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High Frequency of Multiple Autoantibody Positive LADA in Patients with Type 2 Diabetes Mellitus in Iran Population

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ABSTRACT

Background: *Latent autoimmune diabetes of adults (LADA) is a slowly progressive form of autoimmune diabetes initially managed with diet and oral hypoglycemic agents before becoming insulin requiring. Autoantibodies are used for differential diagnosis between LADA and Type 2 Diabetes Mellitus. We assess the frequency of LADA using islet autontibodies in Qom, Iran population with T2DM and characterize clinical and biochemical characteristics of them.*

Methods: *In this cross-sectional study, 86 clinically diagnosed as T2DM patients aged between 27-73 years were tested for presence of autoantibodies by ELISA technique. The GADA, ICA and IA2A assessment are used for diagnosis of LADA patients.*

Results: *Of 86 phenotypically T2DM, 35(40.70 %) were antibody positive and 51(59.30%) persons were negative for any antibody. A significance difference was found between LADA and antibody negative T2DM patients groups in age, duration of disease, family history of diabetes, autoimmune thyroid disease and C- peptide levels. Altogether 21(60.0%) of patients had two or more antibodies, 6(17.14%) were only GADA positive, 7(20%) patient were single ICA positive and 1(2.86%) patient was single IA2A positive. None of the sera were positive for ICA and IA2A in combination.*

Conclusions*: Among our T2DM subjects, islet autoantibody positive patients were not uncommon. Also, multiple autoantibodies were more frequent than single autoantibodies in LADA patients. Seroconversion after disease progress and our methodology can explain our observation.*

Key words: *LADA, Autoantibody, T2DM.*

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INTRODUCTION

Latent Autoimmune Diabetes in Adult (LADA) is a T1DM that unlike classic T1DM manifests in adult patients, hence diagnosed as T2DM[26]. This slowly progressive form of type 1 autoimmune diabetes with an onset in adults is not clinically distinguishable from T2DM. Precise diagnosis of LADA patients is important, because they often need to take insulin earlier than T2DM patients and even in diabetes prevention or intervention trials may respond differently when compared with T2DM patients[8]. LADA is currently identified by three criteria: (i) the onset of diabetes that is at adult ages. Cut-off for LADA varies from 25 to 40 years, but not absolute. It seems the operational minimal age be 30 years; (ii) the presence of circulating autoantibodies. These autoantibodies can be glutamic acid decarboxylase autoantibody (GADA), islet antigen 2 antibody (IA2A) orislet cell autoantibody (ICA). Islet autoantibodies are indicator of autoimmunity that distinguishes LADA from T2DM; and (iii) lack of need to take insulin for at least 6 months after diagnosis. This period of non-requirement to insulin administration after diagnosis can be used to distinguish LADA from classic T1DM[11]. In LADA beta cells destruction as compared to T1DM is slower and the symptoms of disease are later than T1DM so that often misdiagnosed with T2DM. At the beginning, hyperglycemia is limited by oral drugs but finally autoimmune damage of the pancreatic beta cells is overt and needs to insulin is seen within 3-5 years after diagnosis. Many studies on LADA reveal a prevalence of 10- 30% or more and the more frequent autoantibody in these patients have shown to be GADA and or ICA[3]. Studies have shown, without evaluation of islet autoantibodies, it is difficult to discriminate T1DM from T2DM in adult patients. Phenotypically T2DM patients are prone to develop beta cells failure when they have circulating autoantibodies[36]. Autoantibodies to islet antigens help us to distinctly discriminate LADA from antibody negative T2DM patients and also indicate that LADA is autoimmune disorder. In the other hand, these immune markers indicate there is difference in underlying disease process in LADA versus classic T1DM[27]. However, development of autoantibodies against one or more autoantigens show killing beta cells by immune system[31]. In contrast to classic autoantibody negative T2DM, LADA and T1DM patients have autoreactive T cells. In spite of similarity, there are antibody, T cell and genetic differences between T1DM and LADA[28,29].

The prevalence of T2DM is on the rise and if 10% of these patients are positive for islet autoantibodies, then testing for islet autoantibodies as part of the diagnostic assessment in T2DM is relevant to a great number of adult patients, as it may contribute to the rate of progression to insulin requirement, particularly in the absence of gross visceral obesity. Autoantibodies indeed help distinguish adult patients with T1DM, LADA or T2DM[15].

In Iran, several studies have examined the prevalence and clinical characteristics of LADA patents. However, the study patients were tested only for GADA. Thus, these studies had a limitation to represent the frequency of other autoantibodies in Iran. In this study, we measured islet cells autoantibodies in patients with T2DM and compared the clinical characteristics of patients with and without islet cells autoantibodies.

