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Original Article

Characteristics and Prevalence of Latent Autoimmune Diabetes in Adults (LADA) in Torbat-e Heydarieh, Iran

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ABSTRACT

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Key words
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Background and Aims: The prevalence of latent autoimmune diabetes in adults (LADA) among diabetic patients is less recognized and underdiagnosed. The aim of this study was to determine the prevalence of LADA in type 2 diabetic patients and to compare the characteristics of these two groups in Torbat-e Heydarieh, Iran.

Materials and Methods: Totally, 198 male and 277 female patients diagnosed with type 2 diabetes are aged between 35 -70 years were selected and the glutamic acid decarboxylase antibodies (GADA) assessment was used for the diagnosis of LADA in them. GADA in their sera was measured by commercial anti-GAD enzyme-linked immunosorbent assay (ELISA) kit. In addition, blood pressure, sera C-peptide and cholesterol levels was measured and compared in the mentioned two groups. Demographic data including age, gender, age at diagnosis, family history of diabetes, body mass index and need for insulin therapy were collected from subjects and data were analyzed using SPSS software.

Results: Of 475 patients, 53 ones (11.2%) were GADA positive. Significant difference was found between GADA positive and GADA negative patients in terms of mean age, C-peptide levels, cholesterol levels and need for insulin therapy to control the disease. As for gender, family history of diabetes, body mass index value and hypertension there was no significant correlation between these two groups.

Conclusions: The prevalence of LADA in diabetic patients was 11.2%. Presence of GAD antibodies in diabetic patients is related to reduced levels of C-peptide, increased cholesterol levels and the need for insulin during the follow-up.

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Introduction

There has been a dramatic increase in the incidence of diabetes in human societies during the past decade. It's prevalence has particularly raised faster in the low- and middle-income countries than in the high-income countries. Diabetes is an important public health problem and the number of the cases along with the prevalence of diabetes has been steadily increasing over the past few decades [1]. The total number of people with diabetes is predicated to rise from 171 million in 2000 to 366 million in 2030 [2]. Type 2 diabetes is the commonest form of diabetes constituting around 90% of the total this population whereas type 1 diabetes constitutes about 10-15% of the diabetic population. Ethnicity, age, life style, and genetic factors play a major role in the variation of incidence of all types of diabetes [3]. While diabetes is classically divided as type 1 and type 2 diabetes, there are some forms of diabetes which cannot be classified into either of these categories. The subgroup of patients diagnosed with type 2 diabetes bears circulating antibodies to islet cell autoantibodies and more frequently to glutamic acid decarboxylase (GADA) thus distinguishing them from type 2 diabetes [4, 5]. This condition is accompanied by the onset of diabetes after 35 years of age [6-8] and not requiring insulin, during at least the first 6 months after diagnosis thus distinguishes these patients from classic type 1 diabetes [6, 9]. This subset is named latent autoimmune diabetes in adults (LADA) [9, 10] or slowly progressive insulin-dependent diabetes mellitus [11, 12]. Epidemiological studies suggest that LADA may account for 2-12% of all the cases of diabetes [6]. There are numerous reports about the prevalence and characteristic of LADA in the world [3, 7, 8, 13]. Since LADA is often misdiagnosed as type 2 diabetes, it must be properly recognized and managed appropriately at the clinical level. Little is known concerning the prevalence and significance of islet cell autoimmunity in the elderly patients affected with type 2 diabetes in the Middle East. In Iran, there are few reports on the prevalence of LADA [14-17]. In this cross-sectional study, we were looking for investigating the prevalence of LADA and the relationship between occurrence of it and some parameters such as age, gender, family history of diabetes, body mass index (BMI), blood pressure, serum cholesterol and C peptide level in Torbat-e Heydarieh, Razavi Khorasan province, Iran.

Materials and Methods

Subjects

A total of 475 patients (277 females, 198 males) clinically diagnosed with type 2 diabetes as per World Health Organization (WHO) criteria [18] were included in this cross-sectional study. Samples were collected from Muslim Ibn-e-Aqil Medical center and 9 Dey Hospital at Torbat-e Heydarieh, Iran, in 2016. All patients were informed of the nature of the study and gave their written consent and the study protocol was approved by the Ethical

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Committee of Torbat-e Heydarieh University of Medical Sciences. LADA patients were identified based on Immunology of Diabetes Society criteria [12] as follows: 1) the presence of type 2 diabetes and age≥35 years; 2) lack of requirement for insulin at least 6 months after the diagnosis of type 2 diabetes; and 3) serum GADA positivity as tested by enzyme-linked immunosorbent assay (ELISA).

