Association of genetic polymorphism in the BCL2 gene in/with Hemorrhagic Stroke/Aneurysm

Associação do polimorfismo genético no gene BCL2 no Acidente Vascular Cerebral Hemorrágico/Aneurisma

Asociación de polimorfismo genético en el gen BCL2 en/con el accidente cerebrovascular hemorrágico/aneurisma

Anyelle Stephane Nascimento de Lima[1], Caroline Ferreira Fratelli[2], Daniel Oliveira Freire[3], Luzitano Brandão Ferreira[4], Hélia Carla Souza[4], Izabel Cristina Rodrigues da Silva[1,2]

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1. Universidade de Brasília, Faculty of Ceilândia. Brasilia, Federal District, Brazil.

2. Universidade de Brasília, Graduate Program in Health Sciences and Technologies. Brasilia, Federal District, Brazil.

3. Faculdade LS. Brasilia, Federal District, Brazil.

4. Centro Universitário de Brasília. Brasilia, Federal District, Brazil.

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RESUMO

Objetivo: Verificar a distribuição do polimorfismo do gene BCL2 (rs1801018), em sua região codante, em pacientes portadores de Acidente Vascular Cerebral Hemorrágico (AVCh)/Aneurisma. Além de associar o presente polimorfismo as manifestações clinicas da doença. **Método:** O estudo foi conduzido com 158 participantes de pesquisa. Os grupos foram pareados quanto ao sexo e idade. A genotipagem foi conduzida pela técnica PCR-RFLP. Após o cálculo das frequências alélicas e genotípicas de cada grupo, foram utilizados testes estatísticos apropriados para cada tipo de comparação. O nível de significância adotado foi de 5%. **Resultados:** Os dados indicaram que a frequência dos genótipos apresentou uma diferença estatisticamente significante entre o grupo caso e controle, encontrando-se o genótipo ala43ala na maioria dos participantes de ambos os grupos. **Conclusão:** A presença do alelo mutante (trh) foi vista como um fator protetor para AVCh/aneurisma. Porém, estudos em outras populações devem ser realizados para se obter uma melhor compreensão sobre a doença AVCh/aneurisma.

Descritores: Polimorfismo genético; Acidente Vascular Cerebral; Genes bcl-2.

ABSTRACT

Objective: Verify the distribution of the BCL2 gene polymorphism (rs1801018), located in its coding region, in patients with Hemorrhagic Stroke (HS)/Aneurysm (A). Furthermore, to associate this polymorphism with the HS/A clinical manifestations. **Method:** The study was conducted with 158 research participants and the groups matched by sex and age. Genotyping was done by the PCR-RFLP technique. After calculating the allele and genotype frequencies of each group, appropriate statistical tests were performed for each comparison type with the adopted significance level of 5%. **Results:** The data indicated that the frequency of the genotypes showed a statistically significant difference between the case and control group, and the ala43ala genotype was found in most participants in both groups. **Conclusion:** The presence of the mutant allele (trh) was observed as a protective factor for HS/A. However, studies in other populations should be performed to obtain a better understanding of this disease.

Descriptors: Genetic polymorphism; Stroke; Bcl-2 genes.

RESUMEN

Objetivo: Objetivo: Verificar la distribución del polimorfismo del gen BCL2 (rs1801018), en ubicado su región de codificación, en pacientes con accidente cerebrovascular hemorrágico (ACVh)/aneurisma. Asimismo, asociar el presente polimorfismo con las manifestaciones clínicas de la enfermedad. **Método:** El estudio se realizó con 158 participantes, y los grupos fueron agrupados por sexo y edad. La determinación del genotipo se realizó mediante la técnica PCR-RFLP. Después de calcular las frecuencias de alelos y genotipos de cada grupo, se realizaron pruebas estadísticas apropiadas para cada tipo de comparación con el nivel de significación adoptado de 5%. **Resultados:** Los datos indicaron que la frecuencia de los genotipos mostró una diferencia estadísticamente significativa entre el grupo caso y el control, con el genotipo ala43ala encontrado en la mayoría de los participantes de ambos grupos. **Conclusión:** La presencia del alelo mutante (trh) fue visto como un factor protector para el ACVh/aneurisma. Sin embargo, se deben realizar más estudios en otras poblaciones para obtener una mejor comprensión de la enfermedad de ACVh/aneurisma.

