Clinical features of cases of seroconversion of anti-glutamic acid decarboxylase antibody during the clinical course of type 2 diabetes: a nationwide survey in Japan

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Abstract The pathogenesis of type 1 diabetes is different from that of type 2 diabetes, and anti-glutamic acid decarboxylase antibody (GADA) helps to diagnose autoimmune type 1 diabetes. Some studies reported that GADA seroconversion occurs during the clinical course of type 2 diabetes, leading to development of “type 1 on type 2 diabetes”. To clarify the clinical characteristics and

triggers of GADA seroconversion, we performed a nationwide questionnaire survey for clinical cases identified by literature search, and obtained information on 38 cases (24 with insulin therapy and 14 without it). The diabetes duration up to determination of GADA seroconversion was significantly longer in the group with insulin therapy than that without it. This finding was particularly noted in insulin-treated non-obese patients with lower serum C-peptide levels. In these patients, insulin therapy could have masked sudden increases in plasma glucose and HbA1c levels, possibly leading to delayed determination of

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GADA seroconversion. In non-obese patients without insulin therapy, an abrupt rise in the plasma glucose and HbA1c levels was observed at immediately before the determination, a finding which may help to predict GADA seroconversion. From the results of the present survey, we could not determine apparent triggers of GADA seroconversion. Thus, physicians may need to consider the possibility of concurrent type 1 diabetes during the therapeutic course of type 2 diabetes; GADA measurement should be considered when non-obese type 2 diabetic patients not receiving insulin therapy experience unexpected abrupt hyperglycemia and when those receiving insulin therapy show low serum C-peptide levels.

Keywords Anti-glutamic acid decarboxylase antibody · Seroconversion · Type 1 on type 2 diabetes

Introduction

Diabetes mellitus is mainly classified into type 1 and type 2 diabetes based on its etiology and pathophysiology. Most type 1 diabetes is considered to be caused by β -cell destruction through anti-islet autoimmunity [1], and eventually progresses to an insulin-dependent state. Generally, some types of anti-islet-associated autoantibodies, such as anti-glutamic acid decarboxylase antibody (GADA), anti-insulinoma-associated antigen-2 (IA-2) antibody, anti-insulin autoantibody (IAA), and anti-zinc transporter 8 (ZnT8) antibody, are detected in the peripheral blood of patients at the onset of autoimmune type 1 diabetes [2]. On the other hand, type 2 diabetes is caused by a combination of insulin resistance and decreased insulin secretion [3], and does not generally progress to an insulin-dependent state. In principle, anti-islet-associated autoantibodies are not detected in patients with type 2 diabetes. In other words, each type of diabetes has its own unique clinical and etiological features, meaning that there is a difference between type 1 and type 2 diabetes in pathogenesis.

In terms of onset pattern of hyperglycemia, type 1 diabetes can be classified into 3 types; i.e. acute-onset, slowly progressive, and fulminant type 1 diabetes [4, 5], and seroconversion of GADA helps to diagnose type 1 diabetes [6]. In particular, the presence of GADA in diabetic patients in a non-insulin-dependent state strongly suggests diagnosis of slowly progressive insulin-dependent (type 1) diabetes mellitus (SPIDDM) [7].

Suzuki et al. reported on 2 diabetic patients whose anti-islet-associated autoantibodies were initially all negative, and later at least one of these antibodies became positive without insulin or interferon therapy [8]. Eventually, they developed insulin-dependent diabetes. Because type 1

diabetes appeared to have been superimposed on type 2 diabetes, they called these cases “type 1 on type 2 diabetes”. Since then, many similar cases have been reported in Japan. In fact, it is unlikely to be uncommon that physicians encounter such types of diabetes in which GADA seroconversion occurs during therapy for type 2 diabetes. According to the current diagnostic criteria [7], such cases in a non-insulin-dependent state are generally diagnosed as SPIDDM.

