

Role of the autoantibodies and IL- 17 cytokine in the pathogenesis diabetes mellitus

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دور الاجسام المضادة الذاتية والانتريليوكين 17 في امراضية السكري

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المستخلص

داء السكري هو متلازمة معقدة وغير متجانسة متعددة العوامل التي تتميز بارتفاع السكر في الدم الناتج عن عدم كفاية إفراز الأنسولين و / أو خلل في عمل الأنسولين. تلعب الخلايا الليمفاوية والبلعمية دورا هاما في التوسط في تدمير خلايا β وتسبب مرض السكري من النوع الاول.

اجريت الدراسة خلال الفترة من فبراير 2011 و حتى نهاية فبراير 2012، وقد شملت الدراسة 90 مريضا بداء السكري قسموا الى ثلاث مجموعات (النوع 1، النوع 2 و LADA) بواقع 30 مريض في كل مجموعة ممن حضروا الى مستشفى الزهراء التعليمي في مدينة الكوت و 25 شخص كمجموعة سيطرة. الغرض من الدراسة هو لتحديد مدى انتشار الأجسام المضادة لخلايا جزيرة اللانكرهاتز، و حامض الجلوتاميك منقوص الكربوكسيل (ICA و GADA) وكذلك قياس مستوى الحركات الخلوية IL17 في مرضى السكري والغير مصابين السكري من مجموعة السيطرة ممن ليس لديهم تاريخ عائلي لمرض السكري. وقد تم استخدام تقنية الامتزاز المناعي المرتبط بالانزيم ELISA لتشخيص الاجسام المضادة الذاتية في مصل الدم لمجاميع الدراسة، واستخدام تقنية ELISA البيئية التصنيع لتحديد مستويات السيتوكينات في مصل الدم لمجاميع الدراسة. وفقا لنتائج هذه الدراسة، كان انتشار الاجسام المضادة الذاتية من النوع GAD 65 بنسبة عالية في مصل مرضى السكري من نوع 1 مقارنة مع بقية مجاميع الدراسة الأخرى، كما أظهرت الدراسة ان ثمانية عشر مريض (60%) اظهروا ايجابية للاجسام المضادة من نوع GAD65 بالمقارنة مع 6.6% في مرضى السكري من النوع 2 ، 56.7% في LADA و 0% في مجموعة السيطرة، اما بالنسبة لانتشار الاجسام المضادة الذاتية من نوع ICA فان عشرون من المرضى السكري من النوع 1 (66.7%) كانوا ايجابيين بالمقارنة مع النوع 2 (33.33%) ، و (70%) في LADA و (4%) في مجموعة السيطرة. حيث أظهر التحليل الإحصائي فرق معنوي كبير.

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-17cytokine 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 cytokine levels in the sera of all study groups.

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.

the IL-17 level has been elevated significantly in the sera of type 1 (927.9 ± 906.9 pg./ml), type 2 (599.5 ± 75.66 pg./ml) and LADA (388.2 ± 533.5 pg./ml) diabetics patient's positive autoantibodies in comparison with other groups type 1, type 2 and LADA diabetics patients negative autoantibodies and healthy control (62 ± 37.26 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 β -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].

IL-17 cytokines is a newly cytokines discovered and found to be important in autoimmune diseases. Recently it have been demonstrated the imbalance between T helper type 17 (Th17) and T regulatory cells has been reported in autoimmune liver disease [11]. Th17-related cytokines were increased significantly in patients with primary biliary cirrhosis (PBC). In type 1 diabetes, a role of Th17 immunity in human T1D was indirectly suggested by a recent report in which IL-1– and IL-6–secreting monocytes from diabetic patients induced IL-17 production from allogenic memory T cells in vitro. However, increased IL-17 secretion upon T cell activation was only demonstrated in peripheral blood T cells from patients with long-lasting but not in patients with recent-onset T1D [12]. IL-17 immunity is upregulated in human T1D and IL-17 was detrimental to human islet cells by exacerbation of inflammatory and proapoptotic responses in vitro. The role of IL-17 immunity in human T1D, which provides a new view on the pathogenesis of the disease and implies a novel potential therapeutic strategy in T1D, is based on the control of IL-17 immunity. [13]. The mechanism by which cytokines impair β -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. [14, 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.
- **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 done 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 atients.

No.30	Type 1 D.M (%)	Type 2 D.M. (%)	LADA (%)	Control %
GAD65	^{a, b} 18 (60)	^c 2 (6.6)	^{a, b} 17(56.7)	0(0)
ICA	^{a, b} 20 (66.7)	^{a, c} 10(33.33)	^{a, b} 21(70)	2(4)

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-17) cytokines in the sera of type 1 diabetics' patients

The result showed that the level of IL-17 in the sera of Type 1 diabetic's patients positive to pancreatic autoantibodies (927.9 ± 906.9 pg/ml) with Type 1 diabetics patients negative to pancreatic autoantibodies and healthy control groups (87.67 ± 175 , 62 ± 37.26 pg/ml respectively) there was significant difference ($p \leq 0.001$, $p \leq 0.05$ respectively). (Figure 1)