Descriptores: polimorfismo genético; Accidente vascular cerebral; Genes Bcl-2.

ORIGINAL

Introduction

Cerebral aneurysm is characterized by distention and consequently the dilation of a cerebral artery, caused by the weakening and destruction of a vessel. When it ruptures, it causes Hemorrhagic Stroke (HS), which is characterized by blood leakage, in a certain brain region, due to an obstruction or rupture of a vessel.[1–3]

HS is divided into three major groups according with the main risk factors: modifiable, which include systemic arterial hypertension, smoking and diabetes mellitus; non-modifiable factors, such as age, gender, race and family history; and the potential risk group, such as physical inactivity, obesity and alcoholism. Therefore, it is a disease that involves genetic and environmental factors.[4]

The Bcl-2 family (B-cell lymphoma protein 2) is a family of apoptosis-inducing and death-suppressing proteins that actively participate in the regulation of apoptosis. Members of the Bcl-2 family inhibit apoptosis as they prevent the release of cytochrome c, and are called anti-apoptotic regulators.[5]

Located on the human chromosome 18q21.33, genes of the Bcl-2 family have been associated as important mediators of neurotoxic effects in some studies to HS, as they are associated with the apoptotic cascade determined by BAX / BCL2 and caspase 9, which activate inflammation.[6-7]

Thus, we aimed to verify the distribution of the BCL2 gene (rs1801018) polymorphism, in its coding region, in patients with Hemorrhagic Stroke/ aneurysm. In addition, we sought to associate the present polymorphism with the clinical manifestations of the disease.

Method

Subjects

In this observational, cross-sectional and case-control study, the research participants were divided into two groups.

The participants in the case group were selected from the medical records of a hospital in the Federal District (Brazil). All participants were of both sexes and over 18 years of age. Therefore, 78 patients with Hemorrhagic Stroke / Aneurysm were selected.

The control group was formed by research participants who had no history of Hemorrhagic Stroke / Aneurysm and were not related to the case group, in addition, were of both sexes and over 18 years of age. Thus, 80 individuals participated in this group.

This study was approved by the Research Ethics Committee of FEPECS and all participants had a detailed clinical history and clinical evolution followed.

Laboratory methods

After signing the Free and Informed Consent Form, 10 mL of venous blood was collected from all participants. From that, molecular biology techniques were applied.

Deoxyribonucleic acid (DNA) was extracted from venous blood using Invitek's Invisorb Spin Blood Mini Kit (250) (catalog # CA10-0005, lot # 1031100300), reaching an average concentration of 20ng / µL. The genotyping of the BCL2 rs 1801018 gene took place using the Polymerase Chain Reaction (PCR)

technique. The oligonucleotides used were: Senso 5´CCCGTTGCTTTTCCTCTGGGA3´ and Antisenso 5´GGGAGGAGAAGATGCCCGCCGCGGGG3´. The following thermocycling conditions were: initial denaturation at 94ºC for 5 minutes, followed by 35 cycles of denaturation at 94ºC for 45 seconds, followed by 48ºC for 45 seconds for the annealing of oligonucletides and 72ºC for 60 seconds for the extension step. Finally, the final extension process took place at 72ºC for 10 minutes.

At the end of the DNA amplification by PCR and using the 178bp fragment as a product, the enzymatic digestion process was carried out, using the BglI enzyme (New England Biolabs, Inc. Beverly, MA, USA), which cleaves the fragment of the Ala43 allele in two fragments: 157 bp and 21 bp, and is not able to cleave the Thr43 allele, the latter remaining with 178 bp.

Statistical analysis

The Hardy Weinberg equilibrium was performed, in which the control group was considered within the equilibrium. Genotype and allele frequencies were estimated using the SPSS program, version 23.0. For the comparison of frequencies, the Chi-Square test was used. Probabilistic associations of less than 5% were considered.

