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Original Article

Tumour necrosis factor alpha, ınterleukin 10 and ınterleukin 6 gene polymorphisms of ıschemic stroke patients ın south Marmara region of Turkey

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Abstract: Background: Stroke is an important cause of adult mortality and morbidity; however its pathogenesis is still unknown. Several studies have examined to determine the role of genetic polymorphism of proinflammatory cytokines in the occurence of stroke. The objective of this study was to evaluate the relationship between three polymorphisms; including tumour necrosis alpha (TNFα)-238 GA, interleukin(IL-10)-1028 GA (rs1800896), IL-6-(rs1800795) and ischemic stroke in a Turkish population. Methods: Forty two stroke patients and 48 healhty controls were genotyped using PCR analysis for TNFα-238 G/A, IL-10-1028 GA and IL-6-rs1800795 AG polymorphisms. Results: The frequency of the CC and CG, GG genotype of IL-6 gene (rs1800795) were statiscially significiantly higher in IS patients than controls (for C/C genotype, *P*=0.03, OR=4.3; 95% CI: 1.13 to 16.29 and for C/G genotype, *P*=0.04, OR=3.6; 95% CI: 1.03 to 12.95, for G/G genotype, *P*=0.02, OR=0.25; 95% CI: 0.07-0.85 respectively). Conclusion: Il-6 CC genotyped was found strongly associated with ischemic stroke than other two polymorpisms TNF-α and IL-10 in our population.

Keywords: Stroke, TNF-α, Il-6, Il-10, polymorhisms

Introduction

Ischemic stroke (IS) is an important cause of disability and mortality in the worldwide. It accounts for 85 to 90% of all strokes and is closely related with both environmental and genetic factors. Inflamation also is an important mechanism which contributes to ischemic stroke [1, 2]. An increased levels of proinflammatory cytokines has been found in patients with acute ischemic stroke [3]. Cytokines were thougth to play an essential role in immun response and regulates the normal homeostatic environment of central nervous system [4]. In addition to all, recently the genes encoding these inflammatory cytokines were also found associated with the pathogenesis of stroke [5]. Tumour necrosis alpha (TNFα), interleukin (IL)-10 and IL-6 genes are one of these cytokines with antiinflammatory properties, which has been showed taking part in the pathogenesis of IS [6-8].

The present study was aimed to evaluate the association of tumour necrosis alpha (TNFα)-238 G/A, interleukin(IL-10) gene-1028 G/A (rs1800896) and IL-6 gene-(rs1800795) polymorphism with ischemic stroke in a Turkish population.

Material and method

Subjects

The study group enrolled 42 patients with ischemic stroke and 48 healhty age and sex matched control subjects who were admitted to Canakkale 18 Mart University Medical Faculty Neurology clinic. The stroke subjects were obtained from both acute strokes and the ones who had stroke before. All patients were diagnosed by neuroimaging evidence with both CT and MRI according to the World Health Organization's diagnostic criteria for ischemic stroke. Patients with intracranial hemorrhage,

Table 1. Distributions of demographic characteristic

Variable	Stroke (n=42) N (%); mean ± SD	Control (n=48) N (%); mean ± SD	*P* value
Male	21 (50%)	17 (35.4%)	0,16
Female	21 (50%)	31 (64.6%)	
Age (mean ± SD)	63.57±15.3	62.29±12.6	0.77
Diabetes	20 (47.6%)	13 (27%)	0.04
Hypertension	27 (64.3%)	28 (58.3%)	0.56
Smokers	19 (45.2%)	15 (31.3%)	0.17
Alcohol consumers	9 (21.4%)	6 (12.5%)	0.25
TC (mmol/L)	199.34±46.42	166.85±62.53	0.005
TG (mmol/L)	126.13±84.06	104.33±36.84	0.16
HDL-C (mmol/L)	46.66±13.34	49.43±14.03	0.36
LDL-C (mmol/L)	115.80±30.97	101.70±35.14	0.023

SD: standart deviation; TC: total cholesterol; TG: triglycerides; HDL-C: high density lipoprotein cholesterol; LDL-C: low density lipoprotein cholesterol.

brain tumors or transient ischemic attacks were excluded from study. The study was approved by the local ethical committe and written informed constent from each individual have been obtained.

