

LATENT AUTOIMMUNE DIABETES IN ADULTS (LADA)

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ABSTRACT

Background: It is difficult to classify diabetes mellitus (DM) of the young adult (30-50 years) as type 1 or type 2 based solely on clinical criteria and plasma glucose. Measuring C peptide and markers of islet cell autoimmunity may be useful for proper classification and early adequate treatment.

Aim: The aim of the study is to identify and characterize the patients with LADA and to analyze the differences between LADA and type 1, respectively type 2 DM.

Patients and methods: We studied 268 patients aged between 30 and 50 years, diagnosed with type 2 DM according to the clinical criteria. We measured: body mass index, waist circumference, fasting plasma glucose, HbA_{1c}, C peptide, autoantibodies. We additionally recruited a control group (48 healthy subjects) and 202 adult patients with type 1 DM.

Results: In patients from type 2 DM group 10.4% were identified as having LADA, according to the accepted criteria. In LADA subgroup, age at diagnosis was similar with type 2 DM, but higher than in type 1 DM, BMI was lower than in type 2, but higher than in type 1 DM. Plasma glucose at diagnosis was similar with type 1 DM, but higher than in type 2 DM. C peptide was lower than in type 2 DM, and the frequency of antibodies was higher.

Conclusions: C peptide and autoimmune markers are important for a correct classification of DM diagnosed in group 30-50 years.

Key Words: diabetes mellitus, autoimmunity, autoantibodies, C peptide.

BACKGROUND

Diabetes mellitus (DM) is a clinically and pathogenetically heterogeneous disorder with type 1 and type 2 forms comprising the majority of cases. It is well known that about 90% of all cases are type 2 DM, which is characteristic for adults aged above 40-45 years, while 10% are represented by type 1 DM, typical for children and young.¹ Young adults (between 30 and 40 years) with DM represent a heterogeneous mixture of type 1 and type 2 DM, often difficult to differentiate between, because of yet poorly defined clinical and therapeutic criteria.

Latent autoimmune diabetes in adults (LADA) is a special form of autoimmune DM, where beta-cell destruction is less aggressive, leading to a slower development of insulin dependency. Nowadays, the

identification of LADA represents a major interest for many diabetologists, because its prevalence is relatively high and seems to be underestimated. Also, correct diagnosis of LADA patients allows an early and accurate therapeutic intervention.

The major characteristics of LADA patients are represented by: age of diagnosis greater than 35 years, positivity for autoantibodies (ICA and/or GADA), clinical features similar with type 2 DM, glycemic control initially possible only with diet or diet and oral antidiabetic agents. Insulin dependency can occur after a period between a few months or ten years and more.^{2,3}

AIM OF THE STUDY

The aim of the present study is to identify the patients who fit into the criteria for LADA from a group of diabetes patients, initially considered as having type 2 DM, according to the clinical criteria, and to analyze the differences between the patients with LADA, type 1 DM and type 2 DM.

PATIENTS AND METHOD

The study enrolled 268 patients with clinical type 2 DM, with an age of onset between 30 and 50 years

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and with a diabetes duration less than 5 years in the moment of inclusion.

Additionally, we examined other two groups:

-the first group is represented by 48 healthy non-diabetic subjects, matched for age. This group was necessary for establishing the normal range for C peptide and the positivity cut-off for pancreatic autoantibodies;

-the second group consisted of 202 patients with type 1 DM, with age of diagnosis between 30 and 50 years. This group was used for comparison of the clinical and biological data with the study group.

The diagnosis criteria for DM were those proposed by the ADA, in 1997⁴, and adopted by WHO, in 2000.^{5,6}

The clinical criteria for establishing the diagnosis of type 2 DM were: age at onset greater than 30 years, association with obesity (body mass index >30 kg/m²) or overweight (body mass index between 25 and 29.9 kg/m²), waist circumference greater than 88 cm in women and 102 cm in men, absent or moderate classical symptoms, lack of tendency towards ketosis, and favorable initial response to oral antidiabetic treatment.^{7,8}

The following characteristics of the patients with type 2 DM were analyzed:

1. Clinical parameters: height – H (m), weight – W (kg), body mass index – BMI (calculated by the formula $BMI = W/H^2$) (kg/m²), waist circumference (cm).

