EVALUATION OF THE PARAMETERS OF INSULIN RESISTANCE AND METABOLIC SYNDROME IN TWO GROUPS: LATENT AUTOIMMUNE DIABETES IN ADULTS AND TYPE 2 DIABETES

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Abstract: **Latent autoimmune diabetes in adults (LADA) is a progressive autoimmune disease that result slowly in insulin deficiency and the need of insulin injection. The prevalence of this non-classical form of diabetes is growing higher and the evaluation of the main criteria of diagnose and the importance of metabolic syndrome is essential. As a result, we took a sample of the Albanian population with type 2 diabetes and LADA in order to compare the parameters of the metabolic syndrome and insulin resistance in both groups and to evaluate the potential correlation.**

Materials and Methods: The study design was cross-sectional and it involved 149 participants, 50 females and 99 males. All participants met the criteria above: 1) new diagnose of diabetes in patient over 30 years old, 2) onset of disease in less than a year 3) no use of insulin since the diagnose. Anthropometric data, level od C-peptide and fasting insulin were measured in all participants. All data were analysed with SPSS 10.0 and ANOVA One-Way.

Results: The mean age of the participants in the study was $43{,}22 \pm 7{,}1$ years old. Patients with LADA had BMI of $25{,}07 \pm 3{,}82$, lower than in type 2 Diabetes. Fasting insulin concentration was lower in LADA than in type 2 diabetes in our study ($26{,}8 \pm 0{,}4$ versus $31{,}5 \pm 19{,}3$) confirming the insulin deficiency in LADA in comparison with type 2 diabetes where the main event is insulin resistance rather than insulinopenia. The same goes to the level of C-peptide where in the LADA group is lower than in type 2 Diabetes.

The presence of GAD antibodies was significantly higher in LADA ($6.83 \pm 5{,}6$) than in type 2 Diabetes ($0.57 \pm 0{,}09$).

Conclusions: Our observations indicate that metabolic syndrome and insulin resistance are present in LADA but not higher than in type 2 diabetes. Higher BMI was present in type 2 Diabetes but was not significant in diagnose of LADA. Insulin and C-peptide level were lower in LADA and anti-GAD was statistically important in LADA rather than type 2 Diabetes confirming its role in differential diagnose.

Keywords: **Latent autoimmune diabetes in adults (LADA), insulin resistance metabolic syndrome.**

1. BACKGROUND

Latent autoimmune diabetes in adults (LADA) is categorized as a form of autoimmune diabetes which produce antibodies for beta cells and slowly diminishes insulin reserve of the pancreas. The prevalence of LADA is approximately 10 % of subjects with diabetes aged 35-70 years. The frequency is higher (25 %) in patients younger than 35 years old. [1] First described in 1986 as a particular form of type 2 diabetes, LADA shows characteristics of both type 1 and type 2 diabetes and its definition has been largely discussed. As of today, LADA is descried as a type 2 diabetic phenotype combined with islet antibodies and slowly progressive β-cell failure. [1]

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The diagnosis of LADA is currently based on three criteria: (1) adult age at onset of diabetes; (2) the presence of circulating islet autoantibodies, especially anti - GAD (anti-glutamic acid decarboxylase); and (3) lack of a requirement for insulin for at least 6 months after diagnosis. [2]

The autoimmune process that characterises LADA results in insulin deficiency and insulin dependency later on. During the first period of the diagnose, at least 6 to 12 months, treatment consists of diet, regular physical activity and oral hypoglycaemic medications. As insulin deficiency becomes greater, it requires insulin injections. Insulin injections not only are essential later on in LADA, but they help against glucose toxicity since the diagnose so it is advised to start insulin before grave impairment of beta cell function. Along insulin therapy, LADA patients are candidates for immunomodulation and immunotherapy. [3][4]

As per the classification, discussions rise for characteristics that LADA patients have at the moment of the diagnose. Studies have suggested that certain anthropometric measures (BMI > 25 kg/m^2) and elements of metabolic syndrome are often found in LADA patients, like in type 2 diabetics and thus, should be relevant in LADA diagnose. [5]

Concerning this controversial issue, we studied the correlation between metabolic syndrome and LADA criteria of diagnose in the Albanian diabetic population.

2. MATERIALS AND METHODS

We studied 149 adults over 30 years old, 50 females and 99 males, first diagnosed with diabetes within the year. Participants in this study were selected randomly from the ambulatory care of the Endocrinology Clinic in "Mother Teresa" University Hospital Centre and other Endocrinology outpatient care centres in Tirana.

Selection criteria:

Diabetes Mellitus first diagnosed in patient over 30 years old

Clinical complains and time of diagnose within a year

No use of insulin since the moment of diagnose

Exclusion criteria:

Type 1 Diabetes Mellitus

Time of diagnose over 12 months

Positive ketones in patient's urine in the moment of the recruitment

Secondary diabetes

Prediabetes or Glucose intolerance

Patients with serious co-morbidities

Consent

All patients, during their recruitment, were given a consent form about their involvement in the study. The form was approved by the Endocrinology department in "Mother Teresa" University Hospital Centre.

