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Original Article

Polymorphism in the First Intron of Interferon-Gamma Gene *(+874T/A)* in Patients with BCG Adenitis

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Abstract

Background: Cytokines and specially interferon-gamma (IFN-γ) are largely responsible for the regulation of the protective immune response against mycobacterial infections. Several studies have clarified the importance of common variants of *IFN-γ* gene regarding the susceptibility to tuberculosis. Bacille Calmette-Guérin (BCG) vaccine that is used to prevent severe forms of tuberculosis could produce local and systemic side effects. In this study we hypothesized that the *IFN-γ (+874T/A)* polymorphism was associated with development of BCG adenitis.

Methods: Thirty patients with BCG adenitis (18 males and 12 females) and 30 age and sex-matched healthy children, vaccinated with BCG during the first two days of life were chosen. All the patients and controls were of Iranian Fars origin and the study was conducted from 2005 to 2007. DNA samples were obtained from 30 patients with BCG adenitis and 30 age and sex matched healthy vaccinees. Polymorphism at *+874* was identified using allele specific polymerase chain reaction. Allele and genotype frequencies in cases and controls were compared using the χ^2 test and odds ratios (OR) and their 95% confidence intervals (CI) were calculated.

Results: The minor allele (T) frequency was significantly lower in patients with BCG adenitis compared to controls (35% vs. 55%, *P*= 0.02, OR= 0.441, 95% CI= 0.211–0.919). The Armitage trend test revealed a gradually increasing protection from the AA genotype through AT to TT (common odds ratio= 0.49; *P*= 0.037).

Conclusion: Our data suggest that in an Iranian population, the *IFN-γ (+874T/A)* polymorphism is associated with development of BCG adenitis in the vaccinees.

Key words: *BCG, Interferon-gamma, Single nucleotide polymorphism*

Introduction

Bacille Calmette-Guérin (BCG) vaccine has been used to prevent tuberculosis (TB) since 1921, seems to be effective against the disseminated disease and meningitis in childhood TB.

BCG vaccine has a low incidence of serious adverse reactions and is considered to be a safe vaccine. BCG lymphadenitis, defined as the development of ipsilateral regional lymph node enlargement after BCG inoculation, is the most common complication resulting from this vaccination (1, 2). The incidence of this complication depends on many factors, including the type of vaccinee, the age of vaccinee, the used injection technique, and the genetic background of the recipients (1-3).

Cytokines and specially interferon-gamma (IFN-γ) are largely responsible for the regulation of the protective immune response elicited by BCG vaccine and may play an important role in the formation of adenitis (4, 5). Mice or humans that lack components of the IFN-γ signaling pathway are highly susceptible to mycobacterial infections (6). Several studies have documented the importance of polymorphism in cytokine genes in the promoter region and their influence on cytokine production as well as in the susceptibility, severity and clinical outcome of several diseases including infectious ones (7).

A correlation has been reported between the 12-CA-repeat allele in the first intron of IFN-γ gene

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and the presence of the T allele at a single nucleotide polymorphism (SNP) at the +874 position (*+874T/A*) from the translation start site, that might be important in the induction of constitutively high IFN-γ production (8, 9). Therefore, it has been suggested that the T to A polymorphism at +874 directly influences the level of IFN-γ production associated with the CA microsatellite marker.

In the light of the key role played by IFN-γ in BCG-induced immunity, here we aimed to determine whether there is an association between the AA genotype, which has been suggested to be linked to decrease production of IFN-γ, and susceptibility to BCG adenitis.

Materials and Methods

Subjects

Thirty patients with BCG adenitis (18 males and 12 females) and 30 age and sex-matched healthy children, vaccinated with BCG during the first two days of life were chosen. All the patients and controls were of Iranian Fars origin and the study was conducted from 2005 to 2007.

The ages of the patient and control groups were between 2 months and 5 yr. The type and dose of the vaccine were similar in both study groups. BCG adenitis was diagnosed based on clinical examination. All patients matched with the inclusion criteria referred to our infectious disease clinic were selected. The controls were referred for routine check-up. Both groups had normal immunity in clinical work-up, and patients with a defined immunodeficiency syndrome, patients who presented or developed disseminated form of BCG infection during the study or ones with positive culture of lymph node aspiration for bacterial infections other than strains included in BCG vaccine were excluded. Written informed consent for enrolment in the study and for personal data management had been obtained from all parents.

