**Journal of Kerbala University , Vol. 7 No.4 Scientific . 2009**

# The role of anti-islet cell autoantibodies (GADA & IA-2A) in type 2 Diabetes Mellitus.

# دور الأجسام المضادة لخلايا البنكرياس ( GADA و IA-2A ) في مرضى السكري من النوع الثاني

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**الخلاصة**

أجريت دراسة مقارنة لمعرفة مدى شيوع الأجسام المضادة لخلايا البنكرياس الفارزة للأنسولين وهما(Glutamic acid decarboxylase autoantibodies"GADA" & Tyrosin phosphatase –like protein autoantibodies "IA-2A") لدى مرضى السكري من النوع الثاني خلال الفترة من تشرين الثاني ٢٠٠٧ حتى نهاية حزيران ٢٠٠٨ وقد شملت هذه الدراسة ٦٤ مريضا يراجعون مركز الحكيم لبحوث وعلاج السكري في مستشفى الصدر التعليمي في النجف .

تم قياس نسبة السكري في الدم قبل الإفطار لجميع المشمولين بالدراسة وكذلك تحليل مصولهم للكشف عن وجود الأجسام المضادة لل GAD باستعمال تقنية الELISA وللكشف عن الأجسام المضادة لل IA-2 بواسطة تقنية ال IRMA . لقد اظهر ١٥ (٢٣.٤%) و ١٥ (١٠.٦%) من هؤلاء المرضى فحصا موجبا لل GADA وال IA-2A على التوالي وقد لوحظ إن هؤلاء المرضى يتصفون بصفات سريرية تفرقهم عن مرضى النوع الثاني الذين لم تعطي مصولهم فحصا ايجابيا لنفس الأضداد وذلك إن معظمهم ( أي الموجبي المصول ) قد اصيبو بالسكري في فترات مبكرة من أعمارهم قياسا بالسالبي المصول ٣٠.٩٥% مقابل ٩.١% فيما يخص GADA و ٢١.٤٢% مقابل ٤.٥% فيما يخص IA-2A على التوالي .

كذلك وجد إن هؤلاء المرضى ليسوا من ذوي الأوزان الزائدة ( السمنة) ولديهم معيار كتلة جسم BMI اقل من مرضى السكري من النوع الثاني السالبي المصول للأضداد وبفرق معنوي واضح $p<0.001$ .

إن نسبة ذات مدلول معنوي(٨٠.٩٥%) من هؤلاء المرضى يمتلكون تركيز واطئ للأنسولين في مصولهم على العكس من مرضى النوع الثاني سالبي المصول إضافة إلى إن مستوى السكر في الدم قبل الإفطار عند هؤلاء المرضى كان عاليا مقارنة بمرضى النوع الثاني .

## Abstract

A case-control study has been conducted to determine the prevalence of islet cell autoantibodies, (Glutamic acid decarboxylase autoantibodies"GADA" & Tyrosin phosphatase –like protein autoantibodies "IA-2A")in type 2 diabetic patients .

During the period from November /2007 through the end of June /2008, the study enrolled 64diabetic patients who attended AL-Hakeem Centre for Researches and Treatment of DM in AL-Sader Teaching Hospital in Al-Najef city –Iraq.

Fasting blood sugar test was done for every patient , fasting insulin concentration & serological tests for GADA ( by using Enzyme –linked immunosorbent assay "ELISA" )& IA-2A by ( Immuno radiometric assay"IRMA") have been done for all sera of the study groups .

it is noticed that (15, 23.4%) and (10,15.6%) of those patients were positive for GADA and IA-2Arespectively and those patients(Latent autoimmune diabetes of adult "LADA" patients) characterized by certain clinical features that differentiate them from islet cell autoantibodies negatives type 2 diabetics as it was found that the majority of patients with islet cell positivity develop the disease at younger age than those negative patients (30.95% Vs 9.1%) regarding GADA & (21.42% Vs 4.5%) regarding IA-2A

Also it was found that type 2 diabetic patients with islet cell autoantibody tend to be none obese & had lower body mass index "BMI" than those with negative islet cell autoantibodies. Statistical analysis showed high significant correlation between BMI & islet cell autoantibody positivity ($P <0.001$).

When fasting insulin concentration was measured in islet cell autoantibodies positive type 2 diabetic patients, the majority of them(80.95%) had low fasting insulin level & no one of them exhibit hyperinsulinemia in contrast to type 2 diabetic patients where hyperinsulinmia is the predominant feature.

Additionally, islet cell autoantibodies positive patients were significantly of higher FBS levels

than those who were islet cell autoantibodies negative patients and following up of those patients with islet cell autoantibodies reveals that they have high blood glucose levels.