MATERIALS AND METHODS

Subjects

A total 86 phenotypically T2DM subjects were selected from the patients referred to Kamkar hospital in Qom, Iran between February to November 2014. Informed consent was obtained from all participants and the study protocol was approved by the ethical committee of the Qom University of Medical Sciences. Aquestionnaire consisting of a series of items about age, disease history and other information were completed by all participants. Also participants were interviewed and examined by a physician to determine characteristics and medical conditions. The complete history was taken and clinical assessment was done. Anthropometric evaluations were done by trained personnels and weight and height of

each patient were measured and the BMI was calculated using the formula. Participants aged > 30 years (86 individuals) were asked to attend laboratory for collection of blood samples in fasting condition. LADA patients was identified based on the presence of hyperglycemia, age of disease onset 30 years or above ; lack of requirement for insulin at least 6 months after the diagnosis of diabetes; and serum autoantibodies positivity as tested by ELISA. 90 non diabetic age and sex matched healthy selected as control subjects.This group was necessary for establishing the normal range for C peptide and the positivity cut-off for pancreatic autoantibodies

Biochemical measurements

The peripheral blood was drawn from each subject after 10-12 h fasting. Serum was aliquoted following centrifuge and stored at -80 ºC. All samples were run in the same assay. Fasting blood glucose (FBG) by GOD/PAP method was done by using Randox laboratories kit (Hitachi 902). The percentage of glycosylated hemoglobin A1C was calculated using the ion exchange chromatography method by commercial kit (Biosystem, Spain).C-peptidewas determined using commercial ELISA kits (IBL, USA). C-peptidewas determined using commercial ELISA kits (IBL, USA). Intra and inter assay coefficients of variation were less than 10% and the analytical sensitivity was 0.064 ng/mL. C-peptide concentrations were determined using a standard curve derived from known amounts of standard absorbance readings at 450 nm.

Autoantibodies evaluation

The collected sera were kept frozen at -80ºC until laboratory measurement for GADA, ICA and IA2A by commercial kits (EASTBIOPHARM, HANGZHOU EASTBIOPHARM CO., LTD.). Antibodies concentrations were determined using a standard curve derived from known amounts of standard absorbance readings at 450 nm. The minimum and maximum detection levels for the GADA were 1ng/mL and 300 ng/ml respectively. Intra and inter assay coefficients of variation were less than 10% and 12% respectively. The minimum and maximum detection levels for the ICA were 0.5 U/L and 150 U/L respectively and the minimum and maximum detection levels for the IA2A were 1ng/mL and 300 ng/mL respectively. Intra and inter assay coefficients of variation were less than 10% and 12% respectively. Sera with GADA, ICA or IA-2A values above the mean plus three times the standard deviation of the 90 non diabetic control subjects were regarded as positive. Values ≥50 ng/mL for GADA, ≥30 ng/mL for IA2A and ≥20 U/L for ICA considered as positive. All samples measured in duplicates.

Statistical analysis

Statistical analyses were performed using Statistical Package for Social Science (SPSS) 16. The data were expressed as mean ± SD. Proportions were expressed as percentage while significant tests were done with the t-test, ANOVA and correlations done by Pearson or Spearman. Chi-square test has been used to find the significance of study parameters on categorical scale between two or more groups. The result was considered significant at $p < 0.05$.

RESULTS AND DISCUSSION

Of 86 phenotiypically T2DM, 35(40.70 %) were antibody positive and 51(59.30%) persons were negative for any autoantibody. Demographic and biochemical characteristic of patients are presented in Table1.A significance difference was found between LADA and antibody negative T2DM patients groups in age, duration of disease, family history of diabetes, evidence of thyroid disease (Graves disease)and C- peptide levels. No significance difference was found between LADA and antibody negative T2DM patients groups in other characteristics (Table 1). LADA and antibody negative T2DM patients with evidence of thyroid disease (Graves's disease) were female. Distribution of LADA criteria in autoantibody positive T2DM patients was shown in Table2. Blood glucose control in 10 (28.60%) of LADA patients and in14 (27.50%) of antibody negative T2DM patients achieved by insulin and the remaining patients used oral agents, exercise or combination of two for blood glucose control.

The mean (SD) levels of C-peptide in LADA patients (n= 35), antibody negative T2DM patients (n= 51) and healthy controls were 0.69(0.27), 1.00(0.58) and 1.71(0.66) ng/mL respectively.

The mean (SD) level of GADA in GADA positive patients (n= 27) was 88.44(34.36) ng/mL with an level range of 52- 160 ng/mL .The mean (SD) level of ICA in ICA positive patients (n= 27) was 60.13(45.11) IU/L with an level range of 20-150 IU/L . The mean (SD) level of IA2A in IA2A positive patients (n=18) was 79.41(56.57) ng/mL with a level range of 32- 160 ng/mL.

