Role of the autoantibodies and IL-1 cytokine in the pathogenesis of diabetes mellitus

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The abstract

ID diabetes is a complex disease with a genetic background that involves the release of interleukin 1B cytokine and antibodies to its targets. The purpose of this study was to determine the role of autoantibodies and IL-1 cytokine in the pathogenesis of diabetes mellitus.

The study was carried out on a sample of 90 patients with ID diabetes, divided into three groups: type 1 (LADA), type 2, and LADA. The study included testing for autoantibodies (GAD65, ICA) and IL-1 cytokine levels in the blood of patients with ID diabetes.

The results showed that the levels of autoantibodies and IL-1 cytokine were significantly higher in patients with ID diabetes compared to the control group. The highest levels of autoantibodies were found in patients with LADA, followed by type 2 and type 1 diabetes.

The study concluded that autoantibodies and IL-1 cytokine play a significant role in the pathogenesis of diabetes mellitus, with a higher level of autoantibodies and IL-1 cytokine in LADA patients.
Abstract

Diabetes mellitus is a complex multifactorial and heterogeneous syndrome characterized by hyperglycemia resulting from inadequate insulin secretion and/or insulin action. T lymphocytes and macrophages appear to play an important role in mediating β-cell damage and causing Type 1 diabetes.

A case-control study is done during the period from Feb., 2011, through the end of Feb. 2012. The study enrolled 90 diabetic patients divided into three groups Type 1, Type2 and LADA (30 patients in each group) who attended in Al Zahraa Teaching Hospital in Al-Kut city & 25 non diabetic as a control to determine the prevalence of islet cell autoantibodies, glutamic acid decarboxylase autoantibodies (ICAs& GADAs) and measured (IL-1α, IL-4, IL17, TNF α, IFN γ) as well as the frequency of HLA DR was measured in diabetic patients and non-diabetic control with no family history of diabetes. Serological tests for GADA & ICA (by using ELISA) have been done for all sera of the study groups. Dual set ELISA technique is used for estimating the cytokines levels in the sera of all study groups while DNA extracted from whole blood from all study groups and these DNA were used to detected the HLA DR allele by using HISTO SPOT SSO system.

According to the results of this study, Type 1 diabetic patients had higher frequency of GADA 65 autoantibodies compared with other study groups, as eighteen of them (60%) showed GADA positive in comparison to 6.6 % in type 2 DM, 56.7% in LADA and 0% in control group. Furthermore, twenty of type 1 diabetic patients which represent (66.7%) were ICA positive in comparison to 33.33% in type 2 DM, 70% in LADA and 4% in control group. Statistical analysis showed highly significant difference.

Analysis of the serum sample showed that IL-1α levels has been elevated significantly in the sera of Type 1(635.8± 685 pg./ml), type 2 (625.5± 55.86 pg./ml) and LADA (383.2± 533.5 pg./ml) diabetics patients positive autoantibodies in comparison with other groups type 1, type 2 and LADA diabetics patients negative autoantibodies and healthy control (60±35.21 pg. /ml)

Introduction

Diabetes is not a single disease but rather a heterogeneous group of diseases that lead to an elevation of glucose in the blood. Chronic hyperglycaemia and the risk of developing complications are the two unifying properties which have held the notion of diabetes together. During the past decades, however, there has been remarkable progress in understanding diabetes. In 1951, RD Lawrence described "two types of diabetes mellitus, with and without plasma insulin". [1]

Type 1 diabetes is an autoimmune disease characterized by an inflammatory reaction in and around pancreatic islets of Langerhans, The islet cell antibodies (ICAs) were common in the sera of patients with type 1 diabetes (type 1 diabetes) provided strong evidence that the β-cell lesion of type 1 diabetes was autoimmune in nature. Shortly thereafter, it was published that 11% of patients with type 2 diabetes were also positive for ICAs and that this ICA+ subset of type 2
diabetic patients tended to fail sulfonylurea therapy and needed insulin treatment earlier than ICA-type 2 diabetic patients [2]. The frequency of beta cell specific markers, islet cell antibodies (ICA) and glutamic acid decarboxylase antibodies (GADA) are higher among children with Type 1 diabetes than among young adults classified as Type 1 [3,4,5].

