PREVALENCE OF (LADA) AMONG CLINICALLY DIAGNOSED TYPE 2 DIABETIC PATIENTS

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SUMMARY: 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 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.

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 preceeding the onset of the disease, age, BMI. ICA were measured by indirect immunofluorescence on cryostat sections of human pancreas of blood group O. GADab and IA-2ab were measured by ELISA assay.

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) ($P < 0.004$).

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 diabetes (3). Patients with LADA 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.

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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 requirement (7). If defined as a type 2 diabetic phenotype combined with islet antibodies, the prevalence of LADA is around 10% among incident case subjects of diabetes aged 40-75 years (8). A similar prevalence was found among non- insulin- requiring patients older than 35 years at diagnosis with phenotypic type2 diabetes (9). Actually a similar frequency of LADA (10%) was found among type2 diabetic patients of all ages in the U.K Prospective Diabe tes study (10).Other authors have shown that 70-80% of LADA cases tested positive for GADA (11), while Schranz has noted that GADA and IA2 titers are lower in LADA than in type 1 DM patients (12).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 diabetes (13).

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.

MATERIALS 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 "age, sex, age at onset, disease duration, 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 biotenylated 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

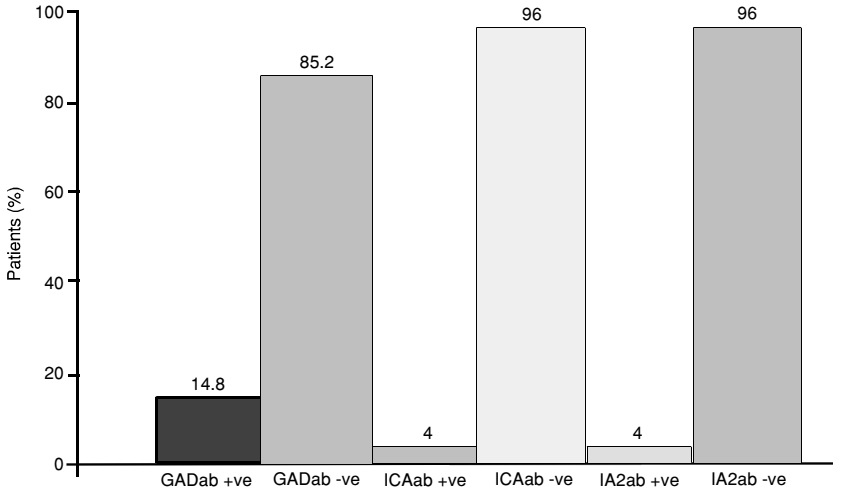
All statistical analyses were performed with Statistical Package for Social Science (SPSS) 7.5. Data were analyzed for mean and standard deviation. Proportions were expressed as percentage while significant tests were done with the t- test. The result was considered significant at $p < 0.05$.

RESULTS

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

At least one kind of serum autoantibody was found

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



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 subjects (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).

Immunological investigations indicated group of 14 patients with serum autoantibodies against pancreatic islet cells. The most common autoantibody was GAD ab(12/14), whereas ICA and IA-2ab were equally distributed (2/14) (Figure 3). There were no significant differences between autoantibody positive and autoantibody negative patients rgarding age, sex and BMI.

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 hyperglycemia (14). 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 autoantibodies found in type 1 diabetes mellitus. In a recent review (15), 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 diagnosis (15). While Juneja *et al.* found that only islet antibodies (islet cell antibodies ICAs or GAD antibodies GADAs defined LADA; not age, BMI, or clinical presentation (16).

In our population with type 2 diabetes mellitus, 60 out of 74 subjects had no autoantibodies but 14 out of 74

Figure 2: Prevalence of coexistence 1, 2 or 3 types of autoantibodies.