Of 35 LADA patients, 26 (74.28%) were positive for GADA, 27 (77.14%) had ICA and 17 (48.57%) had IA2A. Altogether 21(60.0%) of patients had two or more antibodies, 6(17.14%) were only GADA positive, 7(20%) patient were single ICA positive and 1(2.86%) patient was single IA2A positive. None of the sera were positive for ICA and IA2A in combination (Table 3). 26% of LADA patients had BMI <25 Kg/m^2 and 66% of them had age of onset of diabetes <50 years. 8% and 61% of autoantibody negative T2DM individuals had BMI <25 Kg/m^2 and age of onset of diabetes <50 years respectively.

There was significant association between GADA levels but not ICA(p=0.2) or IA2A(p=0.45) and duration of disease at the $p < 0.05$.There was a significant difference between GADA concentration in insulin dependent and insulin independent LADA patients (p= 0.010). The means (SD) of GADA in insulin dependent and insulin independent LADA patients were 96.10 (37.24) and 62.10 (31.70) respectively. There was no significant association between ICA or IA2A autoantibody and insulin requirement in LADA patients. The mean (SD) of GADA in recently diagnosed (1 year) non insulin dependent patients (8 persons) was 55.12(15.56) ng/mL .Only one (12.5%) of the newly diagnosed patients was insulin dependent while the remaining (87.5%) were not.Characteristics of single and multiple autoantibody-positive subjectspresented in Table4. A significance difference was found between single autoantibody positive subjects and multiple autoantibody positive patients in BMI, insulin requirement, FBS and HbA1C. No significance difference was found between single autoantibody positive subjects and multiple autoantibody positive patients in other characteristics (Table4). Of fifteen 3Ab+ persons 7(46.67%) were insulin dependent. Of six 2Ab+ persons 2(33.33%) were insulin dependent and of fourteen 1Ab+ persons only 1(7.1%) were insulin dependent. There was no significant association between age and number of antibodies.

The present study was the first attempt in finding the frequency of LADA patients among T2DM based on detection of GADA, ICA and IA2A in Iran. According to our findings, the frequency of antibody positivity among T2DM patients was 40.70%. Approximately the prevalence of LADA is 10% in diabetic patients of 40-75 years of ages[35,37]. The prevalence of LADA cases among phenotypically T2DM patients varies between 6-50% (Landin Olsson M; 2002) in different populations[20]. Meroj A. Jasem *et al*[17]., found a prevalence of 18.9% in their study. Olufunmilayo O Adeleye *et al*[2]., in Nigerian T2DM patients found a prevalence of 14%. In Tehran city, Iran Jahed et al. reported that the prevalence of LADA based on GADA was 5.44%[16]. But in Kerman city, Iran Gozashti et al. showed that 14.2% were positive for GADA[12]. Recent data from a LADA study[32] in Shiraz city, Iran showed that in persons with T2DM the prevalence of LADA was 28.4%[32]. The prevalence of LADA based on GADA in our study is similar to that reported by previous study on Shiraz city, Iran (28.4%) by Pishdad *et al*[32]., but the prevalence of LADA in present study is lower than a different study that reported by Carina Torn (47%) and patients were positive for ICA, GADA or IA-2A[38]. We think assessment of multiple autoantibodies instead of only GADA, heterogeneity in Qom city population in consequence of high rate of migration and increase in autoimmune diseases due to lifestyle changes, cause high frequency of LADA in Qom. However, the reason of

variable reported prevalence of LADA in the world maybe depends on genetic, criteria of diagnosis, antibody assay method and characteristics of patients[12].

Our major finding was that multiple autoantibodies were more frequent than single autoantibodies in LADA patients. This pattern is a characteristic feature of classic childhood T1DM[27]. Seven LADA patients who were positive for GADA, ICA and IA2A were female and had evidence of thyroid autoimmunity. Thus, it seems the spectrum of autoimmune diabetes that extends across all ages and varies with age at diagnosis[22,38], obesity, disease duration and other factors such as epitope spreading due to chronic inflammation which damages the beta cells and causes antibody production in T2DM patients, and to lesser extent low titre non organ specific autoantibody can explain our results[4].Other explanations for this observation are: First, in some studies only GADA as an autoimmune marker has been assessed. Second, in agreement with Henrik Borg *et al*[7]., associated with decreasing fasting C-peptide levels with disease duration, may ICA developed in antibody negative mostly overweight patients. Although, Henrik Borg et al[7]., shown that most GADA-positive patients remained GADA positive after 12 years. Longitudinal studies observe changing autoantibody status over time and even though the majority of patients are positive for only one type of autoantibody, existing autoantibodies may be lost and other autoantibodies may develop[21].

