Glutamic Acid Decarboxylase-65 and Indices of Insulin Resistance in Latent Autoimmune Diabetic Patients

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Abstract:
Autoimmune diabetes is a heterogeneous disease which can arise at any age. Subjects with adult-onset autoimmune diabetes who do not necessitate insulin-therapy for at least 6 months after diagnosis are defined as having latent autoimmune diabetes in adults. The current work aimed to evaluate cytoplasmic islet cell autoantibodies and glutamic acid decarboxylase-65 in diabetic patients and their roles in insulin resistance. This study was conducted during the period from the end of December 2018 until the end of May 2019. A total of 60 patients were added to 30 healthy individuals for comparison. All patients reviewing Center for Endocrinology and Diabetes at Al-Kindy Hospital. Some tests were conducted at the center, others at Al-Qasim privet laboratory in Al-Sadr City, and the Health and Medical Technical College-Baghdad and the Office of Scientific Progress in Al-Harithiya. There was a substantial rise in fasting serum glucose, glycated hemoglobin, cytoplasmic islet cell autoantibodies, and glutamic acid decarboxylase-65 while a significant decrease was found in serum insulin and c-peptide in diabetic patient as paralleled to the controls. Additionally, diabetic patients had low levels of triacylglycerol and high density lipoprotein cholesterol. It was found in this study that latent autoimmune diabetes in adults' subset of diabetes exhibited metabolic features consistent with both defective insulin secretion and insulin resistance. They were found to be lean with low triacylglycerol and high density lipoprotein cholesterol levels.

Keywords: Latent autoimmune, diabetes, Islet cell autoantibodies, Glutamic acid decarboxylase-65.

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Introduction:
Latent autoimmune diabetes in adults (LADA) is a heterogeneous disease manifested by a less serious autoimmune process and a wide clinical phenotype paralleled to classical type 1 diabetes mellitus (T1DM), sharing features with both type 2 DM (T2DM) and T1DM (1). Insulin resistance (IR) is impairment in the function of insulin targets cells such as liver, adipocytes and musculoskeletal cells to react to the insulin action. It is a group of several clinical metabolic disorders such as T2DM, obesity, dyslipidemia, and hypertension, commonly identified as metabolic syndrome (MS) (2). The IR is the recognition of the long-term process experienced by over-active feeding, when an abundance of glucose and saturated fat enter the
cell, leading to endoplasmic network stress; low-grade inflammation and hypoxia. Islet autoantibodies are proteins produced by the immune system that has been shown to be associated with T1DM. Testing can detect the presence of one or more of these autoantibodies in the blood. The first large-scale studies of the prediction of T1DM relied upon the detection of cytoplasmic islet cell autoantibodies (ICA) assays based on indirect immunofluorescence. High titer cytoplasmic ICA is frequently related with the existence of multiple islet autoantibodies and consequently a high risk of progress to diabetes. Type 1 DM and LADA are related with the presence of serum autoantibodies, which include autoantibodies of glutamic acid decarboxylase-65 (GAD-65), and ICAs but not insulin, which is found mostly in children with T1DM. Furthermore, these autoantibodies are similar in that they tend to be isotype restricted [immunoglobulin (Ig) G1 predominantly] and polyclonal. Cellular immunological variations are not evidently recognized in autoimmune diabetes, although it has been believed that they are the main pathogen mediator. Glutamic acid decarboxylase [EC 4.1.1.15] is a neuronal enzyme complicated in the synthesis of the neurotransmitter gamma-aminobutyric acid (GABA). There are two main types of GAD, GAD65 and GAD67, which stimulate the formation of GABA at different locations in the cell and different periods of development.

The GAD65 antibody is also the main pancreatic islet antibody and an essential serological marker of predisposition to other autoimmune disease occur with T1MD, including thyroid disease, pernicious anemia, premature ovarian failure, and Addison disease. The aim of the present study was to evaluate ICA and GAD65 in LADA patients and their roles in IR.

