Anti-Glutamic Acid Decarboxylase Antibody in Graves’ Disease Is A Possible Indicator for The Unlikelihood of Going into Remission with Antithyroid Agents

AI YOSHIHARA, OSAMU ISOZAKI, YUMIKO OKUBO AND KAZUE TAKANO

Department of Medicine, Institute of Clinical Endocrinology, Tokyo Women’s Medical University

Abstract. The prevalence and titer of glutamic acid decarboxylase antibody (GADAb) in type 1 diabetes mellitus (T1DM) has been reported to be higher in patients with autoimmune thyroid diseases (AITD) than those without them. However, we have no data about the influence of GADAb on AITD. We therefore studied the clinical characteristics of Graves’ disease (GD) with GADAb in order to clarify the influence of GADAb on GD. Twelve GD patients with GADAb were enrolled and were compared to 40 GD patients without DM. The male to female ratio and age of onset of GD showed no statistical difference. The titer of TSH receptor antibody (TRAb) at the onset of GD was similar in both groups. Initial treatment with methimazole (MMI) was started in all patients with GADAb but radioactive iodine (RI) therapy was carried out in five patients because of adverse effects of MMI or poor control of hyperthyroidism. The initial titer of TRAb was significantly lower in patients treated with MMI alone compared to that in RI treated patients but none of the patients treated with MMI alone went into remission after more than 3-years of follow up. We also compared these GADAb-positive patients with 14 patients with diabetes mellitus who had matched clinical features. The number of diabetic patients who remained in possible remission was significantly higher than that of GADAb-positive patients (5 in 14 vs 0 in 12). Moreover, the rate of remission in the diabetic patients was no different from that of 21 control patients without diabetes followed for more than 7 years (5 in 14 vs 7 in 21). These data suggested that GADAb-positive patients are unlikely to go into remission with antithyroid agents. Therefore, definitive therapies might be preferable for the initial treatment of GADAb-positive patients.

Key words: GAD antibody, Graves’ disease, T1DM, Antithyroid agents
clinical characteristics of GADAb-positive patients with GD and compared the patients not only to non-diabetic GADAb-negative patients with GD but also to control diabetic patients with GD in order to clarify the effect of GADAb on remission of GD by medical therapy.

**Subjects and Methods**

**Subjects**

Twelve GD patients with GADAb (2 male, 10 female) were enrolled in this study. GADAb was measured at the onset of DM in patients with GD or at the onset of GD in patients with DM. The diagnosis of GD was confirmed by laboratory findings including elevation of serum free thyroxine (T4) and/or free triiodothyronine (T3), suppression of serum TSH, and the presence of TSH receptor antibodies (TRAb). In some cases, radioactive iodine or thyroidal uptake of $^{99m}$TcO$_4^-$ was carried out for validation of the diagnosis according to the diagnostic criteria of the Japan Thyroid Association [8]. We also enrolled diabetic patients with GD who was matched to the GADAb-positive patients in clinical characteristics. We also randomly selected 40 control GD patients without DM (8 male, 32 female) according to the data of first visit from January to June 2000. These patients were referred to the Endocrine Clinic of Tokyo Women’s Medical University Hospital and were followed up by the clinic. However, some of the control GD patients were followed up by other hospitals by the patient’s choice.

**Serum hormone and antibody determinations**

Serum free T4, free T3 and TSH were determined by automated analyzer (Roche Diagnostics K.K, Tokyo, Japan) and TSH receptor antibody (TRAb: thyrotropin binding inhibitory immunoglobulin) was determined by a commercial radioimmunoassay kit (Yamasa Shoyu Corp., Tokyo, Japan). GADAb was also determined by a commercial radioimmunoassay kit using recombinant human GAD 65 (Cosmic Corp., Tokyo, Japan) and a titer of more than 1.5 units per ml was judged as positive.

**Statistical analysis**

Differences in male-to-female ratio between the two groups were analyzed by Fisher’s exact test. All data are expressed as the median ± standard deviation or the median (minimum, maximum) as indicated. Significance between the values was determined by Student’s t-test or Dunnett’s test for the data with normal distribution. We employed Cox-Box transformation for the values that did not fit the normal distribution. We also used Wilcoxon/Kruskal-Wallis test for non-parametric analysis. Statistical calculations were carried out using a computer program, JMP 5.0 (SAS Institute Inc., Tokyo, Japan). We also used Fisher’s exact test for comparison of remission rate between groups. P values less than 0.05 were considered significant.

