Evaluation of Anti-GAD65 and HbA1c Prevalence among Newly Diagnosed Type 1 Diabetes of Some Iraqi Children

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Abstract

Type 1 diabetes mellitus (T1DM) is a chronic autoimmune illness defined by the gradual and selective destruction pancreatic beta-cells throughout childhood or adolescents. Anti-Glutamic acid decarboxylase 65 (anti-GAD65) is circulating autoantibodies to insulin secreting pancreatic β-cells antigens, and considered as one of the islet cell autoimmunity markers. Hemoglobin A1C (HbA1c) is a blood glucose monitoring measurement that is used as an indication of blood glucose average measurement over a period of several months. According to the significance of prior subjects and in order to have a greater understanding, this study is planned to evaluate the anti-GAD65 and HbA1c frequency and their association among T1DM children. One hundred of new onset T1DM patients consist of males and females with ages ranging from 1-15 years. Blood samples were collected for biochemical and immunological study. The present study results show anti-GAD65 in 87% were with mean concentration (69.03 IU/ml), 14% with mean concentration (3.8 IU/ml) of total T1DM patients. While the results of estimation of HbA1c level in the present study showed a total mean average of HbA1c was (11.22 %) in newly diagnosed T1DM patients. The association between anti-GAD65 and HbA1c showed that positive anti-GAD65 were with (11.75 %) mean level of HbA1c, in compared to (7.66 %) mean level of HbA1c in negative anti-GAD65 T1DM group and that HbA1c levels were significantly higher, according to statistical analysis in positive anti-GAD65 patients than in those who had negative anti-GAD65 at (P < 0.01), suggesting that autoimmunity was found to be involved in glycemic indices reflected by HbA1c levels particularly among very young children who had been developed type 1 diabetes.

1. Introduction

Type 1 diabetes mellitus (T1DM) is a chronic condition characterized by immune-mediated damage of insulin-producing beta-cells (β-cells) in pancreas. That insulin deficiency comes from the loss of β-cells, resulting in a life-threatening hyper-glycemia [1]. It is caused, like other autoimmune illnesses, by the interplay of genetic and environmental variables, both of which have been linked to an increased risk of type 1 diabetes mellitus [2]. The immune system involvement in disease developing has been known for many years, supported by evidence of
antibodies detection against antigens of the islets of Langerhans of susceptible individuals [3]. Anti-glutamic acid decarboxylase 65 (anti-GAD65) is one of these autoantibodies is thought to be a critical antigen for the development of beta cell autoimmunity and has been found in 50-80 percent of T1DM patients [4].

The discovery of Glutamic acid decarboxylase (GAD) as an autoantigen target of T1DM dates back to 1982 as a 64,000 KD immunoprecipitated from islets of T1DM children that were newly diagnosed. At the time of clinical manifestation, autoantibodies against GAD65 are identified in 80% of T1DM patients [5]. GAD catalyzes the synthesis of gamma-aminobutyric acid (GABA), a central nervous system transmitter. GAD65 and GAD67 are two forms of GAD, known to be generated in body tissues of humans, with GAD65 being more prevalent in beta-cells of pancreas [6].

Hemoglobin A1c is a kind of haemoglobin that is chemically attached to a sugar; this process of sugar attachment to haemoglobin is known as glycation [7]. The construction of the sugar-hemoglobin link shows the presence of excessive glucose in the blood-stream, which is frequently associated with diabetes mellitus. The test of HbA1c is restricted to a three-month median since a red blood cell has a four-month lifetime. Because individual red blood cells have different lifespans, the test is restricted to three months [8]. Normal glucose levels result in a normal quantity of glycated hemoglobin; when the average amount of plasma-glucose rises, the proportion of glycated hemoglobin rises predictably [7,9]. Therefore, the aim of this study was for anti-GAD65 (as marker for autoimmunity) and HbA1c (as a marker for glycemic control) prevalence evaluation in some of the newly diagnosed children with T1DM.

2. Experimental Procedure

This study was carried out from beginning of November 2020 to the end of May 2021. A total of one hundred blood samples were collected from newly diagnosed with T1DM children who attended Pediatric teaching hospital, Children’s protection teaching hospital, Al-Alwiya pediatrics hospital and Ibn-Albaladi pediatrics hospital in Iraq. The target population was the new-onset diagnosed T1DM children of all ages ranging from one year old to 15 years old. Five milliliters of whole blood were collected. The drawn blood was immediately separated into two parts; the first part (3ml) was transferred into gel tubes, then centrifuged for 5 minutes at 4000 rpm, the resulting serum was dispensed into three separate plain tubes, one tube for measuring of glucose level, the second for measuring anti GAD65 and the third collected into an anti-coagulated EDTA tube for measuring HbA1c level.

