

Study TNF- α gene polymorphism in Type 1 Diabetic Patients Using Amplification Refectory Mutation System (ARMS) technique

Mona Al-Terehi¹, Rana Ghaleb², Shaimaa A. Al-Oubaidy², Ali H. Al-Saadi¹, Haider K. Zaidan¹

¹University of Babylon, College of Science/ Hilla-Iraq

²Medical college- Babylon University/ Hilla-Iraq

*Corresponding author: E-Mail: Monanajah1981@gmail.com

ABSTRACT

The present study was conducted to study TNF- α gene polymorphisms in type1 diabetes mellitus patients using Amplification Refectory Mutation System (ARMS), The results show that the age mean was (48.5000) and (30.9500) for patients and control respectively and gender was male (100) % in control while in patients(30.77%) (69.230%) for male and female respectively, the genotype Results show non-significant association of TNF- α between patients and control and GG allele was not appear in present study the percentage of AA and GA allele were (4.347%), (95.653%) in patients while it were (5%) and (95%) in control respectively, the present study concluded that there is no-significant association of TNF- α genotype with T1DM.

1. INTRODUCTION

Type 1 diabetes mellitus (T1DM) is autoimmune disease characterized as multifactorial disorder caused by T-cell mediated destruction of the pancreatic β -cells resulted from interaction between genetic and environments factors (Todd 1995) type 1 diabetes is rarely caused by monogenic disorder, which accompanied by multiple other auto immune conditions due to the disruption of common regulatory pathways. Other Studies demonstrate that there are some genes association with type 1 diabetic, there genes have been studied like HLA Genes, Insulin Gene, PTPN22, which encodes the lymphoid protein tyrosine phosphatase (LYP) (57, 402), CTLA-4, interleukin (IL)-2 receptor gene (IL2RA), also immune protein genes can be contributed in auto immune disease developments and pathogenesis like cytokine and interleukin (Radha, 2003).

Some studies reported association of TNF – α levels with some physiological parameters in diabetic patients Jyothi, (2012) found that the TNF- α level was correlated significantly with HOMA IR, HOMA B and insulin.

Swaroop, (2012) suggest the possible role of TNF- α in the pathogenesis of type-2 diabetes mellitus and the importance of reducing obesity to prevent elevated levels of the cytokine and related complications. TNF- α production is thought to play a role in the generation of micro vascular complications associated with diabetes, e.g., by enhancing chronic eye inflammation (Demircan, 2006; Devaraj, 2007).

In addition to triggering acute and chronic inflammation, TNF- α regulates glucose and lipid metabolism and inhibits insulin production in pancreatic beta cells (Pickup, 2010) TNF- α is also produced in adipose tissue.

The multifactorial cytokine, tumor necrosis factor (TNF) is used in the progression inflammatory responses and plays major role in the pathogenesis of inflammatory, autoimmune and malignant diseases (Bazzoni and Beuther, 1996). The role of TNF IN T1D have been under investigators in human but in animal models TNF can be cytotoxic for β -cells supported by both interleukin-1 and interferon- γ (Rabinovitch 1994, Rabinovitch and Suarez-Pinzon 1998). The circulating monocytes and T-cells type 1 pro-inflammatory cytokines are increased in patients with T1D (Hussain, 1996)

The *TNF* gene placed in chromosome 6 in the HLA region class III, 250 kb centromeric of the *HLA-B* and 850 kb telomeric of the class II *HLA-DR* genes in humans (Hamaguchi, 2000). Several *TNF* promoter polymorphisms have been identified and have been implicated in the regulation of TNF transcription (Kroeger, 1997 and Wilson, 1997). Both polymorphisms are G^A substitutions and changes they introduce can alter the transcription-binding site and affect the transcription rate (Kroeger, 1997). Since both promoter polymorphisms have been associated with the transcriptional enhancement rate, it is possible that when acting *in cis*, these two markers show even stronger interaction (Kroeger, 1997 and Kaluza, 2000). As a results of low studies in the TNF role in diabetic type1 and its gene polymorphism the present study was suggested.

2. MATERIALS AND METHODS

- Sample and data collection; about 2 ml of whole blood was collected from patients of type1 from diabetic center in Al-Sadder hospital while control collected from healthy.
- DNA extraction; DNA was extracted from whole blood using Favor gene extraction kit.
- Primers and PCR conditions; primers were used in present study wereF 5'-TCT CGG TTT CTT CTC CAT CG-3, and R 5'-ATA GGT TTT GAG GGG CAT GG-3 for G allele, 5'-AAT AGG TTT TGA GGG GCA TGA-3 for A allele (Al-Rayes, 2011), PCR conditions for ARMS technique were two cycles the first cycle was 10 cycles (94 for 5 min, 94 for 15 sec, 65 for 50 sec, 72 for 40 sec) the second cycle was 25 cycles (94 for 20 sec, 59 for 50sec, 72 for 50 sec, 72 for 7 min)

- Data analysis, PCR product was electrophoresis in 1.5% agarose for 45 min, 70V and 20 mA. The frequency of allele calculated according to hardy-Weinberg law, and the statics analysis implemented using Qi square and odd ration at p value <0.05.

