ORIGINAL ARTICLE

CLINICAL AND IMMUNOLOGICAL ASPECTS OF VERIFICATION OF LATENT AUTOIMMUNE DIABETES IN ADULTS AT EARLY STAGES OF DISEASE MANIFESTATION

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ABSTRACT

The aim: To establish diagnostic markers of LADA at the stage of manifestation based on the analysis of clinical and anamnestic data, the results of immunological examination of patients with different types of DM.

Materials and methods: Study included 121 patients with LADA (1st (main) group), 60 patients with type 1 DM (2nd group), 81 patients with type 2 DM (3d group). The examination included analysis of complaints, medical history, determination of anthropometric data, studies of the level of antibodies to glutamic acid decarboxylase (GAD ab), cytoplasmic antigen (ICA ab), tyrosine phosphatase (IA-2 ab).

Results: Criteria of LADA diagnosis included slow nature of DM course, the average age of the disease onset (45,02±9,96) years, combination of diabetic complaints with gradual weight loss, frequent detection of DM (64,46%) on request, fairly high level of glycemia at diagnosis ((14,12±4,57) mmol/l)), the possibility of ketonuria episodes in a certain amount (23,14%) of cases in the absence of acute ketoacidotic states. The presence of excess body weight and even obesity is not a criterion for excluding LADA.

Conclusions: To verify the diagnosis of LADA it is necessary to study of at least two types of antibodies. The most conclusive is the determination of GAD ab and IA-2 ab.

KEY WORDS: latent autoimmune diabetes in adults, diagnostic criteria, immunological markers

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INTRODUCTION

Nowadays diabetes mellitus (DM) is considered as a heterogeneous pathology and, according to the definition of experts from the World Health Organization, is a group of metabolic diseases characterized by chronic hyperglycemia [1]. Despite of the clear clinical and diagnostic differences between two main types of diabetes (type 1 DM and type 2 DM), it is not always possible to establish the appropriate type of this disease at the stage of its manifestation. It is argued that determination of these two main types does not reflect all possible variants of "diabetic disorders" [2]. This opinion is confirmed by new forms of diabetes identified as nonclassical, which, first of all, should include latent autoimmune diabetes in adults (LADA) [3]. The slow development of clinical symptoms at the onset of the disease and the possibility to achieve compensation using a diet and oral antihyperglycemic drugs during some period of time determine the similarity of LADA with type 2 DM. Such signs of the initial clinical manifestations of LADA are caused by much less aggressive destruction of β – cells of the pancreas and longer preservation of their secretory activity in comparison with that occurs in the classic variant of type 1 DM [4]. This is the reason for the misdiagnosis of type 2 DM at the time of the detection of this disease. However, autoimmune insulitis, which is a pathomorphological substrate of LADA, leads to a progressive depletion of β-cells and, as a consequence, the development of absolute insulin deficiency in the period from 6 months to 5 years from the time of diagnosis, with the need to prescribe insulin therapy [5].

LADA as a special form of DM was identified more than 35 years ago. During this period and till now, large-scale studies are being carried out, such as the Korea National Diabetes Program collaboratory Group – KNDP (Korea), Not Insulin-Requiring Autoimmune Diabetes – NIRAD (Italy), Nord-Trøndelag Health (HUNT) study (Norway), Action LADA (Western European countries), LADA China study (China), A Diabetes Outcome Progression Trial – ADOPT (North America and Europe) etc. However, up to now, many questions remain open. Clear clinical and diagnostic criteria have not been established yet, in particular, age parameters and clinical features of the onset of LADA, homogeneity or heterogeneity of this cohort of patients, as well as pathogenetic factors of its development have not been identified [6, 7]. Treatment tactics have not been adopted also [8, 9].

In addition to the torpid manifestation with the early development of insulin dependence compared to type 2 DM and the presence of antibodies to the structural components of pancreatic β-cells in patients like in type 1 DM, other initially described clinical and diagnostic markers are discussed. There are very different, sometimes conflicting

opinions regarding them. Even the question of the name of this form of the disease remains open. More than 30 definitions of this variant of the course of DM have been found in the literature. The term "LADA" is the most widely used. At the same time, more and more researchers consider the name "slowly progressive autoimmune diabetes in adults" to be more correct, given that diseases at the stage of clinical manifestations cannot be defined by the term "latent" [10]. So, verification of LADA in the detection of DM is an important aspect, since the further tactics of patients` management depends on it.