SPIDDM can be diagnosed if the following 2 conditions are met: (1) presence of GADA and/or islet cell antibodies (ICA) at some point during the disease course, and (2) absence of ketosis or ketoacidosis at the onset (or diagnosis) of diabetes mellitus and no need for insulin treatment for correcting hyperglycemia immediately after diagnosis [7]. The criteria do not refer to the timing of GADA detection during the clinical course of non-insulin-dependent diabetes. Therefore, SPIDDM could be divided into the following two categories: cases where patients test positive for GADA since the onset of diabetes, and cases where patients test negative for GADA early in the clinical course of type 2 diabetes, but test positive for GADA subsequently. The former indicates that the etiology of hyperglycemia is consistently associated with anti-islet autoimmunity since the onset of diabetes; this type of diabetes is usually believed to be so-called SPIDDM. The latter suggests an apparent change in the etiology of diabetes throughout the disease course, that is, from type 2 to type 1 diabetes (type 1 on type 2 diabetes), and the role of anti-islet autoimmunity in the development of type 1 diabetes in these cases may be different from that in the former cases. To our knowledge, the clinical characteristics of such a distinct type of diabetes with GADA seroconversion during the course of type 2 diabetes remain unclear and they are yet to be elucidated in large-scale studies. In the present study, we collected data regarding similar cases reported previously in Japan, and performed a nationwide questionnaire survey to determine the clinical characteristics including factors that trigger GADA seroconversion.

Methods

First, we collected case reports regarding type 1 on type 2 diabetes. In April 2014, we searched the ICHUSHI (Japan Medical Abstracts Society) databases for Japanese- or English-language articles using the keywords of “ichigata tounyoubyou (type 1 diabetes)”, “nigata tounyoubyou (type 2 diabetes)”, “GAD antibody (GAD koutai)”, “tenka or youtenka (positive conversion or seroconversion)” and “yousei (positivity)” in humans published between 1985 and March 2014. We excluded reviews,

letters, editorials, and original articles that did not directly pertain to the topic area. Thereafter, the selection criteria were as follows: (1) history of type 2 diabetes regardless of past or current insulin therapy, (2) GAD antibody initially determined to be negative, then spontaneous seroconversion during period of treatment for type 2 diabetes. We excluded patients with fulminant type 1 diabetes, interferon-induced type 1 diabetes, and type 1 diabetes suspected to be due to insulin allergy in the clinical course of type 2 diabetes.

Next, we conducted a nationwide questionnaire survey by mail. The questionnaire was sent to all first authors who reported the cases satisfying the above selection criteria. They were requested to provide information on clinical characteristics of the cases. If they had encountered similar cases which satisfied the above criteria and had not been reported, we requested them to add their details to the survey responses.

In the questionnaire, the clinical characteristics of all patients [patient's initial, sex, age, family history of diabetes, symptoms accompanying onset of diabetes, past maximal body mass index (BMI), HLA DR and DQ genotypes, last timing of GADA measurement which was confirmed to be negative, presence or absence of hyperglycemic symptoms and/or ketosis, presumed triggers of GADA seroconversion] were recorded. Also noted were information regarding oral hypoglycemic agents, regimen of insulin therapy, and the following laboratory data determined at the onset of type 2 diabetes, at 0, 3, 6, and 12 months before and at 6 months after the timing of determination of GADA seroconversion: BMI; ad lib plasma glucose level; serum C-peptide level; HbA1c level; daily urinary C-peptide excretion level; serum GADA level. C-peptide index was calculated as the ratio of serum C-peptide (ng/mL) to plasma glucose level (mg/dL) (X100). The serum GADA level was measured using an anti-GAD radioimmunoassay (RIA) kit (RSR Ltd, Cardiff, UK). The normal range for GADA is less than 1.5 U/mL. Obesity was defined as having BMI ≥ 25 kg/m² [9]. The study protocol was approved by the Institutional Review Board of Tokyo Saiseikai Central Hospital and the Japan Diabetes Society.

Statistical analysis

Data were presented as the mean $\pm$ SD. Exceptionally, GADA was presented as the mean $\pm$ SE. Group comparisons were performed using Pearson's Chi square test, Fisher's exact test, paired and unpaired *t* tests, and Mann–Whitney *U* test as appropriate. A *P* value < 0.05 was considered significant. Statistical analysis was performed with the SPSS statistics software package.

Results

Patient disposition

The questionnaire was sent to 76 hospitals (102 cases), and information for 58 cases was obtained. Of 58 cases, 20 cases were inadequately recorded. Therefore, a total of 38 cases were identified for the study. We then divided the patients into two groups—a group that received insulin therapy ($n = 24$) and another group that did not receive insulin therapy ($n = 14$) (Fig. 1)—and investigated the patient characteristics in each group. GADA titers ≥ 10 U/mL were considered to be high risk factors for future progression to an insulin-dependent state in SPIDDM [10], suggesting that GADA titers may influence the clinical characteristics of patients. Therefore, we also carried out a subgroup analysis in which patients were divided into two groups according to the GADA titers (those with ≥ 10 U/mL and those with < 10 U/mL).

