

Prevalence of Islet Cell Autoantibodies in Adult Patients Followed for Type 2 Diabetes Mellitus: A Cross-sectional Study

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Abstract

Introduction: This study was aimed to assess the prevalence of islet cell autoantibodies (ICAs) in type 2 diabetes mellitus (T2DM) patients and to compare some clinical and metabolic markers between ICA-negative and ICA-positive groups in South Khorasan province, East of Iran.

Methods: In this cross-sectional study, 384 T2DM patients, all over 30 years old, were selected. None of the patients needed insulin therapy for at least 6 months following diagnosis. Demographic data including gender, age, age at diagnosis, disease duration, health status, family history of diabetes, body mass index (BMI) and treatment type were collected from all subjects. ICA assays were conducted using commercial enzyme-linked immunosorbent assay (ELISA) kits. In addition, blood pressure, as well as cholesterol, serum C-peptide and HbA1c levels were compared between ICA-negative and ICA-positive groups. Data were analyzed using SPSS software and $P < 0.05$ was considered as statistically significant.

Results: ICA was detected in 1.30% of all diabetic patients. Compared with ICA-negative patients, ICA-positive subjects had significantly lower median age ($P = 0.000$), lower median disease duration ($P = 0.001$), lower C-peptide level ($P = 0.006$) and worse glycaemic control (HbA1c, $P = 0.040$). Furthermore, autoantibody-positive patients had more need for insulin therapy ($P = 0.022$). No significant correlation was found between ICA prevalence and family history of diabetes ($P = 0.262$), mean fasting blood glucose (FBG) ($P = 0.282$), BMI ($P = 0.054$), blood pressure ($P = 0.070$) as well as cholesterol levels ($P = 0.888$).

Conclusion: Autoantibody screening is recommended in T2DM patients, especially non-obese patients under 40 years old, showing worse glycaemic control.

Keywords: Islet cell autoantibody, Prevalence, Adult-onset autoimmune diabetes, Type 2 diabetes

Introduction

Although glutamic acid decarboxylase autoantibody (GADA), islet cell autoantibody (ICA), insulinoma-associated antigen-2 autoantibody (IA-2), and zinc transporter-8 autoantibody (ZnT8) are the most important markers in prediction and diagnosis of autoimmune type 1 diabetes mellitus (T1DM), some type 2 diabetes mellitus (T2DM) patients can show these autoantibodies just like T1DM patients.¹⁻³ This type of diabetes is known as latent autoimmune diabetes in adults (LADA)^{4,5} or adult-onset autoimmune diabetes.¹⁻⁵ LADA describe people with slowly progressive insulin-dependent diabetes mellitus.^{6,7} This feature discriminate LADA patients from type 1, because unlike T1DM,

β cell function is maintained in LADA patients, which is defined by measurable fasting C-peptide levels.^{2,8} LADA is also common in patients over 30 years of age at diagnosis.^{8,9} This property along with some other criteria for metabolic syndrome, which leads to insulin resistance in these patients, is more like T2DM.^{10,11} However, unlike T2DM, obesity is not the prominent phenotypic feature in LADA patients.^{12,13} Therefore depending on the dominant factor, the clinical parameters of LADA will be more T1DM-like or T2DM-like.^{14,15} Hence LADA is also called "type 1.5" diabetes.^{9,14,16} Various studies have shown that LADA patients do not require insulin therapy for at least 6 months following diagnosis.^{8,17} However, due to the T cell



“insulinitis”, pancreatic β cells lose their ability to secrete insulin and gradually lose their function^{8,14}; finally making most of these patients insulin-dependent.^{18,19} The presence of islet autoantibodies may illustrate the contribution of insulinitis to the gradual disruption of β -cell function. Therefore, islet-associated antibodies are thought to be key factors in immune system strain rather than pathogens in islet cell destruction.^{8,15} However, the rate of insulin-producing cells destruction and the need for insulin therapy depends on the number and titer of autoantibodies against β -cell antigens.^{2,20}

Due to the importance of this issue, many studies have been conducted in the field of LADA epidemiology.²¹⁻²⁴ It seems that adult-onset autoimmune diabetes accounts for 4%-14% of all diabetic cases.¹⁴ Because LADA occurs in adults, it is very difficult to differentiate it from T2DM, without measuring autoantibodies. After autoantibody to GAD (GADA) as the most prevalent autoantibody in autoimmune diabetes, ICA is the most common autoantibody,²⁰ where its presence increases the likelihood of needing insulin therapy in the future.^{3,25} There is an increased need for early diagnosis of adult autoimmune diabetes in order to preserve β cell function.¹⁶ This study was aimed to estimate the prevalence of ICA in the T2DM subjects, in order to identify autoimmune diabetes in adults and to mediate more effective and specific treatment.

