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

Muntadher Ali Alsaidi   , Hussein Sarhan Al- Janabi*

and Jaafar K. Al-Mousawi*

College of Medicine Wasit University   College of Medicine Kufa University*

دور الاجسام المضادة الذاتية والانترليويكين 1 في امراضية السكري

منتظر علي السعيدي, حسين سرحان الجنابي, جعفر الموسوي

كلية الطب/جامعة واسط , *كلية الطب / جامعة الكوفة

المستخلص

داء السكري هو متلازمة معقدة وغير متجانسة متعددة العوامل التي تتميز بارتفاع السكر في الدم الناتج عن عدم كفاية إفراز الأنسولين و / أوخلل في عمل الانسولين. تلعب الخلايا الليمفاوية والبلعمية دورا هاما في التوسط في تدمير خلايا ß 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 اللانكرهانز،و حامض الجلوتاميك منقوص الكربوكسيل (ICA وGADA) وكذلك قياس مستوى الحركيات الخلوية IL-1 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 اللاجسام المضادة الذاتية في مصل الدم لمجاميع الدراسة، واستحدام تقنية ELISA البيتية 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 مصول 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 و0٪ في مجموعة السيطرة، اما بالنسبة لانتشار الاجسام المضادة الذاتية من نوع ICA فان عشرون من المرضى السكري من النوع 1 (66.7٪) كانوا إيجابين بالمقارنة مع النوع 2 (33.33 ٪) ، و(70٪) في LADA و(4٪)في مجموعة السيطرة. حيث أظهر التحليل الإحصائي فرق معنوي كبير.

وأظهرت نتائج تحاليل للعينات مصول الدم لمرضى السكري ارتفاع في مستوى IL-1α بشكل كبير في مصول المرضى من النوع 1 (685 ±635.8 pg./ml) و النوع 2 (625.5± 55.86 pg./ml) وLADA (383.2± 533.5 pg./ml) من مرضى السكري ممن اظهروا ايجابية للاجسام المضادة بالمقارنة مع المجموعات الأخرى نوع 1، نوع 2 وLADA من مرضى بالسكري ممن اظهروا السلبية للأجسام المضادة ومجموعة السيطرة (60±35.21 pg. /ml)

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 ß-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-1A α , 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-1 α 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 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. [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 Hospitalin 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): Pprevalence of the GADA65, ICA auto antibody in type 1 and others diabetics patients.

| 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).
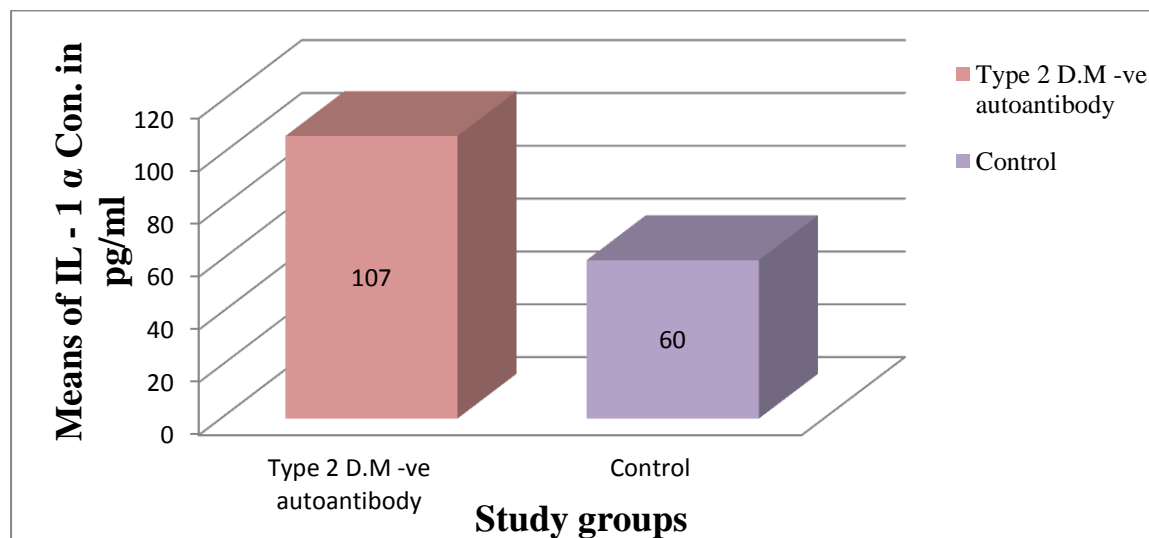**c** statistically significant difference as compared to LADA group (p < 0.001).