Data colection

Data on age, gender, age at diagnosis, health status, treatment and family history of diabetes, were collected from participants at the screening phase by using a questionnaire. Family history of diabetes was defined as having diabetes in any of the following family members: parents, grandparents (either paternal or maternal), and siblings. Blood pressure was measured by a trained physician using a mercury sphygmomanometer using a standardized protocol [19]. Hypertension was defined as systolic blood pressure (SBP) ≥140 mm Hg or diastolic blood pressure (DBP) ≥90 mm Hg.

Laboratory measurements

The peripheral blood (10 mL) was drawn from each subject following overnight 8 h fasting. Sera were aliquoted following centrifuge and stored at -20ºC. All samples were run in the same assay. In all subjects, fasting blood glucose (FBG) was measured by GOD-POD colorimetric method. A fasting blood sugar level less than 100 mg/dL (5.6 mmol/L) is regarded as normal. A fasting blood sugar level from 100 to 125 mg/dL (5.6 to 6.9 mmol/L) is considered prediabetes. A reading of 126 mg/dL (7 mmol/L) or higher on two separate tests indicates diabetes. Total cholesterol was measured by using Cholesterol Assay Kit (CHOD PAP method, Pars Azmun CO. Iran). C-peptide was 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. GAD antibodies were determined in the above-mentioned groups using Isletest GAD diagnostic kit (Diametra Co., Italy cat: DKO-082) with 92.3% sensitivity and 98.6% specificity.

Statistical Analysis

All statistical analyses were performed using Statistical Package for Social Science (SPSS) 7.5. Data were analyzed for mean and standard deviation. Proportions were expressed as percentage while significant tests were conducted with the T-test and $p<0.05$ was considered as statistically significant.

Result

Totally 475 patients with type 2 diabetes in Torbat-e Heydarieh were studied out of whom 198 subjects (41.7%) were male and 277 (58.3%) were female. Results of different parameters evaluated in type 2 and LADA patients are summarized in Table 1. The average age of subjects was 52.51±8.55 years with the age range of 35-70 years. From all, 53 ones (11.2%) were GADA positive and 422 ones (88.8%) were GADA negative. The prevalence of GADA was 54.7% in female and 45.3% in male subjects thus showing no

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significant difference, but slightly higher in women. Age range at diagnosis was 29 to 38 years for the group with type 2 diabetes and 21 to 27 years for the group with LADA. Also the mean age of LADA patients (41.62±6.72) was found to be significantly lower than type 2 diabetes subjects (53.87±7.74).

Table 1. Characteristics of different parameters for type 2 diabetes and LADA

Characteristics	Type 2 diabetes	LADA	P-value
Number (%)	422 (88.8%)	53 (11.2%)	
Gender-wise prevalence			NS
Males (%)	174 (41.2%)	24 (45.3%)	
Females (%)	248 (58.8%)	29 (54.7%)	
Mean age (years)	53.87 ± 7.74	41.62 ± 6.72	<0.001
Age range at diagnosis (years)	29-38	21-27	
Family history of diabetes	207 (49.1%)	27 (50.9%)	NS
BMI			
Normal (20–24.99 kg/m^2)	143 (33.9%)	19 (35.8%)	
Overweight (25–29.99 kg/m^2)	182 (43.1%)	20 (37.7%)	
Obese (30-34.99 kg/m^2)	88 (20.9%)	14 (26.4%)	
Very obese (≥ 35 kg/m^2)	9 (2.1%)	-	
Mean BMI	27.04 ± 3.20	26.43 ± 3.05	0.19
Hypertension (%)	167 (39.6%)	21 (39.6%)	NS
Mean fasting blood glucose (mg/dL)	150.06 ± 12.53	154.62 ± 9.46	0.011
Mean cholesterol levels (mg/dL)	181.23 ± 11.99	187.74 ± 8.66	<0.001
Mean C-peptide levels (ng/mL)	1.37 ± 0.43	0.699 ± 0.27	<0.001
Treatment regimen N(%)			NS
Nothing	12 (2.8%)	-	
Use of OHA	320 (75.8%)	14 (26.4%)	
Insulin therapy with OHA	90 (21.3%)	39 (73.6%)	