The clinical characteristics of patients, such as Hypertension, Diabetes, also had their frequencies calculated. However, variables such as glucose, creatinine and platelets were described by summary statistics (mean and standard deviation).

Results

A total of 158 research participants were divided into two groups. The case group consisted of 78 patients with Hemorrhagic Stroke/Aneurysm, 49 of whom were women (62.8%) and 29 were men (37.2%), with a mean age of 55 ± 6 years. The control group, on the other hand, consisted of 80 research participants considered healthy, 39 women (48.8%) and 41 men (51.2%), with an average age of 54 ± 1 years. It is worth mentioning that there was no statistical difference between the variables gender (p = 0.075) and age (p = 0.186).

Table 1 shows the genotype and allele frequencies of the BCL2 gene, in both groups. The ala43ala genotype was found in 88.5% of the patients in the case group (n = 69) and in 75% of them in control group (n = 60). While the thr43thr genotype was not seen in the case group, it was found in 21.3% of the control group participants. In addition, a statistically significant difference was found between the groups (p = 0.048).

When assessing allele frequencies, we noted that the ala allele was found more frequently in both groups studied. Therefore, the mutant allele, thr, acted as a protective factor against the occurrence of Hemorrhagic Stroke / Aneurysm (p = 0.011; OR = 2.74; CI = 1.22-6.13) (Table 1).

Table 1- Genotype and allele distribution of the BCL2 polymorphism rs1801018 in the case-control study.

	***BCL2* rs1801018**						
	Group						
	HS/aneurysm		**Control**				
	N	**%**	**N**	**%**	**P**	**OR**	**CI**
ala43ala	69	88,5	60	75,0			
ala43thr	9	11,5	17	21,3	0,048*	NA	NA
thr43thr	0	0,0	3	3,8			
Total	78	100,0	80	100,0			
ala43ala	69	69,0	60	75,0			
ala43thr/thr43thr	9	9,0	20	25,0	0,028*	2,55	1,08-6,03
Total	78	78,0	80	100,0			
ala	147	94,2	137	85,6			
thr	9	5,8	23	14,4	0,011*	2,74	1,22-6,13
Total	156	100,0	160	100,0			

Chi-square test; NA = Not applicable; p*<0.05.

After assessing few characteristics, such as Systemic Arterial Hypertension (SAH), Diabetes, Smoking and Alcoholism, only in the participants with Hemorrhagic Stroke / Aneurysm, it was observed that there was no statistical association between the presence of the BCL2 gene polymorphism with the present characteristics (Table 2).

Table 2- Association between the BCL2 rs1801018 polymorphism and the clinical characteristics / habit related to patients in the case group.

Clinical characteristic / habits		***BCL2* rs1801018**				
		ala43ala		***ala43thr/thr43thr***		
		N	**%**	**N**	**%**	**P**
SAH	Yes	55	88.7%	7	11.3%	0.893
	No	14	87.5%	2	12.5%	
Diabetes	Yes	3	100.0%	0	0.0%	0.524
	No	66	88.0%	9	12.0%	
Smoking	Yes	28	90.3%	3	9.7%	0.676
	No	41	87.2%	6	12.8%	
Alcoholism	Yes	18	81.8%	4	18.2%	0.250
	No	51	91.1%	5	8.9%	

Chi-square test; SAH = Systemic Arterial Hypertension.

Table 3 describes summary statistics of some biochemical parameters, such as blood glucose, creatinine and platelets. Analyzing only the platelet parameter, an increase in its mean is observed when associated with the mutant thr allele absence. However, the value is still within the normal range. We highlight that, only for this parameter, a statistically significant difference was found when analyzing the genotypes (p = 0.010).

Table 3- Summary statistics of blood glucose, creatinine and platelets according to the presence of BCL2 polymorphism rs1801018 related to patients in the case group.