2- Levels of (IL-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)
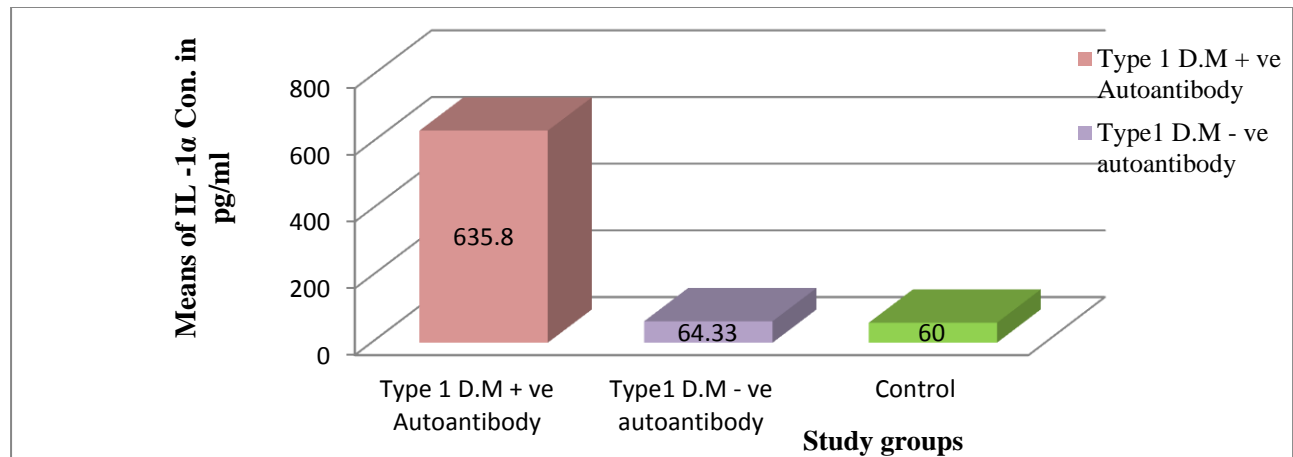
**Figure (1): Mean distribution of IL-1 α cytokine in the type 1 D.M patients.**

T
patients (107±166.2 pg /ml) in comparison with healthy control cases 60±35.21 pg /ml (P≤ 0.05).(figure No. 2)

Figure (2): Mean distribution of IL-1 α cytokine in the type 2 D.M patients.

4- Levels of (IL-1) Cytokines in the Sera of Type LADA diabetics' patients.
It was clear that IL-1 α level has been elevated significantly in the sera of LADA diabetics patients positive autoantibodies (383.2± 533.5 pg/ml) in comparison with other groups (64.33±121.3, 60±35.21 pg /ml LADA diabetics patients negative autoantibodies and healthy control, respectively) (P≤ 0.001).(figure 3)

Figure (3):Mean distribution of IL-1 α cytokine in the type LADA D.M patients.
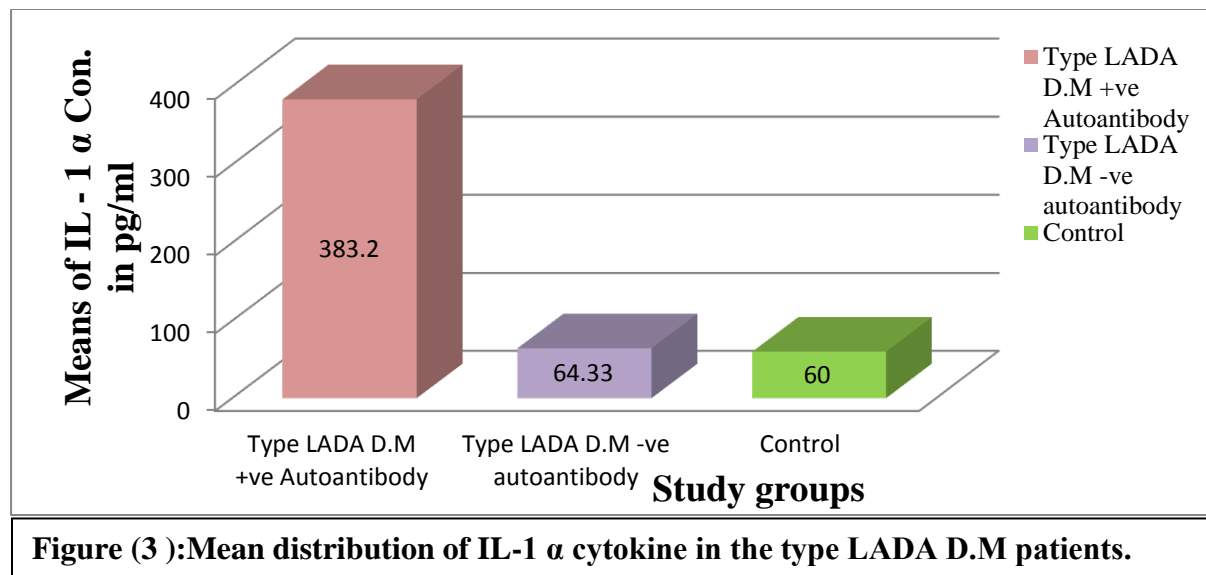
Type 1 diabetes mellitus is a multifactorial disease resulting from destruction of islet beta cells that leads to an absence of intrinsic insulin secretion; autoimmunity is considered the major factor in pathophysiology of type 1 DM [**18, 19**]