In adults, autoantibodies can also appear in patients not classified as Type 1 [6, 7]. In these patients, positivity for ICA or GADA at diagnosis has been proposed to be predictive for later insulin treatment [3, 8].

Current evidence suggests that T lymphocytes and macrophages play a major role in mediating ß-cell damage and causing Type 1 diabetes [9]. Both activated T cells and macrophages operate and interact through the release of soluble factors called cytokines. Cytokines include regulatory proteins of the immune system and are produced by different cell types including lymphocytes and monocytes. Their pleiotropic activities critically affect the level of the immune response and they have therefore been implicated in the pathogenesis of several autoimmune diseases including IDDM [10].

The mechanism by which cytokines impair ß-cell function also includes the expression of the inducible isoform of nitric oxide (NO) synthase (iNOS), resulting in the production of high levels of NO. [11, 12, 13, 14] Interleukin (IL)-1 alone is sufficient to stimulate iNOS expression in rat islets, while IL-1 plus interferon (IFN)-γ are the minimal combination of cytokines required to stimulate iNOS expression and NO production in mouse and human islets [15, 16, 17].

Materials and methods
This study was performed on 115 people who attended to Al Zahraa Teaching Hospital in Al-Kut governorate in the period from Feb., 2011 to Feb., 2012.

- **First group**: Thirty patients with Type 1 Diabetes mellitus of both sexes (14 females and 16 males) their ages <35 years old.
- **Second group**: Thirty patients with Type 2 Diabetes mellitus of both sexes (15 females and 15 males) their ages >35 years old.
- **Third group**: Thirty patients with Type LADA Diabetes mellitus of both sexes (15 females and 15 males) their ages >35 years old, some of them were newly diagnosis and another misdiagnosis as Type 2 D.M and uncontrolled.
- **Fourth group**: Twenty five people as a control group who had no history or clinical evidence of Diabetes mellitus or any acute and chronic disease. Ten milliliters (ml) of venous blood were collected from patients as well as controls by venipuncture.

Serological test for pancreatic auto antibody (GAD 65 and ICA) was donning for every patient (by using ELISA) and also serological test for measuring cytokines level (IL-17 and IL-1) by using du set ElISA.

Results
1-Prevalence of pancreatic autoantibodies (glutamic acid decarboxylase antibody and islet cell antibody) (GADA 65 & ICA) in type 1 diabetic patients & other study groups

Type 1 diabetic patients had higher frequency of GADA & ICA compared with other study groups, as eighteen of them (60%) showed GADA positive in comparison to 6.6% in type 2 DM, 56.7% in LADA and 0% in control group, the difference is highly significant (P<0.001) between Type 1 and type 2 as well as the difference is highly significant (p<0.001) between Type 2 and LADA type and there is no significant difference (p>0.05) between Type 1 and LADA type, table (1).

Regarding ICA, twenty of type 1 diabetic patients which represent (66.7%) were ICA positive in comparison to 33.33% in type 2 DM, 70% in LADA and 4% in control group. (P<0.001). Table (1)

The prevalence of islet cell autoantibodies increased significantly in type 1 diabetic patients and LADA diabetic patients when GADA & ICA are taken together in comparison to type 2 diabetics & control respectively.

Table (1): Prevalence of the GADA65, ICA auto antibody in type 1 and others diabetics patients.

<table>
<thead>
<tr>
<th>No.30</th>
<th>Type 1 D.M (%)</th>
<th>Type 2 D.M. (%)</th>
<th>LADA (%)</th>
<th>Control %</th>
</tr>
</thead>
<tbody>
<tr>
<td>GAD65</td>
<td>a, b 18 (60)</td>
<td>c 2 (6.6)</td>
<td>a, b 17(56.7)</td>
<td>0 (0)</td>
</tr>
<tr>
<td>ICA</td>
<td>a, b 20 (66.7)</td>
<td>a, c 10(33.33)</td>
<td>a, b 21(70)</td>
<td>2 (4)</td>
</tr>
</tbody>
</table>

a statistically significant difference as compared to control group (p < 0.001).
b statistically significant difference as compared to Type 2 DM group (p < 0.001).
c statistically significant difference as compared to LADA group (p < 0.001).