Clinical characteristics of patients

The onset age of type 2 diabetes was significantly lower in the group with insulin therapy than that without it (44.21 ± 12.33 vs. 56.50 ± 14.07 years, respectively) (Table 1). However, there was no significant difference in the age at which GADA was found to be positive between the groups with and without insulin therapy (61.04 ± 13.96 vs. 65.43 ± 14.12 years, respectively). As a result, the diabetes duration up to determination of

Fig. 1 Patient disposition

Table 1 Background of patients with and without insulin therapy

	Total (<i>n</i> = 38)	<i>n</i>	Without insulin therapy (<i>n</i> = 14)	<i>n</i>	With insulin therapy (<i>n</i> = 24)	<i>n</i>	<i>P</i>
Sex (female/male)	22/16	38	7/7	14	15/9	24	NS*
Familial history of diabetes (yes/no)	14/21	35	4/9	13	10/12	22	NS*
Past history of obesity (yes/no)	21/14	35	8/4	12	13/10	23	NS*
Past maximal body weight (kg)	67.55 ± 12.77	35	68.23 ± 10.61	12	67.19 ± 13.98	23	NS**
Past maximal BMI (kg/m ²)	26.63 ± 3.85	35	26.33 ± 3.61	12	26.79 ± 4.04	23	NS**
History of SU or glinide use (yes/no)	27/11	38	10/4	14	17/7	24	NS*
Onset age of type 2 diabetes (years)	48.74 ± 14.15	38	56.50 ± 14.07	14	44.21 ± 12.33	24	<0.01**
Other autoimmune diseases (yes/no)	6/30	36	4/9	13	2/21	23	NS*
Rheumatoid arthritis	1		1		0		
Graves' disease	3		2		1		
Hashimoto disease	2		1		1		
Pancreatic cyst (yes/no)	0/35	35	0/12	12	0/23	23	NS*

Data are shown as mean ± SD

BMI body mass index, *SU* sulfonyl urea, *NS* not significant

*Chi square or Fisher's exact test

**Unpaired *t* test

GADA seroconversion was significantly longer in the group with insulin therapy than that without it (16.83 ± 11.36 vs. 8.93 ± 5.72 years, respectively). The period from the start of insulin therapy to determination of GADA seroconversion was 5.74 ± 4.92 years in the group with insulin therapy (Table 2).

The past maximal BMI was comparable between the groups with and without insulin therapy. The BMI at the timing of determination of GADA seroconversion was also comparable between them. The ad lib plasma glucose level and HbA1c level at the timing of determination of GADA seroconversion tended to be lower in the group with insulin therapy than that without it (225.35 ± 146.39 vs. 320.92 ± 239.72 mg/dL, and 9.76 ± 2.00 vs. 10.41 ± 2.29%, respectively), possibly due to the use of exogenous insulin, although differences between the two groups were not significant. The serum C-peptide level was significantly lower in the group with insulin therapy as compared to that without it, although no significant difference in C-peptide index was observed between the groups with and without insulin therapy (Table 2).

The mean GADA titer was higher in the group without insulin therapy than that with it, although there was no significant difference between the two groups. There was also no significant difference between the groups with and without insulin therapy in the population of patients with GADA ≥10 U/mL and those with GADA <10 U/mL (Table 2).

There were no significant differences in the frequencies of body weight loss, hyperglycemic symptoms, ketosis, and autoimmune diseases between the groups with and without insulin therapy. However, the frequency of ketosis seemed

to be slightly lower in the group with insulin therapy as compared to that without it, which might be attributable to the effect of exogenous insulin (Table 2).

Presumed triggers of GADA seroconversion

Although several presumed triggers of GADA seroconversion were given by authors of the reports, most of them were unknown regardless of the presence or absence of insulin therapy (Table 3), and regardless of the levels of GADA titers (Table 4). Although the majority of patients had a history of sulfonylurea or glinide agent use, it was unknown whether or not there was a direct relationship between insulin secretagogues and GADA seroconversion. The frequency that presumed triggers were unknown was higher in patients with GADA <10 U/mL than those with GADA ≥10 U/mL (13/17 vs. 9/21, respectively; Table 4).