Genotyping assays

2 mL of venous blood was obtained from each subjects. DNA isolation was performed by spin column methods with 400 L of blood. The total reaction volume was prepared in 20 L for each Snp (single nucleotide polymorphism). Spesific primers (T1bMolBiol, LightSNIP) used for TNF Alpha G238A (rs361525), IL6 (rs1800795) and IL10 (rs1800896). 20 l Reaction mixture prepared for each (2 l FastStart DNA Master, 1.6 l $MgCl_2$ for final $MgCl_2$ concentration was 3.0 mM, 1 l primer and 10.4 l H_2O) FastStart DNA Master Hybprobe (Roche Diagnostics) (Taq polymerase, deoxynucleotide triphosphates) and 1-5 l (50 ng) DNA were combined for detection of the each polymorphism. Same protocol used for all three polymorphisms. Real-time polymerase chain reaction (PCR) protocol was carried out using a Light Cycler 20 (Roche) device; denaturationwas at 95°C for 10 min; quantitation was with 45 cycles at 95°C for 10 sec, 60°C for 10 sec and 72°C for 15 sec; melting was at 95°C for 20 sec, 40°C for 20 sec and 85°C in 0.2 continuous mode, with cooling at 40°C. G and A alleles for TNF alpha, C and G allels for IL6 rs1800795 and A and G alleles analysed for IL10 rs1800896 with Melting curve analysis at channel 530 after real-time PCR.

Statistical analysis

All data were analyzed with the Statistical Package forthe Social Sciences (SPSS; SPSS Inc., Chicago, IL, USA). Data analysis was with the Kruskal-Wallis test for determination of normal distribution. Categorical variablesare shown as percentages. Other variables are shown asmean # standard deviation (SD) and median (mini-mum-maximum). Between-group differences were eval-uated by Chi-square test (for categorical values). Student's t-test and Mann-Whitney U-test were used forparametric changes and non-parametric values. Odds ratios were calculated in the IS group for frequency of genotypes and alleles of TNF alpha, IL6 and IL10 genes. *P*-values <0.005 were considered statistically significant.

Results

The demographic characteristics of the study group are shown in **Table 1**. The mean age of the patients was 63.57±15.3 years and 62.29±12.6 in the control group. Out of 42 patients 50% [21] were females and 50% [21] were males. Out of 48 control group 64.6% [31] were females and 35.4% [17] were males. There were no statiscially differences in age and sex between two groups.The patients group had statiscially significiant much more diabetes than control group (P=0.04). The percentage of hypertension was 64.3% among stroke patients and 58.3% in control group. Forty-five percent of stroke patients were smokers and 21.4% were alcohol consumers. There were not significiantly difference between IS patients and control group for hypertension, smoking or alcohol usage.The mean blood total cholesterol and LDL levels of stroke patients were statiscially significiantly higher than controls (respectively *P*=0.005; *P*=0.023) (**Table 1**).

In our study group we genotyped three polymorphisms: (TNFα)-238 G/A, IL-10-1028 G/A and IL-6 in 42 stroke patients and in 48 control subjects (**Table 2**).

Table 2. Genotype frequencies of IL-10, IL-6 and TNF-α polymorphism among IS patients and controls with their odds ratios

Variant		Ischemic stroke (n=42)	%	Controls (n=48)	%	OR (95% CI)	*P*
IL-10	AA	11	26.1	19	39.6	1.27 (0.34-4.63)	0.7
	AG	26	61.9	18	37.5	3.17 (0.94-10.72)	0.06
	GG	5	11.9	11	22.9	0.45 (0.14-1.43)	0.17
Allele	A	48	57	56	58	1.05 (0.58-1.89)	0.87
	G	36	43	40	42		
IL-6	CC	16	38	13	27	4.3 (1.13-16.29)	0.03
	CG	22	52.3	21	43.7	3.6 (1.03-12.95)	0.04
	GG	4	9.5	14	29.1	0.25 (0.07-0.85)	0.02
Allele	C	54		47		1.54 (0.81-2.93)	0.18
	G	26		35			
TNF-α	GA	6	14.2	4	8.3	1.83 (0.48-6.99)	0.37
	GG	36	85.7	44	91.6		
Allele	G	42		48		1.71 (0.45-6.48)	0.42
	A	6		4			

OR: odd raito; CI: confidence interval.

The present study showed a significiant difference in genotype frequencies of the IL-6 between patients and controls. The frequency of the C/C and C/G, G/G genotype of IL-6 gene (rs1800795) were statiscially significiantly higher in IS patients than controls (for C/C genotype, P=0.03, OR=4.3; 95% CI: 1.13 to 16.29 and for C/G genotype, *P*=0.04, OR=3.6; 95% CI: 1.03 to 12.95, for G/G genotype, *P*=0.02, OR=0.25; 95% CI: 0.07-0.85 respectively).

