

# Functional Polymorphism of Interferon- $\gamma$ (IFN- $\gamma$ ) Gene +874T/A Polymorphism is Associated with Pulmonary Tuberculosis in Zahedan, Southeast Iran

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**Abstract:** Concerning the key role of interferon- $\gamma$  (IFN- $\gamma$ ) in the protective immunity against *Mycobacterium tuberculosis*, we aimed to find the possible association between single nucleotide polymorphism of IFN- $\gamma$  +874T/A (rs61923114) and pulmonary tuberculosis (PTB). This case-control study was performed on 142 PTB patients and 166 healthy subjects. Genotype analysis was done using amplification refractory mutation system-PCR (ARMS-PCR). We found that the AA genotype of +874A/T IFN- $\gamma$  is a risk factor for PTB (OR = 3.333, 95% CI = 1.537–7.236,  $p=0.002$ ). The results showed that the +874A allele frequency was higher in PTB than in normal subjects (OR = 1.561, 95% CI = 1.134–2.480,  $p=0.007$ ). In conclusion, significant association was found between the IFN- $\gamma$  +874T/A polymorphism (rs61923114) and susceptibility to PTB in a sample of Iranian population.

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## Introduction

Pulmonary tuberculosis (PTB) is still a major health problem in both industrialized and developing countries and it remains a leading infectious cause of death (Alavi-Naini et al., 2009; Naderi et al., 2009). More than one-third of the world's population is infected with *Mycobacterium tuberculosis* (*M. tuberculosis*), yet only 10% develop the clinical disease (Porter and McAdam, 1994).

Convincing evidence from twin studies shows that host genetic factors are important risk factor for development of tuberculosis (Comstock, 1978). The findings of many different TB susceptibility genes in genome-wide screens supported the possibility of multigenic predisposition to TB (Bellamy et al., 2000; Cervino et al., 2002).

Interindividual variation in levels of cytokine production may be due to a single nucleotide polymorphisms located within the promoter regions which affect gene transcription (van Deventer, 2000).

INF- $\gamma$  produced by natural killer cells and T cells is a key T helper-1 cytokine. It plays a crucial role in macrophage activation for controlling mycobacterium infection. It has been found that in active TB the levels of INF- $\gamma$  production by peripheral blood mononuclear cells are lower than latent infection, and local and systemic INF- $\gamma$  levels associate with the severity of the disease (Condos et al., 1998). Subjects defective in the genes for INF- $\gamma$  or INF- $\gamma$  receptor have been shown to be prone for mycobacterial infections including *M. tuberculosis* (Ottenhoff et al., 1998). Due to the importance role of INF- $\gamma$  against tuberculosis and the functional role of the INF- $\gamma$  +874T/A single nucleotide polymorphism in INF- $\gamma$  production, this study was aimed to find out the INF- $\gamma$  +874A/T gene polymorphism and susceptibility to pulmonary tuberculosis in an Iranian samples.

## Material and Methods

This case-control study was performed from July 2008 to February 2010 in Research Center for Infectious Diseases and Tropical Medicine, Bou-Ali Hospital, Zahedan, Iran. The project was approved by ethics committee of Zahedan University of Medical Sciences and informed consent was taken from all patients and healthy subjects. The subjects who underwent treatment for PTB and newly diagnosed PTB cases were enrolled in the study within the case group. The diagnosis of PTB was based on clinical, radiological, sputum Acid Fast Bacillus (AFB) smear positivity, culture and response to antituberculosis chemotherapy as described previously (Alavi-Naini et al., 2009; Naderi et al., 2009, 2010). Control subjects were selected from the Zahedan population showing no recent signs, symptoms, or history of pulmonary infections.

### DNA extraction

Two millilitres of venous blood drawn from each subjects and genomic DNA was extracted from peripheral blood as described previously (Hashemi et al., 2010).

Polymorphism at +874 position was identified using the Amplification Refractory Mutational System (ARMS) methodology described by Pravica et al. (2000). The primer sequences were as follows: IFN- $\gamma$  generic primer, 5'-tcaacaagctgatactcca-3'; IFN- $\gamma$  primer T allele, 5'-ttcttacaacacaaaatcaaatct-3'; IFN- $\gamma$  primer A allele, 5'-ttcttacaacacaaaatcaaatca 3'.

PCR was performed using commercially available PCR premix (AccuPower PCR PreMix, BIONEER, Daejeon, Korea) according to the manufacturer's recommended protocol. Into a 0.2 ml PCR tube containing the AccuPower PCR PreMix, 1  $\mu$ l template DNA (~100 ng/ $\mu$ l), 1  $\mu$ l of each primer (10 pmol/ $\mu$ l) and 17  $\mu$ l DNase-free water were added. The total volume for the PCR was 20  $\mu$ l.