2. Laboratory investigations: fasting plasma glucose (mg/dL) (measured with glucosidase), glycated hemoglobin – HbA_{1c} (%) (using immune turbidimetric method), fasting basal C peptide (ng/ml) (using ELISA), antiglutamic acid decarboxylase antibodies – GADA, anti-islet cell antibodies – ICA, and ICA-512 – IA2 antibodies (units of optic density) (using ELISA). The cut-off level of positivity for GADA and ICA was defined as an optic density greater than the 97th percentile in the control group. The 97th percentile for GADA is 2.2 units of optic density, and for ICA 0.61 units of optic density. IA2 antibodies were considered positive above the value of 0.9 units of optic density (the cut-off value of the lab method).

C peptide values were considered normal between the 5th and the 95th percentile of the control group, i.e. between 0.58 ng/mL and 2.7 ng/mL.

3. Aspects regarding the evolution of the disease: year of diagnosis, type of therapy.

The statistical analysis was done using Microsoft Excel (MS Office 97), SPSS, Graph Pad Instat 3. A p value below 0.05 was considered significant.

RESULTS

1. Identification and characterization of the LADA patients

According to currently accepted definition of LADA,^{2,3} we identified 28 patients (10.4%) that can be classified in this category by the following criteria: age at onset above 35 years, the presence of at least one antibody, insulin therapy after more than 6 month after diagnosis.

Table 1 lists the clinical and biological features of these patients.

Table 1. The characteristics of LADA subgroup

No.	Parameter	Value
1.	Number of patients	28 (10.4%)
2.	Gender	
	- female	10
	- male	18
3.	Age at diagnosis (years), mean ± SD	44 ± 4
4.	Distribution of the patients in age groups, number (%)	
	- 30-35 years	0
	- 36-40 years	5 (17.8%)
	- 41-45 years	15 (53.6%)
	- 46-50 years	8 (28.6%)
5.	BMI (kg/m ²), mean ± SD	27,5 ± 4,8
6.	Distribution of the patients in BMI groups, number (%)	
	- normal weight	7 (25%)
	- overweight	12 (42.9%)
	- obese	9 (32.1%)
7.	Distribution of the patients by waist circumference, number (%)	
	- female with waist circumference <88 cm	5 (50%)
	- men with waist circumference <102 cm	3 (16.7%)
8.	Fasting plasma glucose at diagnosis (mg/dL), mean ± SD	313.9 ± 132.7
9.	C peptide (ng/mL), mean ± SD	1.5 ± 1.4
10.	Distribution of the patients according to C peptide, number (%)	
	- low C peptid	9 (32.1%)
	- normal C peptid	15 (53.6%)
	- high C peptid	4 (14.3%)
11.	Distribution of the patients by the presence of the autoantibodies, number (%)	
	- GADA+:	22 (78.6%)
	- ICA+:	7 (25%)
	- IA2+	11 (39.3%)
	- 1 antibody present	17 (60.7%)
	- 2 antibodies present	10 (35.7%)
	- 3 antibodies present	1 (3.6%)
	- at least 1 antibody present	28 (100%)
12.	HbA _{1c} (%), mean ± SD	8.5 ± 2.7

SD - standard deviation

2. Comparison between the patients with LADA, type 1 and type 2 DM

Age at diagnosis

Mean age at diagnosis was not different in LADA patients compared to type 2 DM ($p > 0.05$), but it was significantly greater than in type 1 DM ($p < 0.01$) (Fig. 1).

BMI

Mean BMI in LADA (27.5 ± 4.8 kg/m²) patients

was significantly higher than in type 1 DM ($24.64 \pm 3.8 \text{ kg/m}^2$) ($p < 0.01$) and significantly lower than in type 2 DM ($30.9 \pm 5.6 \text{ kg/m}^2$) ($p < 0.01$).

