

Prevalence of Latent Autoimmune Diabetes in Adult (LADA) Among Clinically Diagnosed Type 2 Diabetic Patients

Meroj A. Jasem*

Alia A. Al-Ubaidi*

Anni Admon**

Khadeja N. Zwaer***

ABSTRACT

Background and Objectives: 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.

Aim of the Study: The purpose of this study was the evaluation of the prevalence of latent autoimmune diabetes in adults (LADA) among group of clinically diagnosed type2 diabetic patients.

Methods: Seventy four type 2 diabetic patients were tested for the presence of autoantibodies ICA, GAD, IA-2. All patients were also characterized according to clinical symptoms preceding the onset of the disease, age, BMI. ICA was measured by indirect immunofluorescence on cryostat sections of human pancreas of blood group O. GADab and IA-2ab were measured by ELISA assay.

Results: The presence of at least one autoantibody was revealed in 14/74 patients. That allowed us to recognize LADA in these cases. The coexistence of two antibodies was observed in 3/14 patients. None of the patients had three examined autoantibodies. GADab were detected more frequently (12/14) than GADab and IA-2ab, which were found with the same frequency (2/14) ($P < 0.004$).

Conclusions: Among patients with diagnosed type2 diabetes there is a group with autoimmune diabetes, called LADA. Phenotype similarity between diabetes type 2 and LADA makes the proper diagnosis impossible without more specific tests. The diagnosis of LADA is based on the presence of autoantibodies.

Key words: LADA, GADA, Type2 diabetic patients, Pancreatic islet cells autoantibodies.

INTRODUCTION:

The World Health Organization (WHO) and American Diabetes Association (ADA) acknowledged latent autoimmune diabetes in adults (LADA) as a separate entity by dividing type1 diabetes into an autoimmune and idiopathic form, the former was further subdivided into a rapidly (classic type1 diabetes) and slowly (LADA) progressive form^{1,2}. Latent autoimmune diabetes in adults (LADA) is a disorder in which, despite the presence of islet antibodies at diagnosis of diabetes, the progression of autoimmune β - cell failure is slow, showing clearly different features of both type1 and type2 diabetes³. Patients with

have several features of both type1 and type2 diabetes. Like patients with type1 diabetes, they have a positive test for pancreatic autoantibodies, which indicate the autoimmune nature of the disease. Among these antibodies are glutamic acid decarboxylase antibodies (GADAs) the enzyme that catalyses the synthesis of gamma amino butyric acid (GABA), protein tyrosine phosphatase antibodies (IA2), and antibodies directed against cytoplasmic islet antigen (ICAs)^{4,5,6}.

The presence of ICAs and/or GADA is the best predictor of the early insulin requirement⁷. If defined as a type 2 diabetic phenotype combined with islet antibodies, the prevalence of LADA is

* Biology Dep./College of Science/ AL-Mustansyria Univ.

** Immunology Dep. /Central Public Health Laboratories.

***Biochemistry Unit/ Al-Kindy Endocrinology and Diabetic Center.

among incident case subjects of diabetes aged 40-75 years⁸. A similar prevalence was found among non-insulin-requiring patients older than 35 years at diagnosis with phenotypic type2 diabetes⁹. Actually a similar frequency of LADA (10%) was found among type2 diabetic patients of all ages in the U.K Prospective Diabetes study¹⁰. Other authors have shown that 70-80% of LADA cases tested positive for GADA¹¹, while Schranz has noted that GADA and IA2 titers are lower in LADA than in type 1 DM patients¹². Prospective study of β -cells function showed that LADA patients with multiple islet antibodies develop β -cell failure within 5 years, whereas those with only GAD antibodies (GADAs) or only islet cell antibodies (ICAs) mostly develop β -cell failure after five years. Eventhough it may takes up to 12 years until β -cell failure occur in some patients, impairment in the β -cells response to intravenous glucose and glucogan can be detected at diagnosis of diabetes¹³. Correct diagnosis of LADA is not easy if solely on clinical ground, and since LADA can be often misclassified as type2 diabetes because of the age at onset, it was the aim of this study to evaluate the prevalence of LADA clinical phenotype in patients clinically considered to have Type 2 diabetes.

