Acta Diabetol (2012) 49:327–331
DOI 10.1007/s00592-010-0196-2

ORIGINAL ARTICLE

Insulin autoantibody could help to screen latent autoimmune diabetes in adults in phenotypic type 2 diabetes mellitus in Chinese

Gan Huang · Xia Wang · Zhangwei Li · Hui Li · Xia Li · Zhiguang Zhou

Received: 27 January 2010 / Accepted: 21 April 2010 / Published online: 16 May 2010

Abstract Latent autoimmune diabetes in adults (LADA) is characterized by a relatively mild diabetes onset, autoantibody positivity, and eventual requirement for insulin therapy. Glutamic acid decarboxylase autoantibodies (GADA) or cytoplasmic islet cell autoantibodies (ICA) play a key role in distinguishing LADA from type 2 diabetes mellitus (T2DM) in clinical practice. The aim of our research was to determine whether insulin autoantibody (IAA) has some additional value in diagnosing LADA. We analyzed IAA, GADA, and IA-2A (antibodies to insulinoma-associated antigen-2) in 1,003 newly diagnosed phenotypic T2DM patients, 110 type 1 diabetes mellitus (T1DM) patients, and 317 normal controls to survey the prevalence of IAA in phenotypic T2DM patients and the overlapping positivity of IAA with other autoantibodies. Sera were drawn within 7 days from the start of insulin therapy. Results showed that 3.39% of the newly diagnosed phenotypic T2DM, 0.95% of normal control ($\chi^2 = 5.3$, $P < 0.05$), and 21.82% of T1DM ($\chi^2 = 68.2$, $P < 0.001$) were positive for IAA at diagnosis. The combination frequency of three antibodies was 10.47%, which was higher than any single antibody testing. Combination testing of IAA with GADA and IA-2A could improve LADA diagnose rate by 2.39% than that of GADA and IA-2A. IAA-positive subjects had diabetes family history more common compared to its matched group (67.6% vs. 14.7%, $P = 0.000$). Postprandial C-peptide in IAA-positive group tended to be lower, but the difference was not statistically significant ($P = 0.084$). We concluded that IAA can be used to screen LADA in phenotypic T2DM in the Chinese population.

Keywords Insulin autoantibody (IAA) · Type 2 diabetes mellitus · Latent autoimmune diabetes in adults

Introduction

Autoimmune diabetes is a consequence of progressive destruction of beta cells in the islets of Langerhans, which includes type 1 diabetes mellitus (T1DM) and latent autoimmune diabetes in adults (LADA) [1, 2]. Islet antibodies were identified in approximately 10% of phenotypic type 2 diabetes (T2DM) patients [3–5]. Humoral immune responses to beta-cell antigens such as GADA or cytoplasmic islet cell autoantibodies (ICA) are valuable autoimmune markers for disease activity and are suitable for LADA identification [6]. ICA is usually measured by an indirect immunofluorescence method, which is influenced by many factors and is difficult to standardize. Standardized GADA testing is sensitive and has been recognized as the most effective immune marker for LADA diagnosis [7]. The United Kingdom Prospective Diabetes Study (UKPDS) showed that the combination of ICA and GADA predicted insulin requirement better than either measure on its own [8]. As a consequence, exploring the combination of GADA and other islet antibodies becomes necessary and is beneficial in determining the proper diagnosis.

IAA, which is directed against the insulin molecule, reflects the islet beta-cell destructive process through a variety of mechanisms and is considered to be the most

G. Huang and X. Wang contributed equally to this work.

G. Huang · X. Wang · Z. Li · H. Li · X. Li · Z. Zhou (✉)
Diabetes Center, Institute of Metabolism and Endocrinology, Second Xiangya Hospital, Central South University, 139 Renmin Zhong Road, 410011 Changsha, Hunan, China
e-mail: zhouzg@hotmail.com

useful method for identifying subjects who are at high risk of developing T1DM [9]. Conventional measurement of IAA includes a fluid-phase radioimmunoassay (RIA) and a solid-phase enzyme-linked immunosorbent assay (ELISA). Large serum volume and inability to distinguish IAA from antibodies produced in response to exogenous insulin limited the use of IAA. Williams et al. established a novel micro-assay in 1997 [10], which had higher sensitivity and specificity, required much less serum, could easily be automated, and was high throughput. Diabetes autoantibody standardization program (DASP) workshops found that micro-assays for IAA are superior to conventional RIA and ELISA assays and are particularly useful in young at-risk individuals and infants [11].