Table 2 shows the statistical analysis of the weight status in the three categories (p -value, relative risk).

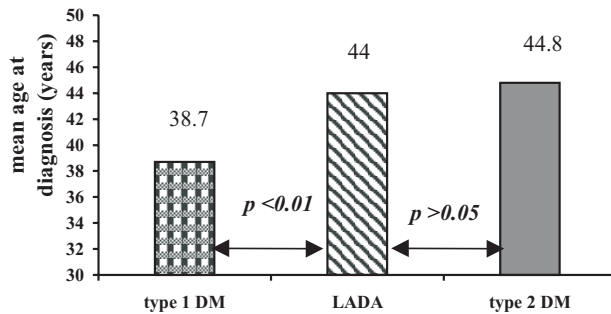


Figure 1. Mean age at diagnosis in patients with type 1 DM, LADA and type 2 DM

Table 2. Statistical analysis of the weight status in the three groups (p -value, relative risk)

	LADA vs type 1 DM	LADA vs type 2 DM
normal weight		
proportion	25% vs 78.2%	25% vs 12.7%
p-value	<0.001	0.03
relative risk	0.3	1.9
overweight		
proportion	42.9% vs 14.4%	42.9% vs 33.9%
p-value	<0.001	0.17
relative risk	2.9	1.2
obese		
proportion	32.1% vs 7.4%	32.1% vs 53.4%
p-value	<0.001	0.02
relative risk	4.3	0.6

Waist circumference

The proportion of women with a waist circumference below 88 cm and of men with a value below 102 cm is different in LADA patients and in those with type 2 DM (Table 3).

Table 3. Statistical analysis of the women with a waist circumference under 88 cm and the men with a waist circumference below 102 cm (p -value, relative risk)

	LADA vs type 2 DM	
	waist circumference below 88 cm (female)	waist circumference below 102 cm (male)
proportion	50% vs 10.8%	16.7% vs 27.3%
p-value	0.003	>0.05
relative risk	4.9	0.6

Fasting plasma glucose at diagnosis

Mean fasting plasma glucose was not different in LADA patients ($313.9 \pm 123.7 \text{ mg/dL}$) and in type 1 DM patients ($324.1 \pm 116.5 \text{ mg/dL}$) ($p > 0.05$), but it was significantly higher than in type 2 DM ($233.7 \pm 90.8 \text{ mg/dL}$) ($p < 0.001$).

HbA_{1c}

Mean HbA_{1c} was similar in LADA patients ($8.5 \pm$

2.7%) and in those with type 2 DM ($8.3 \pm 2.4\%$) ($p > 0.05$).

C peptide

Mean C peptide was significantly lower in LADA patients ($1.5 \pm 1.4 \text{ ng/mL}$) compared to those with type 2 DM ($2.5 \pm 1.7 \text{ ng/mL}$) ($p = 0.0012$).

Table 4 presents the proportion of patients with low, normal and high C peptide in patients with LADA and type 2 DM.

Table 4. Statistical analysis of the C peptide in patients with LADA and type 2 DM (p -value, relative risk)

	LADA vs type 2 DM
low C peptide	
proportion	32.1% vs 7.5%
p-value	0.0005
relative risk	4.3
normal C peptide	
proportion	53.6 vs 57.1
p-value	0.8
relative risk	0.9
high C peptide	
proportion	14.3% vs 35.4%
p-value	0.03
relative risk	0.4

Immune markers

According to the classification criteria for LADA, all patients in this subgroup had at least one antibody present. The proportion of the different antibodies was significantly higher in patients in LADA and type 2 DM (Fig. 2).

