Low Prevalence of Latent Autoimmune Diabetes in Adult Type 2 Diabetic Patients with Age of Onset at 30-39 Years in Taiwan

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Abstract

This study was aimed to determine the prevalence of latent autoimmune diabetes in adult patients clinically classified as type 2 diabetes aged 30-39 years at diagnosis.

Based on an out-patient clinic of one medical center in Taipei, 40 consecutive, eligible patients were studied during a 6-months period. Pancreatic β-cell autoimmunity was tested by measuring serum glutamic acid decarboxylase antibodies (GADA) and islet cell antibodies (ICA 512) levels. β-cell function was tested by measuring serum C-peptide levels before and 6 minutes after glucagon injection. Their clinical characteristics, β-cell autoimmunity and β-cell function were analyzed.

Based on clinical treatment types, group 1 consisting of 24 patients were on oral hypoglycemic agents (OHA) alone. Group 2 consisting of 16 patients were on OHA and insulin. Age, duration of disease, history of diabetes in first degree relatives revealed no significant differences between two groups. Group 2 patients had earlier age of onset (33.4±2.4 yr vs. 35.0±2.3 yr, p=0.02) and were relatively thin as compared with group 1 patients (BMI:23.2±1.8 kg/m2 vs. 25.5±2.2 kg/m2, p=0.02). Group 2 patients also had more impaired β-cell function with lower basal and post-glucagon C-peptide levels as compared with group 1 patients (BCP:2.0±1.7 ng/ml vs. 3.8±2.3 ng/ml, p=0.007; PGCP: 3.5±3.1 ng/ml vs. 6.7±3.7 ng/ml, p=0.005). Of the 40 patients, two cases (5%) were insulin-deficient, and only two cases (5%) were positive for β-cell autoantibodies. One patient in Group 1 was positive for GADA while another patient in group 2 was positive for both GAD and ICA 512 antibodies.

In diabetic subjects with age-onset at 30 to 39 years, 40% of the subjects required insulin supplement albeit only 5% were positive for islet autoantibodies. Although most of them were not absolute insulin-deficient, a poor beta cell function assessed by C-peptide response to glucagon was found in the subjects treated with both OHA and insulin. Other factors than islet autoimmunity might contribute to the decline of beta cell function in this group of patients. (J Intern Med Taiwan 2004; 15: 12-18)

Key Words：Autoimmune diabetes, Glutamic acid decarboxylase antibodies (GADA),
Islet cell antibodies (ICA), C-peptide, glucagon

Introduction

Type 1 diabetes is predominantly caused by chronic autoimmune destruction of insulin-producing β-cells 1. It mostly develops in children and adolescents, however, it can occur at any age 2-3. In fact, in western countries of Caucasian population, type 1 diabetes develops in adults more often than previously expected. If

it develops in adults, the onset is usually slower, symptoms and signs are less acute and severe than in children and adolescents. Progression from oral anti-diabetic medication to frank insulin treatment is insidious and may last several years 4- 6.

Therefore, making correct diagnosis of type 1 diabetes in adults is not easy if solely on clinical grounds. Indeed, adult-onset type 1 diabetes can be often misclassified as type 2 diabetes. In order to feature this particular group of patients, some authors have designated new terms known as latent autoimmune diabetes in adults (LADA), slowly progressive insulin-dependent diabetes mellitus (SPIDDM), or type 1.5 diabetes to distinguish them from type 2 diabetes, by testing β-cell autoimmune antibodies 7-9.

Among β-cell autoimmune antibodies, islet cell antibodies (ICAs), insulin autoantibodies (IAAs) and glutamic acid decarboxylase antibodies (GADAs) are best studied 10-11. ICAs and IAAs are good predictive markers in relatives of patient with type 1 diabetes 12-13. The actual target antigens of ICAs have not been fully elucidated to date. In addition, the measurement of ICAs is technically laborious and is difficult in standardization, thus limiting clinical large scale application. Contrarily, measuring of GADAs can be done with less effort. GADAs are present in up to 80% of patients with newly diagnosed type 1 diabetes 14-15. They can also be detected in the serum of patients up to 10 years before clinical onset of type 1 diabetes and can persist for many years after onset 16-18. As for patients with newly diagnosed type 2 diabetes, GADAs have a 50~100% positive predictive value for later insulin treatment 6,19-20. Meanwhile, measurement of basal C-peptide level (BCP) and postglucagon C-peptide level (PGCP) by performing glucagon stimulation test has been shown to have a high degree of concordance with the clinical classification of diabetes into type 1 or type 2. Moreover, BCP and particularly PGCP levels can also be used to predict the need for later insulin treatment in type 2 patients 21.