However, little data is available concerning the prevalence of IAA in phenotypic T2DM patients. Therefore, we tested IAA using micro-assays, and GADA and IA-2A using radiobinding assays in 1,003 phenotypic T2DM patients and to test whether IAA can help identify LADA patients.

Methods and materials

Subjects

This study was carried out according to the Helsinki guidelines. All patients gave informed consent to participate in the study, and the protocol was approved by the research ethics committee in Second Xiangya Hospital of Central South University. We studied 1,003 patients with phenotypic T2DM at clinical onset (539 men, 464 women, mean age 52.2, range 25–80, mean duration 0.2 years, range 0–1 years) in Second Xiangya Hospital of Central South University from Oct 2003 to Mar 2007, which were randomly selected from the newly diagnosed diabetic patients in our hospital. One hundred and ten cases of T1DM patients (66 men, 44 women, mean age 20.4, range 1.5–60, mean duration 0.14 years, range 0–2 years) were also included for comparison. Sera were drawn within 7 days from the start of insulin therapy. Three hundred and seventeen healthy persons (168 men, 149 women, mean age 36.3, range 2.5–77) with normal OGTT and no family history of diabetes or other autoimmune diseases were included as normal controls.

The inclusion criteria of phenotypic T2DM were as follows: (1) diabetes diagnosed with 1999 WHO definition, (2) onset age more than 25 years old, (3) duration was less than 1 year, (4) no ketosis or ketoacidosis occurrence within half a year, and (5) no history of secondary diabetes or other autoimmune diseases. The inclusion criteria of T1DM patients were as follows: (1) diabetes diagnosed with 1999 WHO definition, (2) ketosis or ketoacidosis occurrence within half a year, (3) insulin dependency since onset, and (4) no history of secondary diabetes or other autoimmune diseases.

IAA assay

As described by Woo et al. [12], 5 μl/well of serum was added to V bottom polypropylene microtiter plates (Costar 3894, Corning Incorporated, USA). Each plate included positive and negative control with each sample run in duplicate. After addition of 20,000 cpm ^{125}I-insulin (3-[^{125}I] iodotyrosyl A14)- rh insulin(PerKinElmer, USA), the plates were gently mixed at 4°C for 72 h on an orbital plate shaker to form immuno-complexes. Immuno-complexes were then transferred to high protein-binding Millipore plates (Millipore Cat MHAB N45, Millipore Corporation, France) coated with protein A (10 mg/dl, Zymed Laboratories, USA). Millipore plates were gently mixed for 45 min at 4°C. Then, 125 μl of TBT buffer (50 mM Tris, 1% Tween-20, pH 7.2) was added to each well and removed by aspiration eight times. After the plates were allowed to dry, 25 μl of scintillation fluid (Otiphase Supermix, Wallac, Finland) was added to each well, and the plates were loaded into the liquid scintillation and luminescence counter (MicroBeta Trilux,Wallac, Finland). An antibody "index" was calculated as: Index = (test serum cpm-negative control cpm)/(positive control cpm-negative control cpm), and the threshold cutoff was defined as 0.06 (99th percentile of 317 normal controls). The intra-assay CV was between 4.8 and 8.9%, and the inter-assay CV was between 6.4 and 10.5%. The sensitivity and specificity in our laboratory were 50 and 97%, respectively, evaluated in the 4th Diabetes Autoantibody Standardization Program (DASP 2005) sponsored by Immunology of Diabetes Society (IDS).

