IL1B - 511 gene polymorphism (rs16944) in Brazilian patients diagnosed with haemorrhagic stroke

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Polimorfismo del gen IL1B - 511 (rs16944) en pacientes brasileños diagnosticados con accidente cerebrovascular hemorrágico

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RESUMO

Objetivo: aferir a possível relação do polimorfismo IL1B (-511 C/T) com o prognóstico do AVEH. **Método:** Para avaliar este questionamento, usou-se uma amostra de 81 indivíduos diagnosticados com AVEH, na qual passaram por análise do polimorfismo IL1B -511 C/T, pela técnica da PCR-RFLP. A análise estatística adotou um nível de significância de 5%. **Resultados:** a pesquisa mostrou que a presença dos genótipos CC ou CT/TT não resultam uma associação estatística entre o polimorfismo da IL1B com o aparecimento do AVEH (P= 0,174). **Conclusão:** O estudo demonstrou que a variação de C e T no polimorfismo do gene rs16944 não está associada com os aspectos clínicos selecionados. Também mostrou não haver associação estatística com a manifestação da doença e a variação genotípica.
Descritores: Interleucina-1; AVEH; Polimorfismo Genético.

ABSTRACT

Objective: To assess the possible relationship between IL1B (-511 C/T) polymorphism and the prognosis of HS. **Method:** To evaluate this questioning, we used a sample of 81 individuals diagnosed with HS, who underwent analysis of the IL1B-511 C/T polymorphism by the PCR-RFLP technique. Statistical analysis adopted a significance level of 5%. **Results:** Research has shown that the presence of CC or CT/TT genotypes does not result in a statistical association between IL1B polymorphism and the appearance of HS (P = 0.174). **Conclusion:** The study demonstrated that C and T variation in rs16944 gene polymorphism is not associated with the selected clinical aspects. It also showed no statistical association with disease manifestation and genotypic variation.
Descriptors: Interleukin-1; HS; Genetic polymorphism.

RESUMEN

Objetivo: evaluar la posible relación entre el polimorfismo IL1B (-511 C / T) y el pronóstico de AVEH. **Método:** Para evaluar este cuestionamiento, utilizamos una muestra de 81 individuos diagnosticados con AHV, que se sometieron a análisis del polimorfismo IL1B-511 C / T mediante la técnica PCR-RFLP. El análisis estadístico adoptó un nivel de significación del 5%. **Resultados:** La investigación ha demostrado que la presencia de genotipos CC o CT / TT no da como resultado una asociación estadística entre el polimorfismo IL1B y la aparición de AVEH (P = 0.174). **Conclusión:** El estudio demostró que la variación de C y T en el polimorfismo del gen rs16944 no está asociada con los aspectos clínicos seleccionados. Tampoco mostró asociación estadística con la manifestación de la enfermedad y la variación genotípica.
Descriptores: Interleucina-1; AVEH; Polimorfismo Genético.

ORIGINAL

Introduction

Noncommunicable Chronic Diseases (NCDs) have become over the years the leading cause of death in the general population, taking the place that once belonged to infectious diseases. Science has been following this trend, and studies on the various predictive factors for these pathologies are increasingly common. Among NCDs, diseases of the circulatory system can be highlighted, which account for an average of 30% of deaths in Brazil.[1] Stroke is a serious pathology of the circulatory system, according to WHO data, it is responsible for about 12.3% of deaths worldwide[2], in Brazil and Latin America stroke is one of the main causes of death and disability.[3]

Stroke is defined as a disorder of blood flow to brain regions, compromising nutrient supply, leading to tissue damage and compromising physiological neurological response.[4] Most risk factors for stroke are preventable, others can be controlled, some risk factors are: high blood pressure, diabetes mellitus, smoking, alcoholism, physical inactivity.[5] Stroke is divided into two types according to its etiology, ischemic and hemorrhagic, the first being the most prevalent, accounting for over 80% of cases.[3] Hemorrhagic Stroke (HAVE), although less common, has higher rates of mortality and morbidity and mortality worldwide.[6]