MATERIAL AND METHODS:

Subjects. A total of 74 patients (44 females, 30 males) (mean of age 41.4 ± 9.8) clinically diagnosed as affected by type2 diabetes was studied. Participating patients were consecutively recruited from Al-Kindy Diabetic Center. Inclusion criteria were (a) age at onset in 30 to 49 years (b) disease duration more than six months (c) treated with diet or hypoglycemic agents. Exclusion criteria were (a) overt type 1 diabetes (b) diabetes secondary to other diseases like hypertension (c) patients with BMI of 30 or more (d) patients with family history of diabetes. A clinical questioner containing personal, historical and clinical data was filled by the participating patients including

family history of diabetes (first degree relatives), treatment, and body weigh and height to measure body mass index (BMI).

Sample collection. Blood samples were taken from patients and control groups after an overnight fasting period. Sera were isolated within 1 h of blood sampling and stored at -20°C until further use.

Islet autoantibody determination:

ICA. Islet cell antibodies were detected by the indirect immunofluorescence technique. Frozen sections of monkey pancreas (EUROIMMUNE/ Germany) are incubated with diluted patients' samples. If the reaction is positive, specific antibodies of classes IgA, IgG and IgM attach to the tissue antigens. In a second step, the attached antibodies are stained with fluorescein-labeled anti-human antibodies and made visible with fluorescence microscope.

GADA. Glutamic acid decarboxylase antibodies were detected by ELISA, human recombinant glutamic acid decarboxylase, isoform GAD65, was used for coating the microplate and preparation of the biotenyated GAD. If the sample is positive, specific antibodies bind to the GAD. Bound antibodies are able to act divalently and form a bridge GAD, which is added in a second incubation step. To detect the bound biotin, a third incubation is carried out using enzyme – labeled avidin, which is capable of promoting a color reaction. The upper limit of the normal range (cut-off value) recommended by (EUROIMMUNE/ Germany) is 10 international per milliliter (IU/ml), so results above 10 (IU/ml) consider positive.

IA-2A. Insulinoma-associated antigen 2 antibodies were detected by ELISA, with a test principle and procedure similar to that of GADAs. The upper limit of the normal range (cut-off value) recommended by (EUROIMMUNE/ Germany) is 20 international per milliliter (IU/ml), so results above 20 (IU/ml) consider positive.

Statistical Analysis: All statistical analyses were performed with 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 done with the t- test. The result was considered significant at $p < 0.05$.

RESULT:

The results of the immunological findings (ICA, GADab, IA-2ab) are presented in (Figure 1)

Figure (2 a, b): represents concentrations of both GAD and IA-A2 autoantibodies in the patients.

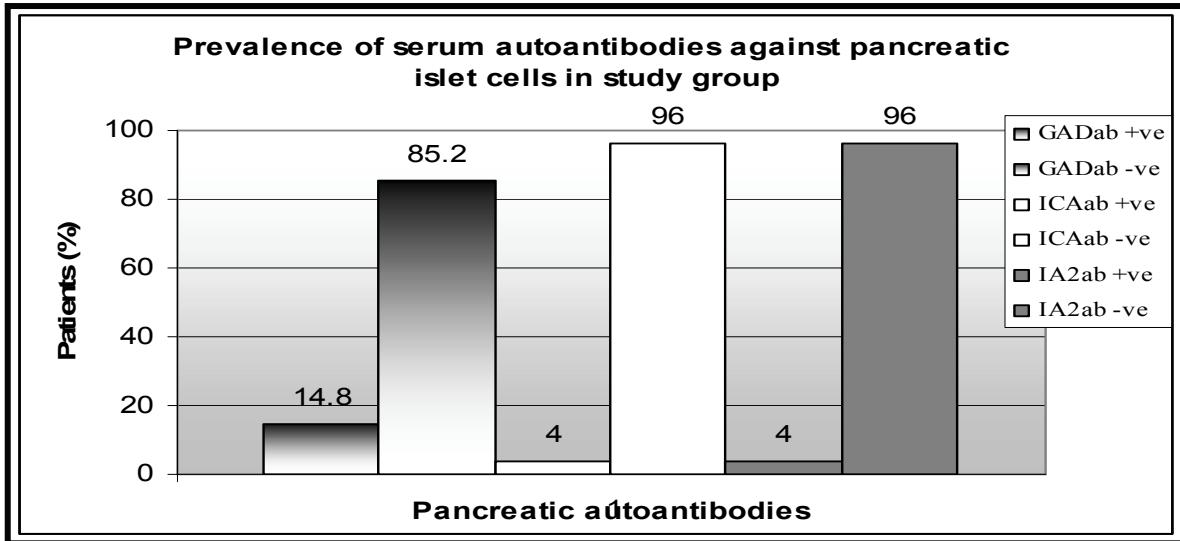


Figure 1: Prevalence of serum autoantibodies against pancreatic islet cells in study group.