GADA and IA-2A assay

GADA and IA-2A were measured by a radiobinding assay based on ^{35}S-labeled recombinant GAD65 or IA-2, as described previously [13], with all samples measured in duplicate. The cutoff point of GADA index was 0.05, and IA-2A index was 0.007, which were determined according to the 99.5% upper limit of 188 healthy controls. The sensitivity and specificity of the GADA assay were 80% and 98%, respectively, and those of the IA-2A assay were 72 and 99%, respectively, which were evaluated in the 4th Diabetes Autoantibody Standardization Program (DASP 2005).

Clinical and biochemical assay

The C-peptide and insulin levels were tested by Bayer Centaur Immunoassay System. HbA1c was tested by

ion-exchange HPLC with the normal range of 3.9–6.1%, and other biochemical measurements were assayed using automatic chemistry analyzer. The homeostasis model assessment of insulin resistance (HOMA-IR) index was calculated based on the conventional formula: HOMA-IR = fasting glucose (mmol/l) × fasting insulin (mU/l)/22.5, and the homeostasis model assessment of insulin secretion (HOMA-IS) index was as follows: HOMA-IS = 20 × fasting insulin (mU/l)/[fasting glucose (mmol/l)−3.5] [14].

Statistics

All analyses were performed using SPSS 13.0 software, the data was presented as mean ± standard deviation. Discrete variables were presented as total number (%) and were compared by chi-square test or the Fisher's exact test when appropriate. One-way analysis of variance (ANOVA) test and two-sample *t*-tests were used for normally distributed data; otherwise Wilcoxon signed rank test was used. The indication of significance is $P < 0.05$.

Results

IAA frequency in phenotypic T2DM, T1DM, and normal controls

Overall, the frequency of IAA in phenotypic T2DM, T1DM, and normal controls was 3.39%(34/1003), 21.82% (24/110), and 0.95%(3/317), respectively. IAA positivity was significantly higher in T2DM than in normal control ($\chi^2 = 5.3$, $P < 0.05$), but was lower than that in T1DM ($\chi^2 = 68.2$, $P < 0.001$).

We had 25 to 34-year, 35 to 44-year, 45 to 54-year, 55 to 64-year, and above 65-year-old groups among 1,003 phenotypic T2DM patients. The frequency of IAA in each different group was 0% (0/56), 4.88% (10/205), 2.90% (9/310), 3.29% (10/304), and 3.91% (5/128), respectively. No significant difference was found among different age groups.

Diabetes autoantibodies in phenotypic T2DM

Among the 34 IAA-positive phenotypic T2DM patients, 70% (24/34) were positive for IAA alone, 21% (7/34) were positive for both IAA and GADA, and 9% (3/34) were positive for IAA, GADA, and IA-2A. The frequency of the three antibody combination was 10.47%, which was higher than that of any single antibody testing and that of IA-2A and IAA combination ($P < 0.05 \sim 0.01$). IAA testing in combination with GADA and IA-2A improves LADA diagnose rate by 2.39% than GADA and IA-2A testing (Fig. 1 and Table 1).

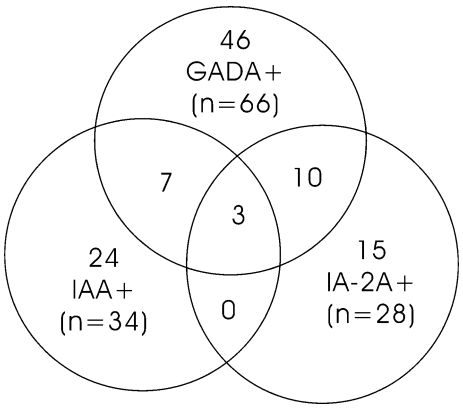

Fig. 1 GADA, IA-2A, and IAA in phenotypic T2DM

Table 1 Frequency of various antibodies combinations in 1,003 phenotypic T2DM patients

	n	Frequency (%)
IAA Positive	34	3.39**
GADA Positive	66	6.58*
IA-2A Positive	28	2.79**
IAA or IA-2A Positive	59	5.88**
GADA or IA-2A Positive	81	8.08
IAA or GADA Positive	90	8.97
IAA or GADA or IA-2A Positive	105	10.47

Compared to frequency of IAA- or GADA- or IA-2A-positive combination,* $P < 0.05$, ** $P < 0.01$