THE AIM

The aim of this study was to establish diagnostic markers of LADA at the stage of manifestation of the disease based on the comparative analysis of clinical and anamnestic data as well as the results of immunological examination of patients with LADA, type 1 and type 2 DM.

MATERIALS AND METHODS

262 patients with DM were observed. The examination included study and analysis of complaints and medical history; determination of anthropometric data (height, body weight, body mass index (BMI), waist circumference (WC), hip circumference (HC)), studies of general clinical and biochemical parameters, as well as the level of antibodies to glutamic acid decarboxylase (GAD ab), cytoplasmic antigen (ICA ab) and tyrosine phosphatase (IA-2 ab) using a qualitative ELISA test for detecting circulating autoantibodies (USA : Biomerica; USA : Medipan). The antibody titer was considered positive if the optical density was higher than that in the control. The control group consisted of healthy people, representative by sex and age, with DM unburdened heredity. As per study design, all patients with DM were divided into three groups. The first (main) group included 121 patients (52 men and 69 women), the average age (48.64 ± 10.07) years, with a preliminary diagnosis of LADA which was made on the basis of the anamnestic data (a slow manifestation of diseases and the development of insulin dependence in the period from 0.5 to 5 years from the diagnosis of DM). Verification of LADA was carried out by determining ICA ab, GAD ab and IA-2 ab. The diagnosis was considered to be confirmed in the presence of anamnestic data in combination with the detection of a positive titer of at least one of the antibodies. The second group consisted of 60 patients with type 1 DM (35 men and 25 women), the average age (34.38 ± 10.88) years, with classical acute manifestation of the disease, in a certain number of cases – with the development of pre-coma or coma. The third group included 81 patients (35 men and 46 women), the average age (52.44 ± 7.47) years, with diagnosis of type 2 DM, in whom the appointment of oral glucose-lowering therapy had good effect, i.e. these patients had the long-term and stable sub- or compensation of carbohydrate metabolism. The second and third groups were comparison groups. Diabetic history in all patients in the observation groups ranged from 0.5 to 6.5 years, and the groups did not differ in this indicator.

Determination of statistically significant differences between the groups was carried out using Student's test with Bonferroni, Sheff corrections. Comparison of variances in one-way analysis of variance was carried out using the Fisher test (F). To assess the association between the trait and the compared groups, the χ^2 test was used taking into account the number of degrees of freedom (df).

RESULTS

In order to determine the features of LADA manifestation, a comparative analysis of the anamnestic data of the disease onset clinical features was carried out. Taking into account the generally accepted differences in the age of manifestation of type 1 and 2 DM, first of all, this parameter was assessed. The average age of patients in the first group at the time of disease manifestation was (45.02 ± 9.96) years, in patients of the second and third groups – (31.75 ± 11.02) and (48.77 ± 7.16) years, respectively. So, patients with LADA in this indicator significantly differed both from patients with type 1 DM ($p = 0.000$) and from patients with type 2 DM ($p = 0.023$).

To identify the other most characteristic clinical signs of LADA early manifestation, a comparative analysis of some other anamnestic data in patients of the observation groups were carried out. Among them the circumstances of the diagnosis (random or targeted examination because of the appearance of diabetic complaints), factors with which the patient associated the DM development, the ratio of the time of appearance of complaints and the time of diagnosis, glycemia and the presence of ketoneuria / coma at the time of diagnosis, body weight dynamics at the manifestation of the disease were assessed. The results are presented in table I.

In addition to the data in Table 1, it should be noted that according to the anamnesis of LADA patients ketonuria was detected in 28 (23.14%) cases at the onset of the disease and in 32 (26.45%) cases on the background of oral hypoglycemic therapy, however in none of these cases, in contrast to patients with type 1 diabetes, the development of pre- or coma was recorded.

