Single Nucleotide Gene Polymorphism of Interleukin 1 Receptor Gene at Position-1970 in Type 1 Diabetes of Iraqi Patients

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Abstract

The aim of this study was to evaluate the frequency of polymorphism of interleukin-1-R gene (IL1R) at position-1970 SNP in T1D and in healthy controls subjects39 of Iraqi patients, (12 males & 27 females;1 5.65 ± 1.79 years) and 21 controls.(7 mail & 14femal ; 14.66 ± 3.43 years) were enrolled in this study the polymorphism of IL1R-1970 was data waved by polymerase chain reaction-specific sequence primer (PCR-SSP) assay. Results revealed that comparing IL1R-1970 genotypes and alleles between T1D patients and controls frequencies of TC genotype and C allele (79.49vs. 68.05%; P =0.419 respectively) were significantly rise in patients contrast to controls, (38.28vs. 40.62%; P =0.094) and the related RR rates were 21.3%and39.8%, respectively. And the associated EF values were 1.71and2.11.Similar observations were made in CC genotype.

In contrast TT genotype and T allele (22.33vs. 38.95%, P =0.706 respectively) frequencies were significantly decreased in patients, compared to controls (42.38vs. 49.38%; P =0.094), and associated PF values were 0.63and0.47, respectively. These findings suggest that IL1R-1970 SNP might have a role in the etiopathogenic mechanism showed associations (positive and negative) with T1D in the samples of Iraqi patients.

Keywords: SNP IL-1R Diabetes.

Introduction

Diabetes is a chronic disease which influences over 3 million people in the UK about 10% Type 1 diabetes and residual 90% have Type 2 diabetes [15, 18].

T1D is a serious autoimmune disease affecting millions of people worldwide. This information is for adults with T1D and parents of children with this condition [15]. T1D usually starts in childhood, adolescence, or early adulthood, but it may also start later in adult life [1, 2].

Everyone needs hormone insulin to keep their blood glucose at a normal level. But with T1D, the pancreatic gland not synthesis insulin or make very little of it [3, 18]. T1D is an autoimmune disease characterized by the destruction of the insulin-producing islet β cells.

Cytokines act as pleiotropic polypeptides regulating inflammatory and immune responses through actions on cells. They provide important signals in the path physiology of a range of diseases, including T1D [14, 5].

There is increasing evidence showing that polymorphisms in cytokine genes may play an important role in modulate the immune response. Numerous cytokines have been shown to participate in the pathogenesis of T1D [4].

Cytokines IL-1, TNF-and IFN-Y that are secreted by macrophages and T cells have broader role in the development of T1DM than previously thought [9].

As gene polymorphisms can influence in cytokine production or function, they may potentially contributed to genetic predisposition to the disease, as at TGF-B1, TNF-a and IL-6 [11, 13].

Mediators of inflammation such as TNF-a, IL-18, the IL-6 family of cytokines, IL-18, and certain chemokine’s have proposed to be involved in the events result in both forms of diabetes.[12,10,6].

Further supply for inflammation to contribute to diabetes comes from researchers to examined the role of
inflammatory cytokines in diabetic such as IL-1 was first implicated in the development of diabetic [14,8]. IL-1 was first described in 1972 as a lymphocyte-activating factor [19] that is mapped to the long arm of chromosome2 (2q14.2) [25].It later was shown to exert a variety of effects including induction of inflammation [22].

The cytokines IL-1 and TNF-α induce B-cell apoptosis in T1D is reported to be rise by elevated glucose and is a known powerful stimulus of extra cellular matrix production. Therefore a study found an increasing level of TNF α and IL-1in both vitreous and serum of diabetic subjects compared to control subjects Interleukin (IL)-1 is produced by activated macrophages and is involved in acute-phase inflammatory responses. Functional IL-1 receptors (IL-1Rs) are present on pancreatic ß-cells.In vitro, [19].For all previous information present study focused on Interleukins-1R.

Materials and Methods

Subjects

The diagnosis and extent of disease was determined by conventional clinical thirty nine patients; (12 males & 27 females) attended the hospital in Baquba for diagnosis and treatment during the period October 2015 – June2016 in addition to twenty one healthy controls (7males and 14females).According to diagnosis after an overnight fasting of 10–12 h in fasting state for all investigations). Blood samples were collected in EDTA .The samples were stored frozen at -20°C. T1D patients and randomly selected healthy controls (HC). The patients age range was 15.65 ± 1.79 years compared subject of health's controls was 14.26 ± 1.43 years, were enrolled in the study.

Detection of IL1R Polymorphism

Genomic DNA was extracted from EDTA blood using Wizard Genomic DNA Purification Kit (Promega, USA). The polymorphism was detected at -1970 positions of the promoter region (IL1R-1970) by polymerase chain reaction-specific sequence primer (PCR-SSP) assay, followed by electrophoresis on 2% a garose-gel by using CTS-PCRSSP Tray Kit (Heidelberg, Germany).

The thermo cycling conditions were: initial denaturation at 94˚C for 2 minutes, followed by denaturation at 94˚C for 15 seconds, and then 10 cycles of annealing and extension at 65˚C for 60 seconds. This was followed by denaturation at 94˚C for 15 seconds, and then 20cycles of annealing 61˚C at 50 seconds and extension at 72˚C for 30seconds.