In our study 5 persons had high titre ICA (>60 U/L), 6 persons had high titre IA2A and 11 persons had high titre of GADA (>80 ng/mL). All ICA and IA2A positive high titre patients also had GADA high titre and had disease duration more than 4 years. In one study[24], 75 percent of patients have disease duration less than 4 years. Third, in contrast to one study[17], BMI of 30 or more and family history of diabetes were not exclusion criteria in our study. Thus, seroconversion after disease progress and our methodology may explain our observation.

All four islet autoantibodies—ICAs, GADA, IA2A, and IAA—and zinc transporter (ZnT8) antibody are found in children with T1DM. Thus, antibody clustering is a feature of classic childhood T1DM. In the other hand, many researchers have demonstrated that GADA and ICA are more frequent than IAA, IA-2A, and ZnT8 antibodies in LADA patients versus T1DM patients[27]. Also, the prevalence of single autoantibodypositivity (ICA or GADA) was higher in subjects with LADA than in those with adult-onset type 1 diabetes and often detected single ICA positivity in patients with LADA. However, the presence of both ICAs and GADAs was a stronger predictor of insulin requirement than GADAs alone among patients older than 45 years of age[14]. Autoantibodies are the major predictors of functional destruction of beta cells, especially at high titers and when existent in combination (GADA and ICA). Rosarioa *et al*[34]., was shown that the high titers GADA persons showed a greater reduction and lower levels of C- peptide and required insulin during follow-up. Our results are consistent with these observations (Table4).

Another study[9] introduced GADA and C- peptide as a diagnostic marker for LADA. Lohmann T *et al*[23]., concluded that high titers and low titers GADA positive patients have characteristic of T1DM and T2DM respectively. Our results are consistent with previous observations and were shown that GADA is not only valuable as a good marker for diagnosis of LADA cases but also a diagnostic tool for requirement to insulin. In one study Mohammed I Hawadd showed that patients with high titer of GADA more likely were female and in contrast patients with low titer of GADA most probably were male[13]. However, our results indicated that there was no significant association between GADA level and sex or age. Our data show that there is significant association between GADA level and duration of disease at the $p < 0.05$. Newly diagnosed LADA patients had lower GADA titers (51.53 ng/mL). We propose that beta cells destruction by immune system is responsible for rise of GADA titers. Maioli M

et al. claimed number of autoantibodies and HLA genotype, more than high titers of GADA, predict insulin dependency in latent autoimmune diabetes of adults[25]. Our results indicated that number of autoantibody can influence the disease progression. *Our data are consistent with other studies*that show the number of islet autoantibodies can predict insulin dependency (p=0.022)(Table4). In our study 7 and 2 out of ten insulin dependent patients had 3 and 2 islets autoantibodies respectively. Rodacki M showed that duration of disease did not affect significantly the prevalence of GADA or its titers in patients with T1DM after one year of diagnosis. It seems the complete beta cells destruction in T1DM patients occurred and GADA titers rise no more[33]. However, in LADA patients' beta cells destruction continues by immune mechanisms and GADA titers rises over time. Our study indicated that there is no significant association between ICA and IA2A levels and age, sex and duration of disease. Some studies show that antibody against insulin and IA2A are inversely correlated with age at onset but GADA has not inverse correlation and in some studies has a positive correlation with age at onset. Therefore GADA is an especially interesting marker for autoimmune diabetes in the adult population[26].Our study indicated that there is no significant association between ICA and IA2A levels and age of onset of disease. But our data show that there is significant association between GADA level and age of onset at the $p < 0.05$.

As mentioned above, all common islet autoantibodies (ICA, GAD, IA-2, IAA) were detected in T1DM patients and many of them were positive for multiple autoantibodies. In fact, increasing number of islet autoantibodies rise the risk of appearance of T1DM[40,41]. In contrast, LADA patients were positive for only one autoantibody (ICA or GADA)[14]. Lohmann *et al*[23]., and Van Deutekom *et al*[39]., showed that there is a correlation between the titre and number of autoantibodies and clinical characteristics of disease in LADA patients. Van Deutekom *et al*[39]., observed that the concurrent presence of multiple autoantibodies and/or a high titer of anti-GADA, compared with single and low-titer autoantibody were associated with an early age of onset. Prospective studies of beta cell function was showed that LADA patients with multiple islet antibodies develop cells failure within 5 years, whereas those with only GADA or only islet cells antibodies (ICAs) mostly develop beta cell failure after 5 years. Even though it may take up to 12 years until beta cells failure occurs in some patients[37]. In our study the mean age of disease onset was a little higher among 3Ab+ subjects compared to 1Ab+ but the difference was not significant. In our study the frequency of IA2A positive patients was fewer than subjects with GADA or ICA. In fact, the presence of IA-2 autoantibodies in both LADA and T1DM is associated with HLA -DR4 and the frequency declines with the age of onset of diabetes in both forms of autoimmune diabetes[15,19]. Also, the mean duration of disease was a little higher among 3Ab+ subjects followed by 2Ab+ patients and 1Ab+ patients.Park Y et al. was reported that after 36 months of follow-up, 3 of 39 patients who were initially classified as LADA have become insulin-dependent and those three were all positive for multiple autoantibodies (GADA, IA-2A and zinc transporter 8 antibody)[30]. Our results indicated multiple autoantibodies positive patients faster than those who positive for one or two autoantibody and or negative for any autoantibody will become insulin dependent (P=0.05).