When studying anthropometric data, significant differences in BMI among patients in the observation groups ($F = 70,810$; $p = 0,000$) were found. Average BMI in patients with LADA (26.06 ± 4.13 kg / m^2) was significantly higher ($t = 3,749$; $p < 0,05$) than that in patients with type 1 DM (23,60 ± 3.42 kg / m^2) and lower ($t = 9.151$; $p < 0.05$) than in patients with type 2 DM (31.59 ± 4.67 kg / m^2). Among patients with LADA people with normal (44.63%) and overweight (36.36%) prevailed (altogether 80.99%). A comparative distribution of BMI in patients with type 1 DM was observed. Most of the patients of the 2nd group (93.33%) had normal (63.33%) and overweight (30.00%). In contrast to LADA, group of patients with type 2 DM was represented mostly (in total 92.59%) by people with overweight (27.16%) and obesity (65.43%). Moreover,

Table I. Comparative analysis of anamnestic data in patients of observation groups

Clinical and anamnestic sign	Group, number of patients					
	LADA 1 group (n = 121)		Type 1 DM 2 group (n = 60)		Type 2 DM 3 group (n = 81)	
	abs.	%	abs.	%	abs.	%
Diagnosis						
Accidentally	43	35,54	1	1,67	58	71,60
On appeals	78	64,46	59	98,33	23	28,40
Factors with which the patient associated the development of the disease						
– infection	8	6,61	11	18,33	4	4,94
– stress	68	56,20	29	48,33	32	39,51
– with nothing (the patient could not specify)	45	37,19	20	33,33	45	55,56
The appearance of diabetic complaints						
– before diagnosis	87	71,90	1	1,67	28	34,57
– simultaneously with the diagnosis	23	19,01	59	98,33	20	24,69
– some time after diagnosis	11	9,09	0	–	33	40,74
– the presence of pre- or comatose states in the manifestation of the disease	0	–	15	25,00	0	–
Dynamics of body weight at the manifestation of the disease						
– stable body weight	7	5,79	1	1,67	48	59,26
– weight loss	114	94,21	59	98,33	17	20,99
– weight gain	0	–	0	–	16	19,75
Glycemia at diagnosis						
blood sugar, mmol / l	14,12 ± 4,57		18,39±5,02		9,33 ± 2,33	

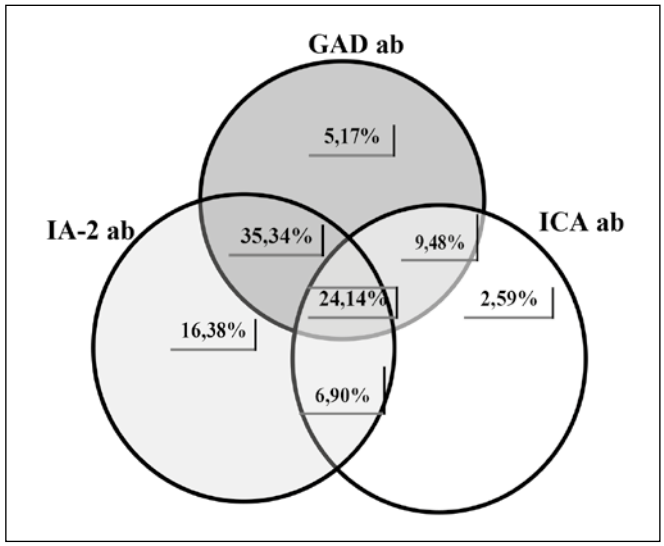

Fig. 1. Frequency of detection of autoantibody positive titer (isolated and in different combinations) in patients with LADA

25 patients (20.66%) with LADA had anthropometric markers of metabolic syndrome (MS) such as BMI greater than or equal to 30.00 kg / m^2 in combination with WC / HC ratio more than 0.85 in women and more than 0.9 in men. Thereby they were diagnosed with MS.

