

## Evaluation of Relation between IL-4 and IFN- $\gamma$ Polymorphisms and Type 2 Diabetes

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### Abstract

#### Objective(s)

Although, type 2 diabetes is the most frequent type of diabetes, its main cause is yet to be clarified. Several environmental and genetic parameters are believed to be involved in diabetes. It has also been established that cytokines play key roles in pathogenesis of diabetes. Expression of cytokines is different from person to person and in different societies. Several studies showed that polymorphisms of +874 of interferon-gamma (IFN- $\gamma$ ) and -590 of interleukin-4 (IL-4) are associated with the regulation of expression of these genes. This study was aimed to find polymorphisms of these regions in type 2 diabetes patients.

#### Materials and Methods

In this experimental study peripheral blood samples were collected from 160 type 2 diabetic patients and 160 healthy controls. DNA was extracted by salting out method. Polymorphisms of +874 of IFN- $\gamma$  and -590 of IL-4 were analyzed by ARMS-PCR and RFLP-PCR.

#### Results

Our findings indicated that TT genotype of IFN- $\gamma$  was increased in type 2 diabetic patients compared to the control but difference was not significant. Our results didn't show any significant difference between IL-4 genotype in diabetic and healthy controls either.

#### Conclusion

Our results suggested that TT genotype of IFN- $\gamma$  can be associated with diabetes. This association can be described by the fact that over expression of IFN- $\gamma$  shifts immune system to Th1; therefore, pancreatic cells can be miscarried by immune cells.

**Keywords:** Interferon-gamma, Interleukin-4, Polymorphism, Type 2 diabetes

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### Introduction

The main cause of type 2 diabetes is unclear but it is well defined that the disease imposes several problems to the patients (1). Several research groups reported that cytokine imbalance is involved in pathogenesis of type 2 diabetes (2). Investigators of this field believe that type 2 diabetes is associated with immune system and in turn is related to the alteration of Th2 to Th1 immune response patterns (3). Gene polymorphisms play key roles in regulation of cytokines expression (4), for example substitution of C by T in -590 position of IL-4 gene causes reduced cytokine expression while TT form up-regulates production of this cytokine (4). IL-4 is a Th2 cytokine which suppresses cellular immunity, thus, prevents immune-mediated destruction of langerhans cells (5). In contrast, IFN- $\gamma$  is a Th1 cytokine which supports immune system to perform cytolysis of target cells (6). Previous studies showed that substitution of T with A in +874 of IFN- $\gamma$  gene leads to a decreased expression of this cytokine, so that TT causes maximum expression, AT mediate expression and AA minimum IFN- $\gamma$  expression (4). Therefore, based on above introductory comments this project was aimed to analyze polymorphisms of -590 region in IL-4 as a Th2 and +874 region in IFN- $\gamma$  as a Th1 response cytokine in type 2 diabetic patients.

### Material and Methods

#### Subjects

In this experimental case-control study peripheral blood were collected from 160 type 2 diabetic patients and 160 healthy controls. Blood sugar was measured before PCR analysis. Fasting blood sugar was measured three times during 9 month in all patients and controls. Patients were selected randomly among diabetic patients of Ali Ebne Abitaleb Diabetes Clinic and control cases were selected from population with same age, sex, ethnic group and socio-economic status after approval by Rafsanjan University of Medical Science ethical committee. All the patients and control cases were from Rafsanjan, Iran.

#### Genotyping

DNA was extracted from anti-coagulant treated whole blood using salting out method

(6). IL-4 (-590C/T) gene polymorphism was analyzed by polymerase chain reaction-restricted fragment length polymorphism (PCR-RFLP) method. The sequences of primers were as follow:

F: 5'-TAAACTTGGGAGAACATGGT-3'

R: 5'-TGGGGAAAGATAGAGTAATA-3'

PCR was performed in 50  $\mu$ l of final volume in the following conditions: 5  $\mu$ l of *Taq* DNA polymerase buffer (10x), 1  $\mu$ l of MgCl<sub>2</sub> (stock concentration 1.5 mM), 1  $\mu$ l of each dNTP ((dATP, dCTP, dGTP, dTTP) stock concentration of 10 mM), 2  $\mu$ l of each primer pair (Table 1 (forward and reverse), stock concentration of 25 ng/ $\mu$ l), 2  $\mu$ l of prepared DNA and sterile double distilled water to a final volume of 50  $\mu$ l. The PCR thermocycler was run with the following program: one cycle of 95 °C for 2 min, 95 °C for 1 min (denaturation), 1 min at 53°C (annealing), 72 °C for 40 sec (elongation) followed by 30 cycles of 94 °C for 20 sec, 65 °C for 20 sec and 72 °C for 2 min. During the last 45 seconds of first stage 0.3  $\mu$ l of *Taq* DNA polymerase was added to the mixture. The PCR product of IL-4 (-590C/T) was a 195 bp fragment and was digested with *Ava*II into 175 and 20 bp fragments. The digested products were then run on a 2.5% agarose gel (Cinnagen-Iran) and visualized on UV transilluminator after staining with ethidium bromide. Polymorphisms at position +874 of IFN- $\gamma$  gene were identified using allele specific polymerase chain reaction (ARMS-PCR) as described by Pravica and colleagues (7). The 262 bp amplified product was then visualized by electrophoresis on 2% agarose gel.