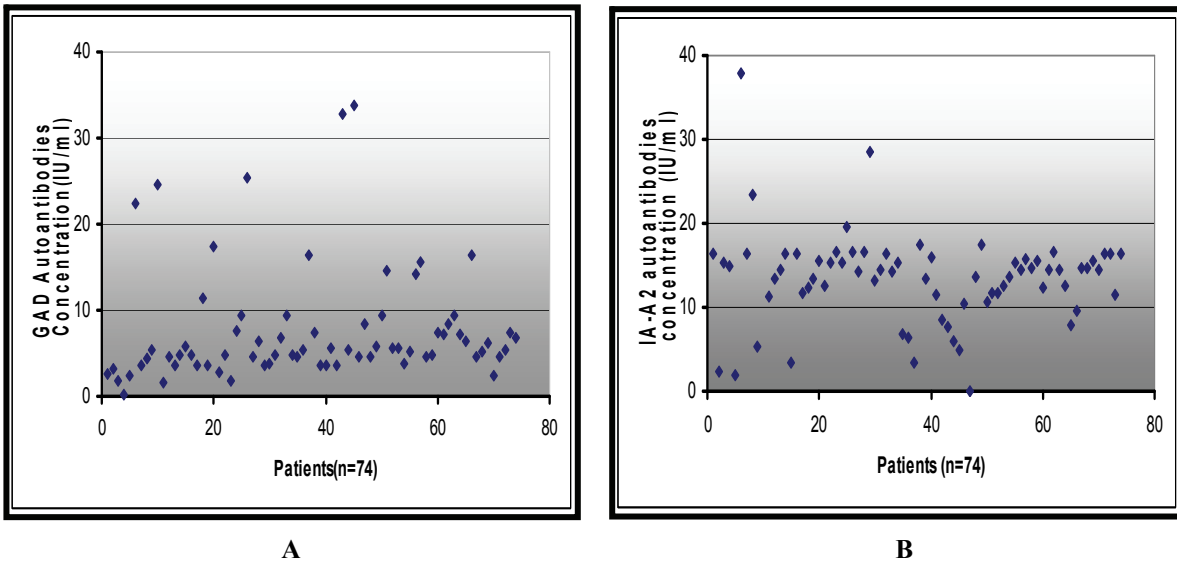


Figure 2: Concentrations of GAD (A) and IA-A2 (B) autoantibodies in total patients

At least one kind of serum autoantibody was found in 14/74 subjects (18.9%). None of autoantibodies was found in 60/74 subjects (81.1%). Only one kind of autoantibody was detected in 10/74

Immunological investigations indicated group of 14 patients with serum autoantibodies against pancreatic islet cells. The most common autoantibody was GAD abs (12/14), whereas ICA and IA-2ab

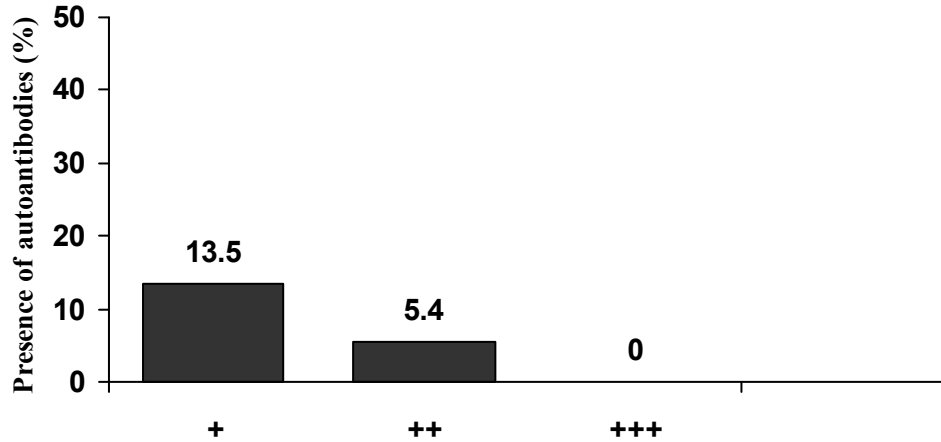


Figure 3: Prevalence of coexistence 1, 2 or 3 types of auto antibodies

(13.5%). The coexistence of two different autoantibodies was noted in 4/74 patients (5.4%). None of the patients had three concomitant autoantibodies (Figure 2).

were equally distributed (2/14) (Figure 3). There were no significant differences between autoantibody positive and auto antibody negative patients regarding age, sex and BMI.

