Interferon gamma (IFN γ) +874A/T gene polymorphism in South Indian ischemic stroke patients

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KEY WORDS

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ABSTRACT

Background: Ischemic stroke is a complex vascular and metabolic process resulting in neuronal death and progression with time. Cytokines play a role in immune response and also maintains the normal homeostatic environment of the central nervous system. IFN γ is one of the key effector cytokines produced by NK and T cells that enhances microbicidal activity of macrophages and neutrophils. **Purpose:** As the association of IFN γ +874A/T gene polymorphism with stroke has not been investigated in Indian population, we wanted to evaluate the association of this polymorphism with ischemic stroke in a South Indian population. **Methods:** We genotyped 171 ischemic stroke patients and 153 age-matched control subjects. **Results:** Statistical analysis showed a significant association of TT homozygote with ischemic stroke (OR=1.9, 95% CI=1.05-3.43, p=0.03), while AA (OR= 0.84, 95% CI=0.54-1.31, p=0.46) and AT (OR=0.80, 95% CI=0.51-1.26, p=0.34) genotypes were not significantly associated. A and T allele frequencies in stroke were 58.78% and 41.22% as against 65.36% and 34.64% in control group, respectively, thus, suggesting no statistically significant differences in the A (OR=0.75, 95% CI=0.54-1.03, p=0.08) and T (OR=1.32, 95% CI=0.96- 1.82, p=0.08) allele frequencies between the two groups. **Conclusion:** We conclude that the IFN γ +874 TT genotype is associated with the increased risk of ischemic stroke.

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Introduction

Ischemic stroke is a complex vascular and metabolic process resulting in neuronal death and progresses with time. Cytokines play a role in immune response and also maintain the normal homeostatic environment of the central nervous system. Inflammatory cytokines play an important role in the etiology of cerebral infarction and they are under strong genetic control. As genetic traits contribute significantly to cerebral infarction variations in the genetic regulation of inflammatory system may increase the risk of the disease from individual to individual.¹ IFN γ has antiviral, immunoregulatory, and anti-tumor properties.² Atherosclerosis is an inflammatory disease, and plaque induced inflammation is considered a cause of intimal erosion and rupture and therefore leads to acute ischemia.^{1,3} IFN γ has important immunoregulatory roles and enhances both antigen specific and non-specific immune responses through actions on monocytes and macrophages.^{4,5}

Complications related to infections such as chest and urinary tract infections, have been reported to occur in 23–65% of all stroke patients within the first few days after stroke.^{6,7} Brain injury was identified as an independent risk factor for infectious complications in trauma patients due to a central nervous shutdown of the immune defense.^{8,9} Howard *et al*, reported association of immunosuppressive state with stroke.¹⁰ IFN γ is one of the key effector cytokines produced by NK and T cells that enhances microbicidal activity of macrophages and neutrophils. Several gene polymorphisms are associated with stroke in humans,¹¹ association between the gene polymorphisms of inflammatory cytokines are meager. In the present study we have examined single nucleotide polymorphism in interferon gamma (IFN γ) at position +874A/T in South Indian ischemic stroke patients.

Methods

Study subjects

The study group comprised of 171 ischemic stroke patients (including both new and recurrent stroke patients) from the major hospitals of Hyderabad, Bhagwan Mahavir Medical Research Centre and Govt. Nizamia General Hospital of (A.P., India). Patients with acute stroke were examined by a qualified stroke neurologist to confirm the diagnosis and the ischemic strokes were differentiated by computed tomography scans and magnetic resonance imaging. Classification of subtypes was done according to TOAST (Trial of Org 10172 in Acute Stroke Treatment) criteria.¹² Patients with hemorrhagic stroke were excluded from the study. The Institutional Ethics Committee approved this study and written informed consent was obtained from all the subjects who participated in the study. Information on demographic characteristics was collected using a standard questionnaire prepared especially for this purpose. Age matched control subjects (153) from the comparable socioeconomic background were selected for comparison. Risk factors including hypertension and diabetes were documented. 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 the 2 blood pressure measurements. Diabetes was diagnosed if fasting plasma glucose was >126 mg/dl in accordance with the American Diabetes Association.¹³ Information was also collected on the number of cigarettes smoked per day and duration of smoking. People consuming one drink in a week were considered as alcohol users.

