) and heterozygous (Del Ins) genotypes are associated with the higher transcriptional activity of the VEGF gene and the increase in its production⁽⁹⁾.

For this purpose, in this study, peripheral blood samples were obtained from 61 SLE patients attended at the Hospital de Base de Brasília and from 33 controls (healthy individuals). These samples were obtained after approval of the Research Ethics Committee of the Health Department of the Federal District [Secretaria de Saúde do Distrito Federal (SES-DF)] – protocol 353/09, opinion 309/2009] and obtaining the Free Prior Informed Consent (FPIC). It is observed that all patients with SLE were female (**Table**). This fact corroborates data from the literature, since it is very well-described that this disease mainly affects women of childbearing age with incidence nine times higher when compared with men⁽¹⁰⁾. Then, the extraction of deoxyribonucleic acid (DNA) was performed using the Invisorb Spin Blood kit (Invitek, Hayward, CA, USA) following the manufacturer's recommendations. Subsequently, the polymerase chain reaction (PCR) was performed according to the following conditions: in each tube were added 1 µl of buffer (10×), 0.5 µl of magnesium chloride (50 nm), 0.2 µl of Taq polymerase (5 U/ul) (Ludwig Biotec, Alvorada, RS, Brazil), 0.1 µl of deoxyribonucleotide triphosphate (dNTPs) (2.5 nm) (Invitrogen, Carlsbad, CA, USA) and 0.5 µl of primers (10 pmol/µl) forward (5'-GCTGAGAGTGGGGCTGACTAGGTA-3') and reverse (5'-GTTTCTGACCTGGCTATTTCAGG-3') (Exxtend, Campinas, SP, Brazil) and 7 µl of DNA (10 ng/µl), totaling 10 µl of final reaction. Thermocycling was performed under the following conditions: 95°C for 5 minutes, followed by 35 cycles at 94°C for one minute, 59°C for one minute and 72°C for one minute and 30 seconds; for final extension, 72°C was used for 10 minutes. The amplification results were evaluated after running a 10% polyacrylamide gel, subjected to a voltage of 100 mV for 10 minutes and 120 mV for 110 minutes. Before applying the gel, 1 µl of GelRed™ (Biotium, Fremont, CA, USA) was added to the sample, and the observation was performed by photodocumentation (Loccus Biotecnologia, Cotia, SP, Brazil). The presence of a 229 pb band represents the homozygous insertion of 18 pb (Ins Ins); for the 211 pb band, the homozygous deletion of 18pb (Del Del); for both bands, the heterozygous (Del Ins). The determination of allele and genotype frequencies was calculated by direct counting using the Microsoft Excel (Microsoft, Redmon, WA, USA). The chi-square test was used to evaluate the difference in allele and genotype frequency between

the groups (SLE × control), and the adopted significance level was 5% ($p < 0.05$). The statistical program used was SPSS version 20.0 (SPSS Inc., Chicago, IL, USA). All results are shown in Table.

Comparison of the genotype frequency between the SLE and control groups showed no statistically significant difference. The same was observed when allele frequencies were compared. These results show that there is no association between 18 pb deletion or insertion alleles of the VEGF gene in the development of SLE. The lack of association between the polymorphism studied and SLE does not rule out the possibility that VEGF gene polymorphisms may influence the development of the disease and its outcomes. Recently, it was observed that the rs10434 polymorphism of the 3'UTR region of the VEGF gene could represent an increase in the susceptibility of neuropsychiatric disorders in patients with SLE⁽¹¹⁾. Another study demonstrated that the presence of the A allele of the rs833070 polymorphism is related to the susceptibility to SLE, whereas the GG genotype presents less susceptibility to arthritis in patients with SLE⁽¹²⁾. Finally, another research has shown that GG genotype of the rs2010963 polymorphism is related to the development of lupus nephritis⁽¹³⁾.

On the other hand, although loss-of-function mutations in isolated genes may result in SLE or SLE-like diseases in a small number of individuals (approximately 1% to 4%), most SLE patients present complex pathophysiological processes that remain misunderstood⁽¹⁴⁾. Although most patients have a genetic predisposition to the development of SLE, the so-called risk alleles are not, by themselves, strong enough to confer the disease in its full form. In these cases, additional factors – including female gender – hormonal factors, environmental factors (infections, medications, chemical agents and toxins), immunological factors and epigenetic events provide additional pathophysiological impact that contributes to the development of the disease⁽⁴⁾.

The search for factors that effectively contribute to the development of the disease may favor the establishment of treatment protocols (individualized or not) and of disease-related biomarkers. The development of new studies involving a larger number of patients and, especially, functional evaluations on VEGF and SLE would be important to fully uncover the role of this growth factor in this pathology.

TABLE – Patient characteristics and allele and genotype frequencies of the VEGF Del/Ins 18pb -2549 polymorphism of in the different groups

Groups	n	Gender (M/F)	Age/range	Alleles		Genotypes		
				Del	Ins	Del Del	Del Ins	Ins Ins
SLE	61	0/61	35.39 ± 13.16 19-73	0.5082	0.4918	0.4429	0.4000	0.1571
Control	33	12/21	52.33 ± 9.56 23-63	0.4848	0.5152	0.2424	0.4848	0.2727
p value				0.8786		0.0554	0.3928	0.2100

VEGF: vascular endothelial growth factor; SLE: systemic lupus erythematosus; M: male; F: female.

RESUMO

O lúpus eritematoso sistêmico (LES) é considerado uma doença autoimune devido à atuação de autoanticorpos, que ocasionam inflamações crônicas em diversos tecidos corporais. Considerando o envolvimento do fator de crescimento vascular endotelial (VEGF) no desenvolvimento da inflamação, este trabalho objetivou avaliar a frequência do polimorfismo na posição -2549 (Ins/Del 18pb, rs35569394) em pacientes com LES, comparando-os com indivíduos saudáveis. Não foram encontradas diferenças estatísticas ao comparar as frequências alélicas e genotípicas entre pacientes e controles, sugerindo que não há associação entre o desenvolvimento de LES e o polimorfismo estudado.

Unitermos: fator de crescimento do endotélio vascular; polimorfismo genético; lúpus eritematoso sistêmico.

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CORRESPONDING AUTHOR

Rodrigo Haddad

Universidade de Brasília, Faculdade de Ceilândia (UnB/FCE); Laboratório de Análises Clínicas Centro Metropolitano, conjunto A, lote 01; CEP: 72220-275; Brasília-DF, Brasil; e-mail: haddad@unb.br