Typing of IFN-γ *gene polymorphism at position +874*

Genomic DNA was obtained from whole blood by the conventional salting out method (10).

Polymorphism at position *+874* of *IFN-γ* gene was identified using allele specific polymerase chain reaction (AS-PCR) as described elsewhere (9, 11). The PCR reaction was performed in total volume of 10 μL, containing 1X reaction buffer (Fermentas-Germany), 200 μM (each) dNTPs (Fermentas-Germany), 3.5 mM MgCl2 (Fermentas-Germany n), 0.5 U Taq DNA polymerase (Cinnagene-Iran), 0.5 μM each specific primers (antisense: TCA ACA AAG CTG ATA CTC CA; sense+874T: TTC TTA CAA CAC AAA ATC AAA TCT; or sense +874A: TTC TTA CAA CAC AAA ATC AAA TCA), 0.2 μM of each internal control primers, and 250 ng DNA template.

Internal control primers amplify a human β-globin sequence (BGF: ACA CAA
CTG TGT TCA CTA GC; BGR: CAA CTT CAT CCA CGT TCA CC). PCR amplification was performed using a touch down method that included initial denaturation at 95 ºC for 5 min followed by two loops; loop 1 which consisted of 10 cycles with the following program: 95 ºC for 30 S, 62 ºC for 50 S, and 72 ºC for 40 S and loop 2 included 20 cycles with the following program: 95 ºC for 30 S, 56 ºC for 50 S and 72 ºC for 40 S and a final extension step at 72 ºC. The amplified products were run on 1.5% agarose gel that was in a buffer containing 0.5 μg/ml ethidium bromides (Fig. 1).

Statistics

Statistical analyses were conducted using SPSS software (SPSS, Chicago, IL, USA; Version 15). Hardy–Weinberg equilibrium was assessed using the genotype data by the χ^2 goodness-of-fit test. Genetic associations were evaluated both at the allelic and genotype level (12).

Allele and genotype frequencies in cases and controls were compared using the χ^2 test and odds ratios (OR) and their 95% confidence intervals (CI) were calculated. Additive genetic model using all three genotype frequencies was tested using Armitage trend test and common odds ratio which provides a single parameter for changes from one genotype category to another was calculated (13, 14).

Results

IFN-γ genotype was determined in 30 infants with BCG adenitis and 30 healthy age and sex-matched vaccinees following AS-PCR amplification of the genomic DNA. The patient group and control subjects were categorized for *+874* polymorphism into three genotypes by discriminating between the presence and absence of the two alleles. The results of genotyping in patients and controls are presented in Table 1.

Both cases and controls did not show any violation of Hardy-Weinberg equilibrium.

The minor allele (T) frequency was significantly lower in patients with BCG adenitis compared to controls (35% vs. 55%, *P*= 0.02, OR= 0.441, 95% CI =0.211–0.919).

The Armitage trend test revealed a gradually increasing protection from the AA genotype through AT to TT (common odds ratio =0.49; *P*= 0.037).

Similar to the allele frequencies, a statistically significant decrease in the frequency of TT genotype was observed in patients with BCG adenitis, 20%, compared to the control group, 26%, (*P*= 0.006, OR= 0.2, 95% CI= 0.060–0.662).

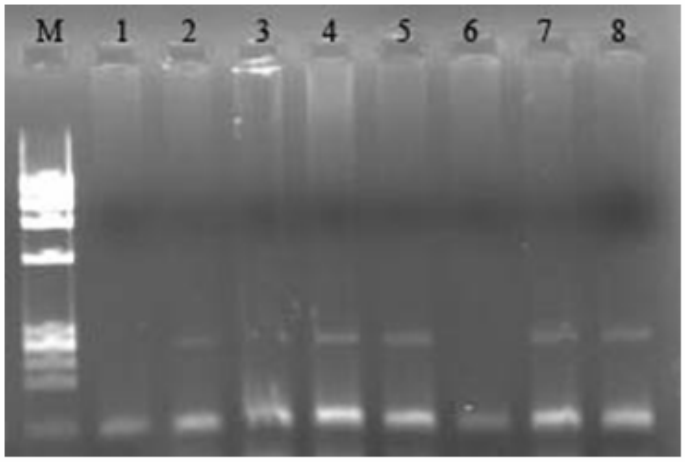

Fig. 1: Agarose gel electrophoresis of AS-PCR products for *IFN-γ (+874T/A)* polymorphism; 262 bp bands correspond to IFN-γ A or T allele and the 100 bp bands correspond to β-globin.