Statistical Analysis

Genotypes of IL1R-1970 SNP were presented as percentage frequencies, and significant differences between their distributions in T1D patients and controls were assessed by two-tailed Fisher's exact probability (P). In addition, relative risk (RR), etiological fraction (EF) and preventive fraction (PF) were also estimated to define the association between a genotype with the disease. These estimations were calculated by using the WINPEPI computer programs for epidemiologists. The latest version of the WINPEPI package is available free online at http://www.brixtonhealth.com.

Result

Genetic polymorphism of IL1Rgene was determined in the promoter region at position -1970(R1970SNP), which was presented with three genotypes (CC, TC and TT) that corresponded to two alleles (Cand T). Among T1D patients, no significant difference was observed between the observed and expected frequencies of the three genotypes (a good agreement with Hardy-Weinberg equilibrium; HWE), while in controls, a departure from HWE was observed (i.e. a significant difference between the observed and expected genotype frequencies they were significantly deviated in controls (P ≤ 0.001).); however comparing patients to controls results some significant differences (Table -1).

The frequencies of TC genotype and Tallele were significantly increased in patients (79.49and 68.05%, respectively) compared to controls (38.28and 40.62%, respectively). The relative risks (RRs) of such positive associations were 21.3%and, 39.8% respectively.In contrast, CC genotype and Callele frequencies were significantly decreased in patients (22.33and 38.95%, respectively) compared to controls (42.38and 49.38%, respectively).

The preventative fractions (PFs) of such negative associations were 0.63and0.47, respectively (Table 2).
Table 1: Observed numbers and percentage frequencies and Hardy-Weinberg (H-W) equilibrium of (IL-1R-1970 genotypes and alleles) in Type 1 Diabetes patients and controls

<table>
<thead>
<tr>
<th>Groups</th>
<th>IL-1R-1970 Genotypes or alleles</th>
<th>H-W X²</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>CC</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>TC</td>
<td></td>
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<td>TT</td>
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<td>C</td>
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<td></td>
<td>T</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Diabetes type -1 (No. = 39)</td>
<td>Observed</td>
<td>No.</td>
<td>%</td>
</tr>
<tr>
<td></td>
<td>5</td>
<td>22.33</td>
<td>79.49</td>
</tr>
<tr>
<td></td>
<td>Expected</td>
<td>No.</td>
<td>%</td>
</tr>
<tr>
<td></td>
<td>2.01</td>
<td>26.89</td>
<td>10.45</td>
</tr>
<tr>
<td>Controls (No. = 21)</td>
<td>Observed</td>
<td>No.</td>
<td>%</td>
</tr>
<tr>
<td></td>
<td>4</td>
<td>42.38</td>
<td>38.28</td>
</tr>
<tr>
<td></td>
<td>Expected</td>
<td>No.</td>
<td>%</td>
</tr>
<tr>
<td></td>
<td>3.44</td>
<td>10.62</td>
<td>7.44</td>
</tr>
</tbody>
</table>

Table 2-1B: Statistical analysis of associations between IL-1R-1970 genotypes or alleles in Diabetes type 1 patients and controls

<table>
<thead>
<tr>
<th>Type of Comparison</th>
<th>Statistical Evaluation</th>
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<tbody>
<tr>
<td></td>
<td>IL-1R-1970 Genotype or Allele</td>
</tr>
<tr>
<td>Diabetes Disease Versus Controls</td>
<td>CC</td>
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<tr>
<td></td>
<td>TC</td>
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<td></td>
<td>TT</td>
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<td>T</td>
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</table>

Discussion

According to the presented results, IL1R-1970 SNP can be highlighted as an important genetic marker in the pathogenesis of T1D was presented with three genotypes (CC TC and TT) that corresponded to two alleles (C and T).

These genotypes were in a good agreement with Hardy-Weinberg equilibrium (HWE) in patients, but they were significantly deviated in controls (P ≤ 0.001).

Present study illustrated that IL-1R-1970 important genetic marker in the pathogenesis of T1D especially if we consider RR values was 21.3% and 39.8% for it was showed that frequency of TC genotype and T allele (79.49% vs. 68.05%; P = 0.419 respectively) were significantly rise in patients contrast to controls, (38.28% vs. 40.62%; P=0.904), and the associated EF values were 1.71 and 2.11, respectively. In contrast, CC genotype and C allele (22.33% vs. 38.95%, P = 0.706 respectively) frequencies were significantly decreased in patients, compared to controls (42.38% vs. 49.38%; P =0.904), and the associated PF values were 0.63 and 0.47 respectively. These findings suggest when increased concentration of interleukin-1R causes increased inflammation of the pancreas cells leading to T1D. According to these finding which agrees with previous results, can concluded that IL1R-1970SNP might have a role in the etiopathogenic mechanism of T1D, one study has been shown recently that single nucleotide polymorphisms (SNPs) associated with T1D [24].

However, other studies investigated other polymorphisms in intron and promoter regions of IL1gene and the results were almost conflicting due to ethnic variations, but they agreed that IL-1 is an important cytokine involved in immunity and its polymorphisms play a critical role in T1D development [14, 8, 17]. Other hand IL1R may decrease insulin secretion.

Deficiency of IL-1R did not protect fetal pancreas grafts from autoimmune attack[19]. Moreover, IL1R inhibits insulin release from previously docked granules in the ß-cells of rats[21]. IL-1R-deficient islets were not protected in vivo[19]. Thus, there is impaired first-phase release of insulin mirroring observations in humans in the early stages of T1D[7]. This is because IL-1Ra binds IL-1R and blocks the activities of IL-1α and IL-1β so it play to control of IL-1 effects on Pancreatic cells [20,25].
References