Materials and Methods

Participants

This cross-sectional survey was carried out between 2019-2020 in the Imam-Reza hospital and health care centers in Birjand, South Khorasan province, Iran. In this study, 384 patients (221 women and 163 men) over 30 years old with type 2 diabetes were enrolled according to World Health Organization (WHO) criteria.²⁶ Diagnosis of autoimmune diabetes in adult patients was carried out based on the criteria of Immunology of Diabetes Society^{9,17} as follows: age over 30 years at diagnosis, no need for using insulin for at least 6 months after initial T2DM diagnosis, and serum ICA positivity. Exclusion criteria included age less than 30 years old, having T1DM, showing ketosis at onset of diabetes, having any other acute or chronic inflammatory illness, pregnancy and history of surgery for at least three months before entering the project.

Data Collection

Demographic data including age, gender, age at diagnosis, health status, treatment type and family history of diabetes were collected at the screening stage. People who did not cooperate properly with the project staff were eliminated at this stage. Family history of diabetes was defined as the presence of diabetes in first-degree relatives, including parents, grandparents, and siblings. Body mass index (BMI) at the time of admission was calculated by

measuring height and weight (kg/height (m²)). According to WHO criteria, normal BMI range for adults is between 18.5 and 24.9. Overweight is defined as the BMI rate of 25 to 29.9, and obesity is defined as the BMI rate of 30 or higher.²⁷

Blood pressure was measured by the standard method mentioned previously¹⁹. People with mean systolic blood pressure (SBP) ≥ 140 mm Hg, mean diastolic blood pressure (DBP) ≥ 90 mm Hg, or those taking antihypertensive drugs were defined as having high blood pressure.¹⁹

Experimental Evaluations

For experimental measurements, 10 mL of venous blood was taken from each patient, fasting for at least 12 hours. Serums were aliquoted following centrifuge and stored at -20°C until further assessment. Fasting blood glucose (FBG) was measured using glucose oxidase kit. By definition FBG ≤ 100 mg/dL is considered as normal. A fasting blood sugar level from 100 to 125 mg/dL is regarded as prediabetes, and FBG ≥ 126 mg/dL on two separate tests illustrate diabetes. The percentage of HbA1c in all patients was measured according to the commercial kit protocol (Pars Azmun CO. Iran). This kit is designed to measure HbA1c percentage within the range of 1.5 to 15%. HbA1c percentage of $\geq 7\%$ (≥ 53 mmol/mol) was considered as poor glycemic control.²⁸

Serum total cholesterol was determined using a Cholesterol measuring Kit (CHOD PAP method, Pars Azmun CO. Iran). Based on the manufacturer's guidelines, the absorbance rate was read at 546 nm. Hyperlipidemia was defined as fasting cholesterol level of > 5.2 mmol/L. C-peptide level was measured using an ELISA-based commercial kit (Diametra Co., Italy cat: DKO-077), with an analytical sensitivity of 0.2-10.0 ng/mL. In practice, using known values, the absorbance rate was read at 450 nm, the standard curve was drawn and samples concentration was determined using the standard curve. ICA level was determined by a commercial ELISA kit (Biomerica, USA). In brief, the absorbance of each well at the wavelength of 405 nm recorded in ELISA reader and calculated the mean optical density (OD) value for reference control, negative control, positive control, which were available in ELISA kit, and samples. The mean OD of the samples and controls were then divided by the mean OD of the reference. The interpretation of results of ratio value was negative if < 0.95 , positive if > 1.05 , and indeterminate (borderline) if 0.95-1.05. All the measurements were carried out in duplicate.

Statistical Analysis

Statistical analysis was carried out using SPSS software version 16.0. Data was represented as average \pm SD and percentage. Comparison of quantitative variables between ICA-positive and ICA-negative groups was performed

using the independent samples *t* test and $P < 0.05$ was considered as statistically significant.

