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

Shehnaz Sultana,1,3 Venkata Kolla K,2 Pranay Penagaluru K,1 Usha Rani P,2 P.P. Reddy1,3

1Bhagwan Mahavir Medical Research Centre, Hyderabad, AP. 2Institute of Genetics and Hospital for Genetic Diseases, Osmania University, Hyderabad, AP. 3School of Human Genome Research & Genetic Disorders, Mahatma Gandhi National Institute of Research and Social Action, Hyderabad, AP, INDIA.

KEY WORDS
Central nervous system immunity response cytokines infections inflammatory

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 maintain 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.

doi: 10.5214/ans.0972.7531.1118305

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 this 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). In brief, each reaction employed a generic antisense primer 5’- TCAACAAAGCTGATACTCCA-3’ and one of the two allele-specific sense primers 5’-TTCTTACAAACAAATCAAATCA-3’ for ‘A’ allele and 5’-TTCTTACAAACAAATCAAATCT-3’ for ‘T’ allele. For evaluation of the PCR amplification 426 bp internal control was amplified using a pair of specific primers 5’-GCCITCCACACACTCTTA-3’ and 5’-TCACCGATTCTGTTGTTCC-3’. The PCR incubation mixture in a total volume of 20µl consisted of 10 mM Tris-HCl, pH 9.0; 50 mM KCl; 400 mM dNTPs; 1.5 mM MgCl2; 0.5 units Taq polymerase; 0.8 mM 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 (χ²) 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 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 ±10 years as against the mean age of 54.19 ±11 years in the control group. The percentage of males among the patients and controls were more in patient group (30.4%) is more compared to controls (19.6%). Family history of hypertension in patients group (50.8%) compared to controls (39.8%). The percentage of diabetes was 30.9% among stroke patients and 20.2% in controls. Family history of hypertension in patients group (50.8%) compared to controls (39.8%). The percentage of diabetes was 30.9% among stroke patients and 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 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 organs and IFN-γ mRNA is increased in rat brain tissue after permanent focal cerebral ischemia. Activation of the SNS and the HPA by proinflammatory cytokines in systemic inflammation results in the release of glucocorticoids and catecholamines, which in-

| Table 1. Demographic characteristics of study group |
|-----------------------------------------------|
|                                | Stroke (n=171) | Controls (n=153) |
|-----------------------------------------------|
| 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 stroke                     | 49(28.6)      | 12(7.8)         |
P-value was calculated by χ² test with 2 x 2 contingency table and <0.05 considered as significant

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

|                | 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    |

A study carried out in Egyptian atopic patients showed a significant association of IFN-gamma gene polymorphism at position +874 A/T.24 Study from China reported a significant association of IFN-gamma +874 A/T gene polymorphism and severe acute respiratory syndrome.25 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.

The article complies with International Committee of Medical Journal Editor’s uniform requirements for the manuscripts.

Competing interests – None, Source of Funding – None

Received Date : 22 May 2011; Revised Date: 9 June 2011

Accepted Date: 5 July 2011

References
1. Woods A, Brull DJ, Humphries SE, et al. Genetics of inflammation and risk of coronary artery disease: the central role of interleukin-6. Eur Heart J 2000; 21: 1574–83.
2. Schroeder K, Hertzog PJ, Ravasi T et al. “Interferon-gamma: an overview of signals, mechanisms and functions”. J Leukoc Biol 2004; 75(2): 163–89.
3. Ross R. Atherosclerosis – an inflammatory disease. N Engl J Med 1999; 340: 115–26.
4. Peleman R, Wu J, Fargeas C, et al. Recombinant interleukin 4 suppresses the production of interferon gamma by human mononuclear cells. J Exp Med 1989; 170: 1751–6.
5. Vercelli D, Jabara HH, Lauener RP, et al. IL-4 inhibits the synthesis of IFN-gamma and induces the synthesis of IgE in human mixed lymphocyte cultures. J Immunol 1990; 144: 570–3.
6. Davenport RJ, Dennis MS, Wellwood I et al. Complications after acute stroke. Stroke 1996; 27: 415–20.
7. Langhorne P, Stott DJ, Robertson L, et al. Medical complications after stroke: a multicenter study. Stroke 2000; 31: 1223–9.
8. Rodriguez JL, Gibbons KJ, Bitzer LG, et al. Pneumonia: incidence, risk factors, and outcome in injured patients. J Trauma 1991; 31: 907–12.
9. Hsieh AH, Bishop MJ, Kubitis PS, et al. Pneumonia following closed head injury. Am Rev Respir Dis 1992; 146: 290–4.
10. Howard RJ, Simmons RL. Acquired immunologic deficiencies after trauma and surgical procedures. Surg Gynecol Obstet 1974; 139: 771–82.
11. Alberts MJ. Stroke genetics update. Stroke 2003; 34: 342–4.
12. Meschia JF. Subtyping in ischemic stroke genetic research. J Stroke Cerebrovasc Dis 2002; 11(5): 208–19.
13. Diagnosis and Classification of Diabetes Mellitus; Definition and 13. Description of Diabetes Mellitus, Diabetes Care 2009; 32: 562-567.
14. Lahari DK, Steve Bye, Nurenberger JJ, et al. A non organic and non enzymatic extraction methods gives high yields of genomic DNA from whole blood samples than do nine other methods tested. J Biochem and Biophisical Methods 1992; 25: 193-205.
15. Madurelo DL, Arnalich F, Serantes R, et al. Interferongamma and Interleukin-10 Gene Polymorphisms in Pulmonary Tuberculosis. Am J Respir Crit Care Med 2003; 167: 970–5.
16. Horie Y, Wolf R, Chervenak RP, et al. T-lymphocytes contribute to hepatic leukostasis and hypoxic stress induced by gut ischemia-reperfusion. Microcirculation 1999; 6: 267–80.