Laboratorial Variable	*BCL2* rs1801018						p
	ala43ala			*ala43thr/thr43thr*			
	Mean	Standard Deviation	N valid	Mean	Standard Deviation	N valid	
Glucose (mg/dL)	107	22	62	95	21	9	0.131
Creatinine (mg/dL)	1.1	1.4	67	0.9	0.1	9	0.578
Platelets (x100.000 mm^3)	330	72	67	262	70	9	0.010*

Chi-square test; p*<0.05.

Discussion

Stroke is a disease that has a higher prevalence of deaths in Brazil, and is also seen as one of the pathologies that causes disability worldwide. As a characteristic, it predominantly affects middle-aged adults and the elderly.[8] This is more frequent in women than men, however in the study by Carvalho et al[9], carried out in Rio Verde - Goiás (Brazil), stroke was more frequent in men than women, being justified by the authors, that this finding is probably due to the fact that the sample is composed, for the most part, by the male gender.

Stroke is a pathology that involves multifactorial causes, which makes its diagnosis more complex, in addition to making it clear that a change in lifestyle is necessary.[10-11] Members of the Bcl-2 family are known for their anti-apoptotic role[5], and suppression of their expression increases the risk of apoptosis and cell necrosis.

In this study, the possible association of the rs1801018 polymorphism in the BCL2 gene and its relationship with the clinical manifestations in patients with Hemorrhagic Stroke / Aneurysm were explored.

When assessing the allele frequency of the polymorphism in the BCL2 gene (rs1801018), it is noted that the ala allele was found in 94.2% of the research participants in the case group, and that this allele was considered a risk factor for the development of Hemorrhagic stroke / aneurysm (p = 0.011; OR = 2.74). In the study by Avetyan et al[12], carried out in Armenia (Asia), the wing (A) or wild allele was seen more frequently in patients with post-traumatic stress. Studies in other diseases, such as thyroid cancer, esophageal cancer and hepatitis C, also found the same result[13–15]

The main risk factors for the development of a stroke/aneurysm are uncontrolled high blood pressure, diabetes, excessive alcohol use and smoking.[16-17] When analyzing the polymorphism of the BCL2 gene associated with the presence of clinical manifestations, SAH and Diabetes, and habits, Smoking and Alcoholism, it was noted that they were not statistically associated with the occurrence of Hemorrhagic Stroke / Aneurysm.

Biochemical and hematological characteristics, such as glucose and platelets, were evaluated. The mean glycemia found is not above the reference values for diabetes risk, and also does not show a statistically significant difference (P = 0.131). It is important to note that the World Health Organization (WHO) diagnostic criteria for diabetes are: fasting plasma glucose ≥126mg / dL or 2-h plasma glucose ≥ 200mg / dL.[18]

However, a relevant fact identified in this study was the statistical difference (p = 0.010) in relation to the mean number of patients' platelets, and the mutant allele (thr) had a reduced number of platelets. In the literature, a study by Xiang et al[19] verified an association between increased pressure and the excess in blood shear tension - this phenomenon causes pores in the vessels, which could activate platelets as a mechanism of thrombosis aneurysm.

Therefore, verifying that, in patients with Hemorrhagic Stroke / Aneurysm, the absence of the mutant allele increases the platelets number, can be a step to understand that the mutation absence is contributing to the anti-apoptotic activation of these cells, which indirectly favors increasing the number of cells. However, gene expression studies should be performed to support this hypothesis.

Conclusion

We observed that in the polymorphism of the BCL2 gene (rs1801018), the thr allele (mutant) is associated with Hemorrhagic Stroke / Aneurysm, which is considered a protective factor for the development of the disease.

The absence of the mutant allele in the studied polymorphism was associated with an average increase in the number of platelets in individuals with Hemorrhagic Stroke / Aneurysm. However, when evaluating the other clinical conditions, habits and laboratory variables, no statistical difference was found.

Studies in other gene regions and other populations should be carried out to better understand the participation of pro and anti-apoptotic mechanisms in the Hemorrhagic Stroke / Aneurysm disease.

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Correspondent Author

Izabel Cristina Rodrigues da Silva.
Universitary Campus, n/n, Metrpolitan Center.
ZIP: 72220-275. Brasilia, Federal District, Brazil.
belbiomedica@gmail.com