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Point mutation of IL-10 1082 gene G/A at promoter region increased susceptibility of T2DM among Iraqi patients

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Abstract: *Several studies have shown that the role of cytokines closely linked with many human diseases. Recent studies have been discovered some relationship between interleukin -10 (IL-10) and type 2 diabetes mellitus (T2DM).On the other hand, some studies have been showed a close correlation between ethnic factors and the physiological reasons for many human diseases included T2DM; therefore, this study aimed to explore the effect of IL-10 G/A gene polymorphism at position 1082 among Iraqi patients, and its correlation with T2DM, through a study of sample consisted of 96 cases with T2DM and an equal number of healthy control subjects by using of allele refractory mutation system polymerase chain reaction (ARMS-PCR) technique. Our findings demonstrated that there is a statistically significant correlation of IL-10 –1082 AA genotype between cases and control (OR=1.63, 95% CI 1.14-2.26, P=0.03) and AA mutant genotype considered as risk factor of disease. Whereas no significant statistically effect of GG and GA genotypes frequency and T2DM disease.*
Keywords: *IL-10, polymorphism, T2DM, Iraqi patients*

I. Introduction

Type 2 Diabetes mellitus is a group of abnormal physiological conditions in which the person produce insulin but the amount of insulin may be not enough or there is enough amount but that insulin not work in that individual's body because precise biological defects (1 ,2). Most of studies summarized diabetes's reasons through two mechanisms. One of them impairs the function of pancreatic islet beta (B) cells and the second is impaired insulin action (1, 3). In 2008 reported that some pro- and anti-inflammatory cytokines secreted by mononuclear cell are one of most critical causes of dysfunction of pancreatic islet beta (B) cells as well as showed that the effect of IL-10 and IL-13 was associated with metabolic syndrome, type-2 DM, type-1 DM, and dyslipidemia (4,5). In 2010 the evidences are increased and improved that cytokines played critical roles in the procedures of pancreatic B cells function (6). That's where some cytokines such IL-1, tumor necrosis factor (TNF), leptine, resistine and adeponectine have been shown diversely adjust islet beta (B) cells function. Pancreatic B cells high chronic exposure to IL-1, TNF, interferon alpha (IFN-α) promote the B cell dysfunction and apoptosis (5, 6).Other researchers demonstrated that pro-inflammatory cytokines like IL-6 and TNF-α associated with metabolic syndrome and T2DM 7, 9). As well as reported that TNF-α played vital role with insulin resistance, given that TNF-α and IL-6 lead to impair insulin action through down regulation of tyrosine kinase activity of the insulin receptor (10,11, 12).On the other hand some studies revealed that IL-6 impairs insulin sensitivity and the liver may be an important target of IL-6 (13).Concerning IL-10 which is the aim of this study is an anti-inflammatory cytokine and play as a immunosuppressive agent as well as act as a regulator of immune response (14). Production of IL-10 results in an efficient autocrine mechanism for regulating of pro-inflammatory cytokine production (15). Several studies reported that there is positive association between serum level of IL-10 and whole-body insulin sensitivity and have been shown that low IL-10 production was associated with hyperglycemia (16, 17). Therefore, in order to support the information series regarding T2DM disease and cytokines, the present study aimed to explore the association between IL-10 gene polymorphism and T2DM among Iraqi patients sample.

II. Materials and Methods

Total sample included 96 cases with T2DM; 51 male and 45 female and an equal number health control subjects; 47 male and 49 female. The mean age for cases and controls was 28.23 and 30.44 respectively. They were collected from consultative clinic of AL-Hashimiah General Hospital. All cases had a diagnosis of established T2DM on the bases of blood, urine tests, medical history, and clinical examinations. Genomic DNA extracted from Leukocytes by using of (Genomic DNA Extraction Kit- Bioneer Company). All samples were genotyped for IL-10 1082 G/A through (ARMS-PCR) technique as described in (Table 1). Amplification was carried out at conditions given in (Table 2).

TABLE 1: Primers used in the present study and their product size

Polymorphism/ Allele location	Primers	Sequence	Product size (bp)
IL-10-1082G/A	Internal control	F: 5'-GTA AGC TTC TGT GGC TGG AGT C-3' R: 5'-TTT CCA GAT ATC TGA AGA AGT CCTG-3'	313
	G & A alleles	F: 5'-AAC ACT ACT AAG GCT TCT TTG GGT A-3' R: 5'-GTA AGC TTC TGT GGC TGG AGT C-3'	161

TABLE 2: PCR conditions for IL-10 1082 G/A gene

S.NO	steps	Temperature	Duration	No. of cycles
1	Denaturation	95 ^{o}C	5 min	1
2	Annealing	95 ^{o}C	30 s	30
3	Annealing	63 ^{o}C	30 s	
4	Extension	72 ^{o}C	5 min	1

III. Statistical analysis

T and Chi square tests were performed to find out whether there exists significant variation in age between cases and controls. The association between the candidate genes and risk of T2MD disease was estimated by computing OR and 95% CI using multivariate logistic regression analysis. The statistical analysis was performed using Epi-Info software (Epi-Info, version 3.5.1. centre for Disease Control and Prevention, Atlanta, GA, USA, August 13, 2008) and software SPSS version 11.5 (SPSS, Chicago, IL). Significance was set at $P<0.05$.

