Absence of association of IL4 polymorphism and clinical characteristics of hemorrhagic stroke

Ausência de associação do polimorfismo IL4 e características clínicas do acidente vascular encefálico hemorrágico

Ausencia de asociación de polimorfismo IL4 y características clínicas del accidente cerebrovascular hemorrágico

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RESUMO

Objetivo: investigar a associação entre o polimorfismo do tipo VNTR, do gene IL4, localizado na região do intron 3, em pacientes diagnosticados com acidente vascular encefálico hemorrágico (AVEH) ou aneurisma intracerebral em uma amostra do Distrito Federal. **Método:** Tratou-se de um estudo observacional, retrospectivo, transversal, com 55 indivíduos, dos quais foram anotadas as características clínicas do prontuário e realizada análise da genotipagem por meio da estratégia de PCR. As frequências genotípicas foram estimadas por contagem direta. O nível de significância adotado foi de 5% e o teste estatístico utilizado foi o Qui-Quadrado. **Resultados:** Foi verificado que o genótipo mais frequente foi o B1/B2 (50,9%; n=28), seguido pelo genótipo ancestral B1/B1 (27,3%, N=15), sendo que o menos frequente foi o genótipo B2/B2 (21,8%, N=12). Não foi encontrada associação estatística entre as variáveis hipertensão arterial sistêmica, diabetes, tabagismo e etilismo e a presença do polimorfismo no grupo estudado. **Conclusão:** A presença do polimorfismo IL4 INTRON 3 VNTR teve associação com a variável sexo, demonstrando que na amostra estudada, o AVEH é mais frequente em mulheres do que em homens, divergindo de estudos nos quais indivíduos do sexo masculino são mais propensos a desenvolverem AVE.

Descritores: Polimorfismo; Interleucina-4; Acidente vascular encefálico.

ABSTRACT

Objective: to investigate the association between The IL4 gene VNTR polymorphism, located in the intron 3 region, in patients diagnosed with hemorrhagic stroke (Stroke) or intracerebral aneurysm in a sample from the Federal District. **Method:** This was an observational, retrospective, cross-sectional study with 55 individuals, from which the clinical characteristics of the medical records were recorded and genotyping analysis was performed using the PCR strategy. Genotypic frequencies were estimated by direct counting. The level of significance adopted was 5% and the statistical test used was Chi-Square. **Results:** It was verified that the most frequent genotype was B1/B2 (50.9%; n=28), followed by the ancestral genotype B1/B1 (27.3%, N=15), and the least frequent was genotype B2/B2 (21.8%, N=12). No statistical association was found between the variables systemic arterial hypertension, diabetes, smoking and alcohol consumption and the presence of polymorphism in the studied group. **Conclusion:** The presence of IL4 INTRON 3 VNTR polymorphism was associated with the gender variable, demonstrating that in the sample studied, AVEH is more frequent in women than in men, diverging from studies in which males are more likely to develop a VENa.

Descriptors: Polymorphism; Interleukin-4; Stroke.

RESUMEN

Objetivo: investigar la asociación entre el polimorfismo VNTR del gen IL4, localizado en la región intrón 3, en pacientes diagnosticados de accidente cerebrovascular hemorrágico (Stroke) o aneurisma intracerebral en una muestra del Distrito Federal. **Método:** Estudio observacional, retrospectivo, transversal, con 55 individuos, del cual se registraron las características clínicas de las historias clínicas y se realizó un análisis de genotipado mediante la estrategia de PCR. Las frecuencias genotípicas se estimaron mediante conteo directo. El nivel de significancia adoptado fue del 5% y la prueba estadística utilizada fue Chi-Cuadrado. **Resultados:** Se verificó que el genotipo más frecuente fue B1/B2 (50,9%; n=28), seguido del genotipo ancestral B1/B1 (27,3%, N=15), y el menos frecuente fue el genotipo B2/B2 (21,8%, N=12). No se encontró asociación estadística entre las variables hipertensión arterial sistémica, diabetes, tabaquismo y consumo de alcohol y la presencia de polimorfismo en el grupo estudiado. Conclusión: La presencia del polimorfismo IL4 INTRON 3 VNTR se asoció con la variable género, demostrando que en la muestra estudiada, AVEH es más frecuente en mujeres que en hombres, divergiendo de los estudios en los que los varones tienen más probabilidades de desarrollar una VENa.

