Detection of GAD$_{65}$ Antibodies in Newly Onset Type 1 Diabetic Children

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Summary:

Background: Glutamic acid decarboxylase (GAD) has been defined as a major target antigen in type 1 diabetes mellitus (T1DM).

Aim of the study: Assessment of GAD$_{65}$ autoantibodies in the serum of T1DM children at onset of the disease.

Patients & Methods: Serum GAD$_{65}$ autoantibodies has been estimated in 60 sera samples of newly diagnosed T1DM children (diagnosed less than 5 months) in comparison with 50 healthy control children using IRMA method.

Results & conclusion: A higher significant proportion of the patients were positive to GAD$_{65}$ autoantibodies (50%) in comparisons with healthy controls (4.76%) in age group ≤10 years old and (6.90%) in > 10 years old (p<0.0001), whereas a significant proportion of girls tested positive for GADA were in age group >10 years old (p<0.05). We concluded that GADA are an excellent diagnostic marker for T1DM.

Key words: GAD autoantibodies, T1DM.

Introduction:

Type 1 Diabetes Mellitus (T1DM) is an organ-specific autoimmune disease characterized by lymphocyte infiltration of pancreatic islets of Langerhans, and by complete destruction of pancreatic β-cells. The disease process occurs over long periods of time, often years eventually reaching a stage where insufficient β-cells are available with insufficient insulin production [1].

The disease generally shows a peak for clinical onset between 10-14 years of age with a sharp drop in late teens. It may be that children susceptible to the disease are exposed to the predisposing factor(s) during their first 14 years of the life and that either exposure subsequently decreases or a large proportion of the susceptible individuals has already developed the disease by the time they reach 20 years of age [2].

The autoimmune mediated destruction of pancreatic β-cells is reflected by the presence of autoantibodies against prominent antigens in the pancreatic β-cells often long periods of time before the disease become clinically manifested [3]. The HLA type of the individual may control the recognition of certain autoantigens including insulin, glutamic acid decarboxylase (GAD$_{65}$ and GAD$_{67}$), membrane proteins that are homologous to tyrosin phosphatase (ICA512 and IA-2), and islet neuroendocrine ganglioside [4], that may occur individually or in combination [5,6].

The frequency of GAD autoantibodies (GADA) has been reported to vary from 0.5 – 3% among children from background population [7], from 6.4 - 13% among siblings of children with T1DM (5), and from 62 - 84% among patients with newly diagnosed disease [9]. More than 80% of prediabetics and most recent onset diabetics have autoantibodies directed against GAD [9]. The data of T-cell reactivity to various diabetes associated antigens in diabetic patients and non-diabetic controls have remained inconsistent. An increased proportion of activated T-lymphocytes in the peripheral circulation was reported in association with the presence of insulin autoantibodies (IAA) [10]. Increased T-cells responses to diabetes associated autoantigens were detected in newly diagnosed patients with T1DM and in antibody positive first degree relatives as compared with healthy controls [11]. Peripheral blood mononuclear cells (PBMs) from approximately one-half of newly-onset T1DM patients were found to respond to GAD [12]. GADAs may play a significant role in the processing and presentation of T-cell epitope from the human GAD$_{65}$ autoantigen to T-cells through increasing the efficiency of antigen capture by antigen presenting cells (APCs) [13]. GADAs are also more frequent in girls than in boys [14] and in individuals more than 10 years of age [15].

In Iraqi patients, we have no available data on the presence of GAD$_{65}$ autoantibodies with the age at onset of the T1DM in children.

Subjects, Materials & Methods:

Subjects:

Sixty Iraqi Type 1 diabetic patients (28 males and 32 females) were subjected to this study. The patients were attending to National Diabetes Center at Al-Mustansiyra University/ College of Medicine
during the period May 2004 to October 2005. Their ages range from 3-17 years, and they were new onset of the disease (diagnosis was from one week up to five months). All the patients were treated with daily replacement doses of insulin at the time of blood sampling. The patients were divided into two groups according to their ages: 36 child equal or less than 10 years and 24 children more than 10 years. For the purpose of comparisons, 50 healthy subjects matched for age (4-17 years old) and sex, and were selected who have no family history or clinical evidence of type 1 diabetes or any chronic diseases and obvious abnormalities as a control group.