Relationship between diabetes duration and GADA seroconversion

To further examine factors contributing to GADA seroconversion in the disease process of type 2 diabetes, we investigated relationships between GADA seroconversion and various clinical parameters. As shown in Fig. 2a and Supplementary Table 1, the diabetes duration up to determination of GADA seroconversion was significantly longer in non-obese patients than obese patients in the group with insulin therapy. By contrast, in the group without insulin therapy, there was no significant difference in the duration between obese and non-obese patients (Fig. 2b and

Table 2 Clinical characteristics at the timing of determination of GADA seroconversion in patients with and without insulin therapy

	Total (<i>n</i> = 38)	<i>n</i>	Without insulin therapy (<i>n</i> = 14)	<i>n</i>	With insulin therapy (<i>n</i> = 24)	<i>n</i>	<i>P</i>
Age (years)	62.66 ± 13.99	38	65.43 ± 14.12	14	61.04 ± 13.96	24	NS**
Body weight (kg)	58.24 ± 11.77	38	60.88 ± 10.85	14	56.70 ± 12.24	24	NS**
BMI (kg/m ²)	22.95 ± 3.86	38	23.73 ± 4.24	14	22.49 ± 3.64	24	NS**
Obesity (yes/no)	11/27	38	5/9	14	6/18	24	NS**
Plasma glucose (mg/dL)	259.86 ± 187.99	36	320.92 ± 239.72	13	225.35 ± 146.39	23	NS**
HbA1c (%)	10.00 ± 2.08	38	10.41 ± 2.29	14	9.76 ± 2.00	24	NS**
Serum C-peptide (ng/mL)	1.125 ± 1.057	34	1.663 ± 0.910	12	0.832 ± 1.033	22	<0.05**
C-peptide index	0.618 ± 0.657	32	0.898 ± 0.679	11	0.471 ± 0.611	21	NS** (<i>P</i> = 0.081)
24 h urine C-peptide (μg/day)	34.55 ± 26.81	26	45.27 ± 30.74	10	27.84 ± 22.50	16	NS**
GADA titers (U/mL, mean ± SE) (normal range, <1.5 U/mL)	3185.24 ± 2285.09	38	7684.79 ± 6119.41	14	560.50 ± 374.53	24	NS ^a
GADA titers (≥10/<10 U/mL)	21/17	38	6/8	14	15/9	24	NS*
Body weight loss (yes/no)	13/23	36	5/8	13	8/15	23	NS*
Symptoms related with hyperglycemia (yes/no)	15/22	37	5/8	13	10/14	24	NS*
Ketosis (yes/no)	5/32	37	3/10	13	2/22	24	NS*
Diabetes duration up to GADA seroconversion (years)	13.92 ± 10.33	38	8.93 ± 5.72	14	16.83 ± 11.36	24	<0.05**
Period from neg. to pos. GADA (years)	3.59 ± 2.55	38	2.13 ± 1.97	14	4.44 ± 2.49	24	<0.01**
Insulin dose (U/day)	–	–	–	–	33.75 ± 18.31	20	–
Insulin dose (U/kg/day)	–	–	–	–	0.59 ± 0.28	20	–
Period of insulin use up to GADA seroconversion (years)	–	–	–	–	5.74 ± 4.92	24	–
Diabetes duration up to start of insulin (years)	–	–	–	–	11.11 ± 10.07	24	–

Data are shown as mean ± SD unless otherwise indicated

BMI body mass index, *GADA* anti-glutamic acid decarboxylase antibody, *neg* negative, *pos* positive, *NS* not significant

*Chi square or Fisher's exact test

**Unpaired *t* test

^a Mann–Whitney *U* test

Table 3 Presumed triggers of GADA seroconversion in patients with and without insulin therapy

Without insulin therapy (<i>n</i> = 14)	<i>n</i>	With insulin therapy (<i>n</i> = 24)	<i>n</i>
Unknown	10	Unknown	12
SU + DPP4 inhibitor	1	Insulin	3
Alcohol	1	Insulin + diet failure, obesity	3
Graves' disease	1	Glinide	1
Viral infection	1	Insulin + anticancer drug	1
		Diet failure	1
		Soft drink ketosis or glinide	1
		Abortion	1
		Influenza vaccine	1

Supplementary Table 1), and the durations were comparable to that in obese patients with insulin therapy (Fig. 2a, b and Supplementary Table 1). Intriguingly, the mean diabetes duration up to the latest timing of GADA testing in which

Table 4 Presumed triggers of GADA seroconversion in patients with GADA titers ≥10 and <10 U/mL

GADA ≥10 U/mL (<i>n</i> = 21)	<i>n</i>	GADA <10 U/mL (<i>n</i> = 17)	<i>n</i>
Unknown	9	Unknown	13
Insulin	2	Insulin + diet failure, obesity	2
Insulin + diet failure, obesity	1	Insulin	1
Glinide	1	Alcohol	1
Insulin + anticancer drug	1		
Diet failure	1		
Soft drink ketosis or glinide	1		
Abortion	1		
Influenza vaccine	1		
SU + DPP4 inhibitor	1		
Graves' disease	1		
Viral infection	1		