It seems that LADA, due to having an autoimmune mechanism, has no strong relationship with risk factors such as age, sex, BMI, family history of diabetes or others. Indeed, in susceptible individuals the breaking of peripheral tolerance due to change in levels of IL-15[1] or vitamin D[6], both frequency and activation status of NK[5] cells and or frequency and function of regulatory T cells[42] or other factors can elicit autoimmunity in adulthood. One of the important LADA development risk factors is diabetes family history. Sofia Carlsson showed that family history of diabetes was associated with a four

times increased prevalence of LADA[10]. Keyhani M *et al*[18]., in a study on T2DM patients and their first degree relatives reported that the mean level of GADA in the patients was 10.76±13.59 ng/mL and in their first degree relatives was 19.71±32.1ng/mL and concluded that the determination of GADA in healthy subjects and their first degree relatives of T2DM patients can be used to predict the development of diabetes and insulin dependency[18]. Some studies propose that LADA patients are unlikely to have a family history of T2DM, but some showed that presence of family history is an important risk factor for the development of LADA[9]. Our data may not confirm Priyanka P Brahmkshatriyastudy and the other related studies that indicated existence of family history can be risk factor for the occurrence of LADA[9].However it seems that single autoantibody positive patients with high BMI are similar to classic T2DM patients. Because the patients who had only one type of autoantibody were fatter than those who were positive for multiple autoantibody (p=0.022).

Table1. Demographic and biochemical characteristic of patients

	LADA(n=35)	T2DM(n=51)	P Value
Age range(years)	36-68	37-73	
Mean age(years)	50.03±9.30	54.18±8.03	0.03
Range of disease duration (years)	1-10	1-25	
Mean of disease duration(years)	3.68±2.04	6.90±6.00	0.001
Range age of disease onset (years)	32-62	31-66	
Mean age of disease onset(years)	46.54±8.84	47.27±8.68	0.7
Sex(M/F)	13/22	20/31	0.85
BMI(Kg/m^2)	29.24±4.84	30.11±3.62	0.34
Dislipidemia (%)	16(45.7)	31(60.8)	0.17
Hypertension (%)	19(54.3)	30(58.8)	0.23
Family history of diabetes (%)	22(62.9)	44(77.19)	0.01
History of autoimmune thyroid diseases (Graves) (%)	7(20)	1(1.96)	0.005
Complications (%)	8(22.9)	12(23.50)	0.94
Insulin therapy (%)	10(28.6)	14(27.5)	0.60
FBS(mg/dL)	151.91±80.53	145.18±78.19	0.70
HbA1c (%)	7.83(2.39)	7.98(1.98)	0.76
C-peptide(ng/mL)	0.69±0.27	1.00±0.58	0.001

n: number, M:Male, F: Female, BMI: Body Mass Index,T2DM:Type 2 Diabetes Mellitus, LADA: Latent Autoimmune Diabetes of Adults; SD: Standard Deviation; HbA1c: Hemoglobin A1c; FBS: Fast blood sugar

Table2. Distribution of LADA criteria in autoantibody positive T2DM patients

LADA criteria	n
Antibody positivity+ Age of onset < 50	23
Antibody positivity + autoimmune thyroid disease	7
Antibody positivity +BMI <25	7
Antibody positivity +Insulin dependency >6 mouths	10
Antibody positivity+ Age of onset < 50+BMI <25	6
Antibody positivity+ Age of onset < 50 + autoimmune thyroid disease	6
Antibody positivity +Age of onset < 50 + Insulin dependency >6 mouth	7
Antibody positivity+ Age of onset < 50+BMI <25 + Insulin dependency >6 mouth	1
Antibody positivity+ Age of onset < 50+BMI <25 + autoimmune thyroid disease	0
Antibody positivity+ Age of onset < 50+BMI <25 + autoimmune thyroid disease + Insulin dependency >6 mouth	0

LADA: Latent Autoimmune Diabetes in Adults; n: number; BMI: Body Mass Index

Table 3. Frequency of islet autoantibodies in LAD Apatients

Autoantibodies	**n**
GADA only	6
ICA only	7
IA2A only	1
GAGA+ICA	5
GADA+IA2A	1
ICA+IA2A	0
GADA+ICA+IA2A	15

n: number; GADA: Glutamic Acid Decarboxylase Autoantibody; ICA: Islet Cell Autoantibody; IA2A: Islet Antigen 2 Antibody