## Introduction

Diabetes mellitus(DM) is a chronic disease which occurs when the pancreas does not produce enough insulin or when the body can not effectively use the insulin it produces, this can lead to an increase of glucose in the blood (hyperglycemia**).**[1]

In 1997, the American diabetic association (ADA) classified DM as type 1, type 2, other specific types of diabetes (e.g., secondary diabetes), and gestational diabetes .[2]

Many studies indicate that as many as 10-15% of patients diagnosed with type 2 diabetes have circulating autoantibodies to either islet cell antigens and they eventually become insulin dependent**,**[3] those patients who are initially misclassified as type 2, are in fact late onset or slow developing type 1 diabetes and some time referred to as latent autoimmune diabetes in adult (LADA) .[4.,5]

Autoimmune markers such as GAD autoantibodies have been detected in the serum of these diabetic patients many years prior to insulin dependency. [6]

The present study aims to: distinguish those who are actually type 1 DM but misclassified as type 2(LADA) by detection one or more than one anti-islet cells autoantibodies and measurement of insulin level in their serum .

## Material &method

During the period from November /2007 through the end of June /2008, the study enrolled 64 type 2 diabetic patients who attended AL-Hakeem Centre for Researches and Treatment of DM in AL-Sader Teaching Hospital in Al-Najef city –Iraq. Descriptive variables of the patients (obtained during collection of blood samples) including: name, age, gender, type of treatment (OHD, or diet), age of onset, other associated diseases ,body weight and length are taken and registered.

Fasting blood sugar test was done for every patient , fasting insulin concentration & serological tests for GADA ( by using ELISA )& IA-2A by( IRMA) have been done for all sera of the study groups

Data have been entered and stored in Microsoft Access Software and analyzed by SPSS version 14, the statistically significant differences have been assessed with chi-square test at two levels of probability ($P \leq 0.05$, $P \leq 0.001$).

## Results

**Prevalence of islet cell autoantibodies among type 2 diabetic patients**

From sixty four type 2 diabetic patients , there were 15( 23.4%) GADA positive ,10(15.6%) IA-2A positive , whereas the percent of positivity increased to 21/64 ( 32.8%) when GADA &/IA-2A positivity were taken together.

**Table(1: Frequency of anti-islet cell autoantibodies in type 2 DM**

| Results / Anti-Islet cell Ab. | Positive | | Negative | | Total |
|---|---|---|---|---|---|
| | No. | % | No. | % | |
| Anti-GAD Ab | 15 | 23.4 | 49 | 76.6 | 64 |
| Anti-IA-2 Ab | 10 | 15.6 | 54 | 84.4 | 64 |
| Anti-GAD &/ Anti-IA-2 Ab | 21 | 32.8 | 43 | 67.2 | 64 |

## Demographic and clinical characteristics of type 2 diabetic patients with islet cell autoantibodies

**Age of onset**

The distribution of type 2 diabetic patients according to the age of onset were illustrated in table (2,3) .

It was shown that those who developed diabetes earlier (before age of 40 years) significantly had higher prevalence of islet cell autoantibodies than those who developed the disease later ( after the age of 40 ), as there were 30.95% of GADA positive & 21.5% IA-2A positive type 2 diabetic patients who developed the disease before age of 40 years in comparison to 9.1% of GADA positive & 4.5% IA-2A positive type 2 diabetic patients who developed the disease after this age .

**Table(2: Distribution of anti- GAD Ab in type 2 DM according to the age of onset**

| Results / Age of onset | Positive | | Negative | | Total |
|---|---|---|---|---|---|
| | No. | % | No. | % | |
| ≤ 40 years | 13 | 30.95 | 29 | 69.04 | 42 |
| > 40 years | 2 | 9.1 | 20 | 90.8 | 22 |
| Total | 15 | | 49 | | 64 |
| $X^2$=3.842 | | df=1 | | P<0.05 | |

**Table (3): Distribution of anti- IA-2A Ab in type 2 DM according to the age of onset**

| Results / Age of onset | Positive | | Negative | | Total |
|---|---|---|---|---|---|
| | No. | % | No. | % | |
| ≤ 40 years | 9 | 21.5 | 33 | 78.5 | 42 |
| > 40 years | 1 | 4.5 | 21 | 95.5 | 22 |
| Total | 10 | | 54 | | 64 |
| $X^2$= 3.852 | df=1 | | P< 0.05 | | |

**Body mass index (BMI)**

Type 2 diabetic patients who were enrolled in this study were classified according to their body mass index (obese , over weight , normal weight and under weight ) , table 4

Type 2 diabetic patients with islet cell autoantibodies had significantly lower BMI than those who were with out islet cell autoantibodies since there was 1(4.76%) of islet cell autoantibodies positive patient who was obese in comparison to 24 (55.81%) patients who were with out islet cell autoantibodies and 12(57.14%) of islet cell autoantibodies positive patients who were normal weight in comparison to 5(11.62%) of islet cell autoantibodies negative patients.