GADA anti-glutamic acid decarboxylase antibody, *SU* sulfonyl urea, *DPP4* dipeptidyl peptidase-4

Fig. 2 Diabetes duration up to determination of GADA seroconversion. Comparison between the presence or absence of obesity in (a) patients with insulin therapy and (b) those without insulin therapy; comparison between patients with GADA titers ≥ 10 and < 10 U/mL in (c) patients with insulin therapy and (d) those without insulin therapy. * $P < 0.05$ by unpaired t test. GADA anti-glutamic acid decarboxylase antibody, SC seroconversion

GADA was negative was 14.92 years in non-obese patients with insulin therapy, which was comparable to the diabetes duration up to the start of insulin therapy (13.59 ± 10.31 years, Supplementary Table 1). In fact, of 18 patients in this group, 14 patients began insulin therapy around 1 year before the latest timing in which GADA was negative to around 5 years after it, meaning that exogenous insulin might contribute to triggering GADA seroconversion.

Relationship between GADA titer and GADA seroconversion

To clarify the influence of GADA titers on the clinical characteristics of the patients, we analyzed clinical data by dividing patients into the high titer GADA group (GADA ≥ 10 U/mL) and the low titer GADA group (GADA < 10 U/mL). As shown in Table 5, there was no significant difference in the background of patients. Moreover, there was no difference in the diabetes duration up to determination of GADA seroconversion between the groups with high and low GADA titers. However, serum C-peptide and 24 h urine C-peptide levels were significantly lower in the high titer GADA group than the low titer one. Although the past maximal BMI and the proportion of patients with a history of obesity were comparable between the groups with high and low GADA titers, BMI and the proportion of patients with obesity at the timing of GADA seroconversion determination were significantly lower in the high titer GADA group than the low titer one (Tables 5, 6).

Subsequently, we performed a subgroup analysis of the above data according to presence and absence of insulin

Table 5 Background of patients with GADA titers ≥ 10 and < 10 U/mL

	Total ($n = 38$)	n	GADA ≥ 10 U/mL ($n = 21$)	n	GADA < 10 U/mL ($n = 17$)	n	P
Sex (female/male)	22/16	38	12/9	21	10/7	17	NS*
Familial history of diabetes (yes/no)	14/21	35	10/9	19	4/12	16	NS*
Past history of obesity (yes/no)	21/14	35	9/10	19	12/4	16	NS*
Past maximal body weight (kg)	67.55 ± 12.77	35	66.51 ± 14.10	19	68.78 ± 11.33	16	NS**
Past maximal BMI (kg/m^2)	26.63 ± 3.85	35	26.17 ± 4.56	19	27.19 ± 2.84	16	NS**
History of SU or glinide use (yes/no)	27/11	38	17/4	21	10/7	17	NS*
Onset age of type 2 diabetes (years)	48.74 ± 14.15	38	46.29 ± 14.56	21	51.77 ± 13.42	17	NS**
Other autoimmune diseases (yes/no)	6/30	36	3/17	20	3/13	16	NS*
Rheumatoid arthritis	1		0		1		
Graves' disease	3		2		1		
Hashimoto disease	2		1		1		
Pancreatic cyst (yes/no)	0/35	35	0/19	19	0/16	16	NS*

Data are shown as mean $\pm$ SD

BMI body mass index, GADA anti-glutamic acid decarboxylase antibody, SU sulfonyl urea, NS not significant

* Chi square or Fisher's exact test

** Unpaired t test

Table 6 Clinical characteristics at the timing of determination of GADA seroconversion in patients with GADA titers ≥ 10 and < 10 U/mL