Table.4 Characteristics of single and multiple autoantibody-positive LADA subjects

	Single antibody (n=14)	Multiple antibody (n=21)	P Value
Age range(years)	**38-66**	**36-68**	
Mean ag(years)	**48.21±9.57**	**51.24±9.15**	**0.35**
Range of disease duration (years)	**1-8**	**1-6**	
Mean of disease duration((years)	**3.43±2.78**	**3.86±1.90**	**0.55**
Range age of disease onset (years)	**33-62**	**32-62**	
Mean age of disease onset((years)	**44.93±9.13**	**47.62±8.70**	**0.38**
Sex(M/F)	**6/8**	**7/14**	**0.57**
BMI(Kg/m^2)	**30.29±3.30**	**26.85±4.96**	**0.029**
Dislipidemia (%)	**8(57.14)**	**8(38.09)**	**0.27**
Hypertension (%)	**9(64.28)**	**10(47.62)**	**0.33**
Family history of diabetes (%)	**11(78.57)**	**11(52.38)**	**0.12**
History of autoimmune thyroid diseases (Graves) (%)	**1(20)**	**6(1.96)**	**0.12**
Complications (%)	**4(28.57)**	**4(19.05)**	**0.62**
Insulin therapy (%)	**1(7.14)**	**9(42.85)**	**0.022**
FBS(mg/dL)	**124.64±40.20**	**170.10±95.43**	**0.06**
HbA1c (%)	**6.96(1.07)**	**8.41(2.84)**	**0.043**
C-peptide(ng/mL)	**0.71±0.27**	**0.68±0.27**	**0.73**

n: number, M:Male, F: Female, BMI: Body Mass Index,T2DM:Type 2 Diabetes Mellitus, LADA: Latent Autoimmune Diabetes of Adults; SD: Standard Deviation; HbA1c:Hemoglobin A1c; FBS: Fast blood sugar

CONCLUSION

In our T2DM subjects, patients who had islet autoantibodies were not uncommon. Also, in our study high frequency of patients who had multiple autoantibodies were detected. In the other hand, sometimes the phenotype of adult autoantibody positive patients resembles that of autoantibody-negative patients. The presence of autoantibodies against beta cells increases the likelihood of beta cells function deterioration, even after sustained improvement of glycemic control by means of insulin treatment. Thus, early and precise diagnosis of LADA patients results in appropriate treatment.

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All authors declare that they have no conflict of interest.

REFERENCES

1. Abadie, V., and Jabri, B., IL-15: a central regulator of celiac disease immunopathology. *Immunol Rev*., **260(1):** 221–234 (2014).