**Results**

The male to female ratio (2/10) in GADAb positive patients were not different from that in the control patients (8/32) by Fisher’s exact test. The age of onset of Graves’ disease in GADAb-positive patients was not statistically different from that of control patients (42.3 ± 16.3 vs 42.0 ± 15.8 years, respectively).

TRAb was positive in all patients with GADAb. The titer of TRAb at the onset of Graves’ disease was similar to that of control patients (61.7 ± 25.4% vs 56.3 ± 26.3%, respectively).

The onset of DM was distributed from 15 years before the onset of GD to 11 years after the onset of GD and the median of the onset of DM was one year before the onset of GD as shown in Table 1. In 50% of the patients, the difference of the onset DM was within 1.75 years; 1.0 year before to 0.75 year after the onset of GD. DM was controlled without insulin in one patient for more than 3 years.

In GADAb-positive patients, the titers of GADAb were 16.4 to 5480 U/ml (median: 232.1 U/ml). As shown in Fig. 1, the titers of GADAb had no correlation with the titer of TRAb before treatment in these patients.

As shown in Table 1, we next classified the GADAb-positive patients into three subgroups: acute type 1 DM (AIDDM; acute insulin-dependent diabetes mellitus), slowly progressive type 1 DM (SPIDDM; slowly progressive insulin-dependent diabetes mellit-
tus), and type 2 DM (T2DM) according the clinical courses. We defined AT1DM as a patient with acute clinical symptoms, including polyuria, polydipsia, and body weight loss and ketosis/ketoacidosis, and in whom insulin therapy was started within 3 months of the diabetic symptoms. The patient with SPIDDM was defined as a patient with T1DM whose insulin therapy was started after 3 months of the initial diabetic symptoms except ketosis/ketoacidosis. The age of onset of GD in patients with AIDDM was younger than that of other patients. However, the difference of the onset of GD and diabetes, titers of GADAb and TRAb showed no statistical difference between these subtypes.

Radioactive iodine (RI) therapy was carried out in 5 patients because of adverse effects of MMI or poor control of hyperthyroidism. None of the patients treated with MMI alone went into remission after 36 months of therapy. However, the initial titers of TRAb in these patients (median: 49.7%, range: 16.6–68.3%) were significantly lower than those in patients treated with RI (median: 93.5%, range: 35.7–97.7%) as shown in Table 2 (P<0.05, Student’s t-test after Box-Cox transformation).

Next we compared these GADAb-positive patients to diabetic patients in order to clarify the impact of GADAb on outcome of GD. We enrolled 14 diabetic patients who had similar clinical characteristics to the case patients. None of the patients were positive for GADAb in the past. Thyroid hormone levels were normal after withdrawal of MMI for more than one year (possible remission by MMI) in 5 out of 14 dia-

### Table 1. Clinical characteristics of GADAb-positive patients (pts) classified according to the types of DM

<table>
<thead>
<tr>
<th>Type of DM</th>
<th># of pts</th>
<th>Gender (M/F)</th>
<th>Age of onset of GD (year)</th>
<th>Onset of DM after GD (year)</th>
<th>GADAb (U/ml)</th>
<th>TRAb (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>AIDDM</td>
<td>4</td>
<td>0/4</td>
<td>27.5 (16, 35) *</td>
<td>−2.5 (−11, −1)</td>
<td>232.1 (16.4, 628.5)</td>
<td>55.75 (16.6, 93.5)</td>
</tr>
<tr>
<td>SPIDDM</td>
<td>7</td>
<td>1/6</td>
<td>47.0 (30, 70)</td>
<td>0.0 (−15, 11)</td>
<td>439.0 (32.8, 5480)</td>
<td>62.1 (37.5, 97.7)</td>
</tr>
<tr>
<td>T2DM</td>
<td>1</td>
<td>1/0</td>
<td>64.0</td>
<td>−1.0</td>
<td>41.6</td>
<td>68.3</td>
</tr>
<tr>
<td>All patients</td>
<td>12</td>
<td>2/10</td>
<td>42.5 (16, 70) *</td>
<td>−1.0 (15, 11)</td>
<td>232.1 (16.4, 5480)</td>
<td>62.1 (16.6, 97.7)</td>
</tr>
</tbody>
</table>

GAD-positive patients were classified into three groups; acute T1DM (AIDDM), SPIDDM, and type 2 DM according the clinical course as described in results.

* The values are median (minimum, maximum).

* The value was significantly lower than that of other types of patients (P<0.05, Dunnett’s test).