MRS measures an individual's neurological ability, sense of lucidity, and independence after stroke. This scale ranges from 0 to 6, where 6 indicates death and 0 the absence of symptoms. The reference scale used as the basis for formulating the MRS has a score of 0 or 1, where score 1 is assigned to patients with score 4 or more on the expanded scale, and score 0 for scores below 4.[7]

During stroke several proinflammatory agents are recruited from the peripheral circulation in order to promote immediate neuroinflammation at the site, some of which are chemokines, free radicals, and various cytokines such as interleukins.[8] Interleukin 1 is an integral part of this process. The interleukin 1 family member is divided into two types, interleukin 1A and interleukin 1B. They act as endogenous pyrogens in the hypothalamic site, in the endothelium are responsible for inducing adhesion molecules to prothrombotic effects.[9-10] Interleukin 1B is encoded by the IL1B gene which is located on chromosome 2q14.1 and has 7 exons.[11]

This study aimed to analyze the association between IL1B - 511 (C / T) polymorphism, rs16944 and AVEH in a Brazilian sample. This research was approved by the Research Ethics Committee of the Federal District Health Department, opinion number: 0095/2010.

Method

This is a cross-sectional, descriptive clinical study, where the participants of the case group were from the population domiciled in the Federal District (Brazil), and were selected through the registration in a hospital that serves the population of the DF, surrounding and neighboring states for highly complex procedures. Eighty-one patients with HAVE participated in this study, of which: 48 women and 33 men; and average age of 53.5 ± 5.9 years.

For genotyping analysis, each patient's samples were obtained by venous puncture for DNA extraction, 10 mL. DNA was extracted from peripheral blood

with the Invisorb Spin Blood Mini Kit (250) for DNA extraction from Invitek (catalog # CA10-0005, lot # 1031100300). DNA integrity was observed in electrophoretic run through ethidium bromide-stained 1% agarose gel, and the mean concentration was estimated on the NanoDrop 2000 / 2000c apparatus (Thermo Fischer Scientific) in which the mean concentration achieved was 20%. ng / μL.

The diluted DNA was submitted to the PCR (Polymorphism Polymerase Chain Reaction) technique. The oligonucleotide sequences used to evaluate the polymorphism were: F 5'-TGG-CAT-TGA-TCT-GGT-TCA-TC-3 'and R 5'-GTT-TAG-GAA-TCT-TCC-CAC- TT-3 '. Thermocycling conditions were 94 ° C for 5 minutes (initial denaturation), followed by 45 cycles of denaturation at 94 ° C for 1 minute, annealing of the oligonucleotides at 55 ° C for 1 minute and 72 ° C for 1 minute for the extension. fragments. Final extension was performed at 72°C for 7 minutes and cooling for 4 minutes. The equipment used was the Techne Thermal Cycler model TC-512.

In each reaction 4.0 μL of genomic DNA was used at the final concentration of 2.5 ng / μL; 2.5 μl 10x buffer (10mM Tris and 50mM KCl); 0.5 μL 50mM MgCl2 (Ludwig Biotec, Alvorada, Rio Grande do Sul, Brazil), 0.5 μL deoxyribonucleotideostriphosphate (dNTPs; 2.5 mM; Ludwig Biotec, Alvorada, Rio Grande do Sul, Brazil); 0.5 μL Taq Polymerase (Ludwig Biotec, Alvorada, Rio Grande do Sul, Brazil), 5 U / μL); 1.5 μL of each foward and reverse oligonucleotide (10 μM, IDT technologies); supplementing with Milli-Q water to a final volume of 25 μL per reaction.