DNA isolation and genotyping

2 ml of venous blood was collected from each participant/subject in an EDTA tube for DNA extraction. DNA was isolated by salting out method.¹⁴ In brief, equal amount of RBC lysis buf-

fer containing Triton-X was added to the whole blood sample, in-order to lyse the RBC and centrifuged to get the pellet. The pellet was lysed with WBC lysis buffer containing 10% SDS, and then high molar concentration of NaCl was added consecutively to separate out the protein fraction. Finally, ice cold ethanol was added to get the DNA which were separated and resuspended in TE buffer and stored at -20°C until the PCR reaction was performed. The polymorphism in interferon gamma (IFN- γ) at position +874A/T was studied using amplification refractory mutation system polymerase chain reaction method (ARMS PCR).¹⁵ In brief, each reaction employed a generic antisense primer 5'-TCAACAAAGCTGATACTCCA-3' and one of the two allele-specific sense primers 5'-TTCTTACAACACAAAATCAAATCA-3' for 'A' allele and 5'-TTCTTACAACACAAAATCAAATCT-3' for 'T' allele. For evaluation of the PCR amplification 426 bp internal control was amplified using a pair of specific primers 5'-GCCTTCCAACCATTCCTTA-3' and 5'-TCACGGATTCTGTGTGTTTC-3'. The PCR incubation mixture in a total volume of 20 μ l consisted of 10 mM Tris-HCl, pH 9.0; 50 mM KCl; 400mM dNTPs; 1.5 mM MgCl₂; 0.5 units Taq polymerase; 0.8mM of each primer; 0.01% gelatin and 40 ng genomic DNA. Amplification was performed with an initial denaturation at 95°C for 1 minutes, 10 cycles were run with denaturation at 95°C for 15 seconds, annealing at 62°C for 50 seconds and extension at 72°C for 40 seconds. The products were analysed on 2% agarose gel stained with ethidium bromide.

Statistical analysis

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). All the statistical tests were two sided, and were considered significant at p value < 0.05. Genotypic frequencies were calculated according to the number of different genotypes observed and the total number of genotypes examined. Yate's correction was applied wherever necessary. Genotype frequencies were checked for deviation from Hardy-Weinberg equilibrium and were not significantly different from those predicted.

Results

The details on the demographic characteristics of the study population are shown in Table 1. The mean age of the patients was 54.22 $\pm$ 10 years as against the mean age of 54.19 $\pm$ 11 years in the control group. The percentage of males among the stroke patients was 71.9% (n=123), which was higher compared to controls 52.9% (n=81), whereas the percentage of females was 28.1% (n=48) in stroke patients and 47.1% (n=72) in the control group. The percentage of hypertension was 72.5% in stroke patients and 43.7% in controls. The percentage of diabetes was 30.9% among stroke patients and 20.2% in control group. The percentage of smokers were more in patient group (50.8%) compared to controls (39.8%). The percentage of alcohol users in patients group (39.7%) was more compared to controls (29.4%). Family history of hypertension in patients group (30.4%) is more compared to controls (19.6%). Family history of diabetes in patients groups was 34.5% as against 20.2% in controls. Family history of stroke was reported in 28.6% of patients and 7.8% of controls.

IFN- γ +874 A/T polymorphism

In our case-control study, we genotyped IFN- γ +874 A/T polymorphism in 171 ischemic stroke patients and in 153 control

Table 1. Demographic characteristics of study group

	Stroke (n=171) No (%)	Controls (n=153) No (%)
Male	123 (71.9)	81(52.9)
Female	48 (28.1)	72(47.1)
Age in years, mean (SD)	54.22(10)	54.19(11)
Hypertension	124(72.5)	67(43.7)
Diabetes	53(30.9)	31(20.2)
Smoking	87 (50.8)	61(39.8)
Alcohol	68(39.7)	45(29.4)
Family history of hypertension	52 (30.4)	30(19.6)
Family history of diabetes	59 (34.5)	31(20.2)
Family history of s stroke	49(28.6)	12(7.8)

subjects. The genotype frequencies of IFN- γ +874 A/T polymorphism among the patients and controls are shown in Table 2. The distribution of genotypes was in Hardy-Weinberg equilibrium among controls. The frequencies of the "AA", "AT", and "TT" genotypes of IFN- γ +874 A/T polymorphism in stroke patients were 39.77%, 38.01%, and 22.22% as against 43.79%, 43.14%, and 13.07% in controls, respectively. The genotypic frequency of "TT" homozygote showed a significant association with ischemic stroke (OR=1.9, 95% CI=1.05-3.43, p=0.03), while AA (OR= 0.84, 95% CI=0.54-1.31, p=0.46) and AT(OR=0.80, 95% CI=0.51-1.26, p=0.34) genotypes were nonsignificant. A and T allele frequencies in stroke were 58.78% and 41.22% as against 65.36% and 34.64% in control group, respectively, thus, suggesting no statistically significant differences in the A (OR=0.75, 95% CI=0.54-1.03, p=0.08) and T (OR=1.32, 95% CI=0.96- 1.82, p=0.08) allele frequencies between the two groups.