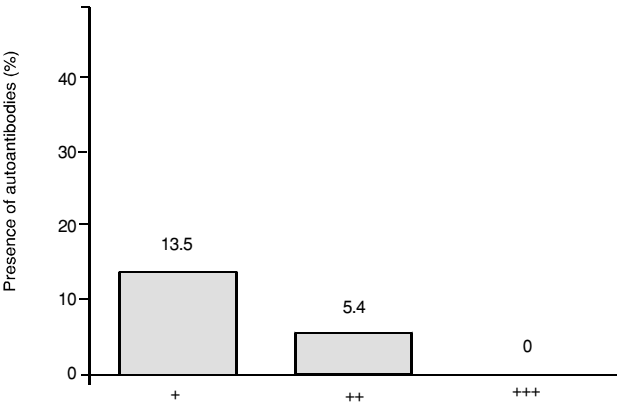
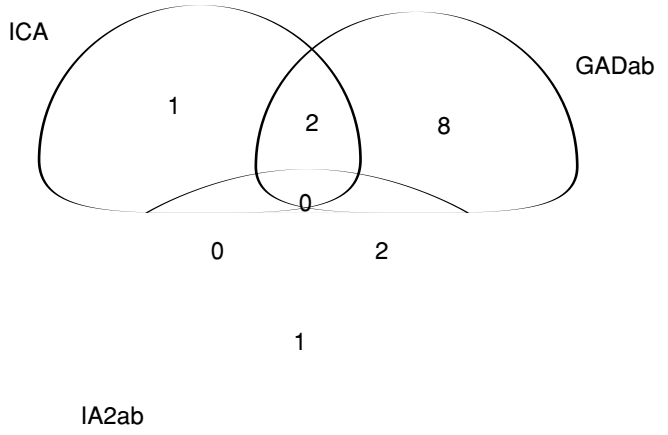


Figure 3: Number of patients in whom the presence of one specific type of autoantibodies was noticed.



(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 by Torn *et al.* demonstrated that 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 Sweden (17). Humphrey *et al.* demonstrated that 37% of adult onset insulin treated patients had positive GADA levels on a population based study in Australia (18). One Japanese multi-center study revealed a prevalence of 4.3% of GADA in 680 clinical type2 patients (19).

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 patients (20). 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 study (21) and so do Thai *et al.* (22). 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 diagnosed (23). 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 artificial (24). 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 LADA (25).

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 autoantibodies which represents the best tool for a correct classification as a necessary prerequisite for a correct therapeutic appraisal.

REFERENCES

1. WHO/ADA Report for the expert commetii on the diagnosis and classification of diabetes militus. Diabetes Care, 20:1183-1197, 1997.

2. Alberti KG, Zimmet PZ: Definition, diagnosis and classification of diabetes mellitus and its complications. Part 1: diagnosis and classification of diabetes mellitus provisional report of a WHO consultation. Diabet Med, 15:539-553,1998.

3. Stenström G, Gottsäter A, Bakhtadze E, Berger B, Sundkvist G: Diabetes. Latent autoimmune diabetes in adults: definition, prevalence, beta-cell function, and treatment, 54 Suppl 2:S68-72, 2005.

4. Borg H, Gottsater A, Landin-Olsson M, Fernlund P, Sundkvist G: High levels of antigen-specific islet antibodies predict future beta-cell failure in patients with onset of diabetes in adult age. J Clin Endocrinol Metab, 86(7):3032-3038, 2001.

5. Fourlanos S, Perry C, Stein MS, Stankovich J, Harrison LC, Colman PG: A clinical screening tool identifies autoimmune diabetes in adults. Diabetes Care, 29(5):970-975, 2006.

6. Van Deutekom AW, Heine RJ, Simsek S. The islet autoantibody titres: their clinical relevance in latent autoimmune diabetes in adults (LADA) and the classification of diabetes mellitus. Diabet Med, 25(2):117-125, 2008.

7. Schiel R, Müller UA: GAD autoantibodies in a selection-free population of insulin-treated diabetic patients: indicator of a high prevalence of LADA? Diabetes Res Clin Pract, 49:33-40, 2000.

8. Pietropaolo M, Barinas-Mitchell E, Pietropaolo SL, Kuller LH, Trucco M: Evidence of islet cell autoimmunity in elderly patients with Type 2 diabetes. Diabetes, 49:32-38,2000.

9. Lohmann T, Keller K, Verlohren HJ, Krug J, Steindorf J, Scherbaum WA, Sessielr J: Titre and combination of ICA and autoantibodies to glutamic acid decarboxylase disrimate two clinically distinct types of latent autoimmune diabetes in adults (LADA). Diabetologia, 44:1005-1010, 2001.

10. Turner R, Stratton I, Horton V, Manley S, Zimmet P, Mackay IR, Shattock M, Bottazzo GF: For UK Prospective Diabetes Study (UKPDS) Group. UKPDS 25: Autoantibodies to islet-cell cytoplasm and glutamic acid decarboxylase for prediction of insulin requirement in type 2 diabetes. Lancet, 350:1288-1293,1997.

11. Differentiating slowly developing type 1 from true type 2? www.medscape.com, Experts and Viewpoint, Medscape Diabetes and Endocrinology, August, 2000.