**Table(4): Correlation between body mass index (BMI) and anti-islet cell autoantibodies**

| Results / BMI | Positive | | Negative | | Total |
|---|---|---|---|---|---|
| | No. | % | No. | % | |
| ≥ 30 (obese) | 1 | 4.67 | 24 | 55.81 | 25 |
| 25 – 29.9 (over weight) | 6 | 28.57 | 12 | 27.90 | 18 |
| 18.5 – 24.9 (normal weight) | 12 | 57.14 | 5 | 11.62 | 17 |
| < 18.5 (under weight) | 2 | 9.52 | 2 | 4.64 | 4 |
| Total | 21 | 100 | 43 | 100 | 64 |
| $X^2$=20.956 | df=3 | | P< 0.001 | | |

**Metabolic Syndrome**

Type 2 diabetic patients with islet cell autoantibodies (LADA) showed low prevalence of obesity, hypertension and dyslipedimia (features of metabolic syndrome) in comparison to type 2 diabetic patients without these autoantibodies,table(5). Statistical analysis showed significant difference ($p<0.05$).

**Table(5): Prevalence of metabolic syndrome features in LADA patients in comparison to classical type 2 DM patients**

| Feature of metabolic syndrome | LADA patients | | Classical type 2 patients | | Total |
|---|---|---|---|---|---|
| | No. | % | No. | % | |
| **Positive** | 2 | 9.52 | 12 | 27.90 | 14 |
| **Negative** | 19 | 90.47 | 31 | 72.09 | 50 |
| **Total** | **21** | **100** | **43** | **100** | **64** |
| **$X^2$=5.44** | | **df=1** | | **P<0.05** | |

**Fasting blood sugar(FBS)**

The fasting blood sugar levels in type 2 diabetic patients were compared in both those islet cell autoantibodies positive and those who were islet cell autoantibodies negative , table 6 .

It was shown that the islet cell autoantibodies positive patients had higher FBS levels than islet cell autoantibodies negative patients. .

Statistical analysis showed significant relationship between islet cell autoantibodies positivity and high FBS ($X^2$=13.444 , P<0.05).

**Table (6) : fasting blood sugar (FBS) in both islet cell autoantibodies positive and negative type 2 diabetic patients**

| Results / FBS mg/dl | Positive | | Negative | | Total |
|---|---|---|---|---|---|
| | No. | % | No. | % | |
| **≤136** | 0 | 0 | 16 | 37.20 | 16 |
| **137-176** | 5 | 23.80 | 10 | 23.25 | 15 |
| **177-216** | 6 | 28.57 | 9 | 20.93 | 15 |
| **217- 256** | 7 | 33.33 | 4 | 9.30 | 11 |
| **257- 296** | 2 | 9.5 | 2 | 4.65 | 4 |
| **297- 336** | 1 | 4.76 | 2 | 4.65 | 3 |
| **Total** | **21** | **100** | **43** | **100** | **64** |
| **$X^2$ =13.444** | | **df=5** | | **P<0.05** | |

**Fasting insulin concentration**

Measurement of fasting insulin concentration in islet cell autoantibodies positive type 2 diabetic patients reveal that the majority of those patients 17(80.95%) had low insulin concentration and 4 (19.04%) had normal insulin concentration while no one of them exhibit fasting hyperinsulinemia, table(7)

**Table (7): Fasting insulin concentration in type 2 DM with anti-islet cell autoantibodies**

| Fasting insulin conc. | Patients | |
|---|---|---|
| | No. | % |
| High (>25 mmol/dL) | 0 | 0 |
| Normal (2-25 mmol/dL) | 4 | 19.04 |
| Low (< 2 mmol/dL) | 17 | 80.95 |
| Total | 21 | 100 |

## Discussion

**Prevalence of latent autoimmune diabetes of adults (LADA) among type 2 DM**

Autoimmune diabetes is subdivided into a rapidly progressive form that is commonly seen in children and early adulthood and a slowly progressive form that is also referred to as latent autoimmune diabetes of adults (LADA) with onset after the age of 30 years and they always misdiagnosed as type 2 DM.[5 , 7]

From the 64 type 2 diabetic patients under study , there were (15, 23.4%) GADA positive, (10, 15.6%) IA-2A positive, and the percentage of these antibodies increased up to 32.8% when GADA & / IA-2A positive results calculated together, table (12)

This finding is going with what was obtained by Turner ***et al.,*** (1997)[8] in United Kingdom Prospective Diabetic Study (UKPDS) and Seissler and Scherbaum **(2006)**[9] who registered a 25-34% and 20%of LADA patients in type 2 diabetics respectively.