The allelic frequency of IL-10-1028 in IS patients was 57% of A and 43% of G where as 58% of A and 42% of G allele in the control group. The genotype frequency of IL-10-1028 A/A homozygotes were found higher in stroke patients compared to G/G homozygotes. Similarly, A/G heterozygotes were also seen higher in patients compared to G/G homozygotes. And G/G homozygotes were higher in controls than patients compared to A/A and A/G genotypes. Our results revealed that A allele was risk, where as G allele was preventive factor for stroke in our population. But these findings were not statiscially significiant (*P*=0.7, OR=1.27, 95% CI: 0.34 to 4.63; *P*=0.06, OR: 3.17 95% CI:0.94 to 10.72; *P*=0.17, OR=0.45, 95% CI: 0.14 to 1.43 respectively).

In our study group there were not any A/A genotype of TNFα-238 in both patient and control groups. TNFα-238 G/A heterozygotes were seen higher in stroke patients than controls compared to G/G homozygotes genotypes with no statiscially significiant (*P*= 0.3, OR=1.83 95% CI: 0.48 to 6.99).

In conclusion, the results showed that IL-6 C/C genotyped is significiantly associated with ischemic stroke in our population.

Discussion

Ischemic stroke is the most common type of stroke and is leading one of major cause of adult disability and mortality. It is important to have knowledge of the pathophysiology of cerebral ischemia for both prophylactic and current theraphies in stroke [2]. Therefore, recently, several epidemiologic studies have investigated genetic component of different population in order to show the genes that underlying the risk for stroke [6, 8, 9].

The present study has demonstrated that IL-6 CC gene polymorphism is significiantly associated with ischemic stroke in our population. There are a lot of studies that have found association between ischemic stroke and IL-6-174 GC polymorphism with different genotypes or alleles in various population. Some of them found G allele or the GG genotype to be associated with ischemic stroke where some found C allele or the CC genotype were frequent in ischemic stroke patients [10].

Many studies were performed for the polymorfism of IL-6 gene in ischemic stroke with consistent results [6, 11, 12]. In many of these studies, IL-6-174 gene polymorphism was found to be a risk factor for ischemic stroke with different genotypes or alleles. A previous study reported that the -174 CC polymorphism had significant risk for ischemic stroke in an Chinese population [13]. Similarly, Chammaro et al. have found an indepently association between -174 CC genotype and lacuner stroke [14]. With consisted these studies our results suggest that C allele may carry a significant risk for ischemic stroke.

IL-10 is a multifunctional proinflammatory cytokine which has been involved in the pathogenesis of ischemic stroke [15, 16]. Previous studies have reported the association between IL-10 gene polymorhism and IS where as some of them have not [7, 17]. Munshi et al. found a significant association of IL-10-1082 GA genotype with stroke [18]. Also in another study, IL-10-2849 AA genotype was found associated with an increased risk for strokes in three seperate study population [19]. Jin et al. analyzed the association between IL-10-1082 AG polymorphism and ischemic stroke risk by their meta-analysis and they indicated that -1082 AG polymorphism was associated with IS and A allele may increase risk for IS in Asians. Consisted with all these studies our results revealed that both homozygotes AA and heterozygotes AG genotype of IL-10-1028 were seen more common in stroke patients than controls and A allele was thougth to be risk factor for IS. But, this findings were not supported statiscially, this may be cause of our relatively small sample size.

The tumor necrosis factor-α gene also play an important role in immune response and inflammation [8]. Previous studies have investigated TNF-α polymorphism in stroke but the results are still controversial [20-22]. Gu et al. indicated that TNF-α 238 GA polymorfism increased the risk of ischemic stroke in adult,Caucasian and overall analysis where as juvenile and Asian population had not have significant association by their meta-analysis [23]. In another study,Sultana et al. have not found any positive correlation between TNF-α-308 GA genotype and stroke [24]. Also, our results did not find significant association between TNF-α and stroke.

In conclusion, IL-6 gene polymorphism is associated with ischemic stroke both homozygous and heterozygous condition. Persons with homozygous CC genotype has 4.3 and heterozygous CG genotype has 3.6 odds ratio for ischemic stroke compared the persons with GG genotype. These findings support the hypothesis that genetic markers of the inflammatory response may be relevant to the pathogenesis of stroke. In addition, IL-6 gene polymorphism was found more important than IL-10 and TNF-α poymorphisms for IS risk in our population. We suggest that it is important to identify the genotypes of IL6 rs1800795 and C allele forischemic stroke patients' first relatives to avoid and be careful about additional risk factors for ischemic stroke.

Disclosure of conflict of interest

None.

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