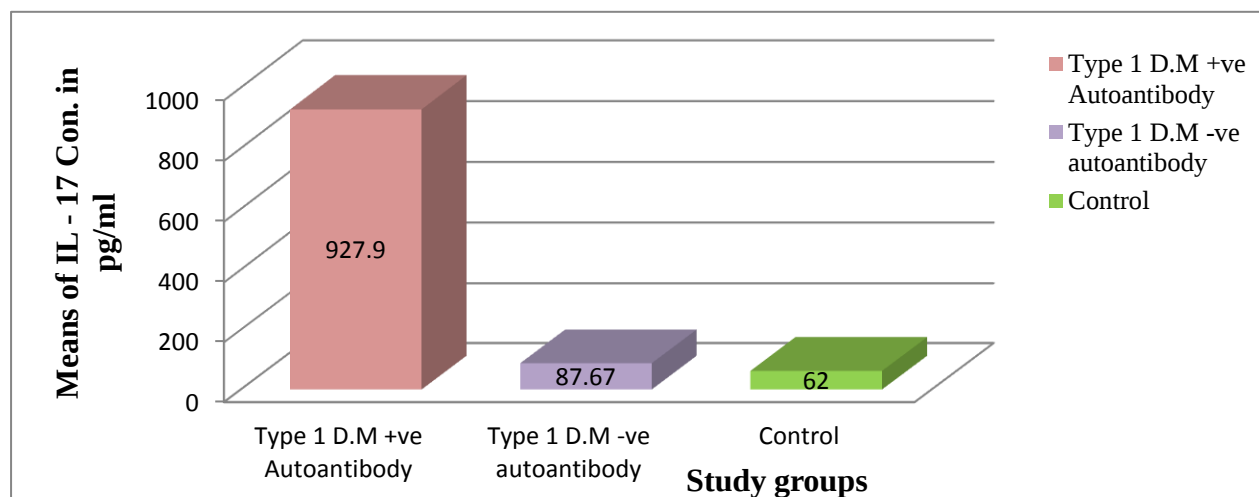


Figure (1): Mean distribution of IL17 cytokine in the type 1 D.M patients.

3- Levels of (IL-17) cytokines in the sera of type LADA diabetics' patients.

The level of IL-17 in the sera of LADA diabetic's patients' positive autoantibodies (388.2 ± 533.5 pg/ml) there was significant elevation ($p \leq 0.001$) in compassion with LADA diabetic's patients' negative autoantibodies and healthy control groups (63.69 ± 77.11 , 62 ± 37.26 pg/ml respectively). (Figure 2)

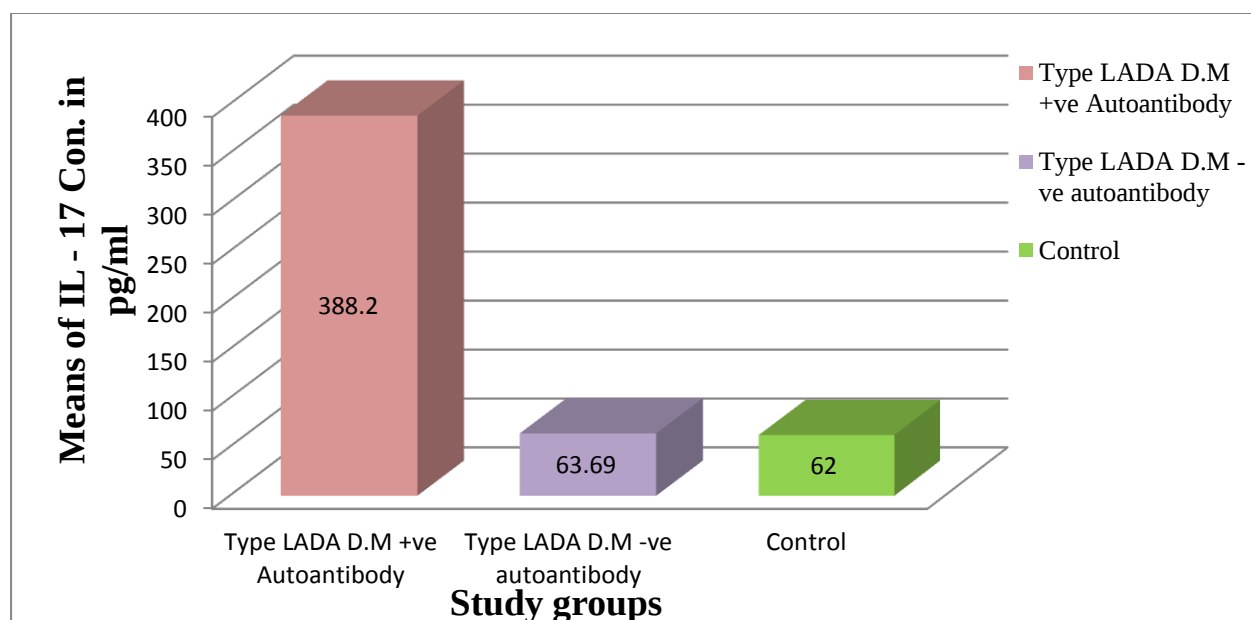


Figure (2) :Mean distribution of IL-17 cytokine in the type LADA D.M patients.

Discussion

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 showed 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 Pardon *et al.*, [21] who found 82.9%, Borg *et al.*, [22] 80.3% and Laadhar *et al.*, 84% [23] 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 ($P < 0.01$) 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]. The roles of Th17 cells in autoimmune disease remain largely unclear. In the present study, we found that, although diabetic T cells produced higher levels of IL-17 than non-diabetic cells, the recently characterized pathogenic Th17 population has been linked to a number of organ-specific autoimmune diseases [28] and is currently being investigated as a clinical therapeutic target in autoimmunity. Although other groups reported that Th17 cells play a less important role in the pathogenesis of diabetes than Th1 cells, [20, 30]. The precise roles of IL-17 and IL-17-producing cells in natural conditions in autoimmune diabetes remain to be investigated. Our results agreed with Jun Zhang, *et al.*, [31] who found a loss of IL-12 results in enhanced pro-inflammatory cytokine production and accelerated pathological damage of the pancreas in NOD mice. This accelerated disease is also associated with an increased number of IL-17-producing T cells.

Our result agrees with Panczel *et al.* [32] 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.

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