	Total (<i>n</i> = 38)	<i>n</i>	GADA ≥ 10 U/ mL (<i>n</i> = 21)	<i>n</i>	GADA < 10 U/mL (<i>n</i> = 17)	<i>n</i>	<i>P</i>
Age (years)	62.66 $\pm$ 13.99	38	60.48 $\pm$ 15.56	21	65.35 $\pm$ 11.66	17	NS**
Body weight (kg)	58.24 $\pm$ 11.77	38	55.45 $\pm$ 11.66	21	61.69 $\pm$ 11.31	17	NS**
BMI (kg/m ²)	22.95 $\pm$ 3.86	38	21.58 $\pm$ 3.25	21	24.64 $\pm$ 3.98	17	$< 0.05^{**}$
Obesity (yes/no)	11/27	38	3/18	21	8/9	17	$< 0.05^{**}$
Plasma glucose (mg/dL)	259.86 $\pm$ 187.99	36	271.05 $\pm$ 215.41	20	245.88 $\pm$ 152.67	16	NS**
HbA1c (%)	10.00 $\pm$ 2.08	38	9.92 $\pm$ 1.88	21	10.10 $\pm$ 2.35	17	NS**
Serum C-peptide (ng/mL)	1.125 $\pm$ 1.057	34	0.745 $\pm$ 0.567	18	1.553 $\pm$ 1.314	16	$< 0.05^{**}$
C-peptide index	0.618 $\pm$ 0.657	32	0.425 $\pm$ 0.345	17	0.836 $\pm$ 0.851	15	NS**
(P = 0.0766)							
24 h urine C-peptide (μ g/day)	34.55 $\pm$ 26.81	26	25.08 $\pm$ 22.31	15	47.46 $\pm$ 27.96	11	$< 0.05^{**}$
Body weight loss (yes/no)	13/23	36	8/11	19	5/12	17	NS*
Symptoms related with hyperglycemia (yes/no)	15/22	37	9/11	20	6/11	17	NS*
Ketosis (yes/no)	5/32	37	2/18	20	3/14	17	NS*
Diabetes duration up to GADA seroconversion (years)	13.92 $\pm$ 10.33	38	14.19 $\pm$ 11.47	21	13.59 $\pm$ 9.06	17	NS**
Period from neg. to pos. GADA (years)	3.59 $\pm$ 2.55	38	3.65 $\pm$ 2.16	21	3.52 $\pm$ 3.03	17	NS**
Insulin dose (U/day)	33.75 $\pm$ 18.31	20	36.36 $\pm$ 15.04	11	30.56 $\pm$ 22.20	9	NS**
Insulin dose (U/kg/day)	0.59 $\pm$ 0.28	20	0.61 $\pm$ 0.23	11	0.55 $\pm$ 0.34	9	NS**
Period of insulin use up to GADA seroconversion (years)	5.74 $\pm$ 4.92	24	5.11 $\pm$ 4.95	15	6.80 $\pm$ 4.99	9	NS**
Diabetes duration up to start of insulin (years)	11.11 $\pm$ 10.07	24	12.78 $\pm$ 10.89	15	8.33 $\pm$ 8.37	9	NS**
Insulin use before GADA seroconversion (yes/no)	24/14	38	15/6	21	9/8	17	NS*

Data are shown as mean $\pm$ SD

BMI body mass index, GADA anti-glutamic acid decarboxylase antibody, *neg* negative, *pos* positive, NS not significant

* Chi square or Fisher's exact test

** Unpaired *t* test

therapy. In the group with insulin therapy, there were no differences in background factors or clinical characteristics other than a significantly higher proportion of patients with a family history of diabetes in the high titer GADA group than the low titer one (Fig. 2c, Supplementary Table 2). In the group without insulin therapy, the diabetes duration up to determination of GADA seroconversion was significantly shorter in the high titer GADA group than the low titer one (Fig. 2d, Supplementary Table 2). Moreover, BMI at the timing of GADA seroconversion determination and the past maximal BMI were significantly lower in the high titer GADA group than the low titer one, although there were no significant differences in serum C-peptide levels and 24 h urine C-peptide levels between the groups with high and low GADA titers. (Supplementary Table 2).

Relationship between time course of diabetes-related markers and GADA seroconversion

Finally, we investigated the time course of changes in the plasma glucose and HbA1c levels at immediately before determination of GADA seroconversion. As shown in

Fig. 3a–d, there was an abrupt rise in the plasma glucose and HbA1c levels at immediately before GADA seroconversion in non-obese patients in the group without insulin therapy (Fig. 3b, d), but no marked change was observed in the other groups.

