J Bras Patol Med Lab. 2020; 56: 1-4.

10.5935/1676-2444.20200031

Statistical association of rs2243250 polymorphism of *IL4* gene and hemorrhagic stroke in Brazilian population

Associação estatística entre o polimorfismo rs2243250 no gene da IL-4 *e o AVC hemorrágico na população brasileira*

Ângelo M. Rolim[1]; Felipe S. A. Borges[2]; Aline R. Barros[2]; Jonathan D. Lima[2]; Fabiana B. A. Silva[2]; Hélia Carla de Souza[3]; Daniel O. Freire[1]; Luzitano B. Ferreira[3]; Izabel Cristina R. Silva[2]

1. Faculdade LS, Brasília, Distrito Federal, Brazil. 2. Universidade de Brasília, Brasília, Distrito Federal, Brazil. 3. Centro Universitário de Brasília, Brasília, Distrito Federal, Brazil.

ABSTRACT

Interleukin-4 (IL-4) has great significance in inflammatory processes in cases of stroke, since it is able to polarize microglia to the anti-inflammatory phenotype called M2. This study analyzed if the variation between TT genotype and the other genotypes (CT and CC), in -589 (rs2243250) polymorphism of *IL4* gene, has association with the prognosis of hemorrhagic stroke (HS) and with clinical aspects which are risk factors for cerebrovascular diseases. The result of this study shows that there is no statistical association of the *IL4* polymorphism with either prognosis or clinical aspects in HS patients.

Key words: interleukin-4; single nucleotide polymorphism; stroke; intracranial hemorrhages, intracranial aneurysm.

RESUMO

A interleucina-4 (IL-4) tem grande importância nos processos inflamatórios em casos de acidente vascular cerebral (AVC), uma vez que ela é capaz de polarizar micróglias para o fenótipo anti-inflamatório chamado M2. Este estudo analisou se a variação entre o genótipo TT e os demais genótipos (CT e CC), no polimorfismo -589 (rs2243250) do gene IL4*, possui associação com o prognóstico de AVC hemorrágico e com aspectos clínicos que são fatores de risco para doenças cerebrovasculares. O resultado deste estudo mostra que não há associação estatística do polimorfismo do* IL4 *nem com prognóstico nem com os aspectos clínicos dos pacientes com AVC hemorrágico.*

Unitermos: *interleucina-4; polimorfismo de nucleotídeo único; acidente vascular cerebral; hemorragias intracranianas; aneurisma intracraniano.*

RESUMEN

La interleucina-4 (IL-4) tiene gran importancia en los procesos inflamatorios en casos de accidente cerebrovascular (ACV), puesto que hace que las microglías sean polarizadas hacia el fenotipo antiinflamatorio M2. Este estudio analizó si la variación entre el genotipo TT y los demás genotipos (CT y CC), en el polimorfismo -589 (rs2243250) del gen IL4*, posee asociación con el pronóstico de ACV hemorrágico y con aspectos clínicos que son factores de riesgo para enfermedades cerebrovasculares. El resultado de este estudio enseña que no hay asociación estadística del polimorfismo del* IL4 *ni con el pronóstico ni con los aspectos clínicos de pacientes con ACV hemorrágico.*

Palabras clave: *interleucina-4; polimorfismo de nucleótide simple; accidente cerebrovascular; hemorragias intracraneales; aneurisma intracraneal.*

First submission on 08/09/19; last submission on 08/19/19; accepted for publication on 08/20/19; published on 04/28/20

INTRODUCTION

Cardiovascular diseases are the leading causes of death globally. In this context, stroke and heart attack are responsible for 85% of those deaths[1]. According to the World Health Organization (WHO), there are approximately 15 million cases of stroke each year worldwide. Of those, 5 million results in death and another 5 million in permanent disability[2]. This can be noticed in Brazil, where stroke is one of the main causes of hospitalization and mortality, with a great majority of surviving patients ending up somewhat disabled[3].