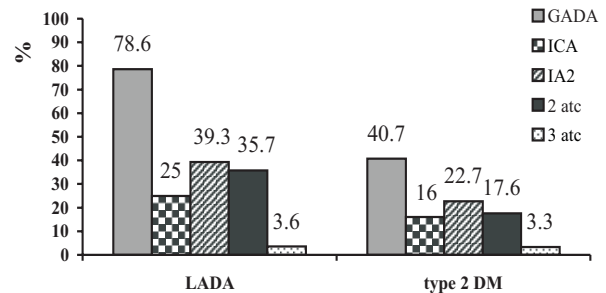


Figure 2. The proportion of antibodies in patients with LADA and type 2 DM

To conclude, patients classified as LADA presented the following features:

- mean age at diagnosis similar with the patients with type 2 DM and higher than in type 1 DM;
- mean BMI higher than in patients with type 1 DM, but lower than in type 2 DM;
- tendency towards obesity higher than in type 1 DM, but lower than in type 2 DM;
- probability of having normal weight lower than in type 1 DM and higher than in type 2 DM;
- mean fasting plasma glucose similar with type 1 DM and higher than in type 2 DM;

- similar HbA_{1c} with type 2 DM patients;
- mean C peptide significantly lower than in type 2 DM;
- immune markers present in all patients;
- all patients eventually required insulin therapy.

DISCUSSIONS

Firstly, the normal range for basal C peptide is not universal but depends on the laboratory method used; furthermore, C peptide values can be reported in nmol/L or in ng/mL (1 nmol/L= 3.9 ng/mL). In March 2001, Marian Parrot, ADA vicepresident for clinical diabetes issues declared that “the role of C peptide is still uncertain... It is difficult to suggest a cut-off that would clearly separate type 1 diabetes from type 2 diabetes. If we were to choose a cut-off value that would include all type 1 diabetes patients, it would be somewhere between 0.8 and 1 ng/mL”.⁹

According to LADA characteristics previously described, 28 out of the 268 patients of our group (10.4%), who are positive for at least one pancreatic autoantibody and have developed insulin dependence after at least six months of diabetes evolution, can be classified as LADA.

The percentage is similar with some of the published data, where the percentage of LADA cases varies as follows: 5-10%,^{10,11} 5-30%,^{12,13} 6-50% (depending on ethnic group, and higher in young people).¹⁴

Schiel et al. have found that 55% from all type 1 DM patients and 21% of insulin-treated type 2 DM patients have GADA, suggesting that the prevalence of LADA is underestimated.¹⁵

Mean age at diagnosis of LADA patients (44 ± 4 years) did not differ significantly when compared with that of type 2 DM patients (44.8 ± 4.5 years) but was significantly greater than in type 1 DM (38.7 ± 6.2 years).

Mean BMI in LADA patients was 27.5 ± 4.8 kg/m², significantly lower than in type 2 DM (30.9 ± 5.6 kg/m²), and significantly higher than in type 1 DM patients (24.6 ± 3.8 kg/m²).

Mean fasting plasma glucose at diagnosis was similar in LADA and in type 1 DM patients (313.9 ± 132.7 mg/dL and 324.1 ± 116.5 mg/dL, respectively), but significantly higher than in type 2 DM patients (233.7 ± 90.8 mg/dL).

These findings are confirmed by other authors. An US study has shown that LADA patients had a significantly lower BMI and C peptide.¹⁶

Pan et al. have found that fasting blood glucose at diagnosis was around 297 mg/dL, while mean basal C peptide was 0.4 nmol/L in LADA patients.¹⁷

Another study compared the characteristics of LADA, type 1 DM and type 2 DM patients and found that BMI and waist-to-hip ratio were similar in type 1 DM and in LADA, HbA_{1c} was comparable in all three groups, while significantly higher C peptide values were encountered in type 2 DM compared to subjects classified as LADA (0.75 pmol/mL vs 0.29 pmol/mL). Furthermore, C peptide differed significantly between LADA and type 1 DM. Insulin deficiency seemed less severe in LADA compared to type 1 DM. In LADA patients, 31.5% tested positive for ICA, 21.1% for GADA, while 15.8% presented both ICA and GADA.¹⁸

