RESEARCH NOTE

Tumour necrosis factor alpha and interleukin 10 gene polymorphisms and the risk of ischemic stroke in south Indian population

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Introduction

Stroke is the leading cause of adult disability and mortality and is the third largest cause of death worldwide (Bonita *et al*. 2004). Prevalence of stroke in India varies in different regions of the country and ranges from 40 to 270 per 100,000 population (Anand *et al*. 2001). Both environmental and genetic factors are involved in the causation of strokes (Bevan and Markus 2004). Cytokines play an important role in immune response and also maintain the normal homeostatic environment of the central nervous system. The key phenomenon in cytokine contribution to ischemic stroke is endothelial transformation altering hemostatic and immunological balance towards the prothrombotic and proinflammatory states (Ross 1993). The genes encoding diverse cytokines may play a vital role in the susceptibility to stroke, and the production of cytokine varies among individuals and depends on cytokine gene polymorphisms. The present study was carried out to evaluate the association of tumour necrosis alpha (*TNFα*) -308G/A and interleukin (*IL*) 10-1082G/A polymorphisms with ischemic stroke in south Indian population and the results showed that the *IL-10* 'GG' genotype is significantly associated with stroke.

Materials and methods

Subjects

The study group comprised 238 ischemic stroke patients from the major hospitals of Hyderabad, India: Bhagwan Mahavir Medical Research Centre and Government Nizamia General Hospital. For comparison, 226 individuals from the same socioeconomic group were recruited for the study as controls. The subjects studied were both new and recurrent stroke patients. Patients with acute stroke were examined by a qualified stroke neurologist to confirm the diagnosis and ischemic stroke cases were differentiated by computed tomography scans and magnetic resonance imaging. Classification of subtypes was done according to TOAST criteria (Meschia 2002). Patients with hemorrhagic cases were excluded from the study. The Institutional Ethical Committee approved this study and informed written consent was obtained from all the subjects before their participation in this study. Hypertension was defined according to Joint National Committee VI–VII, as a systolic blood pressure >140 mm Hg and/or a diastolic blood pressure >90 mm Hg based on the average of two blood pressure measurements. Diabetes was diagnosed if fasting plasma glucose was >126 mg/dL, in accordance with the American Diabetes Association (Diagnosis and Classification of Diabetes Mellitus 2009).

Estimation of lipid profiles, DNA isolation and genotyping

Six mL of venous blood from each subject was collected. Four mL was transferred to a plain test tube for serum and 2 mL was taken in a EDTA tube for DNA extraction. Total cholesterol (TC), triglycerides (TG), high-density lipoprotein (HDL), low-density lipoprotein (LDL) and very low-density lipoprotein (VLDL) were estimated using a semi-automatic analyser (Model, makeTransasia Biomedicals, New Delhi, India) using commercial kits (ERBA, Mumbai, India). DNA was isolated by salting out method (Lahari *et al*. 1992). The polymorphisms in *TNFα* (-308 G/A) and *IL-10* (-1082 G/A) genes were studied using amplification refractory mutation

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system polymerase chain reaction methods (ARMS PCR) (Perrey *et al*. 1999; Louis *et al*. 2000).

Statistical analysis

Lipid profiles of ischemic stroke patients and controls were analysed using independent sample *t*-test. The association between genotypes and stroke was examined by using odds ratio (OR) with 95% confidence interval (CI) and chi square (χ^2) analysis using EPI info 6 software (EPI info 6 CDC; http://www.cdc.gov/epiinfo/Epi6/ei6.htm). All the statistical tests were two-sided, and were considered significant at $P < 0.05$. Genotypic frequencies were calculated according to the number of different genotypes observed and the total number of genotypes examined. Yate's correction (Yates 1934) was applied wherever necessary. The logistic regression was carried out to understand the effect of confounding factors for stroke. Genotype frequencies were checked for deviation from Hardy–Weinberg equilibrium (HWE) and were not significantly different from those predicted.

