Association of Protein Tyrosine Phosphoatase Nonreceptor Type 22 Genes with Type 1 Diabetes Melitus

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ABSTRACT:

BACKGROUND:

Type 1 diabetes is a multifactorial disease caused by a complex interaction of genetics and environmental factors. The Genetics factors involved consist of multiple susceptibility genes such as protein tyrosine phosphoatase nonreceptor 22, which have been associated with T1DM in different populations. Recent studies showed a genetic variation within protein tyrosine phosphoatase nonreceptor 22 gene to be an additional risk factor.

OBJECTIVE:

To analyze the genetic association between protein tyrosine phosphoatase nonreceptor 22 and type 1 diabetes mellitus in some Iraqi population and correlation of Glutamic acid decarboxylase antibodies and interleukin -2 receptor with type 1 diabetes.

METHODS:

A total of 50 type 1 diabetes patients from diabetes center were genotyped for protein tyrosine phosphoatase nonreceptor 22 genes by using an restriction fragment length polymorphism method. Level of Glutamic acid decarboxylase antibodies and interleukin -2 receptor were determined using enzyme linked immunoassays.

RESULTS:

Glutamic acid decarboxylase antibodies were a highly significant increase ($P<0.0001$) in type 1 diabetes patients as compared with healthy control group. Circulating levels interleukin -2 receptor were highly significant in diabetic patients than healthy subjects ($P<0.001$). The frequency of protein tyrosine phosphoatase nonreceptor 2 gene among the 50 patients [CT (33%) , TT(4%) , CT and TT (37%] compared with healthy control [CT (18%) TT(1%), CT and TT (4%)], while the percentage of T allele of patients (21%) and healthy control (10%). In addition, the protein tyrosine phosphoatase nonreceptor 2 genotype was significantly associated with Glutamic acid decarboxylase positivity (CT- 19%, CT and TT -24%).

CONCLUSION:

Strong association between protein tyrosine phosphoatase nonreceptor 2 gene and diabetes type 1. In addition,The protein tyrosine phosphoatase nonreceptor 22 genes were associated with Glutamic acid decarboxylase antibodies.

KEY WORDS: protein tyrosine phosphoatase nonreceptor 22, glutamic acid decarboxylase antibodies.

INTRODUCTION:

Type 1A diabetes is caused by autoimmune destruction of insulin-producing beta-cells in the pancreas in genetically susceptible individuals [(1)]. Identification of genes predisposing to type 1 diabetes is important in establishing effective methods for disease prediction, prevention and intervention. Type 1 diabetes, however, is a multifactorial disease caused by a complex interaction of genetic and environmental factors, with former consisting of multiple susceptibility genes, which makes identification of disease-causing variants very difficult [(2)]. Among the multiple susceptibility genes involved in type 1 diabetes, at least five loci, the HLA class II genes on chromosome 6p21, insulin gene (INS) on 11p15, CTLA4 on 2q33, PTPN22 on 1p13 and the interleukin-2 receptor alpha chain (IL2RA/CD25) region on 10p15, have been shown to be associated with type 1 diabetes in Caucasian populations [(3,4)]. The PTPN22 gene is encodes a lymphoid protein tyrosine phosphatase (LYP) that is important in negative control of T-cell activation and in T-cell development [(5,6)]. This gene is specifically expressed in lymphocytes and through formation of a complex

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with C-terminal Src Kinas (CSK) suppresses the downstream mediators of T-cell receptor signaling [7,8]. later, PTPN22 gene was found to be associated with susceptibility to T1D [9]. Single-nucleotide polymorphism (SNP) 1858 C/T within this gene considered as a risk factor for several autoimmune diseases, such as rheumatoid arthritis (RA), Graves' Disease (GD), systemic lupus erythematosus (SLE), Wegener's granulomatosis (WG) and type 1 diabetes mellitus (T1D) [10,11].

SUBJECTS AND METHODS:

1. Patients group

This group included 50 patients (male, female) suffering from type 1 diabetes mellitus, the diagnosis of TIDM was established by using following criteria (defined by the national Diabetes data group): blood glucose level, Hbc1, and insulin treatment. The diagnosis was confirmed by the presence of major autoantibodies (GAD65 antibodies). They were selected from the out patients of diabetes center.

Apparently healthy group

Fifty Apparently healthy control group with no history of diabetes were recruited.

METHODS:

1. Sampling.

Ten milliliter of venous blood was collected from patients and apparently healthy group, 5 ml of blood in a clean dry tube without the addition of any anticoagulant. It was left to clot for 15 minutes, centrifuge and serum was separated and 5 ml of blood was added in tube with anticoagulant as EDTA to extraction of DNA.

2. Glutamic acid deca 320 e (GAD) antibodies

Measurement of GAD antibodies concentration against glutamic acid decarboxylase (GAD) in serum of studied group. Cut-off value recommended by EUROIMMUN is 10 international units per milliliter (IU\ml) .

EUROIMMUN recommends interpreting results as follows **:**

<10 IU\ml : negative

≥10 IU\ml : positive

2. Serum interlukin -2 receptor (SIL-2R) assay.

The SIL-2R was done by a solid phase sandwich enzyme linked immunosorbent assay using a kit provided by Biosource international, Inc.