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*., 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 ($P < 00.1$) 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 1diabetics 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

1- **Bornstein J and Lawrence Rd.(1951).** Two types of diabetes mellitus with and without available plasma insulin. Br. Med. J. 1:732.

2- **Irvine W.J; Gray R.S.; Mccallum C.J. and Duncan L.J.P.(1977).** Clinical and pathogenic significance of pancreatic-islet-cell antibodies in diabetics treated with oral hypoglycaemic agents. Lancet 1:1025–1027.

3- **Landin-Olsson M, Karlsson A and Dahlquist G. (1989).** Islet cell and other organ-specific autoantibodies in all children developing type 1 (insulin-independent) diabetes mellitus in Sweden during one year and in matched controls. Diabetologia; 32: 387-395; 1989.

4- **Landin-Olsson M, Karlsson FA, Lernmark AÊ and Sundkvist G. (1992).** Islet cell and thyrogastric antibodies in 633 consecutive 15-34 years old patients in the diabetes incidence study in Sweden (DISS). Diabetes; 41: 1022-1027.

5- **Hagopian WA, Sanjeevi CB, Kockum I, Landin- Olsson M. and Karlsen AE. (1995).**Glutamate decarboxylase-, insulin- and islet cell-antibodies and hla typing to detect diabetes in a general population-based study of swedish children. J. Clin. Invest. 95: 1505-1511.

6- **Groop, L. (2005).**Type 1 dm& type 2 dm what do they have in common. Medicographia . 27 (4): 331-337.

7- **Zimmet P, Turner R, Mccarty D. (1999).**Rowley M and Mackay J., Crucial points at diagnosis. Diabetes Care; 22 [Suppl 2]: B59–B63.

8- **Turner R, Stratton I and Horton V E.(1997).** UKPDS 25: Autoantibodies to islet-cell cytoplasm and glutamic acid decarboxylase for prediction of insulin requirement in type 2 diabetes. UK Prospective Diabetes Study Group. Lancet; 350: 1288–1293.

9- **Cahill GF, and McDevitt HO.(1981).** Insulin-dependent diabetes mellitus: the initial lesion. N. Engl. J. Med. 304: 1454.

10- **Mandrup-Poulsen T, Nerup J, Stiller CR, Marner B and Bille G.(1986).** Affinity-purified human interleukin-1 is cytotoxic to isolated islets of Langerhans. Diabetologia 29: 63; 1986.

11- **Southern C, Schulster D and Green IC. (1990).** Inhibition of insulin secretion by interleukin-1 beta and tumour necrosis factor-alpha via a largininedependent nitric oxide generating mechanism. FEBS Lett. 276:42– 44.

12- **Eizirik DL, Bjorklund A and Welsh N.(1993).** Interleukin-1-induced expression of nitric oxide synthase in insulin-producing cells is preceded by C-Fos Induction and depends on gene transcription and protein synthesis. FEBS Lett. 317:62– 66.

13- **Corbett JA, Kwon G, Misko TP, Rodi CP. and Mcdaniel ML. (1994).** Tyrosine kinase involvement in IL-1 beta-induced expression of it by beta-cells purified from islets of langerhans. Am. J., Physiol. 267:C48–C54.