Descriptores: Polimorfismo; Interleucina-4; Accidente cerebrovascular hemorrágico.

ORIGINAL

Introduction

The cerebrovascular accident (CVA) originates through obstruction (ischemic CVA) or disruption (hemorrhagic CVA) of blood vessels in the brain,1 causing interruption of blood flow in a certain brain region,2-5 being an acute event,6 that persists for 24 hours2,3,5 and causes sudden loss of neurological function.[7]

Stroke is classified as ischemic or hemorrhagic, with the first responsible for 80-85% of cases and the second for 15%.8-9 According to the World Health Organization (WHO), in 2019 stroke brain injury was the second leading cause of death in the world, responsible for approximately 11% of the 55.4 million deaths that occurred globally.[10]

During an ischemic stroke, various substances are released from the ischemic nucleus into the penumbra area.[11] These substances can activate microglia and trigger pro-inflammatory responses (M1 phenotype) or anti-inflammatory responses (M2 phenotype).[12]

One of these substances is interleukin-4 (IL-4), a cytokine secreted by T cells, responsible for their differentiation, which is also involved in the tissue repair process.[13,14] IL-4 polarizes macrophages towards the M2 phenotype, which contributes to the reduction of lesions in both ischemic and hemorrhagic strokes.[11]

The gene that encodes IL-4 is located in the chromosomal region 5q31.1.15 This gene has a 70pb VNTR-type polymorphism in intron 3, which may influence the expression of this cytokine and the risk of hemorrhagic stroke. [16-17] This polymorphism has the B1 (two repeats) and B2 (three repeats) alleles. The B2 allele may be more related to the decrease in IL-4 expression.[17-18]

In this context, this study aimed to investigate the association between the VNTR-type polymorphism of the IL4 gene, located in the intron 3 region, in patients diagnosed with hemorrhagic stroke (EVA) or intracerebral aneurysm in a sample from the Federal District.

This research was submitted to the Research Ethics Committee of the Foundation for Teaching and Research in Health Sciences (CEP – FEPECS), being approved under opinion number 0095/2010. All participants signed the Free and Informed Consent Form (TCLE) before the study was carried out.

Method

An observational, retrospective and cross-sectional study was carried out with 55 patients recruited between 2011 and 2012, in a hospital in the Federal District, Brazil. In the studied sample there were 38 women and 17 men.

Inclusion criteria for participation in the study were age over fifty years and diagnosis of stroke, according to the criteria of the World Health Organization (WHO), which was confirmed through computed tomography (CT) and magnetic resonance imaging. Magnetic (RM). Undiagnosed patients aged less than 50 years were excluded from this study.

The clinical characteristics of the patients were noted and from this information was obtained, such as the diagnosis of hypertension or diabetes, and lifestyle habits such as smoking and alcohol consumption. The Modified Rankin

Scale (MRS)17 was used to assess patients' recovery.

For laboratory analysis, 5mL of venous blood was collected from each patient, from which DNA was extracted, using the PureLink®Genomic DNA Mini Kit, by Invitrogen (Waltham, Massachusetts, USA; catalog #K1820-02, batch #19339891). DNA concentrations were obtained from spectrophotometry with the NanoDrop® equipment (Thermo Fisher Scientific Inc., Waltham, Massachusetts, USA). Samples obtained from patients were processed at the Laboratory of Clinical Analysis at the Faculty of Ceilândia, University of Brasília (UnB), Brazil.