Results

Demographic characteristics of the study population are shown in table 1. Mean (±SD) age of patients was 53.72 ± 11 years as against the 54.06 ± 10 years in the control group. Out of 238 patients studied, 68.9% (164) were males and 31.1% (74) were females. Out of 226 control subjects selected, 53.5% (121) were males and 46.5% (105) were females. The percentage of diabetes was 32% among stroke patients and 29% in control group. Seventy-four per cent of the patients and 55% of the control subjects were hypertensives, indicating statistically significant difference in the frequency of hypertension between patients and control groups ($P = 0.05$). Forty-three per cent of stroke patients were smokers and 30.7% were alcohol consumers. The mean (±SD) blood level of total cholesterol among ischemic stroke patients was 238.5 ± 24.5 mg/dL against 164.6 ± 16.2 mg/dL in controls. Blood levels of triglycerides in patients was 109.6 ± 27.1 mg/dL and 98.5 ± 31.2 mg/dL in controls. High density lipoprotein was 35.6 ± 7.1 mg/dL in patients and 43.4 ± 5.2 mg/dL in controls, whereas the mean blood levels of low density lipoprotein was 185.5 ± 11.2 mg/dL in patients and 93.6 ± 16.5 mg/dL in controls. Very low density lipoprotein levels in patients were 37.6 ± 4.7 mg/dL and 31.7 ± 3.2 mg/dL in controls. TC, TG, HDL, LDL and VLDL levels were significantly high in patients compared to controls ($P < 0.01$) (table 1).

In our case–control study we genotyped two SNPs: *TNFα*-308G/A and *IL-10* -1082G/A in 238 stroke patients and in 226 control subjects. The genotype frequencies of the two SNPs studied are shown in table 2. Genotype frequency obtained from *TNFα* gene analysis in patients with stroke revealed that 88.7% were G/A heterozygotes followed by G/G homozygotes (7.1%) and A/A homozygotes (4.2%). In the control group, 91.6% were G/A heterozygotes followed by 5.3% G/G homozygotes and 3.1% A/A homozygotes. The allelic frequency in patient group was 48.5% of G and 51.5% of A, as against 48.9% of G allele and 51.1% of A allele in the control group. The statistical analysis of the data showed that the differences in the allelic and genotypic frequencies between the two groups are not significant for *TNFα* (table 2).

The results of the present study, showed a significant difference for genotype frequencies of the *IL10* -1082 variants between stroke and control subjects ($P = 0.0016$). The genotypic frequency of *IL-10* -1082 G/G was significantly high among stroke patients (16.8%) compared to control subjects (7.1%). The control group showed 72.1% of A/A and 20.8% of G/A genotypes, while in the patient group the frequency was 64.7% of A/A and 18.5% of G/A genotypes The allelic frequency of *IL-10* -1082 G was significantly high among patients with stroke (26.1%) compared to control subjects (17.5%) ($P = 0.0016$, OR 1.66; 95% CI: 1.21–2.28). Statistically significant association was observed in only homozygous G/G genotype among patients with stroke ($P = 0.0013$, OR 2.6, 95% CI: 1.43–4.88).

Table 1. Demographic characteristics of the study population.

Variable	Stroke patients ($n = 238$)	Control ($n = 226$)	*P* value
Males	164 (68.9)	121 (53.5)	–
Females	74 (31.1)	105 (46.5)	–
Age (mean ± SD)	53.72 ± 11.11	54.06 ± 10.98	0.74
Diabetes	78 (32.8)	66 (29.2)	0.54
Hypertension	177 (74.4)	126 (55.8)	0.05*
Smokers	96 (40.3)	73 (32.3)	0.22
Alcohol consumers	73 (30.7)	69 (30.5)	0.98
Total cholesterol	238.5 ± 24.5	164.6 ± 16.2	0.0001
Triglyceraldehydes	109.6 ± 27.1	98.5 ± 31.2	0.01
High density lipoprotein	35.6 ± 7.1	43.4 ± 5.2	0.0001
Low density lipoprotein	185.5 ± 11.2	93.6 ± 16.5	0.0001
Very low density lipoprotein	37.6 ± 4.7	31.7 ± 3.2	0.0001

Values are *n* (%). **P* value < 0.05 considered as significant.

Table 2. Allele and genotype distribution of the TNFα and IL10 polymorphic markers in the study groups.