Discussion

This study aimed to determine the clinical characteristics and factors that trigger GADA seroconversion during the clinical course of type 2 diabetes. We found that the period from the diagnosis of type 2 diabetes to determination of GADA seroconversion was significantly longer in the group that received insulin therapy than that in the group that did not receive insulin therapy. It was suggested that GADA measurement may have been delayed or missed in a group with insulin therapy, because insulin therapy could mask abrupt exacerbation of hyperglycemia. Indeed, the last time it was confirmed that GADA was negative was 2.13 ± 1.97 years previously in the group without insulin therapy and 4.44 ± 2.49 years previously in that with it

Fig. 3 Time course of plasma glucose and HbA1c levels according to the presence or absence of obesity. Plasma glucose in (a) patients with insulin therapy and (b) those without insulin therapy; and HbA1c levels in (c) patients with insulin therapy and (d) those without insulin therapy at 3–6 months before and at the timing of GADA seroconversion determination. *Black filled circle* Non-obese patients. *Open circle* obese patients. * $P < 0.05$ by paired t test. GADA anti-glutamic acid decarboxylase antibody, M months, SC seroconversion

($P < 0.01$ by unpaired t test; Table 2), suggesting reduced opportunities for GADA measurement in the group with insulin therapy.

Tanaka et al. previously performed a nationwide survey and reported the characteristics of SPIDDM in contradistinction to type 2 diabetes and acute-onset type 1 diabetes [11]. Compared with their data, regardless of the presence or absence of insulin therapy, the mean past maximal BMI of our patients was higher than that of their patients with SPIDDM ($24.3 \pm 2.7 \text{ kg/m}^2$; $n = 49$) and tended to be close to that of their patients with type 2 diabetes ($28.0 \pm 5.0 \text{ kg/m}^2$; $n = 425$). However, the BMI at the timing of determination of GADA seroconversion was decreased and comparable to the BMI of patients with SPIDDM at the initial visit ($22.1 \pm 3.2 \text{ kg/m}^2$). These findings suggest that insulin resistance in the early phase of the disease, but impaired insulin secretion after GADA seroconversion, may contribute to the development of diabetes. Moreover, the onset age of type 2 diabetes was lower in our patients, particularly in those with insulin therapy, as compared to their patients with

SPIDDM and type 2 diabetes (51.1 ± 14.4 and 53.4 ± 14.3 years old, respectively), suggesting that a small portion of relatively-young patients with type 2 diabetes requiring insulin therapy to correct hyperglycemia may have GADA seroconversion in the future. To clarify this issue, a long-term prospective study should be performed in the future.

The frequencies of ketosis and body weight (BW) loss were significantly lower in patients with insulin therapy as compared to those without it. The finding suggests that exogenous insulin may mask occurrence of hyperglycemia and deficiency in endogenous insulin secretion, possibly leading to a delay in the timing GADA measurement. Regardless of the presence or absence of ketosis or BW loss, physicians should consider the possibility of complicating type 1 diabetes and try to measure GADA and serum C-peptide levels at an appropriate time.

In the present study, we could not determine apparent triggers of GADA seroconversion. A possible reason for this may be a discrepancy between the timing of seroconversion and that of determining GADA positivity. In fact, the periods from the last determination of GADA negativity to the determination of GADA positivity were 2.13 ± 1.97 years in the group without insulin therapy and 4.44 ± 2.49 years in that with it (Table 2). Such a long interval may have led to the difficulty in identification of the triggers and timing of GADA seroconversion.

Wilkin et al. previously proposed an accelerator hypothesis, which provides a missing link between type 1 and type 2 diabetes [12]. They presumed that hyperglycemia or glucose toxicity caused by insulin resistance induces apoptosis of β -cells and anti-islet autoimmunity, leading to failure of insulin secretion from β -cells. Even in type 1 diabetes, insulin resistance may contribute to anti-islet autoimmunity, resulting in β -cell destruction. In this regard, we found that the diabetes duration up to determination of GADA seroconversion was significantly shorter in the obese patients than non-obese patients in the group with insulin therapy (Fig. 2a), suggesting the possibility that a heavier BW promoted by exogenous insulin may place a burden on β -cells through insulin resistance and contribute to the induction of anti-islet autoimmunity. Alternatively, despite the fact that being overweight, a requirement for insulin therapy to correct hyperglycemia in early stage of the disease process of type 2 diabetes might imply a preexisting burden on β -cells due to insulin resistance or glucotoxicity, leading to induction of anti-islet autoimmunity.

By contrast, the diabetes duration up to determination of GADA seroconversion was significantly longer in the non-obese patients than obese ones in the group with insulin therapy (Fig. 2a). However, the serum C-peptide level was significantly lower in the non-obese patients than the obese

ones (0.530 ± 0.431 vs. 1.858 ± 1.778 ng/ml, respectively; $P < 0.01$ by Mann–Whitney U test; Supplementary Table 1). The finding suggests that exogenous insulin may mask abrupt exacerbation of hyperglycemia and deficiency in endogenous insulin secretion, possibly leading to a delay in determination of GADA seroconversion in non-obese patients with insulin therapy. Therefore, physicians should be careful not to miss GADA seroconversion in non-obese type 2 diabetic patients with insulin therapy and lower serum C-peptide levels.