The samples were obtained based on clinical symptoms such as increased urine, acute hunger, thirst, and sudden loss of weight, as well as a fasting blood glucose test (above 126 mg/dL) and a random blood glucose test (above 200 mg/dL). The information of patients including age, gender, family history of the T1DM and other chronic disease, were taken from patient’s parents or companion during the questionnaire.

Anti-GAD65 was detected as a biomarker in serum of the patients using an enzyme-linked immunosorbent assay (ELISA) quantitates kit (Medizym, Germany); Procedure was performed according to the kit instructions. Detection of HbA1c in T1DM patients’ serum had been performed by Multicare analyzer (SD BIOSENSOR, Korea), quantitative reflectometry and immunoassay technologies were used. The test kit employs an anti-HbA1c antibody that recognizes the first few amino acid residues of the glycated chain of hemoglobin A. The Statistical Analysis System - SAS (2012) program had been performed for detection difference factors consequence in this study parameters. T-test used for comparison between means suggestively.

3. Results and Discussion

Measuring of Anti-GAD65 Auto Antibodies: The present results showed anti-GAD65 in 87% (87/100) were with mean concentration (69.03±46.14 IU/ml), 14% (14/100) with mean concentration (3.8±0.89 IU/ml) of total T1DM patients in all three age groups. The age group (6-10) years old has the highest positive anti-GAD65 antibodies percentage of 41% (41/100), with lowest mean (50.21 ±42.07 IU/ml). While, the age group (1-5) years old 21% (21/100) has the lowest percentage, with the highest mean (82.04 ±55.48 IU/ml). According to the statistical examination of the results, there is a substantial difference at (P<0.01) between age groups, as shown in Table (1).
Table (1). The distribution of T1DM patients with positive and negative anti-GAD antibodies by age group.

<table>
<thead>
<tr>
<th>Patients group</th>
<th>Positive anti-GAD65 No. (%)</th>
<th>Negative anti-GAD65 No. (%)</th>
<th>Positive anti-GAD65 Mean cons. ± SD (IU/ml)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1-5 years</td>
<td>21 (21.00%)</td>
<td>3 (3.00%)</td>
<td>82.04 ±55.48 a</td>
</tr>
<tr>
<td>6-10 years</td>
<td>41 (41.00%)</td>
<td>9 (9.00%)</td>
<td>50.21 ±42.07 b</td>
</tr>
<tr>
<td>11-15 years</td>
<td>24 (24.00%)</td>
<td>2 (2.00%)</td>
<td>58.07 ±48.21 b</td>
</tr>
<tr>
<td>Total</td>
<td>86 (86.00%)</td>
<td>14 (14.00%)</td>
<td>48.28 ±43.12</td>
</tr>
<tr>
<td>P-value</td>
<td>0.0001 **</td>
<td>0.04762 *</td>
<td>0.0277 *</td>
</tr>
</tbody>
</table>

* (P≤0.05), ** (P≤0.01).

In Regarding to gender, Table (2) shows that the patient groups' male and female distributions did not differ substantially. Totally, there were 47% (47/100) with mean (55.01±47.77 IU/ml) in female group, while the males were constituted 39% (39/100) with mean (67.22±49.08 IU/ml).

Table (2). The distribution of T1DM patients with positive and negative anti-GAD65 by gender groups.

<table>
<thead>
<tr>
<th>Patients group</th>
<th>Positive Anti-GAD65 No. (%)</th>
<th>Negative Anti-GAD65 No. (%)</th>
<th>Positive anti-GAD65 Mean ± SD (IU/ml)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Female</td>
<td>47 (47.00%)</td>
<td>12 (12.00%)</td>
<td>55.01 ±47.77</td>
</tr>
<tr>
<td>Male</td>
<td>39 (39.00%)</td>
<td>2 (2.00%)</td>
<td>67.22 ±49.08</td>
</tr>
<tr>
<td>P-value</td>
<td>0.2317 NS</td>
<td>0.094 NS</td>
<td>0.291 NS</td>
</tr>
</tbody>
</table>

NS: Non-Significant.

The presence of circulating anti-GAD65 antibodies have been proposed as predictive markers of T1DM [10]; The result of the present study goes with results of an Iraqi study demonstrated 88.6% of T1DM patients were positive to anti-GAD65 [11]; The results also in accordance with Tunisian study, 84.6 percent of children with newly diagnosed diabetes had seropositive anti-GAD65 antibodies (within six months of diagnosis) [12] and accords with abroad study that found (89%) of seropositivity rate were also among the patients with new-onset T1DM using the anti-GAD65 ELISA test [13]. In contrast, other Iraqi studies exhibited a lower percentage of anti-GAD65 seropositivity [14]and [15]. Those conflicting results might be explained by the varying cut-off values used in diagnostic kits to determine test sensitivity [11] and/or varying the autoimmunity progression level from person to person among different populations [16].