2- Levels of (IL-1) Cytokines in the Sera of Type 1 diabetics’ patients

It was clear that IL-1 α level has been elevated significantly in the sera of Type 1 diabetics patients positive to pancreatic autoantibodies (635.8± 685pg/ml) in comparison with other groups (64.33±121.3, 60±35.21 pg /ml for Type 1 diabetics patients negative to pancreatic autoantibodies and healthy control cases respectively) (P ≤ 0.001). (Figure 1)
levels of (IL-1) cytokines in the sera of type 2 diabetes patients. The result showed that IL-1α level was no significant difference in the sera of type 2 diabetes patients (107±166.2 pg/ml) in comparison with healthy control cases 60±35.21 pg/ml (P≤ 0.05).(figure No. 2)

levels of (IL-1) cytokines in the sera of type LADA diabetes patients. It was clear that IL-1α level has been elevated significantly in the sera of LADA diabetes patients positive autoantibodies (383.2±533.5 pg/ml) in comparison with other groups (64.33±121.3, 60±35.21 pg/ml LADA diabetes patients negative autoantibodies and healthy control, respectively) (P≤ 0.001).(figure 3)
Type 1 diabetes mellitus is a multifactorial disease resulting from destruction of islet beta cells that leads to an absence of intrinsic insulin secretion; autoimmunity is considered the major factor in pathophysiology of type 1 DM \[18, 19\].

Type 1 diabetic patients show significant difference (p<0.001) in prevalence of pancreatic islet cell autoantibodies (GADA, ICA) in comparison to Type 2 diabetics patients and control group while there was no significant differences (p>0.05) in comparison to LADA diabetics group for both autoantibodies. (Table 1)

This result is agreement with George \[20\] who founded that there was significant difference in the prevalence of GADA65 between type 1 and type 2 diabetics patient, and this agrees with Pardoni et al., \[21\] who found 82.9%, Borg et al., \[22\] 80.3% and Laadhar et al., \[23\] 84% and agrees with McDonald et al. \[24\] who found 80/98 (82%).

The above findings demonstrate the important role of islet cell autoimmunity in the pathogenesis of the disease & clarify that autoimmune diabetes (type 1a) is still more prevalent than idiopathic type (type 1b).

Regarding the prevalence of pancreatic autoantibodies (GADA, ICA) in type 2 diabetic patients in comparison with other study groups (type 1 diabetics, LADA type diabetics & control). From thirty type 2 diabetic patients, it was found that there were 2(6.6%) GADA positive and 10 (33.33%) ICA positive type 2 patients. These results agree with Carina Törn et al. \[25\] who found that there is no significant distribution of autoantibody in the type 2 diabetic patients however when we compare between LADA diabetics group with other study group (type 1, type 2 and control) in the prevalence of pancreatic autoantibodies (GADA, ICA). This result showed that from thirty LADA diabetics patients there were 17 patients (56.7 %) GAD positive and 21 patients (70%) ICA positive and there were significant difference (P < 00.1) compared between LADA diabetic group with type 2 diabetics group and control and there was no significant difference (P>0.05) when it compared with type 1 diabetics patients.
The occurrence of autoimmune type 1 diabetes in adult life (LADA) is more common than formerly believed. According to the literature, it can be assumed that LADA may constitute up to 50% of cases of non-obese type 2 diabetes. [26, 27]

Our results showed that IL-1 α level has been elevated significantly in the sera of Type I diabetics’ patients whom were positive autoantibodies (635.8± 685pg/ml) in comparison with other groups (64.33±121.3, 60±35.21 pg /ml for Type 1 diabetics patients whom were negative autoantibodies and healthy control, respectively) (P≤ 0.001). This result agreed with Eastgate et al., [28] and Kramer & Wick, [29], who reported that these cytokine was elevated in the patients with autoimmune disease suggesting that production of these cytokines at the site of target organ may be reflected by measurable levels in circulation. These results disagreed with Cavallo et al. [30] who reported that there was no significant difference in the level of IL-1α cytokine between type 1 D.M and control in the early stage of diagnosis. Our result agrees with Panczel et al. [31] who noted that there is a subgroup called latent autoimmune diabetes of adults (LADA), which is immunologically similar to T1DM, and usually affects adults, developing slowly. Presumably, 10% of T2DM patients are in fact LADA patient. There is a hypothesis based on animal models that early insulin treatment could save beta cells. If this is true for humans, early treatment with insulin should be of benefit.

References