Dessailloud et al. followed up 61 patients with the initial diagnosis of type 2 DM who presented at least one symptom suggesting slowly-progressive type 1 DM (weight loss, normal weight status, secondary failure of oral antidiabetic drugs) and found that ICA were present in 43% of the cases, IA2 in 16% of the cases, GADA in 62%, while 15.7% of the patients were positive for all three antibodies. Patients positive for autoimmune markers had a lower BMI and required earlier insulin therapy. No relationship was noted regarding the presence of antibodies and C peptide or basal insulinemia. The authors suggested that GADA and ICA could represent useful markers of future need for insulin therapy.¹⁹

Lohman et al. measured pancreatic autoantibodies in 312 DM patients with age at diagnosis greater than 35 years and found that 16.3% of them presented at least one antibody. Patients positive for antibodies had a lower C peptide compared with those without antibodies. Subjects with two or more antibodies present showed beta-cellular insufficiency, were thinner and had phenotypic features of type 1 DM (the so-called type 1 LADA). Patients who presented one antibody had similar phenotypic features with type 2 DM (type 2 LADA). Apparently, IA2 were not effective in differentiating patients with LADA.²⁰

Juneja et al. studied 125 patients with recent onset DM, diagnosed after the age of 30 and not treated with insulin. Twenty-nine percent of the cases had at least one antibody present and 26% were positive both for ICA and GADA. The authors did not find any clear correlation between antibody positivity and clinical parameters (younger age at diagnosis, BMI, diabetes symptoms), and concluded that only antibody measurement could be of help in identifying LADA cases.²¹

Other authors have shown that 70-80% of LADA cases tested positive for GADA.²² Schranz has noted that GADA and IA2 titres are lower in LADA than in type 1 DM patients.²³

In a recent study carried out in Canada, 80% of LADA cases presented GADA, similarly with type 1

DM patients, while ICA were found in 43%. LADA subjects were insulin-treated in 83%, compared to 53% of type 2 DM patients negative for autoantibodies. Furthermore, BMI in LADA cases was similar with type 1 DM patients, but significantly lower than in the antibody-negative type 2 DM cases. Insulin resistance measured with HOMA method was significantly lower in LADA and type 1 DM patients compared to the antibody-negative type 2 DM patients.²⁴

A study carried out by Hosszufalusi et al. measured the immune markers in 54 LADA patients, 57 type 1 DM cases and 190 type 2 DM patients. The results showed that 59% of the LADA patients had one antibody present. In type 2 DM, ICA was found in 2.6% and GADA in 2.1%. The LADA cases had lower BMI, waist-to-hip ratio, total cholesterol and triglycerides, and higher HDL cholesterol than type 2 DM patients, whereas high blood pressure was less frequent. According to these authors, overweight does not exclude LADA.²

CONCLUSIONS

By correlating the presence of anti-islet antibodies with the clinical and biological features (plasma glucose, HbA_{1c}, C peptide) of the patients with type 2 DM diagnosed between 30 and 50 years, out of the group of 268 cases, we identified 28 patients who fulfilled the criteria for LADA. The percentage of LADA cases is similar with the data published by other authors.

In the LADA subgroup, mean age at diagnosis was similar with that in type 2 DM, but higher than in type 1 DM. Regarding body weight (mean BMI, waist circumference and the proportion of normal weight, overweight and obesity), we noticed that LADA is intermediate between type 1 DM (lower mean BMI and higher proportion of normal weight) and type 2 DM (higher mean BMI and lower proportion of normal weight).

Fasting plasma glucose at diagnosis in LADA patients was similar to type 1 DM and clearly higher than in type 2 DM. HbA_{1c} is similar in LADA and type 2 DM patients. Mean C peptide is lower in LADA than in type 2 DM patients. It is obvious that the proportion of GADA+, ICA+ and IA2+ is higher in the LADA group than in type 2 DM.

Our opinion, based on a complex analysis of the immunological and biochemical data is that about 10% of the patients diagnosed with type 2 DM between 30 and 50 years of age are, in fact, type 1 DM cases.

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