2. Adeleye, O.O., Ogbera, A.O., Fasanmade, O., Ogunleye, O.O., Dada, A.O., et al., Latent Autoimmune Diabetes Mellitus in Adults (LADA) and it's characteristics in a subset of Nigerians initially managed for type 2 diabetes. *Int Arch Med*., **5:** 23 (2012).
3. Aggarwal, S., Goel, A. and Jain, A., Role of C- peptide in identification of patients suspected of having LADA in north Indian type 2 diabetes mellitus population. *International Journal of Pharma and Bio Sciences*, **1**(**3):** (2010).
4. Ahn, I.M., Izumi, M. and Nagataki, S., Islet cell surface antibodies in Graves' disease; as organ non-specific antibodies. *Korean J Intern Med* **3(1):** 38-44 (1998).
5. Akesson, C., Uvebrant, K., Oderup, C., Lynch, K., Harris, R.A., Lernmark, A., Agardh, C.D. and Cilio, C.M., Altered natural killer (NK) cell frequency and phenotype in latent autoimmune diabetes in adults (LADA) prior to insulin deficiency. *Clinical and Experimental Immunology*, **161:** 48–56 (2010).
6. Alfonso, B., Liao, E., Busta, A., et al., Vitamin D in Diabetes Mellitus – A New Field of Knowledge poised for D-velopment. *Diabetes Metabolism Research and Reviews*, **25:** 417-419 (2009).
7. *Borg, H., Gottsater, A., Fernlund, P. and Sundkvist, G., A 12-year prospective of the relationship between islet antibodies and β-cell function at and after the diagnosis in patients with adult onset diabetes. Diabetes*, **51:** 1754 *–1762 (2002).*
8. Bottazzo, G.F, Bosi, E., Cull, C.A., Bonifacio, E., Locatelli, M. and Zimmet, P. et al., IA-2 antibody prevalence and risk assessment of early insulin requirement in subjects presenting with type 2 diabetes (UKPDS 71). *Diabetologia*, **48(4):** 703–708(2005).
9. Brahmkshatriya, P.P., Mehta, A.A., Saboo, B.D. and Goyal, R.K., Characteristics and Prevalence of Latent Autoimmune Diabetes in Adults (LADA). *ISRN Pharmacol*., 580202 (2012).
10. Carlsson, S., Midthjell, K. and Grill, V., Influence of Family History of Diabetes on Incidence and Prevalence of Latent Autoimmune Diabetes of the Adult. *Diabetes care*, **30(12):** 3040 (2007).
11. Fourlanos, S., Dotta, F., Greenbaum, C.J., Palmer, J.P., Rolandsson, O., Colman, P.G. and Harrison, L.C., Latent autoimmune diabetes in adults (LADA) should be less latent. *Diabetologia*, **48(11):** 2206–2212 (2005).
12. Gozashti, M.H., Maryam, S., Saeed, E., Hamid, N. and Mahdieh, M., The prevalence of latent autoimmune diabetes in adults and its correlates in patients with type 2 diabetes in Kerman, Iran. *Diabetes & Metabolic Syndrome: Clinical Research & Reviews*, **9:** 104–107 (2015).
13. Hawa, M.I., Kolb. H., Schloot, N., Beyan, H. and Paschou, S.A., et al., Adult-Onset Autoimmune Diabetes in Europe Is Prevalent With a Broad Clinical Phenotype: Action LADA 7. *Diabetes Care*, **36(4):** 908-13 (2013).
14. Hosszufalusi, N., Yatay, A., Rajczy, K., Prohaszka, Z., Pozsonyi, E., Horvath, L., Grosz, A., Gero, L., Madacxsy, L., Romics. L., Karadi, I., Fust, G. and Panczel, P., Similar genetic features and different islet cell autoantibody pattern of latent autoimmune diabetes in adults (LADA) compared with adult-onset type 1 diabetes with rapid progression. *Diabetes Care*, **26:** 452–457 (2003).
15. Itariu, B.K. and Stulnig, T.M., *Autoimmune aspects of type 2 diabetes mellitus - a mini-review*. *Gerontology*, **60:** 189–96 (2014).
16. Jahed, A., Hosseinpanah, F. and Azizi, F., Prevalence and predictive factors of LADA "latent autoimmune diabetes in adults" in newly diagnosed diabetics of Tehran lipid and glucose study. *Iranian Journal of Diabetes and Lipid Disorders*, ***7*(1):** E5 (2007).
17. Jasem, M.A., Al-Ubaidi, A.A., Admon, A. and Zwaer, K.N., Prevalence of Latent Autoimmune Diabetes in Adult (LADA) Among Clinically Diagnosed Type 2

Diabetic Patients. *Zanco J Med Sci*., **14:** 181-7 (2010).