The procedure

The study included a form with a list of biochemical, hormonal and immunological tests. It also included a questionnaire regarding the clinical history, medical and family history, harmful behaviours (use of alcohols or drugs, smoking), profession, physical activity and gynaecological history in women.

It was established as normal physical activity walking 150 minutes a week or 30 minutes a day with no more than two days off in a row.

Gynaecological anamnesis:

It involved the clinical story of gestational diabetes, treated or not, in every woman who participated in the study. The story included information about childbirth weight of more than 4 kilograms, feto morto, miscarriages etc.

Diagnose of type 2 diabetes mellitus and its classification:

The diagnose of type 2 diabetes were made based on the criteria of World Health Organisation (WHO) [6]:

Fasting plasma glucose (FPG) >126 mg/ dl

2-hour plasma glucose level > 200 mg/ dl

Patients diagnosed with diabetes, were classified according to immunological test in two groups: anti-GAD positive (later classified as LADA) and anti-GAD negative (diabetes mellitus type 2).

Anthropometric data:

Weight was measured in kilograms. The patients were naked and without shoes on.

The height and abdominal perimeter were measured in centimetres.

The Body Mass Index (BMI) was calculated based on the formula: weight/ height ^{2} (kilograms per square meters).

Metabolic Syndrome Definition:

Metabolic syndrome was assessed according to the NCEP ATP III definition: Metabolic syndrome is present if three or more of the following five criteria are met: waist circumference over 40 inches (men) or 35 inches (women), blood pressure over 130/85 mmHg, fasting triglyceride (TG) level over 150 mg/dl, fasting high-density lipoprotein (HDL) cholesterol level less than 40 mg/dl (men) or 50 mg/dl (women) and fasting blood sugar over 100 mg/dl. [7] [8]

Collection and processing of blood sample

The blood sample of 5 millilitres was taken by the brachial vein. All participants (patients and not) should have been fasting for more than 12 hours at the moment of the sampling. 1.5 millilitres of the sample were in a heparin tube for biochemical analyses and 3.5 millilitres were centrifuged for 1-2 hours in a plain tube. The extracted serum was kept in -20 grade Celsius for later utilised to measure the plasmatic level of insulin. Insulinemia and C-peptide were measured with radioimmunoassay method.

A level of anti-GAD $\geq$ 0,9 UI/ml was accepted as positive.

Statistical analyses

Collected data were analysed with SPSS 10.0. Correlation between numerical variables was determined by Spearmen and Pearson coefficient. For continuous variables were utilised ANOVA One-Way. All results were shown in tables and graphs. For each parameter was calculated a mean value and it was compared with the same value from different groups.

Data are expressed as mean $\pm$ SE or in percentages.

It was considered significant the value of $p < 0.05$ (or 5%).

3. RESULTS

149 participants took part in this study. It involved 50 females and 99 males, first diagnosed with Diabetes from less than a year. The mean age of the participants was $43,22 \pm 7,1$ years old. All participants were divided in two groups: the one with LADA and the one with type 2 diabetes.

Patients with LADA had an average abdominal perimeter lower than patients with type 2 diabetes as shown in table 1. The overall body mass index in type 2 diabetes was higher ($29.02\pm 5,1$) than LADA ($25,07\pm3,82$). Both results were statistically significant. (see table 1)

Table 1: Anthropometric data distributed by groups

Group	Abdominal perimeter (cm)	BMI
LADA	97,4 $\pm$ 7,4	25,07$\pm$3,82
Type 2 Diabetes	100,06$\pm$10,6 **	29.02$\pm$ 5,1 *

        **p<0.043   *p<0,040

The LADA group showed fasting insulin concentration of 26,8$\pm$0,4 µIU/ml and the group with type 2 Diabetes showed a level of 31,15$\pm$19,3 µIU/ml.

There was a slight difference between the males and females of the LADA group. In this group, insulinemia was higher in females (34.5 ± 3,1 μIU/ml) compared to males (22,9 ± 5,9 μIU/ml). The same trend was present in the type 2 Diabetes group where insulinemia in males resulted 30,14 ± 6,2 μIU/ml and in females 32.75 ± 2,1 μIU/ml. (see table 2)

Table 2: Fasting insulin concentration

Group	Fasting Insulin Concentration in males (μIU/ml)	Fasting Insulin Concentration in females (μIU/ml)	Fasting Insulin Concentration (total) (μIU/ml)
LADA	22,9 ± 5,9	34.5 ± 3,1	26,8 ± 0,4
Type 2 Diabetes	30,14 ± 6,2	32.75 ± 2,1	31,5 ±19,3

Overall the level of insulinemia in patients with type 2 Diabetes was higher (31,5± 19,3 μIU/ml) than in LADA patients (26,8 ± 0,4 μIU/ml). This result confirms once again the pathophysiology mechanism of LADA which lies in insulinopenia as an autoimmune process rather than in hyperinsulinemia and insulin resistance like in type 2 diabetes.