BMI= body mass index; OHA= oral hypogylcemic agents
Data are presented as Mean±SD

In regard to the family history of diabetes, among LADA patients, 50.9% (27 out of 53) had and 49.1% (26 out of 53) did not have any family history of diabetes. On the other hand, among type 2 diabetic subjects, 49.1% (207 out of 422) had a family history of diabetes, therefore there was no significant relationship between the prevalence of family history of diabetes in autoantibody positive and negative patients. BMI values of patients with type 2 diabetes showed that most of them (182 of 422) were overweight (43.1%), 20.9% (88 of 422) of these patients were found to be obese and 2.1% (9 of 422) very obese. Among LADA patients, 37.7% (20 of 53) were overweight, 26.4% (14 of 53) were obese but

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none of them were very obese. Mean BMI value for these two groups was calculated and there was no significant difference (p=0.19) observed between subjects with LADA and non LADA subjects in regard with mean BMI (26.43±3.05 vs. 27.04±3.20). In LADA subjects 39.6% (21 of 53) and in type 2 diabetic patients, 39.6% (167 0f 422) suffered from hypertension, but no significant difference observed between the two groups. A significance difference was found between LADA and non LADA subjects in FBG (p=0.011). Mean FBG in LADA and non LADA subjects were (154.62±9.46) and (150.06±12.53) respectively. The mean cholesterol levels were measured in patients and data showed mean cholesterol levels were significantly higher in LADA patients (187.74±8.66) than type 2 diabetic subjects (181.23±11.99) (p<0.001). The results of the C-peptide determination showed significantly low levels of C-peptide in LADA patients (0.699±0.27 ng/mL) compared to non LADA patients (1.37±0.43 ng/mL) (p<0.001). The results are shown in Fig. 1.

Based on medical information of patients, among type 2 diabetic patients, 320 out of 422 (75.8%) were treated with oral hypoglycemic agents (OHA) while 21.3% (90 of 422) received a combination of insulin and oral hypoglycemic agents. In this group, 12 subjects (2.8%) had not received any medications prior to the time of study. Interestingly, insulin therapy with combination of oral hypoglycemic agents was used in 73.6% (39 of 53) of LADA patients and 14 patients (26.4%) were treated only with OHA. Therefore LADA patients more need insulin therapy than non-LADA subjects.