IV. Results

- **Characteristic of study subjects**

Regarding mean age and gender distribution, there was no statistically significant difference among cases and controls.

- **Distribution of 1L-10 -1082 G/A genotypes**

Table 3. Summarize the genotypes and allele frequencies for IL-10 G/A gene at position (1082) between cases and normal healthy controls subjects. Comparison results showed no significant association between cases and controls concerning heterogenotype G/A and the risk of disease (OR= 1.10, 95% CI 0.57-2.22, p< 0.75).While the relationship between mutant homozygous A/A genotype in cases and control was statistically significant (OR=1.63, 95% CI 1.14-2.26, P < 0.03). As well as the study showed a risk factor between A allele frequency and disease but statistically non significant (OR= 1.22, 95% CI 0.76-1.95, p< 0.38). It was found out that the frequencies of genotypes and alleles in cases and controls in the present study were in Hardy-Weinberg equilibrium.

Table 3. Distribution of genotypes and Alleles of IL-10 gene.

IL-10 G/A	Cases n= 96	Control n =96	OR(95%-CI)	P-value
G/G	47	49	reference	0.1
G/A	38	36	1.10(0.57- 2.22)	0.75
A/A	11	2	1.63(1.14-2.26)	0.03*
Alleles frequency				
G	132	134	reference	0.1
A	60	40	1.22 (0.76 – 1.95)	0.38

***Indicate significant p value**

V. Discussion

Polymorphism is one of the most important ways that are used to determine point mutations in the genotypes. Single-nucleotide polymorphism (SNPs) is one of a pattern of polymorphism, which is defined as a variation of a DNA sequence nucleotides in the human genome. Such kinds of genetic variation occurring when a single nucleotide — A , T, C or G — in the genome differs between individuals of a biological species or matching chromosomes in a human (17, 18). For example, two sequenced DNA fragments from different individuals, AAGC**C**TA to AAGCTTA, contain a difference in single nucleotide sequencing. SNPs play a serious responsibility in the shifting of the amino acid sequence of protein then subsequently change of the proteins or enzymes functions (18, 19). Whereas, these genetic variations at coding parts of the genome underlie our susceptibility to disease (20, 21). DNA nucleotide sequence variations of humans genome can affect how humans susceptible and/or progress diseases and react with pathogens; chemicals, drugs vaccines,

and other agents (22). Several studies showed that this type of genetic variation resulted in the changing of the quality and quantity of proteins; so from this biological entry we can access to interpret the results of this study, which showed that people with genetic formula AA genotypes they have a risk of disease (OR=1.63, 95% CI 1.14-2.26, P=0.03). We believe that outcome of such this risk factor was due to firstly; the low production of IL-10 in such AA genotype individuals consistently with previous studies which showed that individuals with IL-10 (-1082) AA genotype produce low levels of IL-10 than GG and GA genotypes that's where plasma Interleukin-10 concentration is positively related to insulin sensitivity in healthy Individuals.(23) So IL-10 may made opposite action for effect of other Interleukins like IL-6 and TNF; as we mentioned in the introduction to the this study that high levels of TNF-α and IL-6 lead to impair insulin action through down regulation of tyrosine kinase activity of the insulin receptor while high levels of IL-10 can cause upregulation of tyrosine kinase activity of the insulin receptor (24, 25, 26, 27, 28).Secondly; may be through the correlation between IL-10 production capacity, high glucose and HbA since the association between IL-10 production capacity and glucose metabolism is real due to postprandial changes in HbA(29).Thirdly; may be through the effect of IL-10 as stimulator of insulin receptor substrate 2/P13-kinase/AKT pathway this proven by Jain and Singh (30,31,32). All these interpretations may be non-curative. So more future studies concerning the interaction of genes (apistasis) and the net work of balance between pro and anti-inflammatory cytokines action and it's correlation with T2DM, certainly will give us clear vision about the real role of each cytokine and T2DM disease.

VI. Conclusion

We concluded that there is a significant correlation between IL-10 A/A genotype at position 1082 and T2DM disease among Iraqi patients.

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