18. Keyhani, M., Firoozrai, M., Gharanlar, J. and Nakhjavan, M., Anti-gad autoantibody levels in type II diabetes patients and their first degree relatives. *Razi journal of medical sciences*, **10:** 1-2 (2003).
19. Kulmala, P., Savola, K., Petersen, J.S., et al., Prediction of insulin-dependent diabetes mellitus in siblings of children with diabetes. A population- based study. The Childhood Diabetes in Finland Study Group. *J Clin Invest* **101(2):** 327-36 (1998).
20. Landin-Olsson, M., Latent autoimmune diabetes in adults. *Annals of the New York Academy of Sciences*, **958**: 112–116 (2002).
21. Laugesen, E., Ostergaard, J.A. and Leslie, R.D., Latent autoimmune diabetes of the adult: current knowledge and uncertainty. *Diabetic Medicine*, **32:** 843–852 (2015).
22. Lin, J., Zhou, Z.G., Wang, J.P., Zhang, C. and Huang, G., From Type 1, through LADA, to type 2 diabetes: a continuous spectrum? *Ann N Y Acad Sci*., **1150:** 99–102 (2008)
23. Lohmann, T., Kellner, K., Verlohren, H.J., Krug, J., Steindorf, J., Scherbaum, W.A. and Seissler, J., Titre and combination of ICA and autoantibodies to glutamic acid decarboxylase discriminate two clinically distinct types of latent autoimmune diabetes in adults (LADA). *Diabetologia*, **44:** 1005 –1010 (2001).
24. Lutale, J., Thordarson, H., Holm, P., Eide, G. and Vetvik, K., Islet cell autoantibodies in African patients with Type 1 and Type 2 diabetes in Dar es Salaam Tanzania: a cross sectional study. *Journal of autoimmune diseases*, **4:** 4 (2007).
25. Maioli, M., Pes, G.M. and Delitala, G., et al., Number of autoantibodies and HLA genotype, more than high titers of glutamic acid decarboxylase autoantibodies, predict insulin dependence in latent autoimmune diabetes of adults. *Eur J Endocrinol*., **163(4):** 541-549 (2010).
26. Maruyama, T., Shilpa, O., Shimada, A., Christiane, S. and Hampe, GAD65 autoantibody responses in Japanese latent autoimmune diabetes in adult patients. *Diabetes Care*, **31:** 1602-7 (2008).
27. Naik, R.G. and Palmer, J.P., Latent autoimmune diabetes in adults (LADA). *Rev Endocr Metab Disord*., **4:** 233 –241 (2003).
28. Naik, R.G., Brooks-Worrell, B.M. and Palmer, J.P., Latent Autoimmune Diabetes in Adults. *J. Clin. Endocr & Metab*., ***94(12):*** *4635-44 (2009).*
29. Palmer, J.P., Hampe, C.S., Chiu, H., Goel, A. and Brooks–Worrell, B., Is latent autoimmune diabetes in adults distinct from type 1 diabetes or just type 1 diabetes at an older age. *Diabetes*, **54:** 62–67 (2005).
30. Park, Y., Hong, S., Park, L., Woo, J., Baik, S., Nam, M., Lee, K. and Kim, Y., LADA prevalence estimation and insulin dependency during follow-up. *Diabetes Metab Res Rev*., **27(8):** 975-9 (2011).
31. Pihoker, C., Gilliam, L.K., Hampe, C.S. and Lernmark, A., Autoantibodies in diabetes. *Diabetes*, 54**(2):** S52–S61 (2005).
32. Pishdad, G.H.R. and Sarveram, A., A. Survey of Anti-GAD anti body level in type 2 diabetic adults, in comparison with healthy individuals in Shiraz. Thesis Submitted To The Graduate Studies In Partial Fulfillment Of The Requirements For The Degree Of Subspecialty In: Endocrinology and metabolism. 2011 Shiraz University of Medical Science Shiraz-Iran.
33. Rodacki, M., Zajdenverg, L., Albernaz, M.S., Bencke-Gonçalves, M.R., Milech, A. and Oliveira, J.E., Relationship between the prevalence of anti-glutamic acid descarboxilase autoantibodies and duration of type 1 diabetes mellitus in Brazilian patients. *Braz J Med Biol Res*., **37(11):** 1645–50 (2004).
34. Rosario, P.W., Reis, J.S. and Fagundes, T.A., Latent autoimmune diabetes in

adults (LADA): usefulness of anti-GAD antibody titers and benefit of early insulinization. *Arq Bras Endocrinol Metabol*., **51:** 8-10 (2007).

35. Singh, P., Sharma, P.K., Garg, V.K., Singh, A.V. and Mondal, S.C., A Review on Prevalence of LADA. *Int J Res Phytochem Pharmacol*., **1(3):**12-123 (2011).
36. *Stenstrom, G., Berger, B., Borg, H., Fernlund, P., Dorman, J.S. and Sundkvist, G., HLA-DQ genotypes in classical type 1 diabetes and in latent autoimmune diabetes of the adult.* Am J Epidem., **156***:* 787 *–796 (2002).*
37. Stenstrom, G., Gottsater, A., Bakhtadze, E., Berger, B. and Sundkvist, G., Latent Autoimmune Diabetes in Adults Definition, Prevalence, β Cell Function, and Treatment. *Diabetes*, **54(Suppl 2):** S68–S72 (2005).
38. Torn, C., Landin-Olsson, M., Ostman, J., Schersten, B., Arnqvist, H., Blohme, G., Bjork, E., Bolinder, J., Eriksson. J., Littorin, B., Nyström, L., Sundkvist, G. and Lernmark, A., 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(6):** 442-47 (2000).
39. Van Deutekom, A.W., Heine, R.J. and Simsek, S., The islet autoantibody titers their clinical relevance in latent autoimmune diabetes in adults (LADA) and the classification of diabetes melllitus. *Diabetic medicine*, **25:** 117–125 (2008).
40. Verg, C.F., Gianani, R., Kawasake. E., Yu, L.P., Pietropaolo, F., Jackson, R.A., Chase, H.P. and Eisenbarth, G., Prediction of type 1 diabetes in first-degree relatives using a combination of insulin, GAD, and ICA512bdc/IA-2 autoantibodies. *Diabetes*, **45:** 926–933 (1996).
41. Verge, C.F., Gianani, R., Kawasake, I., Yu, L.P., Pietropaolo, F., Chase, H.P. and Eisenbarth, G.S., Number of autoantibodies (against insulin, GAD or ICA512/IA2) rather than particular autoantibody specificities determines risk of type 1 diabetes. *J Autoimmun*., **9:** 370–383 (1996).
42. Yang, Z., Zhou, Z., Huang, G., Ling, H., Yan, X., Peng, J. and Li, X., The CD4+ regulatory T-cells is decreased in adults with latent autoimmune diabetes. *Diabetes Research and Clinical Practice*, **76:** 126–131 (2007).