14- **Corbett JA and Mcdaniel ML.(1995).** Intraislet release of interleukin 1 inhibits beta cell function by inducing beta cell expression of inducible nitricoxide synthase. J. Exp. Med. 181:559 –568.

15- **Mandrup-Poulsen T. (1996).**The Role of Interleukin-1 in the Pathogenesis of   IDDM. Diabetologia 39:1005–1029.

16- **Heitmeier H. and Corbett JA. (2000).**Cytotoxic role of nitric oxide in diabetes.in nitric oxide biology and pathobiology. San Diego, CA, Academic, P.785–810.

17- **Thomas HE. , Darwiche R., Corbett JA. and Kay TW. (2002).**Interleukin-1 Plus Interferon Induced Pancreatic Cell Dysfunction Is Mediated By Cell Nitric Oxide Production. Diabetes 51:311–316.

18- **Gale, E.A. (2001).**The Discovery of Type 1 Diabetes. Diabetes. 50: 217 – 226.

19- **Winter, W.E.; Harris,N. And Schatz, D. (2002).**Type 1 Diabetes Islet Autoantibody Markers. Diabetes Technol. Ther. 4:817-839.

20- **George Biesenbach, Martin Auinger, Martin Coldi and Fridrich Prischl.(2005).** Prevalence of LADA and frequency of GAD antibodies in diabetic patients with end-stage renal disease and dialysis treatment in Austria. Nephrol. Dial. Transplant. 20: 559–565.

21- **Pardoni, V.C.; Mourao DM. Nascimento, P.D.; Vivolo, M.A.; Ferreira, S.R.G. And Pardoni,H. (1999).**Frequency of islet cell autoantibodies (IA-2A, GAD) in young Brazilian type 1 diabetic patients. Brazilian J.of Med. & Biolo. Res., 32(10): 1195-1198.

22- **Borg, H.; Marcus, C.; Fernalund, P.And Sundkvist, G. (2002).**Insulin autoantibodies are of less value compaired with islet antibodies in clinical diagnosis of autoimmune type 1 diabetes in children older than 3 years of age. Pediatric Diabetes. 3: 149- 154.

23- **Laadher, L.; Zitouni, M.; Kallel-Sellami,M. ; Bouguerra, R. ; Chaabouni, H. and Makni, S. (2007).**Spectrum of autoantibodies in tunisian adult type 1 diabetes mellitus. Ann. N. Y. Acad. Sci. 1107: 356- 362.

24- **McDonald T. J., Colcough K., Brown R., Shields B.,Shepherd M., Bingley P. and Williams A. (2011).**Islet autoantibodies can discriminate maturity-onset diabetes of the young (MODY) from type 1 diabetes. Diabetic Medicine 1028-1033.

25- **Carina Törn. Mona Landin-Olsson.(2000).** Glutamic acid decarboxylase antibodies (GADA) is the most important factor for prediction of insulin therapy within 3 years in young adult diabetic patients not classified as type 1 diabetes on clinical grounds. Diabetes metab. Res. Rev.; 16: 442-447.

26- **Zimmet P, Turner R, Mccarty D, Rowley M and Mackay J. (1999).**Crucial points at diagnosis. Diabetes Care; 22 [Suppl 2]: B59–B63.

27- **Schiel R, Muller UA.(2000).** GAD Autoantibodies in a selection-free population of insulin-treated diabetic patients: indicator of a high prevalence of LADA? Diabetes Res. Clin. Pract; 49: 33– 40.

28- **Eastgate, J.A., Symous, J.A., Wood, N.C., Grinlinton, F.M., Di Giovine, M.F. & Duff, G.W. (1988).**Correlation of plasma interleukin I levels with disease activity in rheumatoid arthritis. Lancet, 706.

29- **Kraemer, G. & Wick, G.(1989).** The role of Il-2 in Autoimmunity. Imnmunol. Today. 10, 246.

30- **Cavallo M. G., Pozzilli P. and Bird C. (1991).**Cytokines in sera from insulin-dependent diabetic patients at diagnosis. Clin. Exp. Immunol. 86, 256-259.

31- **Panczel P., Hosszufalusi N., Bornemisza B., Horvath L., Janoskuti L. and Fust G. (2001).**latent autoimmune diabetes in adults (LADA): Part of the clinical spectrum of type-1 diabetes mellitus of autoimmune origin. ORV. Hetil. 142: 2571–2578.