12. Schranz DB, Bekris L, Landin-Olsson M: Newly diagnosed latent autoimmune diabetes in adults (LADA) is associated with low level glutamat decarboxylase (GAD65) and IA-2 antibodies. Diabetes Incidence Study in Sweden (DISS). Horm Metab Res, 32:133-138, 2000.

13. Borg H, Gottsäter A, Fernlund P, Sundkvist G: 12-Year Prospective Study of the Relationship Between Islet Antibodies and ß-Cell Function at and After the Diagnosis in Patients With Adult-Onset Diabetes. Diabetes, 51(6): 1754-1762, 2002.

14-. David D, Leslie G, Williams R, Pozzilli P: Type 1 Diabetes and Latent Autoimmune Diabetes in Adults: One End of the Rainbow The Journal of Clinical Endocrinology and Metabolism, 91(5):1654-1659, 2006.

15. Fourlanos S, Dotta F, Greenbaum CJ, Palmer JP, Rolandsson O, Colman PG, Harrison LC: Latent autoimmune diabetes in adults (LADA) should be less latent. Diabetologia, 48:2206-2212, 2005.

16. Juneja R, Hirsch IB, Naik RG, Brooks-Worrell BM, Greenbaum CJ, Palmer JP: Islet cell antibodies and glutamic acid decarboxylase antibodies, but not the clinical phenotype, help to identify type 1(1/2) diabetes in patients presenting with type 2 diabetes. Metabolism, 50:1008-1013, 2001.

17. Torn C, Landin-Olsson M, Ostman J: Glutamic acid decarboxylase antibodies (GADA) is the most important factor for prediction of insulin therapy within 3 years in young adult diabetic patients not classified as Type 1 diabetes on clinical grounds. Diabetes Metab Res Rev, 16:442-447, 2000.

18. Humphrey ARG, McCarty DJ, Mackay IR, Rowley MJ, Dwyer T, Zimmet PZ: Autoantibodies to glutamic acid decarboxylase and phenotypic features associated with early insulin treatment in individuals with adult-onset diabetes mellitus. Diabet Med, 15:113-119, 1998.

19. Takeda H, Kawasaki E, Shimizu I, Konoue E, Fujiyama M, Murao S, Tanaka K, Mori K, Tarumi Y, Seto I, Fujii Y, Kato K, Kondo S, Takada Y, Kitsuki N, Kaino Y, Kida K, Hashimoto N, Yamane Y, Yamawaki T, Onuma H, Nishimiya T, Osawa H, Saito Y, Makino H: Clinical, autoimmune, and genetic characteristics of adult-onset diabetic patients with GAD autoantibodies in Japan (Ehime Study). Diabetes Care, 25:995-1001, 2002.

20. Lutale JJK, Thordarson H, Holm PI, Eide GE , Vetvik K. Islet cell autoantibodies in African patients with Type 1 and Type 2 diabetes in Dar es Salaam Tanzania: a cross sectional study. J Autoimmune Dis, 4:4-8, 2007.

21. Park Y, Lee HK, Koh CS, Min H, Rowley M, Mackay IR: The low prevalence of immunogenetic markers in Korean adult-onset IDDM patients. Diabetes Care, 19:241-245,1996.

22. Thai AC, Ng WY, Loke KY, Lee WRW, Lui KE and Cheah JS. Anti-GAD antibodies in Chinese patients with youth

and adult-onset IDDM and NIDDM. Diabetologia, 40:1425-1430, 1997.

23. Maioli M, Alejandro E, Tonolo G, Gilliam LK, Bekris L, Hampe CS, Obinu D, Manconi A, Puddu L, Lynch C Lernmark A: Epitope-Restricted 65-Kilodalton Glutamic Acid Decarboxylase Autoantibodies among New-Onset Sardinian Type 2 Diabetes Patients Define Phenotypes of Autoimmune Diabetes The Journal of Clinical Endocrinology and Metabolism, 89(11):5675-5682, 2004.

24. Gale EA: Latent autoimmune diabetes in adults: a guide for the perplexed. Diabetologia, 48:2195-2199, 2005.

25. Yang L, Zhou ZG, Tan SZ, Huang G, Jin P, Yan X, Li X, Peng H, Hagopianb W: Carboxypeptidase-H Autoantibodies Differentiate a More Latent Subset of Autoimmune Diabetes from Phenotypic Type 2 Diabetes among Chinese Adults Immunology of Diabetes V: Ann NY Acad Sci,1150:263-266, 2008.

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