17. Li HL, Kostulas N, Huang YM, et al. Link H. IL-17 and IFN-gamma mRNA expression is increased in the brain and systemically after permanent middle cerebral artery occlusion in the rat. J Neuroimmunol 2001; 116: 5–14.

18. Bernik TR, Friedman SG, Ochani M, et al. Pharmacological stimulation of the cholinergic antiinflammatory pathway. J Exp Med 2002; 195: 781–8.

19. Tracey KJ. The inflammatory reflex. Nature 2002; 420: 853–9.

20. Asadullah K, Woiciechowsky C, Docke WD, et al. Immunodepression following neurosurgical procedures. Crit Care Med 1995; 23: 1976–83.

21. Prass K, Meisel C, Höflich C, et al. Stroke-induced immunodeficency promotes spontaneous bacterial infections and is mediated by sympathetic activation reversal by poststroke T helper cell type 1-like immunostimulation. J Exp Med 2003; 198: 725-36.

22. Ishikawa M, Zhang JH, Nanda A, et al. Inflammatory responses to ischemia and reperfusion in the cerebral microcirculation. Front Biosci 2004; 9: 1339–47.

23. Yenari MA, Kunis D, Sun GH, et al. Hu23F2G, an antibody recognizing the leukocyte CD11/CD18 integrin, reduces injury in a rabbit model of transient focal cerebral ischemia. Exp Neurol 1998; 153: 223–33.

24. Ishikawa M, Cooper D, Arumugam TV, et al. Platelet-leukocyte–endothelial cell interactions after middle cerebral artery occlusion and reperfusion. J Cereb Blood Flow Metab 2004; 24: 907–15.

25. Perry L, Love CP. Screening for dysphagia and aspiration in acute stroke: a systematic review. Dysphagia 2001; 16: 7–18.

26. Finegold SM. Aspiration pneumonia. Rev Infect Dis 1991; 13: 5737–42.

27. Marik PE. Aspiration pneumonitis and aspiration pneumonia. N Engl J Med 2001; 344: 665–71.

28. Hussein YM, Ahmad AS, Ibrahim MM, et al. Interferon gamma gene polymorphism as a biochemical marker in Egyptian atopic patients. J Investig Allergol Clin Immunol 2009; 19(4): 292-8.

29. Chong WP, Eddie WK, Wan Tso GH, et al. The interferon gamma gene polymorphism +874 A/T is associated with severe acute respiratory syndrome. BMC Infectious Diseases 2006;6:82.

30. Kim K, Cho KS, Sestak A, et al. Interferon-gamma gene polymorphisms associated with susceptibility to systemic lupus erythematosus. Ann Rheum Dis 2010; 69: 1247-50.

31. Lai HC, Chang CC, Lin YW, et al. Genetic polymorphism of the interferon-gamma gene in cervical carcinogenesis. Int J Cancer 2005; 113: 712-8.

32. Agnes f, Anna J, Agnes A et al. Association Study of Interferon-?, Cytosolic Phospholipase A2, and Cyclooxygenase-2 Gene Polymorphisms in Alzheimer Disease. American Journal of Geriatric Psychiatry 2010; 18: 983-7.