** The value was not significantly different from that of the control GD patients without DM.

### Table 2. Clinical characteristics of patients treated with radioactive iodine (RI)

<table>
<thead>
<tr>
<th>Case #</th>
<th>Type of DM</th>
<th>Initial TRAb (%)</th>
<th>GADAb (U/ml)</th>
<th>Reasons for RI therapy</th>
<th>Final treatment of GD</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>AIDDM</td>
<td>93.5</td>
<td>16.4</td>
<td>liver dysfunction induced by MMI</td>
<td>l-thyroxine replacement</td>
</tr>
<tr>
<td>2</td>
<td>SPIDDM</td>
<td>35.7</td>
<td>60.8</td>
<td>poor control of hyperthyroidism</td>
<td>None</td>
</tr>
<tr>
<td>3</td>
<td>SPIDDM</td>
<td>93.7</td>
<td>122</td>
<td>fever and arthralgia induced by MMI</td>
<td>l-thyroxine replacement</td>
</tr>
<tr>
<td>4</td>
<td>SPIDDM</td>
<td>74.2</td>
<td>439</td>
<td>poor control of hyperthyroidism</td>
<td>MMI</td>
</tr>
<tr>
<td>5</td>
<td>SPIDDM</td>
<td>97.7</td>
<td>1450</td>
<td>poor control of hyperthyroidism</td>
<td>l-thyroxine replacement</td>
</tr>
<tr>
<td>Sum of RI treated cases</td>
<td>–</td>
<td>(35.7, 97.7)*</td>
<td>(16.4, 1450)**</td>
<td>–</td>
<td>–</td>
</tr>
</tbody>
</table>

* The values are median (minimum, maximum) of the five cases treated with radioactive iodine.

* The value was significantly higher than that of patients with GADAb who were not treated with RI (P<0.05, Student’s t-test after Box-Cox transformation).

** The value was not statistically different from that of patients with GADAb who were not treated with RI.
Therefore the possible remission rate by MMI was significantly lower in GADAb-positive GD patients compared to that in diabetic patients (0 in 12 vs 5 in 14, P<0.05 in Fisher’s exact test). On the other hand, the number of diabetic patients who went into possible remission was similar to that of control patients without DM after 7-year-follow up (5 in 14 vs 7 in 21, P>0.05 in Fisher’s exact test).

### Discussion

The presence of GD and T1DM in the same subject is not usual, but the molecular etiology for this condition has been identified in only a small number of patients such as APS-1 [3]. One Japanese study on GADAb in patients with both GD and T1DM suggests a close immunological relationship between the two diseases in one patient rather than an incidental complication [9]. In support of this close immunological relationship hypothesis, their study showed that the onset of GD and T1DM was simultaneous in 6 out of 14 patients, and that the onset of GD was earlier in 8 out of their 14 patients with GADAb. In our study, the difference of onset of GD and DM was less than one year in 50% of the patients with GADAb. These data also support the close immunological relationship theory. However, it should be remembered that these observations do not necessarily suggest simultaneous

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### Table 3. Comparisons of GADAb-positive, diabetic and control patients (pts) with Graves’ disease

<table>
<thead>
<tr>
<th></th>
<th>GADAb-positive</th>
<th>Diabetic</th>
<th>Control</th>
</tr>
</thead>
<tbody>
<tr>
<td># of pts</td>
<td>12</td>
<td>14</td>
<td>40</td>
</tr>
<tr>
<td>Male/Female</td>
<td>2/10</td>
<td>4/10</td>
<td>8/32</td>
</tr>
<tr>
<td>Age of onset of Graves’ disease (year)*</td>
<td>42.5 (16, 70)</td>
<td>42.5 (18, 58)</td>
<td>44.5 (19, 80)</td>
</tr>
<tr>
<td>TRAb at the onset of Graves’ disease (%)*$</td>
<td>62.10 (16.6, 97.7)</td>
<td>69.45 (26.96, 107.0)</td>
<td>51.22 (20.12, 110.0)</td>
</tr>
<tr>
<td>Types of DM*</td>
<td>4/7/1</td>
<td>3/0/11</td>
<td>–</td>
</tr>
<tr>
<td>Therapy (Tx) for DM*</td>
<td>11/0/1</td>
<td>5/7/2</td>
<td>–</td>
</tr>
<tr>
<td>Onset of DM after or before Graves (year)*</td>
<td>–1.0 (-15, 11)</td>
<td>–1.25 (-10, 20)</td>
<td>–</td>
</tr>
<tr>
<td>Final treatment of GD (MMI/RI/Operation)*</td>
<td>7/5/0</td>
<td>13/1/0</td>
<td>16/4/15</td>
</tr>
<tr>
<td># of pts in possible remission</td>
<td>0/12</td>
<td>5/14</td>
<td>7/21</td>
</tr>
<tr>
<td>Duration of MMI therapy (year)*</td>
<td>4.0 (3, 7)</td>
<td>6.0 (1, 12)</td>
<td>7.0 (7, 8)</td>
</tr>
</tbody>
</table>

* The values are median (minimum, maximum).