M: DNA size marker; lanes 1 and 2 show homozygosity for A allele; lanes 3 and 4 show heterozygosity for A and T alleles; lanes 5 and 6 show homozygosity for T allele; lanes 7 and 8 show heterozygosity for A and T alleles.

Table 1: *IFN-γ (+874T/A)* allele and genotype frequencies (n, %) in cases and controls

	Patients (n=30)	Controls (n=30)
A allele (%)	39(65)	27(45)
T allele (%)	21(35)	33(55)
AA (%)	15(50)	5(16)
TA (%)	9(30)	17(56.6)
TT (%)	6(20)	8(26.6)

Discussion

The knowledge of the genetic basis of susceptibility to disease is an expanding field (15, 16). Now, a growing body of evidence indicates that both the risk of acquiring infection and the risk of developing severe complications are related to genetic variability of cytokine loci, especially within known or putative regulatory regions that have a significant effect on transcription (7).

Considering the association between *IFN-γ (+874)* gene polymorphism and cytokine production (8, 9), and the central role IFN-γ plays in immunity to BCG, it was considered important to investigate the relationship between *IFN-γ* polymorphism and BCG adenitis.

Our aim was to determine whether there is an association between having A allele, which has

been suggested to be linked to a decreased production of IFN-γ, and susceptibility to BCG adenitis. Our results indicate an association between decreased +874 T allele frequency and BCG adenitis.
As this SNP coincides with a putative NF-κB binding site, the role of this polymorphic marker in susceptibility to pulmonary TB as well as BCG adenitis becomes more noticeable (9).
There are little investigations on the genetic basis of BCG adenitis. Polymorphisms in the genes encoding TNF-α and IL-4 was not shown to be significant in susceptibility to BCG adenitis, however the frequency of -592 AA genotype of IL-10 which results in more IL-10 production is found in BCG adenitis patients compared to controls (17, 18).
To the best of our knowledge, there is no published study assessing association between the *IFN-γ (+874T/A*) polymorphism and BCG adenitis, but many studies have looked for an association of TB susceptibility with *IFN-γ (+874T/A)* polymorphism. In previous studies the A allele, thought to produce less IFN-γ, was more common in patients with TB, whereas the T allele was more common in controls (14, 19).
The increase in the chances of having TB in an individual with an adenine at +874 compared to the chances in a patient with two thymidines at +874 (TT) varied from ~ 1.5 to 4.6 fold. In Croatia, an association was found with disease severity rather than susceptibility to TB (20), and no association emerged from works performed in Turkey (21), and Malawi (22).
Published studies can only suggest that polymorphisms in the IFN-γ gene might influence susceptibility to TB in some populations, but not all. It is possible to suggest that differences in genetic background or in study design or selection of controls can account for contradictory findings in distinct populations.
Based on the common genetic basis of immunity to *Mycobacterium tuberculosis* and BCG strains, we postulated that the same polymorphic changes may susceptible children to BCG adenitis.

BCG adenitis, which is a frequent complication after BCG vaccination, should be differentiated from disseminated BCG infection that develops in patients with some cellular immunodeficiencies as well as defects of IFN-γ/IL-12 signaling pathway. Those deficiencies are single gene defects with Mendelian inheritance. However, development of BCG adenitis seems to be polygenic, including the type, dose and concentration of vaccine, the age of the recipient, the use of proper intradermal injection techniques (1, 2). Our results showed that the presence of TT at *IFN-γ(+874)* is a protective factor against development of BCG adenitis.
The impact of these findings should be substantiated in other populations and with a larger number of subjects.

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