Results

Out of a total of 384 participating patients, 221 (57.6%) were female and 163 (42.4%) were male. The age range of the subjects was between 32 - 70 years old, with the mean age of 52.33 ± 8.51 years old. Among 384 patients, 1.30% (5 of 384) of the subjects were ICA-positive including two men and three women ($P = 0.911$), which was not statistically significant. The mean age of autoantibody-positive subjects was 35.80 ± 2.39 years old, which is significantly lower than the mean age of autoantibody-negative patients (52.55 ± 8.34) ($P = 0.000$). The median of disease duration in ICA-positive and ICA-negative subjects was 2.02 ± 1.87 and 29 ± 4.97 years, respectively ($P = 0.001$). Results of various parameters studied in ICA-positive and negative subjects are presented in Table 1.

A family history of diabetes was reported in 80% (4 out

Table 1. Clinical Characteristics of ICA-Positive and ICA-Negative Patients

Characteristics	ICA-Positive Patients	ICA-Negative Patients With T2DM	P Value
Number	5 (1.30%)	379 (98.7%)	
Gender			
Females (%)	3 (60%)	218 (57.5%)	
Males (%)	2 (40%)	161 (42.5%)	
Mean age (y)	35.80 ± 2.39	52.55 ± 8.34	0.000
Median of disease duration (y)	2.02 ± 1.87	6.29 ± 4.97	0.001
FBG (mg/dL)	156.80 ± 2.95	150.88 ± 12.26	0.282
Family history of diabetes (%) ^a	4 (80%)	208 (54.9%)	0.262
Hypertension (%)	0	151 (39.8%)	0.070
Total cholesterol (mg/dL)	12.498 ± 183.80	183.092 ± 11.127	0.888
HbA1c (%)	8.66 ± 2.03	7.93 ± 1.98	0.040
Mean C-peptide levels (ng/mL)	0.73 ± 0.37	1.311 ± 0.47	0.006
BMI (kg/m ²)			
Normal (18.5–24.99 kg/m ²)	5 (100%)	122 (32.2%)	
Overweight (25.0–29.99 kg/m ²)	0	163 (43.0%)	
Obesity (≥ 30.0 kg/m ²)	0	94 (24.8%)	
Mean BMI	0.44 ± 24.22	27.06 ± 3.28	0.054
Treatment regimen, No. (%)			
Nothing	0	9 (2.4%)	
Use of OHA	1 (20%)	250 (66%)	
Insulin therapy with OHA	4 (80%)	120 (31.7%)	0.022

Abbreviations: BMI, body mass index; OHA, oral hypoglycemic agents; FBG, fasting blood glucose; ICA, islet cell autoantibody; T2DM, type 2 diabetes mellitus.

Data are presented as median or No. (%).

^a Is defined as family history of any types of diabetes among first or second-degree relatives.

of 5) of ICA-positive and 54.9% (208 out of 379) of ICA-negative subjects ($P = 0.262$). This indicates no significant correlation between family history of diabetes and having islet cell antibodies.

Measuring BMI in T2DM patients showed that the majority of patients (163 out of 379) are overweight (43.0%), 122 of 379 (32.2%) patients were normal, and 94 patients (24.8%) were obese. Although the BMI of all five ICA-positive patients was within the normal range, however, mean BMI value for these two groups were (27.06 ± 3.28) and (0.44 ± 24.22) respectively ($P = 0.054$), which was not statistically significant.

Another parameter evaluated in patients was blood pressure. While, hypertension was not observed in any of the ICA-positive subjects, 39.8% (151 out of 379 patients) of ICA-negative group showed high blood pressure ($P = 0.070$), though the difference was not statistically significant. Assessment of total serum cholesterol in ICA-positive and ICA-negative patients showed the mean cholesterol level of 12.498 ± 183.80 and 183.092 ± 11.127 , respectively, results which were not statistically significant ($P = 0.888$).

Measuring fasting blood glucose, the mean FBG in ICA-positive and ICA-negative patients was observed as 156.80 ± 2.95 and 150.88 ± 12.26 respectively. The results showed no significant difference ($P = 0.282$), though it was numerically higher in ICA-positive than ICA-negative patients. On the other hand, there was a significance difference ($P = 0.040$) in average HbA1c percentage between autoimmune and other subjects (8.66 ± 2.03 vs 7.93 ± 1.98 respectively).