The present study was aimed to evaluate both the β-cell function and autoimmunity in a clinical subgroup that consisted of young adults with recent onset of clinical type 2 diabetes.

Subjects and Methods

During a 6-month period, 40 patients were selected consecutively in a diabetes clinic of one medical center. Inclusion criteria are (1) age at onset in 30 to 39 years and (2) disease duration more than 6 months. Exclusion criteria are (1) overt type 1 diabetes with ketoacidosis episodes, and (2) diabetes secondary to other causes. They were subsequently referred to one laboratory assistant, where sex, age, age at onset, disease duration, family history of diabetes (first degree relatives), and body mass index were attained. This study was approved by the institutional review board.

Subsequently, glucagon stimulation test was performed to determine both basal C-peptide (BCP) and post-glucagon C-peptide (PGCP) levels to evaluate β-cell function in a morning fasting state for each patient. C-peptide was measured before and 6 minutes after an intravenous injection of 1 mg of glucagon. Oral antidiabetic medication and insulin were withheld since the previous evening before test. Different thresholds for BCP and PGCP have been reported, we adopted BCP<0.6 ng/ml and PGCP<1.0 ng/ml as the cutoff levels in differentiating between insulin deficiency and non-insulin deficiency 21-24. Serum C-peptide was measured by radioimmunoassay (C-peptide kit Daiichi III Tokyo Japan) with an intra-assay CV of 3.3% and inter-assay CV of 4.4%. The lowest detection limit was 0.02 ng/ml. Their serum samples were also frozen for later test

for β-cell autoimmune markers GADA and ICA-512 by immunoprecipitation radioligand method 25. Cut-off value of positive GADA was 13.38U, and ICA-512, 9.20U. Data were presented as means and standard deviation (S.D.). Student's t test and Fisher's exact test were used to analyze the data, where appropriate. A p value of less than 0.05 was considered statistically significant.

Results

Subject characteristics

We categorized patients into group 1 and group 2 based on treatment modality (Table 1). Group 1 consisted of 24 patients who were treated with oral hypoglycemic agents (OHA) alone while group 2 consisted of 16 patients who were on OHA and insulin combination treatment. Compared with group 1 patients, those with combination treatment had an earlier onset and thinner BMI. There was no difference in age, duration of disease and family members with diabetes in first degree relatives between two groups.

β-cell function study

To evaluate β-cell function of the patients in two groups, glucagon-stimulated C-peptide response was performed in all of these subjects (Table 2). We found that group 2 patients had significantly lower BCP and PGCP levels (3.8±2.3 ng/ml vs. 2.0±1.7 ng/ml, p=0.007, 6.7±3.7 ng/ml vs. 3.5±3.1 ng/ml, p=0.005, respectively). The increments of CP in group 2 patients were also significantly smaller than those in group 1 patients (3.0±1.9 ng/ml vs. 1.6±1.5 ng/ml, p=0.01). Of the 40 patients, 2 patients (5%) had absolute insulin deficiency. One had BCP level of 0.2 ng/ml and PGCP level of 0.3 ng/ml. (See case presentation, case 2). The other had BCP level of 0.4 ng/ml and PGCP level of 0.5 ng/ml.

Study of β-cell autoantibodies

Of the 40 patients, two subjects (5%) were po-sitive for β-cell autoimmunity. One has GADA positivity in group 1 and the other had positivity for both GADA and ICA512 antibody in group 2 (Table 3), see case presentation in the following.

Case presentation

Case 1

This was a 38-year-old male patient in group 1. Disease duration was 3 years. He had no family history of diabetes. His BMI was 23.7 kg/m2. BCP and PGCP levels were 2.5 ng/ml and 4.5 ng/ml respectively (and therefore, he was not insulin deficient). He had a positive GADA with a titer of 80.6 units but was negative for ICA512 antibody. He was currently treated with large dose of OHA, i.e. glimepiride 3 mg b.i.d , metformin 1000 mg b.i.d, acarbose 50 mg b.i.d and rosiglitazone 4 mg q.d. Levels of HbA1c were around 7.0-9.0%. Although he never suffered from episodes of diabetic ketoacidosis, insulin treatment had been suggested but declined.