#### Statistical analysis

Allele and genotype frequencies were calculated in patients and healthy controls by direct gene counting. Statistical analysis of the differences between groups was determined by  $\chi^2$  test using EPI 2000 and SPSS software version 13. The *P* value less than 0.05 was considered significant. The study power was also calculated for each allele and genotype.

### Results

In this study IFN- $\gamma$  and IL-4 gene polymorphisms were analyzed in 160 type 2

diabetic patients and 160 healthy controls. The demographic information such as age, sex, race and socio-economic status of patients and healthy controls are shown in Table 1. As it is obvious from Table 1 the mean age of patients and healthy controls is between 38±9 and 38±8, respectively. There is not a significant difference between the age of patients and healthy controls. 40% of patients were female

and 60% were male while 41% of healthy controls were female and 59% male. As shown in Table 1, there was not a significant difference in socio-economic status of patients compared to healthy controls either. Polymorphisms results of +874 region of IFN-γ and -590 region of IL-4 are shown in Tables 2 and 3.

Table 1. Demographic data including age, sex and socio-economic status of diabetic patients and healthy controls.

Number	Variant	Healthy control	Patient
1	SD of age	38±8	38±9
2	Sex	64 (40%)	66 (41%)
		96 (60%)	94 (59%)
3	Socio-economic status	38 (24%)	34 (21%)
		70 (44%)	78 (49%)
		52 (32%)	48 (30%)

Table 2. Polymorphisms results of +874 region of IFN-γ. Chi-Square: 2.321, df: 2, P: 0.313.

Polymorphisms	TT n (%)	AT n (%)	AA n (%)	Total n (%)
Condition				
Patient	85 53.1%	51 31.8%	24 15.1%	160 100%
Healthy control	80 50%	54 33.7%	26 26.3%	160 100%
P value	P>0.3	P>0.3	P>0.3	

Table 3. Polymorphisms results of -590 region of IL-4. Chi-Square: 2.321, df: 2, P: 0.16

Polymorphisms	CC n (%)	CT n (%)	TT n (%)	Total n (%)
Condition				
Patient	112 70%	35 21.8%	13 8.2%	160 100%
Healthy control	116 72.5%	33 20.6%	11 6.9%	160 100%
Total	P>0.1	P>0.1	P>0.1	

## Discussion

Immune system plays key roles in pathogenesis of type 2 diabetes (2). Cytokines are key mediators which regulate immune response and it is well established that expression of cytokines by immune cells depends on several factors such as infection, inflammation, hormonal condition and also cytokines gene polymorphisms (1, 8). In this study, we analyzed gene polymorphisms of IFN-γ as Th1 and IL-4 as Th2 cytokines in type 2 diabetic patients. We selected the gene regions which influence expression of these genes for comparison in type 2 diabetic patients and healthy controls. As it is obvious

in Table 1 the two groups had same age, sex, ethnic group and socio-economical condition. We could not find a significant difference between patients and control group regarding -590 region of IL-4 gene (Table 2). Our study showed that in +874 of IFN-γ gene, TT alleles is more expressed in diabetic patients than healthy controls but the difference was not significant but it seems that it would be significant if more samples had been analyzed. Tsiavou and colleagues showed that in +874 region of IFN-γ gene, polymorphism is associated with type 2 diabetes and AA allele is significantly higher in diabetic patients in Greece (9). Their results are interesting

because they showed that the presence of this allele is associated with down-regulation of IFN- $\gamma$ . Several research teams showed increased expression of IFN- $\gamma$  in type 2 diabetic patients (9, 10); thus, complementary studies are needed in type 2 diabetes to cover the gap of knowledge in this field. Although, our results did not show any significant difference in TT allele in IFN- $\gamma$ , it was increased in diabetic patients. Therefore, it is probable that with a larger sample an association of this polymorphism with type 2 diabetes can be found. To the extent of our knowledge, there is not much information related to these polymorphisms and diabetes, however association between these polymorphisms and brucella (11), leishmaniasis (12), tuberculosis (13) and malaria (14) was reported. Hanck *et al*, analyzed IFN- $\gamma$  polymorphisms in patients with alcoholic chronic pancreatitis but did not find any association between the different genotypes and the clinical course of the disease. Therefore, they assumed that these genetic variants did not play an important role in alcoholic chronic pancreatitis (15).

In another report Rafinejad *et al*, examined the relation between IFN- $\gamma$  gene polymorphisms and type 1 diabetes and found

a significant difference between patient and control groups in TT genotype (16). Overall these results and other research teams' investigations give perspectives to the etiology of diabetes.

For future studies, analysis of other cytokine gene polymorphisms such as IFN- $\gamma$ , TNF- $\alpha$  and IL-12 as the main cellular immunity cytokines is suggested, since it seems that Th2 cytokines (like IL-4) suppress the cellular immunity and may not play a clinical role in etiology and genetic of diabetes.

### Conclusion

The results of this study demonstrated that there is not any relation between IFN- $\gamma$  and IL-4 polymorphisms and type 2 diabetes.

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