One of the most important parameters for assessing pancreatic beta cell activity is measuring C-peptide levels in LADA patients. The mean C-peptide level in ICA-positive and ICA-negative patients was 0.73 ± 0.37 and 1.311 ± 0.47 , respectively, which was considered as statistically significant ($P = 0.006$).

Data on treatment options, showed that among 379 non-autoimmune diabetic patients, 250 (66.0%) subjects were treated with oral hypoglycemic agents (OHA), 120 (31.7%) subjects received a combination of oral hypoglycemic agents and insulin and 9 (2.4%) subjects, received no treatment prior to this study. In the other group (ICA-positive group), one patient (20%) used only oral medications for treatment, and the remaining four (80%) used insulin therapy in addition to oral hypoglycemic drugs. Therefore ICA-positive patients showed more insulin therapy requirement to control and treat their disease than ICA-negative subjects ($P = 0.022$). The clinical features of all five ICA-positive patients are summarized in Table 2.

Discussion

Various studies have shown that the prevalence of adult-onset autoimmune diabetes varies in different

Table 2. Clinical Features of five ICA-Positive Patients

Characteristics of ICA+Patients	Patient 1	Patient 2	Patient 3	Patient 4	Patient 5	Mean
Gender	Women	Women	Women	Men	Men	
Age (y)	39	37	36	34	33	35.80±2.39
Median of disease duration (y)	2	3	1.5	1.9	1.7	2.02±1.87
HbA1c (%)	7.8	8.02	9.2	8.59	9.7	8.66±2.03
C-peptide (ng/mL)	1.3	0.9	0.45	0.45	0.55	0.73±0.37
Treatment	OHA	OHA+I	OHA+I	OHA+I	OHA+I	-

Abbreviations: OHA, oral hypoglycemic agents; ICA, islet cell autoantibody; I, Insulin therapy

regions and populations. LADA studies in Tianjin¹⁸ and China,²² showed LADA prevalence of 9.2% and 5.9%, respectively. Moreover, the LADA prevalence of 3.8% has been reported in Ehime population, Japan.²⁹ In all of these studies, only GADA has been measured in the population study, but in some other studies, other autoantibodies such as IA-2, ZnT8 or ICA have been measured, as well. For example in United Arab Emirates, LADA prevalence of 2.6% was reported by measuring GAD and/or IA-2 autoantibodies,²³ or in Action LADA study, the LADA prevalence of 9.7% has been reported, using GAD and/or IA-2, ZnT8 as biomarkers.²¹ LADA prevalence in United Kingdom was reported as 12% by measuring GAD and/or ICA autoantibodies.²⁵ Therefore, one of the most important reasons for the discrepancy in LADA prevalence among different populations is the difference in study design and the number of measured antibodies. The prevalence of ICA in subjects of this study was reported as 1.30%. Previously, the proportion of ICA-positive T2DM patients in UK Prospective Diabetes Study 25 (UKPDS 25) was reported as 6%.²⁵ In another study on 86 T2DM patients, Khoshroo et al reported that 27 patients (31.4%) are ICA-positive.²⁰ The prevalence of ICA in our study seems to be lower than previous reports and is more similar to the two China studies, reporting an ICA prevalence of 3.03%³⁰ and 5.55%,³¹ respectively. The reason for such discrepancies can be due to the size of the study population and the ethnic and genetic differences. Differences in inclusion criteria and the sensitivity and specificity of antibody measurement kits are also involved.

Of the five ICA-positive patients in this study, three (60%) were female and two (40%) were male, showing no significant difference in ICA prevalence between opposite genders. Our results are consistent with those previously reported by Al-Zubairi et al.⁵ Similarly, there are other studies showing no significant correlation between patient gender and LADA prevalence.^{22,24} Although autoimmune diseases are generally more common in women,²⁴ some other studies have reported a higher LADA prevalence in men.^{18,32} This could be due to demographic differences, racial distribution or the involvement of genetic and environmental factors.