Comparison of clinical characteristics between IAA-positive alone T2DM group and IAA-negative matched group

We chose 68 phenotypic T2DM subjects matched for age, sex, duration, GADA, and IA-2A status with 34 IAA-positive subjects at a ratio of 1:2. IAA-positive subjects had diabetes family history more common compared to their matched group [67.6% (23/34) vs. 14.7% (10/68), $P = 0.000$]. Postprandial C-peptide in IAA-positive group tended to be lower, but the difference was not statistically significant [864 (137–3,559) pmol/L vs. 1,307 (242–3,494) pmol/L, $P = 0.084$]. Other clinical characteristics showed no significant difference between two groups.

Furthermore, we compared clinical characteristics of three single antibody-positive groups. The level of HbA1C was lower, and total cholesterol was higher in the IAA only group, with statistically significant differences from those of GADA-positive only group (*P* value was 0.010 and 0.017, respectively), and no significant differences between IA-2A only and the antibody-negative group. In addition, GADA only group had a lower WHR, triglycerides, and total cholesterol than the antibody-negative group (*P* value was 0.04, 0.000, and 0.002, respectively).

Discussion

Although multiple islet autoantigens are recognized by T lymphocytes and many autoantibodies could be detected before the development of autoimmune diabetes, there is increasing evidence that autoimmunity to insulin may be central to disease pathogenesis [15]. IAA was first identified in the sera of newly diagnosed insulin-naive T1DM patients in USA by Palmer et al. [16], who proposed that they were immune markers of beta-cell damage. IAA now has been demonstrated as an important autoimmune marker for the diagnosis and prediction of T1DM [17]. Nevertheless, phenotypic T2DM with IAA can also be classified as LADA in line with IDS (2005) [18], little data has been available concerning the prevalence of IAA in phenotypic T2DM patients so far. To our knowledge, our study is novel in that there are no such studies from a large Chinese population like this and little in the way of IAA in other countries.

Our study has shown that the prevalence of IAA in phenotypic T2DM patients is 3.39%, and the frequency of IAA, GADA, and IA-2A in combination was 10.47%, which was similar to 11.6% of ICA, GADA, and IA-2A combination in European from UKPDS [5]. IAA testing in combination with GADA and IA-2A improves LADA diagnose rate by 2.39% than GADA and IA-2A testing, which gives further evidence to support IAA increases LADA diagnosis sensitivity. The combination of IAA *plus* GADA, GADA *plus* IA-2A, and IAA *plus* IA-2A was 85.7, 77.1, and 56.2%, which infers that the IAA *plus* GADA combination is the most effective among the two-antibody combinations.

Family history in LADA patients with IAA is significantly different from that in matched group, which infers that LADA patients with IAA have a strong genetic background. LADA patients with IAA tended to have lower C-peptide levels, which may indicate IAA-positive LADA patients have more limited beta-cell function than T2DM. We compared the clinical characteristics of the IAA only group, the GADA only group, the IA-2A only group, and the triple negative group. We observed that the IAA alone group had a lower level of HbA1C than the GADA alone group, which reflects that patients with single IAA positivity had a better glucose control than GADA alone patients. No statistical difference could be found in the other clinical characteristics among the IAA alone group, the IA-2A alone group, and the three-antibody negative group, which infers that LADA patients with IAA share similar clinical characteristics with T2DM patients.

Our results showed that no difference was found between IA-2A-positive patients and T2DM patients, but GADA patients were thinner with lower cholesterol than T2DM patients. According to the literature, our results of GADA-positive patients were consistent with those of Europeans [5, 19], all the evidence showed that GADA identifies the "classical" phenotype of LADA. The phenotype of LADA with IA-2A positivity or IAA positivity was less clear. One study in Europeans (Italian) showed that IA-2A-positive patients had low BMI, higher HbA1c, and worse beta-cell function [8], and the results were inconsistent with ours, which may be due to the sample difference. Even limited studies on the clinical features of IAA-positive LADA were found, ours here showed they may have the more latent and slower process than GADA-positive LADA. But more evidence should be needed to confirm this.