The DNA obtained was analyzed using the Polymerase Chain Reaction (PCR) technique for genotyping. 4.0µL of genomic DNA were used at a final concentration of 2.5ng/µL; 12.5µL of 10x buffer (10mM Tris and 50mM KCl); 3.8µL of 50mM MgCl2 (Ludwig Biotec, Alvorada, Rio Grande do Sul, Brazil), 10µL of 2.5mM deoxyribonucleotide triphosphate (dNTPs) (Ludwig Biotec, Alvorada, Rio Grande do Sul, Brazil); 2µL of Taq-Polymerase 10U/µL (Ludwig Biotec, Alvorada, Rio Grande do Sul, Brazil); 5µL of each oligonucleotide (10µM, IDT technologies); completing with Milli-Q water to a final volume of 25µL per reaction.

The oligonucleotides used to amplify the sequence of interest of the IL4 VNTR gene polymorphism intron 3 70pb were: forward 5'-AGCTGAAAGGGGGAAAGC-3' and reverse 5'-CTGTTCACCCTCAACTGCTCC-3'. Amplification of the studied fragment was performed using a thermal cycler programmed under the following conditions: initial denaturation at 94ºC for 5 minutes, followed by 35 cycles of denaturation at 94ºC for 20 seconds, annealing at 58ºC for 20 seconds, extension at 72ºC for 20 seconds and final extension at 72ºC for 10 minutes. The PCR products were electrophoretic run on a 3% agarose gel with ethidium bromide for 1 hour at 80W. After the run, the fragments were visualized on a transilluminator (L-PIX Touch) with an ultraviolet source, and the genotypic frequency was determined by directly counting the amplicons. Bands of 183 bp were defined as genotype B1/B1, bands of 253 bp were defined as genotype B2/B2, and bands having both 183 bp and 253 bp were defined as genotype B2/B2.

After the results of the PCRs, the statistical analysis of the data was performed using the statistical program SPSS version 25.0, which aims to compare the genotype and clinical characteristics of the patients. The adopted significance level was 5%, and the statistical test performed for the association analyzes was the Chi-square.

Results

In the studied sample, alleles B1 (two repetitions of 70 bp) and B2 (three repetitions of 70 bp) were found. After the statistical analysis of the samples, it was found that the most frequent genotype was B1/B2 (50.9%, N=28), followed by the ancestral genotype B1/B1 (27.3%, N=15), with the least frequent was the B2/B2 genotype (21.8%, N=12), as shown in Table 1.

Table 1- Distribution of IL4 INTRON 3 VNTR genotype among stroke patients.

Genotypes		N	%
IL4	B1/B1	15	27,3
	B1/B2	28	50,9
	B2/B2	12	21,8

IL4 = gene; B1/B1, B1/B2, B2/B2 = genotypes; N = number of patients corresponding to different genotypes; % = percentage of patients matching the different genotypes.

From the analysis of the data presented in Table 2, between the different genotypes, it was demonstrated that there was no statistical association between the variables SAH (systemic arterial hypertension) (p=0.505), diabetes (p=0.545), smoking (p=0.875) and alcoholism (p=0.725).

There was an association between the genotypic distribution and the gender variable in the studied sample (p=0.039), but this did not influence the stroke prognosis (MRS p=0.929).

Table 2- Distribution of IL4 INTRON 3 VNTR genotypes according to the variables gender, systemic arterial hypertension (SAH), diabetes, smoking, alcoholism and Modified Rankin Scale (MRS) of patients with stroke.

		IL4						P
		B1/B1		B1/B2		B2/B2		
		N	%	N	%	N	%	
Sexo	Feminine	7	46,7	20	71,4	11	91,7	0,039*
	Masculine	8	53,3	8	28,6	1	8,3	
HAS	Yes	14	93,3	23	82,1	11	91,7	0,505
	No	1	6,7	5	17,9	1	8,3	
Diabetes	Yes	0	0,0	2	7,1	1	8,3	0,545
	No	15	100,0	26	92,9	11	91,7	
Tabagismo	Yes	4	26,7	9	32,1	3	25,0	0,875
	No	11	73,3	19	67,9	9	75,0	
Etilismo	Yes	4	26,7	8	28,6	2	16,7	0,725
	No	11	73,3	20	71,4	10	83,3	
ERM	Bad Prognosis	2	13,3	5	17,9	2	16,7	0,929
	Good prognosis	13	86,7	23	82,1	10	83,3	

* P<0.05; Chi-Square Test. IL4 = gene; B1/B1, B2/B2 and B1/B2 = genotypes; N = number of patients corresponding to the genotypes; % = percentage of patients matching the genotypes.