	Stroke patients ($n = 238$)		Controls ($n = 226$)			
	No.	%	No.	%	OR (95% CI)	*P* value
TNFα -308						
Allele						
G	231	48.5	221	48.9	0.98 (0.76–1.27)	0.91
A	245	51.5	231	51.1	–	
Genotype						
G/G	17	7.1	12	5.3	1.37 (0.64–2.94)	0.41
G/A	211	88.7	207	91.6	0.71 (0.38–1.33)	0.29
A/A	10	4.2	7	3.1	1.37 (0.51–3.66)	0.52
IL10 -1082						
Allele						
G	124	26.1	79	17.5	1.66 (1.21–2.28)	0.0016*
A	352	73.9	373	82.5	–	
Genotype						
G/G	40	16.8	16	7.1	2.65 (1.43–4.88)	0.0013*
G/A	44	18.5	47	20.8	0.86 (0.54–1.36)	0.53
A/A	154	64.7	163	72.1	0.70 (0.47–1.05)	0.086

**P value* was calculated by χ^2 test with 2 × 2 contingency table and considered <0.05 as significant.

Table 3. Multivariable logistic regression analysis of IL-10 and TNF in stroke patients.

	Dominant		Recessive		Additive 1		Additive 2	
Variant	*P*	OR (95% CI)	*P*	OR (95% CI)	*P*	OR (95% CI)	*P*	OR (95% CI)
IL-10	0.004	1.91 (1.22–2.99)	0.087	0.70 (0.47–1.05)	0.007	0.37 (0.18–0.76)	0.969	0.99 (0.62–1.58)
TNF	0.416	0.73 (0.34–1.56)	0.53	1.37 (0.51–3.66)	0.398	0.72 (0.33–1.54)	0.502	0.71 (0.26–1.91)

The multiple logistic regression analysis of genotypes using dominant, recessive and additive models was performed to elucidate the effects of different clinical variables on stroke patients for both the cytokines (table 3). *IL-10* 'GG' genotype showed a statistically significant effect on the stroke, when the dominant model analysis was performed. The other clinical parameters, do not show significant effect on stroke. The results showed that the *IL-10* 'GG' genotype is significantly associated with stroke.

Discussion

Extensive studies were carried out to understand the various gene polymorphisms with stroke in various ethnic groups all over the world, and established that factor XIII and apolipoprotein E are associated with an inceased risk of ischemic and hemorrhagic stroke (Catto *et al*. 1998; Rastenyte *et al*. 1998; O'Donell *et al*. 2000). Studies on *TNFα* in stroke in other parts of the world by Bis *et al*. (2008) and Achal *et al*. (2006) in the USA, Karahan *et al*. (2005) in Turkey, Byung-Cheol *et al*. (2004) in South Korea did not indicate any association between *TNFα* and stroke. Also our results did not find any association between *TNFα* and stroke. A study carried out by Banerjee *et al*. (2008) in north India also could not find any association. Tong *et al*. (2010) in China showed the protective role of *TNFα* in ischemic stroke. However, Hoppe *et al*. (2007) in the USA found an association between *TNFα* and stroke.

IL-10 is an anti-inflammatory cytokine that may regulate the complex network of reactions that occur in acute cerebral ischemia. The difference in anti-inflammatory profile is determined not only by the levels of IL-10 production with neurological deterioration and also by the functional polymorphisms of the gene. Several functional *IL-10* gene polymorphisms have been described (Koch *et al*. 2001). A study in south Indian population showed association of *IL-10* with diabetic peripheral neuropathy (Kolla Venkata *et al*. 2009). The role of *IL-10* in the causation of stroke is not well understood. Very few reports are available on the association of allele polymorphisms or mutations in the human *IL-10* gene with the risk of stroke. The association of *IL-10* gene polymorphism at the position -1082 with hemorrhagic stroke was studied by Bis *et al*. (2008) and Pawlikowska *et al*. (2004) and they did not find any significant association. A study carried out by Munshi *et al*. (2010) in a south Indian population (Andhra Pradesh) found a significant association of *IL-10* 'GA' genotype with stroke, whereas our

study showed an association between *IL-10* 'GG' genotype and stroke. Although the reasons for variation in results are not well understood, it may be attributed to the differences in selection of patients.

The overall studies on *TNFα* and *IL-10* in stroke all over the globe showed, differences which might be due to ethnic variation. The present study was carried out on the association of pro and anti inflammatory markers, *TNFα* and *IL-10* respectively with ischemic stroke. We found that *IL-10* 'GG' genotype was susceptible to disease with three times at risk at the promoter position 1082 ($P < 0.001$, OR 3.25).

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