There are two types of stroke: the ischemic stroke, which is the most common form (80%-85% of cases); and the hemorrhagic stroke (HS), the unusual form of the disease (15%-20% of cases)[4].

After an ischemic stroke, several harmful agents are released from the ischemic core to the ischemic penumbra. Those agents are able to initiate proinflammatory responses that may cause damage to the local nervous tissue, but they can also activate the microglia present in the environment[5]. The activation of microglia, resident in the central nervous system, is a heterogeneous process, since it can result in two subtypes: the pro-inflammatory phenotype M1, by the classical activation; and the anti-inflammatory phenotype M2, by the alternative activation[6, 7].

Interleukin-4 (IL-4), which is released from the neurons in the ischemic penumbra, plays a significant role in this process, as it is able to polarize microglia from the phenotype M1 to the phenotype M2. This happens because IL-4 can induce expression of genes responsible for the M2 phenotype. IL-4 can also enhance the expression of the IL-4 receptor on microglia[5]. The great majority of studies that show the role of IL-4 in stroke cases deal with the ischemic stroke, but the few studies related to HS show IL-4 promoting the activation of phenotype M2 in microglia[8].

The human gene responsible for IL-4 codification is located on chromosome 5q31.1[9]. Studies have pointed that polymorphisms in genes regulating the inflammatory response can be associated with increased risk of both types of stroke[10]. In the promoter region of *IL4* gene, there is the rs2243250 polymorphism (C/T) at position downstream -589[11]. This single nucleotide polymorphism (SNP) is pivotal, since it was demonstrated that the risk of HS is influenced by the alleles of rs2243250[9]. Considering that this topic is poorly understood, our study aimed to investigate the correlation between variation in *IL4* -589 alleles in Brazilian HS patients and five clinical aspects of these patients [sex, systemic arterial hypertension (SAH), diabetes, alcohol consumption, smoking status], besides the prognosis of HS.

We recruited a total of 21 patients for the study (14 females and 7 males), all of them diagnosed according to the WHO definition of stroke, and the results were confirmed by imaging [computed tomography (CT) or magnetic resonance image (MRI)][12]. The samples were obtained from a hospital-based case-control study undertaken from January 2011 to December 2012.

This study was submitted to and approved by the institutional Ethics Committee and, before information collection, we obtained the informed consent from all the participants.

Clinical evaluations were made in all the patients, who also had their detailed history analyzed. The following aspects are common risk factors for cerebrovascular diseases and they were evaluated for each patient: sex, current smoking, alcohol consumption, SAH (blood pressure was measured), diabetes and the HS prognosis. This last aspect was assessed with the modified Rankin scale (MRS), which is widely applied to evaluate recovery from stroke[13]. This scale is used to measure the degree of dependence in daily activities or disability in patients[14, 15].

The deoxyribonucleic acid (DNA) of each patient was obtained from 5 ml peripheral venous blood sample using the Invisorb Spin Blood Mini Kit (250) by Invitek (catalog #CA10-0005, batch #1031100300). Polymerase chain reaction (PCR) combined with restriction fragment length polymorphism analysis was used to perform the genotyping of *IL4* -589 C/T (rs2243250) polymorphism. The primers used to amplify the rs2243250 polymorphism were 5'-AAA CTA GGC CTC ACC TGA TAC G-3' forward and 5'-TGC ATA GAG GCA GAA TAA CAG G-3' reverse. The amplification was conducted using the following cycling program: start with denaturation step at 94°C for 30 seconds, followed by the annealing step at 55°C for 30 seconds and finally the extension step at 72°C for 60 seconds. The products of PCR were sequenced by the Genetic Center of Universidade de São Paulo (USP), Brazil.

The allele frequencies of the HS patients were compared among them, specifically, with each one of the five clinical aspects previously mentioned and with the HS prognosis. This comparison was drawn using the chi-squared test in recessive and dominant variations of the SNP.