Case 2

This was a 39-year-old female patient in group 2. Duration of diabetes was 4 years. She had no family history of diabetes. Her BMI was 20.1 kg/m2. BCP and PGCP levels were 0.2 ng/ml and 0.3 ng/ml respectively (and therefore she was defined as insulin-deficient). Her GADA was positive with a titer of 66.8

units, and positive ICA512 antibody positive with a titer of 39.9 units. She had been on maximal OHA for the first 3 years, however, levels of HbA1c were mostly around 8.0~10.0%. She never suffered from episodes of diabetic ketoacidosis despite poor control of diabetes. She was then received insulin injections with Mixtard (N70/R30) twice a day together with acarbose 50 mg b.i.d . The levels of HbA1c dropped to around 6.0~7.0% since initiation of insulin injection.

Discussion

Diabetes consists of heterogeneous groups of patients. The classification of young adult diabetic patients poses a challenge to clinical physicians because type 2 diabetes was believed to occur mostly in patients aged over 40 while type 1 before age 30. However, the number of apparent type 2 diabetic patients diagnosed in their 30s of age or even younger has been rising remarkably nowadays26.

Previous study on βcell autoimmunity has been reported for patients with typical type 1 diabetes with their age of onset before 30 years25. They had found that GADAs were present in 58.71% of patients, 8% of their relatives, and 1.82% in healthy control subjects25. In the present study, GADA was found in only 2 patients (5%) and ICA512 antibody in one (2.5%) among the young-onset diabetes subjects with age-onset at 30-39 years. Our data suggest that the prevalence of LADA is very low in our study patients. In contrast, Ko et al in Hong Kong showed a higher rate of 12.2% of positive GADA in both type 1 and type 2 young adults (aged 16-39 years) with a onset age at 4-35 years 27. However, their study cohort had a younger average age of disease onset and included both type 1 and type 2 patients as compared to ours. Thai et al in Singapore found the even higher presence of GADA in 16.1% of 168 clinical type 2 patients, with average age at diagnosis is 38.4 ±15.3 years28. One Japanese multicenter study revealed a prevalence of 4.3% of GADA in 680 clinical type 2 patients 29. Park et al revealed a low prevalence of GADA of 1.7% in newly diagnosed type 2 Korean patients aged over 30 years from a population-based study 30. (Table 4).

Contrary to studies in Asian diabetes patients, Caucasian diabetes patients possess high autoimmune antibodies prevalence. 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 β-cell autoimmune antibodies in Sweden 6. Humphrey et al demonstrated that 37% of adult-onset insulin-treated patients had positive GADA levels on a population-based study in Australia 20. Compared to the above studies, our results suggested that our young adult diabetes patients, with the more specified range of age of disease onset, seemed to have little autoimmune component. Owing to the small number of patients (2 patients) in our study, we were limited to further analyze the potential differences between antibody-positive and negative patients. Admittedly, this is a small-sample-sized and hospital-based study, therefore we can hardly extrapolate our data to the general population affected with diabetes.

Although LADA only contributes a few cases in our series, it is surprising to find that a large portion (around 40%) of the young-onset diabetes was treated with insulin in addition to maximal OHA within 3 years after the diagnosis of diabetes. Of these 16 patients, 5 had only one parent with diabetes and 1 had both parents with diabetes. Furthermore, this group of subjects had significant lower BMI, younger age at onset, and lower BCP and PGCP levels than those treated with OHA alone. These data suggest a subgroup of patients who are clinically similar to those of LADA but without a positive beta cell autoantibody in our population. The beta

cell reserves of those patients without antoantibodies were only mildly impaired as compared to those with antibodies. We suggest the acronym of RPDA (rapid-progressive diabetes in adult) to identify this subgroup of patients who are at a rather rapid disease-progressive status. Whether their disease progression is related to β-cell autoimmunity other than existing autoantibodies or influenced by other genetic factors remains unclear. In one large-scale survey from Taiwan, the percentage of diabetic subjects treated with insulin or insulin plus OHA was around 14% and 10% respectively 31 . Therefore, the percentage of group 2 (receiving insulin plus OHA) was higher in the present study. One possible reason to explain the discordance might be that we might have underestimated the rate of LADA in our patients (particularly those in group 2). The antibody-positive rate may decline with disease duration, and there might have been many years before type 2 diabetes could be diagnosed 32.