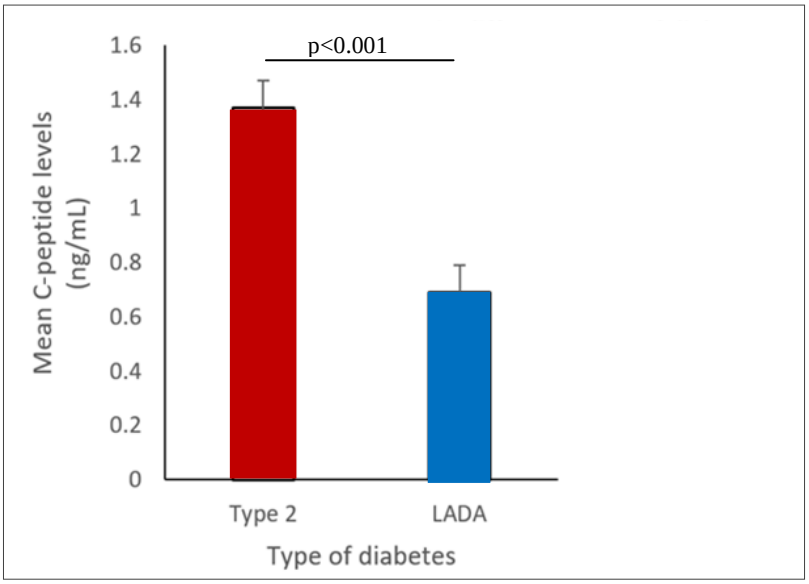

Fig.1. Mean C-peptide levels in type 2 and LADA subjects

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Discussion

The main objective of this study was to determine the prevalence and characteristics of LADA in type 2 diabetic patients in Torbat-e Heydarieh, Iran. In this study the prevalence rate of LADA was 11.2%. According to our findings, gender had no important role in the development of this disease. Whereas China study indicated that female gender is a risk factor of LADA [3], in India, the survey mentioned that the prevalence of LADA significantly higher in males than in females [13]. Comparison of age range in the autoantibody positive group showed a tendency toward the lower age range compared to autoantibody negative subjects. On the other hand, mean age of GADA positive patients was significantly lower than GADA negative subjects which is agreement with studies performed in India, Korea and Europe [13, 20, 21]. Our study showed no significant difference in family history of diabetes, BMI values and hypertension between LADA and non LADA subjects. However, our findings indicate that family history of diabetes is an important risk factor in the incidence of both types of diabetes. This finding is consonant with the result obtained from Kerman study in 2015 [16] and Japan in 2002 [22] while studies in China show that family history of diabetes may be associated with the occurrence of LADA [3]. Our data in the case of BMI value is contrary to the information obtained from recent studies [3, 7, 13, 23]. In these studies, LADA patients had a lower BMI values than type 2 diabetic patients. Hypertension results are similar to the results in Kerman and Qom cities, Iran [16, 17], but contrary to the data obtained from the study in Tianjin of China [3]. A significance difference was found between $GADA^+$ and $GADA^-$ patients in FBG, C-peptide levels, total cholesterol and use of insulin for disease control. Mean FBG in LADA group is significantly more than non LADA group ($p=0.011$). These data indicated that the severity of the disease is higher in autoantibody positive group than autoantibody negative group. Also mean cholesterol levels were significantly higher in LADA patients than non-LADA patients that are contrary to the result of the study in Korea. In the Korea study, it was shown that there is a negative correlation exists between LADA and total cholesterol [20]. Our finding indicated that C-peptide levels are significantly low in autoantibody positive patients than negatives. This is in agreement with this fact the LADA is an autoimmune type of diabetes and progressive β-cell destruction may occur in this case. This result is confirmed in several studies [10, 13, 20, 24]. On the other hand, in LADA group, 73.6% use insulin for better control of disease, but in type 2 diabetic patients only 21.3% use insulin therapy. Although LADA patients do not require insulin therapy early in the diagnosis of diabetes, within a few years, β-cell function is severely impaired, leading to insulin dependency in most LADA patients [25]. These data are in agreement with other reports

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in insulin requirement in LADA patients [6, 7, 24, 26, 27]

Conclusion

The report showed that the prevalence of LADA among type 2 diabetic patients of Torbat-e Heydarieh, Iran is 11.2% which is relatively high compared to its prevalence elsewhere in the world. According to different studies, prevalence of LADA is approximately 2-12% of diabetic cases [6]. The frequency of GAD antibody in Europe [21], North America [28], China [3], Korea [20], and Japan [22] has been 3.7%, 4.7%, 9.2%, 8.7% and 3.8% respectively, while in Kerman (a province of Iran), it has been reported as 14.2% [16]. Differences in the prevalence of LADA worldwide may be relevant to age at diagnosis, criteria of diagnosis, disease duration at study, and the numbers of patients under the study [29]. However the limitation of this study is the relatively small number of patients included which can affect the results. On the other hand, although GADA was the most common reported autoantibody in autoimmune diabetes, there has been shown that presence of other autoantibodies toward β- cells such as IA-2A (tyrosine phosphatase antibodies), ZnT8 (zinc transporter 8) and IAA (insulin autoantibody) in some type 2 diabetic patients could be an important marker of autoimmunity in diabetes and combination of antibodies increases the prevalence of LADA [9]. Therefore all major autoantibodies should be measured in the screening process. In addition to the number of antibodies, there is an association between the antibody titer and the need for insulin therapy. Failure to comply with this is another limitation of our study. However, our data confirmed that the presence of GADA was significantly associated with the need for insulin therapy, and low C-peptide among adult diabetic patients, and measurement of these parameters in type 2 diabetic patient could help physicians to identify and better control and treat LADA disease.

Conflict of Interest

We have no conflict of interest.

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