In conclusion, there is certain frequency of IAA in Chinese phenotypic T2DM patients, and more patients will be diagnosed as LADA if the combination testing of GADA, IA-2A, and IAA is performed. The islet beta-cell function of those IAA-positive patients was worse to some extent, so starting insulin treatment earlier may be helpful in preserving beta-cell function.

Acknowledgments This study was partially supported by a grant from the National High Technology Research and Development Program of China (863 Program, No. 2006AA02A409) and Key Program of Changsha Bureau of Science and Technology (No. K061025-32). We thank Dr. Bill Hagopian for providing the recombinant hGAD65 and IA-2 plasmid.

References

1. Knip M, Siljander H (2008) Autoimmune mechanisms in type 1 diabetes. Autoimmun Rev 77:550–557
2. Seissler J (2008) Latent (slowly progressing) autoimmune diabetes in adults. Curr Diab Rep 8:94–100
3. Desai M, Clark A (2008) Autoimmune diabetes in adults: lessons from the UKPDS. Diabet Med 25(Suppl 2):30–34
4. Li X, Zhou ZG, Huang G et al (2005) Study on the positive frequency and distribution of glutamic acid decarboxylase antibody in phenotypic type 2 diabetic patients. Zhonghua Liu Xing Bing Xue Za Zhi 26:800–803
5. Davis TM, Wright AD, Mehta ZM et al (2005) Islet autoantibodies in clinically diagnosed type 2 diabetes: prevalence and relationship with metabolic control (UKPDS 70). Diabetologia 48:695–702
6. Groop L, Tuomi T, Rowley M et al (2006) Latent autoimmune diabetes in adults (LADA)—more than a name. Diabetologia 49:1996–1998
7. Davies H, Mannan S, Brophy S et al (2008) Routine glutamic acid decarboxylase autoantibody (GADA) testing: patients' perspective. Fam Pract 25:176–180
8. Bottazzo GF, Bosi E, Cull CA et al (2005) IA-2 antibody prevalence and risk assessment of early insulin requirement in subjects presenting with type 2 diabetes (UKPDS 71). Diabetologia 48:703–708
9. Taplin CE, Barker JM (2008) Autoantibodies in type 1 diabetes. Autoimmunity 41:11–18
10. Williams AJ, Bingley PJ, Chance RE et al (1997) A novel microassay for insulin autoantibodies. J Autoimmun 10:473–478

11. Bingley PJ, Bonifacio E, Mueller PW (2003) Diabetes antibody standardization program: first assay proficiency evaluation. Diabetes 52:1128–1136
12. Woo W, LaGasse JM, Zhou Z et al (2000) A novel high-throughput method for accurate, rapid, and economical measurement of multiple type 1 diabetes autoantibodies. J Immunol Methods 244:91–103
13. Petersen JS, Hejnaes KR, Moody A et al (1994) Detection of GAD65 antibodies in diabetes and other autoimmune diseases using a simple radioligand assay. Diabetes 43:459–467
14. Matthews DR, Hosker JP, Rudenski AS et al (1985) Homeostasis model assessment: insulin resistance and beta-cell function from fasting plasma glucose and insulin concentrations in man. Diabetologia 28:412–419
15. Zhang L, Nakayama M, Eisenbarth GS (2008) Insulin as an autoantigen in NOD/human diabetes. Curr Opin Immunol 20:111–118
16. Palmer JP, Asplin CM, Clemons P et al (1983) Insulin antibodies in insulin dependent diabetes before insulin treatment. Science 222:1337–1339
17. Hummel M, Bonifacio E, Schmid S et al (2004) Brief communication: early appearance of islet autoantibodies predicts childhood type 1 diabetes in offspring of diabetic parents. Ann Intern Med 140:882–886
18. Fourlanos S, Dotta F, Greenbaum CJ et al (2005) Latent autoimmune diabetes in adults (LADA) should be less latent. Diabetologia 48:2206–2212
19. Buzzetti R, Di Pietro S, Giaccari A et al (2007) High titer of autoantibodies to GAD identifies a specific phenotype of adult-onset autoimmune diabetes. Diabetes Care 30:932–938