Discussion

The statistical association between the gender variable and the IL4 gene polymorphism in the studied sample is considered a new finding in relation to existing studies.

In a study carried out at Hospital de Base de São José do Rio Preto, São Paulo, the authors observed that among the medical records analyzed there was a higher prevalence of stroke in males (56.04%) than in females (43, 96%).[19]

A cross-sectional study carried out with 223 patients diagnosed with stroke, who were treated at a hospital in Belo Horizonte, Minas Gerais, Brazil, from January to June 2015, demonstrated that most affected individuals were men (123 patients - 55 %), in an age range of 33 to 93 years, where the average age of these individuals was 64.3 years.[20]

Male individuals, under 85 years old, are the most affected by stroke. This fact is also linked to the pre-existing systemic arterial hypertension, which is more prevalent in men aged up to 50 years. Above the age of 70, women become more likely to develop stroke due to hormonal factors (menopause).[21]

Genetic polymorphisms can alter the cytokine release profile, and the polymorphism of the IL4 intron 3 VNTR gene may be one of those responsible for altering the release of IL-4.[22] This polymorphism has the B1 (two repeats) and B2 (three repeats) alleles. The B2 allele may be more related to the decrease in IL-4 expression.[16,18]

Previous studies showed that interleukin-4 is able to modulate the immune response and polarize microglia to the M2 phenotype, which is associated with tissue repair and the release of anti-inflammatory cytokines, which would benefit the recovery of ischemic stroke and AVEH.[23-25]

In a study with mice, Zhao et al.11 obtained positive results in the recovery of these animals after ischemic stroke. The administration of IL-4 resulted in a reduction in the ischemic injury and positively influenced the neurological recovery of these animals, in addition to inducing the polarization of microglia to the M2 phenotype, which is capable of reducing inflammation and is related to tissue repair.[23 .24]

Yang et al.14 induced intracerebral hemorrhage, one of the types of stroke, in Sprague Dawley rats to test the effect of IL-4 injection on microglia polarization and on the immune response to the damage caused. The results demonstrated that the administration of IL-4 was capable of inducing microglia polarization to the M2 phenotype, in addition to improving the anti-inflammatory response and, consequently, the recovery of the rat after intracerebral hemorrhage. However, they emphasized that more studies need to be done in order to find the ideal dose and the best moment for administration of IL-4, whether in the initial or final phase of the stroke.

Rolim et al.17 analyzed the rs2243250 single nucleotide polymorphism (SNP), which had two alleles, C and T, which were related to the risk of developing stroke. In data analysis, the allelic variation of C and T was not related to risk factors for stroke, nor was there a statistical association between stroke prognosis and the allele frequency of the studied polymorphism.

Park et al26 investigated two IL4 SNP polymorphisms, rs2070874 and rs2243250, finding the CC, TT and TC genotypes. The C allele was associated with reduced risk for stroke and the T allele was associated with increased IL-4

expression, with a statistical association between these polymorphisms and stroke. However, in the same study, they pointed out the sample used as a limitation and suggested that more studies need to be carried out in order to elucidate the role of IL-4 in stroke.

Conclusion

There are few studies demonstrating the relationship between interleukin-4 and AVE, because in most cases, IL-4 is related to AVE. This study showed that the presence of the IL4 INTRON 3 VNTR polymorphism was associated with the gender variable, demonstrating that in the studied sample, AVEH is more frequent in women than in men, diverging from studies in which male individuals are prone to develop BIRD.

Regarding the other clinical characteristics of hemorrhagic stroke (systemic arterial hypertension, diabetes, alcoholism, smoking and prognosis), no statistical association was found.

More detailed studies are needed to better understand the role of this polymorphism in the studied population, evaluating other characteristics of the patients, such as lipid, inflammatory and biochemical profiles, and analysis of medical records.

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