**Blood collection:**
Two milliliter of blood was collected into plain test tubes, then the serum was separated by centrifugation at 2500 rpm for 10 min. and kept at -20°C until used.

**Assessment of serum anti-GAD$_{65}$ autoantibodies:**
Serum anti-GAD$_{65}$ autoantibodies were measured by Immunoradiometric assay (IRMA) using anti-GAD IRMA kit (Immunotech Beckman Coulter, France). Values are calculated by interpolation from the standard curve. Values below 1U/ ml were considered normal, whereas values above 1U/ ml should therefore consider as pathological.

Statistical analysis was performed by using Chi Square test and P value equal or below 0.05 is considered as significant.

**Results:**
The proportion of index cases positive for the both age groups in comparison with controls was shown in table (1). A higher significant proportion of the patients was positive to GADA in both age groups (18/36, 50% and 12/24, 50% respectively) as compared to control groups (1/21; 4.76% and 2/29; 6.9% respectively). This differences were highly significant $P_1=0.0001$.

GADA were detected in 30 of Iraqi children with newly diagnosed T1DM (50%). A higher significant proportion of the girls tested positive for GADA (5/9; 55.6%) were observed in age group >10 years old than of girls ≤10 years old (11/23; 47.8%), ($P_2=0.049$); while the proportion of boys tested positive for GADA was higher in age group ≤10 years old than >10 years old (7/13; 53.8% vs 7/15; 46.7%), but this difference was not significant ($P_2=0.804$), as shown in table (2). No statistical differences were observed between males and females in each age group ($P_1=0.729$ and 0.673 respectively).

<table>
<thead>
<tr>
<th>Age</th>
<th>Groups</th>
<th>No.</th>
<th>Sero positive</th>
<th>Sero negative</th>
<th>$P_1$</th>
<th>$P_2$</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>No.</td>
<td>%</td>
<td>No.</td>
<td></td>
<td></td>
</tr>
<tr>
<td>≤10 years</td>
<td>Controls</td>
<td>21</td>
<td>1</td>
<td>4.76</td>
<td>20</td>
<td>95.24</td>
</tr>
<tr>
<td></td>
<td>T1DM</td>
<td>36</td>
<td>18</td>
<td>50.00</td>
<td>18</td>
<td>50.00</td>
</tr>
<tr>
<td>&gt;10 years</td>
<td>Controls</td>
<td>29</td>
<td>2</td>
<td>6.90</td>
<td>27</td>
<td>93.10</td>
</tr>
<tr>
<td></td>
<td>T1DM</td>
<td>24</td>
<td>12</td>
<td>50.00</td>
<td>12</td>
<td>50.00</td>
</tr>
</tbody>
</table>

Table 1: Differences of sero positive / negative of GADA between control and T1DM patient groups.
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Table 2: Differences of sero positive / negative of GADA between T1DM males and females patients.

<table>
<thead>
<tr>
<th>Parameter</th>
<th>≤10 years (n=36)</th>
<th>&gt;10 years (n=24)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>GADA+</td>
<td>GADA–</td>
</tr>
<tr>
<td>Males</td>
<td></td>
<td></td>
</tr>
<tr>
<td>No.</td>
<td>7</td>
<td>6</td>
</tr>
<tr>
<td>%</td>
<td>53.8</td>
<td>46.1</td>
</tr>
<tr>
<td>Females</td>
<td>11</td>
<td>12</td>
</tr>
</tbody>
</table>

Discussion:
GAD autoantigen is neither beta-cell nor islet specific and is expressed predominantly in the nervous system and other tissues, including the testes, ovary, adrenal, pituitary, thyroid and kidney\(^{16}\).

The present results indicated that older children were more often tested positive than younger ones in females (55.6 vs 47.8%). This difference seems to be not significant between males in both age groups (table 2). This result is in disagreement with Sabbah, (2000), but in agreement with Graham et al. (2002) which indicated that GADA was less affected by age at clinical onset in patients than other autoantibodies marker.

Islet cells reactivity as judged by the presence of antibodies to the GAD65 were observed in 50% of the patients studied in both age groups (table 1), 55.6% in >10 years old group were females. Our observation is in consistent with other studies and supports the notion that autoimmunity is more common among females more than 10 years old\(^{14, 15}\).

In conclusion GADA were present in 50% of diabetic children. Older children were tested positive for GADA more than younger ones, especially females.

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