The PCR products in question were 304bp fragments, subsequently digested with the restriction enzyme Aval (New England Biolabs, Inc. Beverly, MA, USA). Allele 1 (C) creates a new restriction site, and the 304bp fragment is cleaved into two of 190bp and 114bp; Allele 2 (T) is not cleaved by the enzyme, and thus the polymorphism was divided into cleavage genotype, or ancestor homozygote (CC), heterozygote (CT) and non-cleavage genotype, or recessive homozygote (TT). The digestion system was assembled using: 10.0 μL PCR; 2.0 μl 10x NEB4 buffer (Biolabs); 1 μL of AvaI enzyme (10 U / μL), supplemented with Milli-Q water to a final volume of 20 μL per reaction. The system was kept at 37 ° C for 3 hours. The digestion products were electrophoretically run on a 3% agarose gel with 0.1% ethidium bromide at a power of 100W for 20 minutes.

Results

The clinical characteristics of the patients were compared in the study of the statistical association between the polymorphism of Table 1, adopting the critical value of 0.050. Sex, systemic arterial hypertension, diabetes, smoking and alcoholism did not show statistical association with the genotypic distribution of the polymorphism. The rs16944 polymorphism was not statistically associated with the onset of HS in patients.

Table 1 – Association between IL1B-511 (C/T) rs16944 genetic polymorphism and clinical features.

		IL 1B-511				
		CC		**CT+TT**		**P**
		N	**%**	**N**	**%**	
Gender	Female	20	57.1%	28	60.9%	0.740
	Male	15	42.9%	18	39.1%	
SAH[1]	Yes	26	74.3%	34	73.9%	0.999
	No	9	25.7%	12	26.1%	
Diabetes	Yes	1	2.9%	2	4.3%	0.999
	No	34	97.1%	44	95.7%	
Smoking	Yes	15	42.9%	17	37.0%	0.590
	No	20	57.1%	29	63.0%	
Alcoholism	Yes	10	28.6%	12	26.1%	0.806
	No	25	71.4%	34	73.9%	
ERS[2]	Bad prognosis	2	5.7%	8	17.4%	0.174
	Good prognosis	33	94.3%	38	82.6%	

[1]Systemic arterial hypertension; [2]Modified Rankin Scale; Chi-square test.

Discussion

Data point to the relationship of IL-1β with the transcriptional activation of the NF-kB gene that would be related to the synthesis of adhesion molecules in the endothelium, contributing to the endothelial inflammatory cell conglomerate. Also, IL-1β elevates macrophage activity, present in inflammatory disease processes, such as Arteriosclerosis.[12]

The prognosis did not show a statistical relationship between IL1B (-511 C / T) polymorphism and neurological impairment profile, according to the Modified Rankin Scale of the evaluated cases.

Another aspect assessed was risk factors, a term that can also be used to define clinical characteristics or lifestyle habits that could increase the likelihood of developing disease.[13] We selected for the study hypertension, diabetes, smoking and alcoholism.

SAH is the main modifiable risk factor for the development of stroke, increasing the risk three to four times and being directly responsible for at least half of the cases.[5] We believe that Diabetes Mellitus (DM) is related to the acceleration of the atherosclerosis process, and is therefore recognized as a risk factor for stroke, with higher occurrence in ischemic episodes when compared to hemorrhagic episodes.[14] Proinflammatory cytokine IL-1β has the ability to negatively impact insulin-induced glucose transpote.[15-16]

Although they are admittedly important in the outcome of AHVH / Aneurysm, there was no association between IL1B-511 / T polymorphism and clinical characteristics/habits: SAH, diabetes, smoking and alcoholism (P> 0.05).

Stroke, according to WHO data, is the second leading cause of death in the world, affecting mainly elderly and middle-aged people. In Brazil, it is the leading cause of death and disability, being responsible for high mortality and sequelae that will accompany the patient for the rest of his life.[17] Understanding the molecular mechanisms and the influence of risk factors broadens the

medical perception to identify people at high risk of suffering clinical complications from stroke, especially the HS.

Conclusion

The study demonstrated that C and T variation in rs16944 gene polymorphism is not associated with the selected clinical features. It also showed no statistical association with disease manifestation and genotypic variation. However, the study was limited to a small number of patients, a larger amount would be needed to be sure, because the statistical value in correlation with the onset of the disease was close to the established limit.

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