In summary, in diabetic subjects with age-onset at 30 to 39 years, only few were positive for islet autoantibodies. Although most of them were not insulin-deficient, a poor beta cell function assessed by C-peptide response to glucagon was found in the subjects treated with both OHA and insulin. Although the percentage of islet autoimmunity might be underestimated, other factors than islet autoimmunity might contribute to the decline of beta cell function in this group of patients.

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台灣 30 至 39 歲之第 2 型糖尿病人自體免疫性糖尿病盛行率低

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摘　　要

本研究想了解 30 歲至 39 歲被診斷爲糖尿病的病人中，自體免疫性糖尿病人的百分率。在一醫學中心，六個月內共研究 40 名合格病人。每個病人皆測量血液 GAD 和 ICA-512 抗體，靜脈注射升糖激素 1mg 前，與 6 分鐘後的血液 C-peptide 數值。40 名病人中，有 24 位接受口服降血糖藥治療，其餘 16 位接受口服降血糖藥加上胰島素治療。2 位病人(5%)被歸類於胰島素缺乏，2 位病人(5%)有 GAD 或是 ICA-512 抗體。16 位接受口服藥加胰島素治療的病人中，胰島細胞功能較另外 24 位僅接受口服藥治療的病人差。可能有除了自體免疫因素外的原因造成他們的胰島細胞功能不良。

Table 1. Clinical characteristics of adult onset diabetes based on treatment modality

Parameter	Treatment		P
	Group 1 （OHA only）	Group 2 （OHA plus insulin）	
N	24	16	
Age（yr）	35.9±3.3	36.3±3.0	ns
Age of onset（yr）	35.0±2.3	33.4±2.4	0.02
Duration（yr）	2.1±1.5	2.8±1.9	ns
Diabetes in first-degree relatives（n）	10（42%）	6（38%）	ns
BMI（kg/m^2）	25.5±2.2	23.2±1.8	0.02
Fasting plasma glucose (mg/dl)	206.5±80.5	218.0±59.2	ns
HbA_{1c} (%)	8.4±2.4	9.6±2.2	ns

ns：non-significant statistically

Table 2. Comparison of the beta cell function by glucagon stimulation test according to treatment modality.

Parameter	Treatment		P value
	Group 1 （OHA only）	Group 2 （OHA plus insulin）	
BCP（ng/ml）	3.8±2.3	2.0±1.7	0.007
PGCP（ng/ml）	6.7±3.7	3.5±3.1	0.005
ΔCP（6'-0'）	3.0±1.9	1.6±1.5	0.01

OHA：oral hypoglycemic agent

BCP：basal C-peptide

PGCP：post-glucagon C-peptide

ΔCP：increment of C-peptide

Table 3. Positive β-cell autoimmunity based on treatment modality

Parameter	Treatment		P
	Group 1 （OHA only）	Group 2 （OHA plus insulin）	
GADA（+）（n）	1	1*	ns
1CA512 antibody(+)(n)	0	1*	ns

P was tested by Fisher's exact test

* see case presentation in text

Table 4. Different series of study on the prevalence of β-cell autoantibodies in clinical type 2 diabetes.

	N	Ethnicity	Age (yr)	Age at onset (yr)	Duration (yr)	BMI (kg/m^2)	GADA (+), n	ICA (+), n
Ko et al[27]	140 type 1 and 2	Asian	30.0±5.8	25.9±7.2	4.6±4.7	25.6±5.1	17 (12.1%)	
Thai et al[28]	168 type 2	Asian	40.6±15.3	38.4±15.3	2.3±4.4	24.4±4.0	27 (16.1%)	8 (4.8%)
Tsuruoka et al[29]	680 type 2	Asian	46.9±19.49				29 (4.3%)	
Park et al[30]	121 newly diagnosed type 2	Asian	57.4±12.5			25.2±3.8	2 (1.7%)	
Tőrn et al[6]	110 type 2, 71 unclassified	Caucasian	15-34				52 (47%) in type 2, 42 (59%) in unclassified	
Humphrey et al[20]	Adult-onset, insulin-treated 192 male, 182 female	Caucasoid		M 46.3±0.74 F 46.8±0.77	M 16.9±0.63 F 18.0±0.72		M 69 (35.9%) F 70 (38.5%)	
Present Study	40 type 2	Asian	36.9±3.4	34.4±2.5	2.4±1.8	24.6±3.7	2 (5%)	1 (2.5%)

M：male

F：female