Association of IFN-γ Gene Polymorphism with Type 1 Diabetes in Iranian Patients

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ABSTRACT

Background: Type 1 Diabetes (T1D) is a chronic and progressive autoimmune disorder. Cytokines play a critical role in the pathogenesis of T1D. **Objective:** IFN-γ polymorphism was investigated in T1D and compared with normal controls. **Methods:** Thirty patients suffering from T1D and 40 normal controls were studied simultaneously using PCR technique. IFN-γ gene was evaluated at position 5'UTR +5644. **Results:** There was a significant difference between patient and control groups in TT genotype ($P<0.05$). **Conclusion:** In this study, we found a negative association between IFN-γ gene at position 5'UTR +5644 and T1D in Iranian patients pointing to T allele as a protective factor against T1D.

Key words: Cytokine, Polymorphism, Type 1 Diabetes Mellitus

INTRODUCTION

Type 1 Diabetes (T1D) is a chronic autoimmune disease which develops as a result of the interaction between genetic, environmental and immunologic factors that ultimately destroy the pancreatic beta cells and usually leads to insulin deficiency (1). Several mechanisms may contribute to the beta cells destruction including delayed type hypersensitivity reactions mediated by CD4+ Th1 cells reactive with specific islet antigens, cytolytic lymphocyte-mediated lysis of islet cells, local production of proinflammatory cytokines and autoantibodies (2,3). Proinflammatory cytokines play a fundamental role in the initial stages of T1D and in the development of severe complications of the disease. Cytokine and cytokine receptor genes are generally highly conserved in terms of exon sequences (4,5), but polymorphisms within the 5' and 3'

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regulatory sequences or introns of genes may have a significant effect on gene transcription and lead to alteration of cytokine function and production. Considering the pivotal role of cytokines in the pathogenesis of T1D, we have evaluated the possible association between polymorphisms in the promoter regions of IFN-γ and the susceptibility or the resistance to type 1 Diabetes.

PATIENTS AND METHODS

Patients. Thirty patients suffering from T1D (16 females and 14 males) were studied. Duration of disease was between 3 months to 18 years. 40 normal controls were tested simultaneously. High molecular weight DNA was prepared from peripheral venous blood using EDTA as anticoagulant, DNA was isolated by salting out method (6).
PCR Technique. Cytokine gene typing was performed by a polymerase chain reaction with sequences specific primers (PCR-SSP) assay (7), which uses identical amplification and detection conditions, enabling analysis of multiple polymorphisms. The PCR-SSP tray kits were supplied by Heidelberg University (Germany). Amplification was carried out using a PCR Techne apparatus under the following conditions: initial denaturation at 94ºC for 2 min followed by 10 cycles of denaturation at 94ºC and 1 min of annealing and extension at 65ºC. The next 20 cycles included 10s of denaturation at 94ºC 50s of annealing at 61ºC, and 30s of extension at 72ºC, 30s. The absence or presence of PCR products was visiualized by 2% agarose gel containing ethidium bromide, electerophoresis. After electerophoresis the gel was placed on a UV transilluminator and a polaroid picture for interpretation and documentation was taken. Each of the primer mixes contained a primer pair that amplified either a part of the beta globulin gene or a part of C-reactive protein gene as internal control (8). The allele frequencies of IFN-γ gene at position (A/T 5'UTR +5644), was determined.

RESULTS AND DISCUSSION

The data indicated that there was a significant decrease in the TT genotype among patient compared to control ($P<0.05$, $OR = 0.23$, $0.1<OR<0.53$). The association between T1D and IFN-γ gene at position 5'UTR+5644 was a negative association due to $OR<1$ (table 1).
This study characterizes IFN-γ gene polymorphisms in patients with Type 1 Diabetes Mellitus. We have found that IFN-γ gene polymorphism at position 5'UTR+5644 has a negative association with the occurrence of T1D in Iranian patients. Therefore, it may be considered a predisposing genetic marker in resistance to T1D. The importance of pro-inflammatory cytokines in the pathogenesis of T1D is well established (9). IFN-γ has an immunomodulatory activity, influences cell-mediated mechanisms of cytotoxicity and is a modulator of T cell growth and differentiation. In addition, by producing IL-

Table 1. A/T allele frequencies at position 5' UTR +5664 in IFN gamma gene.

IFN gamma 5'UTR +5644	Patient	Control
Adenine (T allele)	11*	41
Thymine (A allele)	46	39

* There is a significant association between IFN gamma 5'UTR +5644 and T1D in patient group (P value: 0.0001, RR: 0.39, OR: 0.23)

2, IFN-γ activates macrophages which are the main participants of many pathological circumstances. In this investigation, we found a negative association between IFN-γ T allele in position 5'UTR +5644 and T1D. This is in accordance with the results of a previous study (10) which demonstrated a decreased frequency of IFN gamma TT genotype in T1D. Awata et al. (11) suggested that the IFN-γ gene region may contribute to the pathogenesis of T1D and could be a genetic marker for this disease. Pociot et al. (12) studied the polymorphism of IFN-γ gene in Danish patients and indicated that there is little support for the hypothesis that the IFN-γ gene microsatellite is associated with T1D. The different result from various studies may indicate the variation between different populations that have been studied.

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