In the group without insulin therapy on the other hand, the mean diabetes duration up to determination of GADA seroconversion was shorter in the non-obese patients than obese patients, although the difference was not significant. Moreover, HbA1c levels were significantly elevated at immediately before GADA seroconversion in the non-obese patients (Fig. 3d). These findings suggest that insulin secretory capacity may be lower in non-obese patients, contributing to earlier appearance of overt hyperglycemia through anti-islet autoimmunity. Indeed, the means of C-peptide index and 24 h urine C-peptide levels tended to be lower in this group (Supplementary Table 1). Thus, especially in non-obese patients, the appearance of unexpected overt hyperglycemia during therapy other than insulin therapy may imply superimposition of type 1 on type 2 diabetes. In this case, GADA should be immediately measured, and if type 1 on type 2 diabetes is diagnosed, insulin therapy should be considered.

Previous observational studies regarding SPIDDM showed that diabetes in the non-insulin-dependent state with GADA titers ≥ 10 U/mL is a higher risk for progression to an insulin-dependent state as compared to patients with GADA titers < 10 U/mL [13, 14]. Considering these findings, the shorter duration between diabetes diagnosis and determination of GADA seroconversion observed in the high GADA titer group that did not receive insulin therapy might indicate higher activity of anti-islet autoimmunity. Meanwhile, in patients receiving insulin therapy, this duration was comparable between patients with high and low GADA titers, indicating that exogenous insulin may mask abrupt exacerbation of hyperglycemia and lead to a loss of opportunities to measure GADA in both groups.

In this study, the timing of GADA measurement was left to the discretion of each physician. We investigated the reasons for the primary physicians' decisions to measure GADA during the clinical course and found that of 38 patients, 32 underwent the GADA test owing to poor glycemic control, 2 underwent the GADA test incidentally, regardless of the level of glycemic control, and 1 patient underwent the test as a screening test before initiation of interferon therapy against chronic hepatitis C infection. Unfortunately, we could not obtain details of

the remaining 3 cases due to lack of information. Thus, this survey found that poor glycemic control was a factor leading to GADA measurement in the majority of our cases. For reference, there were very few differences in the clinical findings and outcomes of the 32 cases and those of the original 38 cases (data not shown). Besides, there was no case in which GADA seroconversion was found through regular repetitions of GADA tests, regardless of glycemic control.

The present study has several limitations. First, because this is a case series study, it is difficult to generalize our results. Second, to clarify the clinical characteristics of diabetes patients showing GADA seroconversion during the course of type 2 diabetes, it is essential to establish appropriate control subjects (e.g., patients with type 2 diabetes who were found to test negative for GADA twice consecutively during intervals similar to those in our cases). However, we could not collect data regarding such cases using the ICHUSHI database. Third, the response rate for the questionnaires was low, which may have affected the interpretation of the data obtained to some extent. Moreover, for the data we collected on diabetic patients with GADA seroconversion, the continuity or reproducibility of GADA values after the first determination of GADA seroconversion could not be fully confirmed. We may have included patients with transient seroconversion of GADA seroconversion who should have been considered as having type 2 diabetes, possibly leading to misinterpretation of data. Furthermore, the assay used in Japan for measuring GADA was changed from a RIA to an enzyme-linked immunosorbent assay (ELISA) in December 2015. If we had analyzed the clinical characteristics by using GADA levels determined by the ELISA method, our results may have been different. Finally, although there was a possibility of an association between HLAs susceptible for type 1 diabetes and duration up to determination of GADA seroconversion data, HLA data were not fully retrieved from questionnaires.

Conclusion

In this nationwide survey, we could not determine apparent triggers of GADA seroconversion during the clinical course of type 2 diabetes, regardless of the presence or absence of insulin therapy. Physicians may need to consider the possibility of concurrent type 1 diabetes during the therapeutic course of type 2 diabetes; in particular, measurement of GADA levels should be considered when non-obese type 2 diabetic patients not receiving insulin therapy experience unexpected abrupt hyperglycemia and when those receiving insulin therapy show lower serum C-peptide levels.

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Compliance with ethical standards

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Ethical standards All procedures followed were in accordance with the ethical standards of the responsible committee on human experimentation (institutional and national) and with the Helsinki Declaration of 1964 and later versions. Informed consent or substitute for it was obtained from all patients for being included in the study.

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