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![Fig. 1.](image-url) Relationship between GADAb and TRAb in GADAb-positive patients with GD. The initial titer of TRAb before therapy was expressed as % of inhibition according to the manufacture of the radio-receptor assay kit. Titer of GADAb as U/ml was expressed in log scale. Linear regression analysis was conducted between the Box-Cox transformed titers of TRAb and GADAb as described. The analysis did not show any significant correlation between the titers.
development of DM and GD in GADAb-positive patients because we measured GADAb only at the onset of DM in GD patients and at the onset of GD in diabetic patients. Therefore, patients who used to have GADAb only at the onset of DM may have been overlooked in this study, which is often observed in patients with the acute type of T1DM [4]. However, GADAb in patients with GD may suggest the presence of common contributing factors in the pathogenesis of DM and GD in these patients.

The relatively higher titer of GADAb in our GADAb-positive patients is compatible with previous reports on GADAb in patients with autoimmune thyroid diseases [5, 6]. However, the titer of TRAb at the onset of GD was similar in both groups and had no correlation with the titer of GADAb. These observations suggest that the production of GADAb and TRAb may be regulated at least partially by some common mechanisms but not by the same mechanism in these patients. These data are also compatible with a previous report [9].

The observation that none of the GADAb-positive patients in our study went into remission with MMI therapy in spite of the lower titer of TRAb compared to that in patients treated with RI also suggested that GADAb itself had no effect on initial disease activities of GD, but that disease activity in patients with GADAb may persist for long periods. A similar observation is also reported in GD patients with SPIDDM [4]. They reported that eight GD patients with GADAb (titer more than 40 U/ml) before therapy had higher titers of both GADAb and TRAb after 6-year-treatment with MMI [10]. They therefore recommended RI therapy or subtotal thyroidectomy for GD patients with high titer of GADAb. Compatible with the previous report the number of patients who went into remission in the present study was significantly less in GADAb positive patients compared to that in control diabetic patients.

Persistence of the disease activity of GD in GADAb-positive patients may also be suggested by the fact that the titer of GADAb itself is sustained for a prolonged period in some diabetic patients. GADAb in SPIDDM is usually higher in titer and it is sustained in higher titer for longer periods compared to that in AIDDM [4, 10]. Sustained high titer of GADAb is also reported in T1DM patients with AITD [6]. Moreover, a recent study revealed that the epitope regions of GADAb in SPIDDM are different from that in AIDDM [11]. Therefore, the epitope regions of GADAb in patients with AITD may different from those in patients without AITD, and the difference of GADAb might contribute not only to the higher titer of GADAb but also to the sustained GADAb production. However, there is no data about the difference of epitope of GADAb in GD patients so far.

The sustained activity of GD in GADAb-positive patients may be explained by genetic markers related to autoimmunity. According to previous reports the frequency of GG genotype or G allele at position 49 of CTLA-4 polymorphism is significantly higher in GADAb-positive patients with both GD and TIDM compared to GADAb-negative patients [9, 12]. With respect to the CTLA-4 polymorphism, one study demonstrated that the GG genotype is associated with significantly longer time until remission with antithyroid drugs [13]. Taken together, it is reasonable to assume that GADAb in GD patients may be a possible marker indicating the unlikelihood of going into remission with antithyroid agents.

Recently Miya et al. reported a case of SPIDDM and GD which was complicated with MMI-induced liver dysfunction and agranulocytosis [14]. We have no evidence to indicate that GADAb may increase the incidence of adverse reaction to MMI, but RI therapy might be preferable in patients who should continue to take antithyroid agents for a prolonged period in order to avoid the adverse effects of antithyroid agents. The fact that we should abandon MMI-therapy in two out of 12 patients because of its adverse effects also supports the choice of definitive therapy as initial treatment. Therefore, definitive therapy might be preferable for GD in T1DM patients with GADAb.

In sum, this study suggested that GD patients with GADAb are unlikely to go into remission with antithyroid therapy. Therefore, definitive therapies might be preferable for these patients at the start of therapy. However, prospective studies with longer observation periods and with larger numbers of patients with or without GADAb are necessary to clarify the exact role of GADAb in GD.
References