3. RESULTS AND DISCUSSION

The results of present study included demographic study and TNF (308) gene polymorphism, the demographic study show significant differences between patients and control in age and gender (table 1). the incidence of diabetic type 1 dependent on many factors like risk and ethnicity, gender and age, environments factors and genetic susceptibility Dorman, (2000). Life style have been stilled occupies a prominent place in the science field for DMT1 incidence in last decades, investigators and nutrition's scientist improved that the nutrition system, food quality and exercise are very important in families which have genetic predisposition of diabetic incidence, however the balance food and intake food supplements may be had role in immune system developments in childhood which avoid some disease like diabetic.

The differences between male and female don't deal with most of the published studies reported that no significant difference between the type 1 diabetes incidence in boys and girls (Abellana, 2009; Svensson, 2009; Samardzic, 2010; Teeaar, 2010). Other groups found small and thereby not relevant sex-related differences only for subgroups (Shaltout, 1995).

Table.1.Demographic study of study groups

Categories	Control	Patients	Statics	P- value
Age	30.9500±12.44134	48.5000±13.26560	4.3881*	0.0001
Female	0%	30.77%	32.855**	0.0001
Male	100%	69.230%		

Statics at (p<0.05), *Qi square, ** t-test

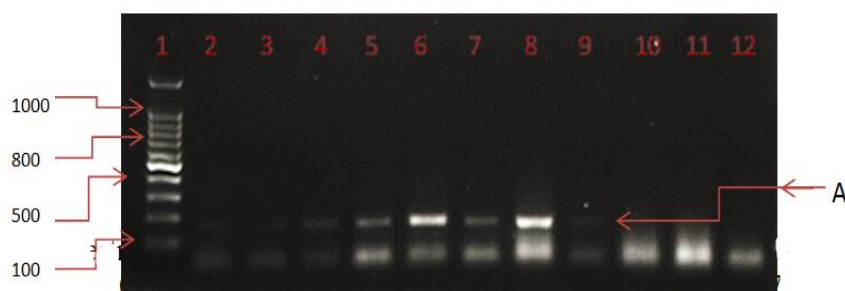


Figure.1.Electrophoresis pattern of TNF genotyping using ARMS technique

Exp. conditions: 1.5% agarose, 70V, 20mA, for 60 min, lane 1 (100 bp) DNA marker, lane 2-7 TNF genotype for patients, lane8-12 TNF genotype for control. Lane 2-9 GA pattern, lane 10-12 AA genotype.

Table.2.The genotype differences of TNF genotype in study group

Genotype	Patients%	Control%	X	P- value	Odd ratio
GG	0	0	0.010	0.9193	0.8636 0.0505 to 14.7672
AA	4.347	5			
GA	95.653	95			
A	0.521	0.525			
G	0.478	0.475			

The results show tow alleles AA and GA as in table (2) and figure (1), GG allele was disappeared in present study, there is non-significant variation between patient and control in TNF- α gene polymorphisms. Also the association between gene polymorphisms and demographic study show no significant variation between patients and control in gender and age categories, this may be because that the loci of amplification was not effect or changes in patients thus other loci must be study or use other technique like DNA sequences to determinate SNPs. The *TNF* gene is located within HLAIII loci which included about 10 times more genes than other regions in the genome and is considered to be the densest region in the human genome, Beside *HLA DR/DQ* locus of the *HLA* region II, *HLA* class III region, particularly around the *TNF* gene, has been regarded as a susceptible locus for T1DM (Nishimura, 2003).

In present study ARMS technique was used to detected genotyping of *TNF* premotor because it easy, have short time and low cost, this technique dependent on touchdown PCR technique and three primers (Saste, 2012) .

The present study deal with study performed by Boraska, (2008) they were the first investigate two *TNF* gene promoter polymorphisms (-308 and -238) in a case control sample from South Croatia they found that with a

limited significance, a higher frequency of *TNF* -308 A allele and a specific (-308A -238G) haplotype in T1DM patients. However, they did not find strong evidence of association of *TNF* promoter polymorphisms with T1DM. Manuel, (2013) found that the genotype and allele frequencies of both polymorphisms *TNF* > 308G/A and 238G/A did not differ significantly between the women with GDM and the controls.

Abdu-Azize, (2001) found that *TNF* level were elevated in patients with type 1 DM in Egypt. Thus the present study needs more investigation about *TNF* role in DMT2 like epigenetics which is considered as an important project to understand other factor role in *TNF* association with DMT2 complications this was suggested in early study by Ratter, (1999) they found effect of the cellular methylation state in *TNF* - α mediated cytotoxicity.

The present study concluded that there was no association between *TNF*- α genotype with diabetic patient's type 1 in the promoter of this gene, however other gene loci must be analyzed in Iraqi patients

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