Regarding the age distribution of anti-GAD65 autoantibodies, several studies have shown that the incidence of anti-GAD65 autoantibodies reduced as the period of T1DM increased, with the prevalence of anti-GAD65 in patients with disease duration less than 5 years being 78.3 percent, and beginning to decline in those with disease duration more than 12 years, attributable to the depletion of islet cell autoantibodies with time [17]. While gender distribution: accorded with other studies that found no significant differences in mean anti-GAD65 levels between males and females of Type 1 diabetic patients, although there was a slight trend towards higher values in females [15,18].

Estimation of Glycated Hemoglobin Level: The results of estimation of glycated haemoglobin (HbA1c) level in the present study showed a total mean average of HbA1c was (11.22±2.69) % in newly diagnosed T1DM patients. Distributed as (11.38±2.78) % in females and (12.28±1.71) % in males, according to the statistical analysis, there has been no considerable difference between males and females (P =0.698), as shown in Table (3).
Table (3). The distribution of HbA1c level according by gender.

<table>
<thead>
<tr>
<th>Gender group</th>
<th>HbA1c level (mean±SD) %</th>
</tr>
</thead>
<tbody>
<tr>
<td>Female</td>
<td>11.38±2.78</td>
</tr>
<tr>
<td>Male</td>
<td>12.28±1.71</td>
</tr>
<tr>
<td>Total</td>
<td>11.22±2.69</td>
</tr>
</tbody>
</table>

T-test (P-value) = 2.631 NS (0.698)

NS: Non-Significant.

Estimation the levels of HbA1c considered an important biochemical test which is commonly used as a screening tool and monitoring for diabetes mellitus [1]. Current results demonstrated an elevation above normal standard values in all patients included in this study. According to the recommendation of ADA, values < 6.5% are considered an indicator for the diagnosis of diabetes mellitus [19]. The difficulty in attaining glycemic control in T1DM patients has been linked to the fact that T1DM develops owing to beta cell death instead of insulin resistance as in classic type 2 diabetes-mellitus [20].

Parallel studies measured HbA1c level in T1DM patients, such as study which reported Above 7.8% HbA1c level in newly diagnosed diabetic children [21], besides, a study that exhibited HbA1c levels > 9.4 % at newly diagnosed T1DM patients prior starting insulin treatment [22], and study which showed that HbA1c >10% [23].

The results of current study and those previous studies may suggest a poor control of HbA1c in those diabetic patient attributing that to undiagnosed diabetes in those patients until they developed a sever clinical symptoms or developed a diabetic ketoacidosis (DKA) condition.

The Association between Anti-GAD65 and HbA1c: The results of current study showed that positive anti-GAD65 T1DM patients' group were with (11.75±2.40 %) mean level of HbA1c, in compared to (7.66±0.95 %) mean level of HbA1c in negative anti-GAD65 T1DM group. The statistical analysis revealed that HbA1c values were substantially higher in anti-GAD65 autoantibody-positive individuals than in those who had anti-GAD65 autoantibody-negative at (P < 0.01), as demonstrated in Table (4).

Table (4). The association between anti-GAD65 and HbA1c.

<table>
<thead>
<tr>
<th>Patients group</th>
<th>HbA1c % (Mean)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Positive anti-GAD65</td>
<td>11.75±2.40</td>
</tr>
<tr>
<td>Negative anti-GAD65</td>
<td>7.66±0.95</td>
</tr>
<tr>
<td>T-test (P-value)</td>
<td>1.893 ** (0.0078)</td>
</tr>
</tbody>
</table>

** (P≤0.01).

The current data might be seen as significant evidence of the involvement of autoimmunity in glycemic indices represented by HbA1c levels in T1DM. That were similar with study suggests that the patients who were positive for the autoantibodies displayed significantly higher HbA1c than those who were autoantibody-negative [24].

At the other hand, these findings contradicted the findings of two other studies [25,26], which found no significant difference in HbA1c levels between patients with positive anti-GAD65 antibodies and those with negative anti-GAD65 antibodies.

4. Conclusions
The results of anti-GAD65 indicated that the anti-GAD65 could be considered as an important diagnostic marker for identification the T1DM patients and these autoantibodies are more occurrence in earlier affected T1DM patients. Moreover, the results showed an elevated consecration levels of anti-GAD65 associated with high HbA1c levels compared with lower levels of anti-GAD65 at onset T1DM patients.
References