3.Genotyping methods

For genotyping, DNA was isolated from 5 ml peripheral blood using Extra – Gene kit (BAG Health care, Germany) and 0.5 µg DNA were used in the PCR reactions. For the determination of the *PTPN22* alleles, PCR-based restriction fragment length polymorphism (RFLP) analysis was performed as described previously (16). Briefly, a fragment of the *PTPN22* gene was amplified by PCR using the forward primer 5'-TCA CCA GCT TCC TCA ACC ACA-3' and the reverse primer 5'-GAT AAT

GTT GCT TCA ACG GAA TTT A-3'. Each 25µl amplication reaction contained 2µl of DNA,2.5 µl 10x PCR began with an initial denaturation at 94ºC for 5min. followed by 30 cycles consisting of 30sec. at 94ºC,60 sec at 62ºC 60 sec at 72 ºC and it ended with a final elongation step at 72ºC for 5 min. Amplified products were digested using Xcm I (Applied Biosystems, USA) overnight at 37 ºC. Digested products were was electrophoresed on a on 3% agarose gel, stained with ethidium bromide and visualized by UV (12).

Statistical analysis

Data were analyzed by using the statistical program SPSS v12 (13). Mean and standard deviation was used to describe IL-2R among patients and control. P-value of less than 0.05 was considered statistically significant.

RESULTS:

Levels of GAD antibodies were assessed in sera of studied groups. It was clear that there was a highly significant increase (P<0.0001) noted in T1DM patients as compared with healthy control group, as publicized in table (1) .

Table 1: The Mean GAD antibodies among the sera of the studied groups.

Autoantibodies	No.	Mean	Sd.	P value	Significant
GAD antibodies T1DM	50	27.47	22.80	<0.0001	H.S
Healthy group	50	7.28	30.40		

Circulating interleukin- 2 receptor was estimated in sera of T1DM patients and apparently healthy group were shown in (Table 2). The results indicated that serum IL-2 R levels were highly significant in diabetic patients than healthy subjects (P<0.001).

Table 2: The Mean of sIL-2R level among the sera of the studied groups (T1DM patients and apparently healthy control).

Cytokines	No.	Mean	Sd.	P value	Significant
IL-2R T1DM	50	779.0	467.07	<0.0001	H.S
Healthy group	50	254.33	173.0		

The distribution of genotypes and the resulting frequencies of the resulting allele frequencies of variant *PTPN22* 1858 alleles in T1DM patients and apparently healthy control are shown in (Table 3). The frequency of *PTPN22* 1858 variant among the 50 patients [CT (33%) and TT(4%)] compared with fifty healthy control [CT (18%) and TT(1%)] . The CT and TT genotype was present in 37% of patients and 19% of healthy control group. In addition the percentage of T allele of patients and healthy control (21% and 10% respectively).

Table 3: Genotype and allele frequencies of *PTPN22* 1858 T/C SNP in studied groups.

Studies groups	Genotype frequency			Allele frequency
	CT%	CT and TT%	TT%	T
T1DM	33	37	4	21
Apparently healthy control	18	19	1	10

To evaluate the association between PTPN22 genotype and GAD positivity (Table 4). The PTPN22 genotype was non significantly associated with GAD positivity (CT- 19% , CT and TT -22%, TT%-3%).

Table 4: Genotype frequencies of PTPN22 1858 T/C SNP in T1DM patients, stratified by GAD autoantibody.

Autoantibody	Genotype frequency		
	CT%	CT and TT%	TT%
GAD +	19	22	3
GAD -	14	15	1

DISCUSSION:

Different studies have shown that PTPN22 gene is correlate with the development of type 1 diabetes and other autoimmune diseases. This gene encodes a lymphoid protein tyrosine phosphatase (LYP) which by dephosphorylation of Src family kinases negatively regulates T-cell receptor (TCR) signaling [5]. The association between the PTPN22 polymorphism and type 1 diabetes are widely accepted, our study was the first genetic analysis to pin point this association in Iraqi patients. In present work, there was a highly significant association of GAD autoantibodies with T1DM (P<0.0001), as compared with healthy control group. Data of the present study showed that serum cytokines (sIL-2R) levels were significantly higher in T1DM than healthy control. These results are in agreement with other studies, which revealed that the inflammatory activity is increased in individuals with type-1 diabetes, and this may be due to hyperglycemia and the formation of advanced glycation end products (13, 14). Many interleukins play important role in the etiopathogenesis of T1DM, among these is the binding to specific interlukine-2 receptors that present on the surface of T-cells [15]. In addition, IL-2 system which involves IL-2 production , IL-2R expression and response to IL-2 are associated with autoimmune phenomena. Immunological abnormalities including autoimmune phenomena are belived to contribute to the pathogenesis of T1DM [16]. It was found that the percentage of Il-2R was significant increased in diabetic children than non diabetic group [17]. The frequency of PTPN22 variant among the 50 patients [CT (33%) , TT(4%) , CT and TT (37%] compared with fifty healthy control [CT (18%) TT(1%), CT and TT (4%)]. These results disagreed with other study [18] who reported that association of *PTPN22* 1858 variant among diabetes patients[CT (25%) , TT(2%) , CT and TT (47%) , CC(73%)] . These difference may be explained by variation in PTPN22 gene frequencies in different ethnic groups or due to the small sample number .In addition, The PTPN22 genotype was not significantly associated with GAD positivity (CT- 19% , CT and TT -22%, TT%-3%) in study groups. This result was similar to previous findings that the impact of this gene on the presence of GADA may only be observed in patients with long disease duration. Based on their detailed study of autoantibodies in T1DM patients in association PTPN22, Ladner and coworker proposed that the presence of GADA may be a marker of general autoimmunity [19]. Our result support this hypothesis. Additional studies are needed to explore further the role of this gene in onset of disease, as well as on the prevalence of autoantibodies in the T1DM and other autoimmune diseases.

CONCLUSION:

Strong association between protein tyrosine phosphoatase nonreceptor 2 gene and diabetes type 1. In addition,The protein tyrosine phosphoatase nonreceptor 22 genes were associated with Glutamic acid decarboxylase antibodies.

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