PCR cycling was performed at 95 °C for 1 min, followed by 10 cycles at 95 °C for 15 s, 62 °C for 40 s and 72 °C for 40 s, and 20 cycles at 95 °C for 30 s, 56 °C for 40 s and 72 °C for 50 s (Corbett research, Australia). The amplified products were separated by electrophoresis on a 2% agarose gel containing 0.5  $\mu$ g/ml ethidium bromide.

The statistical analysis of the data was performed using the SPSS 17.0 software. Genotypes and alleles were compared between groups by use of  $\chi^2$  test.

## Results

The study consisted of 142 PTB (54 male, 88 female; ages 50.1  $\pm$  20.9) and 166 healthy subjects (82 male, 84 female; ages 50.7  $\pm$  13.3). We have evaluated the genetic frequencies of the +874T/A IFN- $\gamma$  polymorphism in healthy and PTB subjects. A significant difference was observed among case and control groups regarding +874T/A IFN- $\gamma$  polymorphism ( $\chi^2 = 11.58$ ,  $df=2$ ,  $p=0.003$ ). As shown in Table 1, the AA genotype is a risk factor for PTB (OR = 2.567, 95% CI = 1.439–4.580,  $p=0.002$ ). In addition, the A allele frequency was significantly higher in PTB than in normal subjects (OR = 1.56, 95% CI = 1.134–2.48,  $p=0.007$ ) (Table 2).

**Table 1 – Distribution of genotype frequencies of IFN- $\gamma$  gene (+874T/A) among patients with pulmonary tuberculosis (PTB) and control subjects**

Genotypes	PTB	Normal subjects	OR	95% CI	P value
TT	12.6% (18/142)	19.9% (33/166)	1	referent	–
AT	59.2% (84/142)	66.9% (111/166)	1.378	0.731–2.633	0.342
AA	28.2% (40/142)	13.2% (22/166)	3.333	1.536–7.236	0.002

**Table 2 – Distribution of allele frequencies of IFN- $\gamma$  gene (+874T/A) among patients with pulmonary tuberculosis (PTB) and normal subjects**

Allele	PTB	Normal subjects	OR	95% CI	P value
T	42.3% (120/284)	53.3% (177/332)	1.561	1.134–2.48	0.007
A	57.7% (164/284)	46.7% (155/332)			

## Discussion

In the present study, the allelic and genotypic frequencies of IFN- $\gamma$  gene (+874T/A) were evaluated among PTB patients and a healthy individuals in a sample of Iranian subjects. We found that SNP in the intronic region of IFN- $\gamma$  gene (+874T/A; rs61923114) may be associated with susceptibility to tuberculosis in our population. The frequency of +874AA genotype as well as +874A allele was significantly higher in PTB than normal subjects. To the best of our knowledge this is the first report from Iran. Our results are in agreement with the findings from other studies with different population which found an association between IFN- $\gamma$  +874T/A polymorphism and susceptibility to tuberculosis (Pravica et al., 2000; Lio et al., 2002; Lopez-Maderuelo et al., 2003; Rossouw et al., 2003; Tso et al., 2005; Etokebe et al., 2006; Sallakci et al., 2007; Amim et al., 2008; Ansari et al., 2009; Vallinoto et al., 2010). However, some studies showed no association between IFN- $\gamma$  +874T/A polymorphisms and vulnerability to tuberculosis (Fitness et al., 2004; Vidyarani et al., 2006; Selvaraj et al., 2008; Onay et al., 2010). Hwang et al. (2007) have found no association between IFN- $\gamma$  +874T/A polymorphism and susceptibility to non-tuberculosis pulmonary disease.

It has been reported that the +874T allele correlates with high IFN- $\gamma$  expression, whereas +874A allele correlates with low IFN- $\gamma$  expression (Pravica et al., 2000). The patients with tuberculosis carrying the genotype +874A/A showed significantly lower IFN- $\gamma$  plasma levels than those with +874A/T and +874T/T genotypes (Vallinoto et al., 2010).

Given the important role of IFN- $\gamma$  in the control of host defence against mycobacterial pathogens, this suggested that +874AA homozygosity may be an important genetic risk marker for the development of PTB, whereas the TT genotype might be associated with protection against TB.

In conclusion, our results show that IFN- $\gamma$  polymorphism at +874T/A confers susceptibility to pulmonary tuberculosis in a sample of Iranian population.

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