The clinical characteristics of the patients had no statistical association with the polymorphism, as shown in the **Table** (p-value > 0.05). The Table compares the TT genotype with the others (CC homozygote and CT heterozygote); this form of representation was chosen due to the reason that the -589T allele was able to increase IL-4 expression[16]. Sex has no association with the genotypic distribution of the polymorphism. The same result was shown for HS and diabetes, but it is important to mention that although diabetes was one of the evaluated aspects, since it is well known that

diabetes can increase the stroke risk promoting changes in cerebral blood vessels[17], after the analyses, we observed that none of the patients was diabetic. Alcohol consumption and smoking status also had no statistical association with the SNP. Smoking status presented a small variation between the groups with different alleles ($p = 0.128$), but this result was not statistically significant. The MRS results, as well as smoking status, show a small variation in patients with different alleles. However, this variation in rs2243250 also had no association with prognosis in HS patients ($p = 0.624$), which is the most important aspect of this study.

TABLE – Association between *IL4* -589 (C/T) rs2243250 polymorphism genotypes and clinical aspects in the model

		IL4 C/T (rs2243250)						
		TT		CT + CC				
		n	%	*n*	%	*p*	OR	CI (OR)
Sex	Female	3	60	11	68.8	0.999	0.68	0.09-5.45
	Male	2	40	5	31.3			
SAH	Yes	4	80	13	81.3	0.999	0.92	0.07-11.54
	No	1	20	3	18.8			
Diabetes	Yes	0	0	0	0	0.999	NA	NA
	No	5	100	16	100			
Smoking status	Yes	2	40	1	6.3	0.128	10	0.67-149.05
	No	3	60	15	93.8			
Alcohol consumption	Yes	1	20	4	25	0.999	0.75	0.06-8.83
	No	4	80	12	75			
MRS	Poor prognosis	1	20	6	37.5	0.624	0.42	0.04-4.66
	Good prognosis	4	80	10	62.5			

SAH: systemic arterial hypertension; MRS: modified Rankin scale; OR: odds ratio; CI: confidence interval; NA: not applicable.

According to genome-wide association studies (GWAS), genetic factors have great importance for risk and prognosis of HS[18]. Other studies, such that by Yamada *et al*. (2006)[19], revealed the role of pro-inflammatory cytokine SNP in the susceptibility to strokes. Among them, the IL-6 polymorphism was significantly associated with HS. Borges *et al*. (2018)[20], in a Brazilian population study, underlined the SNP tumor necrosis factor alpha (TNFα)-308 A/G as a possible protective factor against HS. Park *et al*. (2011)[21], in a study with 79 Korean HS patients, found a significant statistical relation between two SNPs (rs2243250 and rs2070874) of *IL4* gene; however, they also call attention to the fact that the sample used in their study was an important limiting factor. Until the present moment, there are few studies about the relation between polymorphisms and the *IL4* gene; therefore the conclusions concerning the genetic background related to this gene and its connection with HS are limited.

This study shows that, in the Brazilian population, the variation of C and T in rs2243250 polymorphism is not associated with the common risk factors for HS, the least frequent type of stroke. Another important aspect is that there is no statistical association between HS prognosis and the allele frequency in this SNP. However, the major limitation of this study was the small number of analyzed individuals. Further studies with a larger number of patients are necessary to report with more confidence the absence of association between those clinical aspects of HS patients and the allele variation in rs2243250 SNP.

REFERENCES

1. Cardiovascular diseases (CVDs) [Internet]. [cited 2019 May 1]. Available at: https://www.who.int/en/news-room/fact-sheets/detail/cardiovascular-diseases-(cvds).

2. Katan M, Luft A. Global burden of stroke. In: Mackay J, Mensah G, editors. Vol. 38. Atlas Heart Dis Stroke. 2004; 50-1.

3. Almeida SRM. Análise epidemiológica do acidente vascular cerebral no Brasil. Rev Neurociencias. 2012; 20(4): 481-2.

4. Porcello Marrone LC, Diogo LP, de Oliveira FM, et al. Risk factors among stroke subtypes in Brazil. J Stroke Cerebrovasc Dis. 2013; 22(1): 32-5.