As already mentioned, verification of the diagnosis of "LADA" was carried out by determining the titers of diabetes – associated antibodies. The results of the GAD ab, ICA ab, IA-2 ab study indicate the presence of a total positive titer of each of the antibodies (isolated and / or in various combinations) in all patients who, according to the anamnesis, were previously diagnosed with LADA. The obtained results not only confirmed this form of diabetes, but also became a basis for verification of LADA diagnosis. The overall frequency of positive antibody titers in patients with LADA was quite high: IA-2 ab was found in 81.89% of cases, GAD ab – in 76.03%, ICA ab – in 42.37% (Fig. 1).

It should be noted that most patients with LADA had a combination of different types of antibodies. In examining of all three groups of antibodies (GAD ab, ICA ab, IA-2 ab), the presence of an isolated positive titer of only one species was observed in 28 people (24.14%). However, in patients with LADA, a positive titer of only IA-2 ab was found in more cases than isolated positive titers of GAD ab and ICA ab, which was 16.38% vs. 5.17% and 2.59%, respectively (see Fig. 1). At the same time the positive GAD ab titers (isolated and in combination with other antibodies) were

determined in 74.14% of cases, ICA ab – in 43.10% of cases, IA-2 ab – 82.76% of cases. A positive GAD ab titer and a positive IA-2 ab titer (isolated and in combination with other antibodies) were found in most patients (97.41%) in the presence of clinical signs of LADA. Meanwhile, a positive titer of none of the antibodies was determined in 100% of cases.

DISCUSSION

In our study it was found that the onset of type 1 DM occurred at a young and middle age, and did not occur in the older age group. There were no cases of type 2 DM manifestation in people under 25 years old, and the maximum incidence of type 2 DM onset was found in middle age. At the same time, unlike the classical variants of type 1 DM and type 2 DM, the manifestation of LADA was observed in all age cohorts. Isolated cases of the onset of the disease with a subsequent diagnosis of LADA were revealed both in people under 25 years old and in patients of the older age. The possibility of developing LADA-type diabetes at any age is recognized and, accordingly, such forms as Latent autoimmune diabetes in the young (LADY) and Latent autoimmune diabetes in the children (LADC) are distinguished today [11, 12. 13]. Meanwhile, the maximum number of patients in the first group (87 people) had the age between 35 and 54 years old (71.90%) at the time of disease manifestation. In spite of the statements of other authors about the comparability of the incidence of LADA among all age groups, the maximum number of cases of this form of the disease, they also determined in people of this age group [14]. Therefore, in our opinion clinicians' vigilance regarding LADA should be maximized in case of the development of slow-onset DM in patients aged 35 to 54 years.

Among the factors with which patients associated the development of the disease, stressful situations experienced on the eve of the onset of DM were highlighted, in particular, viral infection and food poisoning. According to this criterion, patients with LADA differed from patients in the comparison groups. Patients of the first group reported infection as a provoking factor significantly less often than patients of the second group ($df = 1$; $\chi2 = 4.685$; $p = 0.030$), and probably more often associated the development of diabetes with a stressful situation than patients of the third group ($df = 1$; $\chi2 = 4.761$; $p = 0.029$). Food poisoning was considered as a provoking factor of DM in only 2 patients with LADA. People who could not associate the development of the disease with anything were significantly more common in the third group ($df = 2$; $\chi2 = 9,110$; $p = 0,011$) in the absence of corresponding difference between patients of the first and second groups ($df = 1$; $\chi2 = 0.119$, $p = 0.730$) (see Table 1).

In contrast to type 1 DM with usual rapid and pronounced development of clinical signs, type 2 DM is characterized by a fairly long asymptomatic course of manifestation. Due to these circumstances, a lot of patients with type 2 DM do not seek medical help on their own, and the disease is detected by random examination [15]. According to the data obtained, the diagnosis of LADA was established upon targeted appeal due to the appearance of diabetic complaints nearly in 2/3 of cases. Incidental diagnosis in patients with LADA (first group) was observed significantly more often than in patients of the second group ($df = 1$; $\chi2 = 23\ 201$; $p = 0.000$), and much less often than in patients of the third group ($df = 1$; $\chi2 = 23.825$; $p = 0.000$) (see Fig. 1).