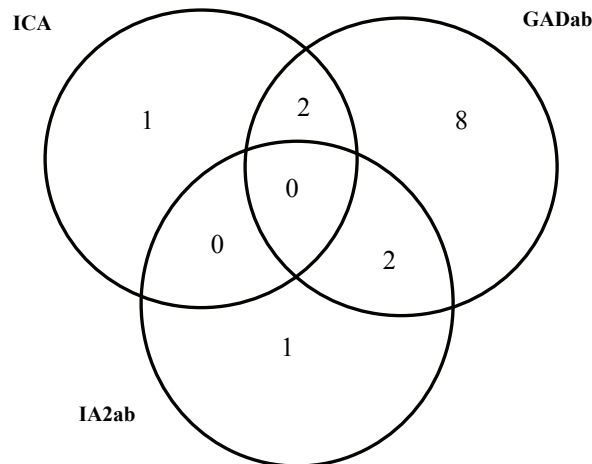


Figure 4: Number of patients in whom the presence of one specific type of auto antibodies was noticed

DISCUSSION:

The diagnosis of LADA is difficult due to a lack of defining features; most authors propose that LADA should have three features including: adult age at diagnosis, the presence of diabetes-associated autoantibodies, and delay from diagnosis in the need for insulin therapy to manage hyperglycemia¹⁴. However, the first and last are not categorical traits, being dependent on the mode of ascertainment and decision making by physicians. The second feature lacks disease specificity because it is based on positivity for auto antibodies found in type 1 diabetes mellitus. In a recent review¹⁵, it was suggested that LADA patients should be diagnosed with non-insulin-requiring diabetes at age 30 yr or older and that age (range 30–70 yr) was also used in a major European Union initiative (www.actionlada.org); in addition, both defined LADA to include patients who had 6 months without insulin treatment after diagnosis¹⁵. While Juneja et al. found that only islet antibodies (islet cell antibodies ICAs or GAD antibodies GADAs defined LADA; not age, BMI, or clinical presentation¹⁶. In our population with type 2 diabetes mellitus, 60 out of 74 subjects had no autoantibodies but 14 out of 74 (18.9%) had at least one serum autoantibody. In 3 patients out of those 14, concomitant two autoantibodies were found and none of the patients had all three autoantibodies. Using the above described criteria we may say that the prevalence of LADA in our patients is about 18.9%. This prevalence in patients phenotypically diagnosed as type2 diabetes is in agreement with prevalence of LADA in patients reported by Torn et al. demonstrated that 47% of type 2 and 59% of unclassifiable patients at diagnosis in the age range from 15 to 34 years had B- cell autoimmune antibodies in Sweden¹⁷. Humphrey et al demonstrated that 37% of adult onset insulin treated patients had positive GADA levels on a population

GADA in 680 clinical type2 patients¹⁹. In contrast, Lutale et al found low prevalence of GADA and ICA2 autoantibodies (7.3%) among the young onset diabetes subjects of African origin in Dar esSalaam, Tanzania. concluding low prevalence of LADA among these patients²⁰. Park et al also revealed low prevalence of GADA of 1.7% in newly diagnosed type 2 Korean patients age over 30 years from a population based study²¹ and so do Thai et al²². The prevalence of LADA in Italy was estimated in a population based study and it was 2% of all cases of adult diabetes and 2.8% of those diagnosed²³. Nevertheless, it should be noted that the results of the majority of studies on the prevalence of autoantibodies in type 2 diabetic patients are not consistent. Probably this is due to nonhomogenous populations studied with regard to the number of patients, age and ethnicity. Unfortunately, LADA remains clinical controversy. In a recent editorial, Gale questions LADA as a distinct disease entity, he states that there is currently no reason to assume that the etiology underlying LADA is any different from that of DM1. Thus, the current subdivision of autoimmune diabetes into DM1 and LADA is artificial²⁴. In the other hand Yang et al investigated the possibility of using carboxypeptidase-H antibody which is an insulin granule component serving in proinsulin processing, and involved in the production of peptide hormones and neurotransmitters, as a differentiation tool to detect subsets within LADA²⁵. In conclusion, this study, which was carried out on patients with adult-onset clinically diagnosed type2 diabetes, had indicated a proportion of autoimmune diabetes among them. Since the clinical phenotype alone may not help to characterize patients with adult- onset autoimmune diabetes (LADA), it is our recommendations that physician should request for the pancreatic auto antibodies which represents the best tool for a correct classification as a necessary prerequisite for a correct therapeutic

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