5. Zhao X, Wang H, Sun G, Zhang J, Edwards NJ, Aronowski J. Neuronal interleukin-4 as a modulator of microglial pathways and ischemic brain damage. J Neurosci [Internet]. 2015; 35(32): 11281-91. Available at: http://www.jneurosci.org/cgi/doi/10.1523/JNEUROSCI.1685-15.2015.

6. Tang Y, Le W. Differential roles of M1 and M2 microglia in neurodegenerative diseases. Mol Neurobiol. 2016; 53(2): 1181-94.

7. Schwartz YS, Svistelnik AV. Functional phenotypes of macrophages and the M1-M2 polarization concept. Part I. Proinflammatory phenotype. Biochem. 2012; 77(3): 246-60.

8. Yang J, Ding S, Huang W, et al. Interleukin-4 ameliorates the functional recovery of intracerebral hemorrhage through the alternative activation of microglia/macrophage. Front Neurosci. 2016; 10(Mar): 1-8.

9. IL4 interleukin 4 [Homo sapiens (human)] [Internet]. 2019; 114-9. Available at: https://www.ncbi.nlm.nih.gov/gene/3565.

10. Wei H, Feng G, Pei W, Gang W, Yuelin Z. Association between interleukin-4 genetic polymorphisms and the risk of cerebral infarction in a population of China. Biomed Res. 2017; 28(21): 9198-203.

11. Anovazzi G, Medeiros MC, Pigossi SC, et al. Functionality and opposite roles of two interleukin 4 haplotypes in immune cells. Genes Immun [Internet]. 2017; 18(1): 33-41. Available at: http://dx.doi.org/10.1038/gene.2016.47.

12. Hatano S. Variability of the diagnosis of stroke by clinical judgement and by a scoring method. Bull World Health Organ. 1976; 54(5): 533-40.

13. Banks JL, Marotta CA. Outcomes validity and reliability of the modified Rankin scale: implications for stroke clinical trials: a literature review and synthesis. Stroke [Internet]. 2007; 38(3): 1091-6. PubMed PMID: 17272767.

14. Dewilde S, Annemans L, Peeters A, et al. Modified Rankin scale as a determinant of direct medical costs after stroke. Int J Stroke. 2017; 12(4): 392-400.

15. van Swieten JC, Koudstaal PJ, Visser MC, Schouten HJA, van Gijn J. Interobserver agreement for the assessment of handicap in stroke patients. Stroke. 1988; 19(5): 604-7.

16. Paffen E, Medina P, de Visser MCH, et al. The -589C>T polymorphism in the interleukin-4 gene (IL-4) is associated with a reduced risk of myocardial infarction in young individuals. J Thromb Haemost. 2008; 6(10): 1633-8.

17. Chen R, Ovbiagele B, Feng W. Diabetes and stroke: epidemiology, pathophysiology, pharmaceuticals and outcome. Am J Med Sci. 2017; 351(4): 380-6.

18. Chauhan G, Debette S. Genetic risk factors for ischemic and hemorrhagic stroke. Curr Cardiol Rep. 2016; 18(124): 1-11. Available at: https://doi.org/10.1007/s11886-016-0804-z.

19. Yamada Y, Metoki N, Yoshida H, et al. Genetic risk for ischemic and hemorrhagic stroke. arteriosclerosis, thrombosis, and vascular biology. 2006; 26(8): 1920-5.

20. Borges SAB, Renata SF, Rafael MM, et al. TNFA gene in Brazilian patients with hemorrhagic stroke or cerebral aneurysm. J Bras Patol Med Lab. 2018; 54(3): 164-9.

21. Park HJ, Kim MJ, Kang SW, et al. Association between interleukin-4 gene polymorphisms and intracerebral haemorrhage in Korean population. Int J Immunogenet. 2011; 38(4): 321-5. Pubmed PMID: 21501388.

CORRESPONDING AUTHOR

Izabel Cristina Rodrigues da Silva ID 0000-0002-6836-3583
e-mail: belbiomedica@gmail.com