Discussion

Interferon gamma (IFN- γ) is an important cytokine in cellular immunity and the presence of thymidine at + 874 correlates with microsatellite repeats associated with high cytokine production. In the present study we examined single nucleotide polymorphism in interferon gamma (IFN- γ) at position +874A/T and found a significant association of "TT" genotype with ischemic stroke. Infectious complications in particular, bacterial pneumonia and their relevance for mortality are well known in acute stroke. The high incidence of infections in stroke patients is likely to be a result of an impaired immune function. A functional role of neutrophils in the development of stroke-associated injury remains controversial, and the contribution of specific lymphocyte subpopulations and their products to the pathogenesis of ischemic stroke are not clear. T-cell derived interferon- γ (IFN- γ) has been shown to contribute to the injury elicited by ischemia-reperfusion in other organs¹⁶ and IFN- γ mRNA is increased in rat brain tissue after permanent focal cerebral ischemia.¹⁷ Activation of the SNS and the HPA by proinflammatory cytokines in systemic inflammation results in the release of glucocorticoids and catecholamines, which in-

Table 2. Genotype distribution of IFN- γ +874 A/T polymorphism in ischemic stroke patients and control subjects

IFN- γ +874A/T	Stroke Patients (n=171)		Controls (n=153)		OR	95%CI	P-value
	No.	%	No.	%			
Genotype							
AA	68	39.77	67	43.79	0.84	0.54-1.31	0.46
AT	65	38.01	66	43.14	0.80	0.51-1.26	0.34
TT	38	22.22	20	13.07	1.9	1.05-3.43	0.03
Allele							
A	201	58.78	200	65.36	0.75	0.54-1.03	0.08
T	141	41.22	106	34.64	1.32	0.96-1.82	0.08

P- value was calculated by χ^2 test with 2 x 2 contingency table and <0.05 considered as significant

hibit further production of proinflammatory mediators. Vagus nerve activation by inflammatory cytokines during endotoxemia was found to inhibit macrophage cytokine production through release of acetylcholine^{18,19} and rapid activation of these pathways in inflammatory conditions protects the organism against any adverse effects of an overwhelming immune response. However, an excessive activation of inhibitory neuroendocrine pathways without systemic inflammation can inappropriately suppress the immune system and increase the risk of infections. Intrathecal release of proinflammatory cytokines is associated with signs of systemic immunodepression and a high incidence of infections in neurosurgical patients.²⁰ According to Konstantin *et al* stress mediator blockade underlines the importance of functional defects in IFN- γ production in the control of infectious complications after stroke.²¹ $\gamma\delta$ T cells are essential for pulmonary bacterial clearance and $\alpha\beta$ T cells are more critical in the peripheral blood in stroke induced infections. Inflammation is an early and rate-determining step in the microvascular dysfunction and tissue injury associated with cerebral ischemia- reperfusion (I/R) is supported by several reports that describe a reduction in brain edema and infarct size in animal models of stroke treated with antibodies that block leukocyte adhesion.^{22,23} The microvasculature of post-ischemic brain assumes an inflammatory phenotype that is manifested as endothelial activation and barrier dysfunction, enhanced generation of oxidants and inflammatory mediators, and the recruitment of adherent leukocytes and platelets.²⁴ Aspiration due to dysphagia is a known risk factor for pneumonia after severe strokes and other factors that might predispose stroke patients to pneumonia is an impaired immune responsiveness.^{25,26,27}

A study carried out in Egyptian atopic patients showed a significant association of IFN-gamma gene polymorphism at position +874 A/T.²⁸ Study from China reported a significant association of IFN-gamma +874 A/T gene polymorphism and severe acute respiratory syndrome.²⁹ A significant association was observed between interferon-gamma gene polymorphisms and systemic lupus erythematosus suggesting that elevated interferon gamma is associated with increased systemic erythematosus susceptibility.³⁰ Lai *et al* reported that genetic polymorphism of IFN-gamma gene is associated with individual susceptibility to cervical carcinogenesis.³¹ Feher *et al* could not find any association between IFN- γ +874 A/T gene polymorphism and Alzheimer disease.³² Our study found a significant association

of 'TT' genotype of IFN- γ +874 gene polymorphism and ischemic stroke in South Indian population.

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