A targeted examination is carried out, as it is known, when a patient turns to a doctor about complaints that have arisen. The results of this study indicate that diabetic complaints in patients of the first group (LADA) occurred before diagnosis in most cases (71.90%). Patients of the second group (type 1 DM) had an acute onset of diabetes with the development in 5 people (20%) of pre- or comatose states, and the appearance of complaints and the diagnosis were almost simultaneous (98.33%). In 40.74% of patients of the third group (type 2 DM) who were diagnosed with diabetes incidentally, complaints arose some time after the detection of the disease (see Table I).

Thirst, dry mouth, general weakness were the most common complaints at the manifestation of DM. In contrast to patients of the third group, the vast majority of people of the first and second groups also indicated weight loss (see Table I). However, the rate of weight loss in patients with LADA was much slower. Weight gain in the onset of diabetes was noted only by patients of the third group.

According to the level of glycemia at the time of diagnosis, there were also significant differences among the observation groups. This indicator in patients with LADA was significantly higher than that in patients with type 2 DM ($p < 0.05$) and lower than that observed in patients with type 1 DM ($p < 0.05$). Unfortunately, the level of HbA1c at the stage of disease manifestation was known in isolated observations. As already mentioned, in a certain number of LADA patients, ketonuria was detected at different stages of the disease without the development of acute ketoacidotic conditions. In the literature, initially the possibility of detecting ketonuria in LADA was denied, but later a number of authors determined the development of ketonuria in a certain percentage of patients with this form of diabetes [16, 17].

Thus, on the basis of comparative analysis of clinical and anamnestic data, the peculiarities of LADA manifestation are highlighted. First of all, this refers to the age of the disease, the nature of the complaints, the level of glycemia and the presence of ketonuria at diagnosis. Such differences in the clinical onset of LADA and classical types of DM can be explaned by the pathomorphological substrate of this form of the disease, namely autoimmune insulitis, but with a slower destruction of the pancreatic insular apparatus in comparison with type 1 DM.

The primary view of normal or slightly reduced BMI in patients with LADA is refuted by a number of further studies that have been conducted in different populations [18, 19]. According to the results of the current study, the predominance of people with normal and overweight among patients with LADA was established, the identity of the distribution of BMI of patients with LADA and type 1 DM was confirmed. At the same time, a certain number

of obese patients (18.18%) were observed among patients with LADA. Anthropometric markers of MS were detected in 25 (20.66%) of these people. This is also consistent with the results of certain studies that indicate the phenotypic heterogeneity of patients with LADA with the development of MS in a certain proportion of them [20-22].

Thus, the obtained data indicate that neither overweight nor obesity can be considered as criteria for excluding the diagnosis of LADA. Our conclusion coincides with the point of view of other authors [23].

The established presence of a combination of different types of antibodies in most patients with LADA is a confirmation of autoimmune stroke, and reflects a certain level of autoimmune aggression in this group of patients. Meanwhile, the absence of a positive titer of any of the antibodies in 100% of cases indicates the inadequacy of determining only one type of antibody and justifies the need to study at least two types of antibodies to verify the diagnosis of LADA. According to our data the most convincing are GAD ab and IA-2 ab, which allows us to recommend the determination of these indicators for the final confirmation of the development of this form of DM.

The results of our study indicate the need for careful and accurate collection of anamnesis in patients with newly diagnosed DM. This will allow early suspicion and then using autoantibodies detection to timely confirm the LADA diagnosis.

CONCLUSIONS

1. On the basis of retrospective comparative analysis of anamnestic data together with the slow nature of the DM course, the clinical signs of LADA include: the average age of onset of the disease (45.02 ± 9.96) years, a combination of specific diabetic complaints with gradual weight loss, frequent detection of the disease (64.46%) on request, a fairly high level of glycemia at diagnosis (14.12 ± 4.57 (mmol / l)), the possibility of episodes of ketonuria in a certain number (23.14%) of cases in the absence of acute ketoacidotic conditions.
2. The presence of excess body weight and even obesity is not a criterion for excluding the diagnosis of LADA.
3. To verify the diagnosis of LADA it is necessary to study of at least two types of antibodies. The most conclusive is the determination of GAD ab and IA-2 ab.

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Conflict of interest:

The Authors declare no conflict of interest.

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