The mean age and the median of disease duration of ICA-positive patients were significantly lower than that

of ICA-negative ones. Previously Turner et al reported that ICA and GADA is more frequent among younger patients than older ones.²⁵ Nevertheless, in some studies, no difference has been reported between the antibody positive and negative groups in terms of age or duration of diabetes.^{30,33}

Our results showed no significant difference in family history of diabetes and mean BMI between ICA-positive and ICA-negative groups. These results may suggest that autoimmune patients and T2DM share the same risk factors. Although no significant difference in median BMI was observed between these two groups, but none of the ICA-positive subjects were obese. Previously, the UKPDS 25 study indicated that ICA-positive patients younger than 40 years old have lower BMI than ICA-negative ones.²⁵ The average age of ICA-positive patients in our study was 35.80±2.39 years old, which is consistent with the results of the study. In addition, there was no statistically significant difference in hypertension and hyperlipidemia prevalence, as the markers of metabolic syndrome. Our results may suggest that the presence of metabolic syndrome does not necessarily exclude adult-onset autoimmune diabetes. This is in accordance with the results of other studies showing the vital role of insulin resistance in the pathogenesis of adult autoimmune diabetes, in addition to autoimmunity.^{15,17} Nevertheless, this cannot be confirmed with certainty due to the small number of LADA patients in our study.

Out of five ICA-positive patients, four subjects used insulin therapy to control their disease, with the exception of one 39 years old ICA-positive patient who had suffered from the disease for 2 years. Another interesting point was that the amount of C-peptide in this patient was more than the other four ICA-positive subjects (Table 2). None of the other four patients required insulin therapy for at least the first six months of their illness and the following decision to take insulin therapy was definitely dependent on the attitude of their personal physician. These results confirm previous data suggesting that ICA may be associated with progressive beta cell dysfunction and the need for future insulin therapy.^{25,30} Consistent with our results, it has shown that there is a greater need for insulin therapy in young non-obese autoantibody-positive patients.^{25,34} Despite insulin therapy, our four ICA-positive patients

had worse glycaemic control than T2DM subjects. This, along with the shorter median duration of the disease, can indicate a more severe form of diabetes. These results have been confirmed in previous studies, as well.^{3,28}

Given the size and population of Iran and its racial diversity, so far few studies have been conducted on the prevalence of the adult-onset autoimmune diabetes in Iran. Moreover, most of the studies have only investigated the prevalence of GAD autoantibody,^{19,35,36} with very few ones studying the prevalence of ICA, as well.²⁰ To the best of our knowledge, this is the first study to investigate adult-onset autoimmune diabetes in South Khorasan province, Iran, using ICA biomarker. However, this survey has some limitations, as well. Firstly is the relatively small size of the study population and the small number of ICA positive subjects that makes comparison between two groups difficult. Secondly, since GADA is the most abundant and sensitive autoantibody in autoimmune diabetes,^{16,17} its measurement is necessary to estimate the true rate of adult-onset autoimmune diabetes. Therefore, measuring the prevalence of ICA alone, it is not possible to estimate the prevalence of LADA in the study population. Another point to mention is that the ICA-positive and negative patients in this study may have other autoantibodies, as well; therefore, it cannot be said with certainty that the observed characteristics in both study groups are related to the presence or absence of ICA alone. The final issue is that none of the patients were investigated at follow up. Therefore, assessment of other autoantibodies (GADA, IA-2, IAA or ZnT8) in a larger sample size is recommended.

Conclusion

Our results revealed the ICA prevalence of 1.30% in T2DM patients. In this regard, autoantibody screening is recommended in T2DM patients, especially non-obese patients under 40 years old, showing worse glycaemic control. This is because oral medications alone may not be suitable for treating adult-onset autoimmune diabetes, and if insulin therapy is not started in time, the rest of β -cells function will be lost. Due to the small size of the study group and also the lack of measurement of other autoantibodies, especially GADA, the results of this study cannot be generalized with certainty only to the presence or absence of ICA. Assessment of other autoantibodies (GADA, IA-2, IAA or ZnT8) in a larger sample size and clinical parameter examination at follow-up is recommended.

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Authors' Contribution

RN performed laboratory activities and collected data. MahM contributed to article collection and data analysis. MaM

participated in project design, project guidance, article writing. ML contributed to data analysis and consulting on project advancement.

Competing Interests

The authors have no conflict of interest to declare.

Ethical Approval

This study was carried out in accordance with the Helsinki Declaration principles and approved by the Sistan and Baluchestan University ethics committee, with ethics code of IR.USB.REC.1398.016.

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