However other studies like Botnia study.[10] Ehime study in Japan.[11] and Goswami (2003)[12] showed a lower percentage of islet cell positivity (9.3%, 3.8% &13% respectively); this difference could be attributed to the difference in population ethnicity and age of onset of the disease since (UKPDS) study had taken those with age of onset 25-34 years while the later studies involved older ages (up to 45 years).

## Demographic and clinical characteristics of type 2 diabetic patients with islet cell autoantibodies (LADA)

**Age of onset**

When age of onset is taken in consideration, it was found that the prevalence of these autoantibodies was more common in younger patients who develop disease before age of 40 years in comparison with those who developed diabetes after age of 40 years which is statistically significant ($P<0.05$) ,table (2,3).

This result is in agreement with Elbein *et al*., (1997)[13], Turner *et al*., (1997)[8], and Nabhan *et al.,* (2005)[6] who estimated a significantly higher prevalence of these antibodies (GADA, IA-2A) in young patients with type 2 DM.

The explanation of this finding may be due to the facts that those patients who develop the disease before age of 40 years have HLA-DR3/DR4 and show significant higher frequencies of

anti-islet cell antibodies while those who begin the disease after 40 years were HLADRB1/DRQB1 which show much less evidence of autoimmunity.[14,15,16]

**Body mass index**

When the body mass index (BMI) is taken in consideration in relation to islet cell autoantibodies positivity in type 2 diabetics , it was found that the majority of those who are GADA & IA-2A positive patients(LADA) showed lower BMI (18.5-24.9 &25-29.9 ) in comparison to those who are negative to these autoantibodies with high significant difference ($p<0.001$) , table (4).

This finding is similar to what was obtained by Pozzil**li** &Mario(2001)[15] , ( Melchionda *et al.*, 2002)[17] **(**and Genovese *et al*., (2006)[18] who concluded that, LADA patients are non obese in contrast to those who are actually type 2 DM as they are mostly obese with BMI >30.

**Metabolic Syndrome**

Indicators of metabolic syndrome such as increase body weight, hypertension & dyslipidemia are a common features of type 2 DM .[19]

In comparison with truly type 2 diabetics (negative for islet cell autoantibodies), these features are significantly less frequent in diabetic patients with islet cell autoantibodies (LADA) as there were 12(27.9%) of truly type 2 diabetic patients had features of metabolic syndrome versus 2(9.52%) of LADA patients who were with these features, table(5).

This results is more or less similar to what was found by other researchers who demonstrated a low prevalence of metabolic syndrome in LADA patients .[8,18,20]

The most accepted explanation for this result is again attributed to the difference in pathogenesis of diabetes in both groups, since LADA patients develop the disease due to autoimmune destruction of beta cells while type 2 diabetics develop the disease due to insulin resistance which may be the common aetiological factor for the individual components of metabolic syndrome.[21]

**Fasting blood sugar (FBS)**

During estimation of FBS in type 2 diabetic patients, it was found that those with islet autoantibodies had higher levels of FBS more than those without ($X^2=13.444$, $p<0.05$), table (6).

This is similar to the results reported by Arkan *et al.*, (2005)[22]&Nabhan *et al.*, (2005**).**[6]

The difficulty in achieving glycemic control despite oral hypoglycemic drugs (OHD) is attributed to the fact that pathogenesis of diabetes in LADA patients are due to beta cell destruction rather than insulin resistance as in classical type 2 DM , although they initially respond to OHD as these agents increase insulin sensitivity & decrease the need for insulin at the early stages when insulin concentration is not so deficient or low but they (OHD) do not do this as the destruction progress and insulin deficiency become more obvious so the patients express poor glycemic control & they may be in need for insulin replacement therapy .[23]

**Fasting insulin concentration**

Uncontrolled blood sugar level as well as islet cell autoantibodies positivity in those patients prompt for other tests to evaluate beta cell function like insulin concentration and C-peptide level .[2]

In the present study , fasting insulin concentration (FIC)was measured in islet cell autoantibodies positive patients and the results showed that the majority of patients (80.95%) showed low fasting insulin concentration,(table7)

This finding is more or less the same as that found by others, who mentioned that a low FIC was found in similar patients and they concluded that LADA patients share features with type 1 DM as they complain from progressing (but slow) defect in beta cell function due to autoimmune destruction of pancreatic islets manifested by low insulin level, in contrast to type 2 diabetic patients where hyperinsulinemia is the predominant feature .[20,25,26]

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