The level of C-peptide in patients with LADA was lower (0.42± 0,21 ng/ml) than in type 2 diabetic patients (0.84± 0,56 ng/ml) as shown in table 3. (see table 3)

Table 3: Distribution of C-peptide and insulinemia according to groups

Groups	C-peptide (ng/mL)	Fasting Insulin Concentration (μIU/ml)
LADA	0.42± 0,21	26.8 ± 0.4
Type 2 Diabetes	0.84± 0,56 *	31,15 ±19,3

*p< 0.05

The higher level of C-peptide, associated with a higher abdominal perimeter and BMI, was significant with the diagnose of type 2 Diabetes. On contrary, patients with LADA demonstrated a lower level of C-peptide, accompanied with a lower abdominal perimeter and BMI. (see table 4)

Table 4: The correlation between C-peptide level, abdominal perimeter and BMI in LADA and Type 2 Diabetes

Groups	C- peptide	Abdominal Perimeter (cm)	BMI
LADA	0,42±0,21	97,4 ± 7,4	25,07±3,82
Type 2 Diabetes	0,84±0,56 ***	100,06±10,6 **	29.02± 5,1 *

***p<0.001   **p<0.043   *p<0,040

There was a significant correlation between the diagnose, fasting insulin concentration and GAD antibodies. In LADA patients insulinemia was lower and Anti-GAD was higher (6.83 ± 5,6) compared to type 2 diabetic patients (Anti-GAD 0.57 ±0,09). (see table 5)

Table 5: Comparison of Insulinemia and GAD antibodies in LADA and Type 2 Diabetes Groups

Groups	Fasting Insulin Concentration (μIU/ml)	Anti-GAD
LADA	26.8 ± 0,4	6.83 ± 5,6
Type 2 Diabetes	31.15± 0,56*	0.57 ±0,09 **

*p < 0.05   ** p < 0,001

From the results above and from other studies it is believed that more than 20 % of people diagnosed with type 2 diabetes have LADA. [9] [10] If we would base our study in the level of insulin secretion, insulin concentration and C-peptide level and not in immunological data, then the percentage of LADA within type 2 Diabetes would be higher, at almost 30 %. It would mean that 30 % of them would be insulinopenic and 70% would have hyperinsulinemia. This higher level of insulinopenia in our study comes partially (19.46 %) as a result of including in the study patients with type 2 diabetes where primary hyperinsulinemia associated with insulinic resistance is substituted with hypoinsulinemia later on. This process damages the correlation between insulin secretion and metabolic syndrome. For this reason, it is recommended that criteria for LADA diagnose should include C-peptide level, insulin concentration and GAD antibodies.

4. DISCUSSION

A cross-sectional study was realized with 149 patients with diabetes, diagnosed within the first year of the onset of the disease. For all participants were taken anthropometric data, insulin plasma concentration, C-peptide level and anti – GAD level. According to the presence of anti-GAD, the participants were divided in two groups: anti-GAD positive (the LADA group) and anti-GAD negative (type 2 Diabetes) as suggested from the worldwide recognised criteria for LADA. [2]

The mean age of the participants in the study was $43,22 \pm 7,1$ years old. Patients with LADA had BMI of $25,07 \pm 3,82$, lower than in type 2 Diabetes. This result has been confirmed by other studies as well. [5] In 2001, Pozzilli and Di Mario published a review about LADA and the characteristics of patients with this diagnose. It was described as a form of diabetes that was present usually in lean bodies and BMI less than 25 kg/m^2 was part of the clinical diagnose. [11] Later studies such as a 2004 study by Zinman et al. demonstrated that even in LADA patients the BMI was higher than 25 (31.4 $\pm$ 0.466 kg/m^2). The mean BMI in our study was similar to the 2001 study and it confirmed the tendency of BMI being higher in type 2 diabetes rather than in LADA. [12]

Fasting insulin concentration was lower in LADA than in type 2 diabetes in our study ($26,8 \pm 0,4$ versus $31,5 \pm 19,3$) confirming the insulin deficiency in LADA in comparison with type 2 diabetes where the main event is insulin resistance rather than insulinopenia. This result is similar to previous studies. [13] The same goes for the level of C-peptide where in the LADA group is lower than in type 2 Diabetes.

The presence of GAD antibodies was significantly higher in LADA ($6.83 \pm 5,6$) than in type 2 Diabetes ($0.57 \pm 0,09$), confirming the importance of the immunological criteria for the diagnose of LADA. [14]

Our observations indicate that metabolic syndrome and insulin resistance are present in LADA but not higher than in type 2 diabetes as suggested from previous studies. [15]

5. CONCLUSIONS

As a conclusion, late adult-onset autoimmune diabetes is a heterogeneous form of diabetes with clinical, metabolic and immunological features that varies from the classical forms of type 1 and type 2 Diabetes. Patients with LADA develop gradually insulin deficiency as a result of the autoimmune pathological process of the beta-cells of the pancreas. Some parameters of metabolic syndrome and insulin resistance are present in LADA, but in a lesser degree than in type 2 diabetes. Nonetheless these parameters are significantly relevant and their evaluation is important in diagnosing LADA.

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