**Patients and Methods:** This revision was done during the period from the end of December 2018 until the end of May 2019. A total of 60 patients were added to 30 healthy individuals for comparison. The BMI was normalized between (18-25). All patients reviewing Center for Endocrinology and Diabetes at Al-Kindy Hospital. The patients were asked about the period of time they were infected with DM, the type of treatment they take, weight, height, and waist circumference (WC) as indicated in the questionnaire. Some tests were conducted at the center, others at Al-Qasim privet laboratory in Al-Sadr City, and the Health and Medical Technical College-Baghdad and the Office of Scientific Progress in Al-Harithiya. They were divided into three groups:

- The first group (GI) was patients who take medications as tablets.
- Second group (GII) was Patients who take medications in the form of tablets and insulin by injection.
- Third group (GIII) was healthy people as control.

**Anthropometric Estimation:** The weight and WC were measured for patients and healthy people. Body mass index (BMI) was calculated by applying the following equation:

\[
BMI = \frac{Weight \ (Kg)}{(Height \ (m))^2}
\]

**Biochemical Measurements:**

Fasting serum glucose (FSG), glycated hemoglobin (HbA1c), and serum lipid profile comprising: [total cholesterol (TC), triacylglycerol (TAG), high density lipoprotein cholesterol (HDL-C), low density lipoprotein cholesterol (LDL-C), and very low density lipoprotein (VLDL)] were measured using chemical analyzer. Serum insulin, ICA and GAD65 were measured using enzyme-linked immune-sorbent assay (ELISA) technique.
Estimation of Insulin Resistance:

Insulin resistance was estimated by homeostasis model assessment for IR (HOMA-IR), homeostasis model assessment for β-cell function (HOMA-β), and quantitative insulin sensitivity check index (QUICKI) using the Matthews equation as shown below:

\[
\text{HOMA-IR} = \frac{\text{fasting glucose (mg/dl)} \times \text{fasting insulin (mU/ml)} - 405}{405}
\]

\[
\text{HOMA-}\beta = \frac{20 \times \text{fasting insulin (mU/ml)}}{\text{fasting glucose (mg/dl) - 3.5}}
\]

\[
\text{QUICKI} = \frac{1}{\log \text{fasting glucose (mg/dl)} + \log \text{fasting insulin (mU/ml)}}
\]

Statistical Analysis:

Study analysis of data are presented as means ± SD. Excel 2010 was done to equate the variance in means values between two groups. The \( p \)-value < 0.05 was deliberated substantial.

Results:

Clinical measurements and anthropometric of the LADA groups and the control group are listed in table (1). There was a highly significant increase in weight for the GI group compared to the GIII group, as well as a significant in waist circumference for GI group compared to the GIII group. In addition, there was a substantial variance in BMI of the group GII compared to the group GIII.

Table (1): Clinical measurements and anthropometric of the LADA groups and the control group

<table>
<thead>
<tr>
<th>Parameters</th>
<th>GI</th>
<th>GII</th>
<th>GIII</th>
</tr>
</thead>
<tbody>
<tr>
<td>Number</td>
<td>30</td>
<td>30</td>
<td>30</td>
</tr>
<tr>
<td>Age (years)</td>
<td>43.8±5.695</td>
<td>43.566±5.870</td>
<td>43.0±4.160</td>
</tr>
<tr>
<td>Height (cm)</td>
<td>170.066±4.982</td>
<td>168.266±4.471</td>
<td>168.633±5.404</td>
</tr>
<tr>
<td>Weight (Kg)</td>
<td>72.9±8.506</td>
<td>63.966±4.382</td>
<td>62.666±4.816</td>
</tr>
<tr>
<td>WC (cm)</td>
<td>93.768±7.330</td>
<td>89.232±3.844</td>
<td>90.220±5.873</td>
</tr>
<tr>
<td>BMI (Kg/m²)</td>
<td>23.039±2.196</td>
<td>23.073±0.929</td>
<td>22.345±1.549</td>
</tr>
</tbody>
</table>

GI: Patients who take medications as tablets, GII: Patients who take medications in the form of tablets and insulin by injection, GIII: The controls.*Substantial variances which \( p \)-value < 0.05, high substantial variances which \( p \)-value < 0.001, No substantial variances which \( p \)-value > 0.05.
It is clear from the metabolic profile of patients and control group explained in the table (2) there was a highly significant increase in FSG and HbA1c levels in both GI and GII groups compared to GIII group. The CRP had no effect on both GI and GII groups compared to GIII group. Additionally, there was a substantial reduction in insulin and c-peptide concentrations for both GI and GII groups compared with GIII. There was a significant decrease in HOMA-IR for GI when compared to GIII. There was a significant decrease in GI and GII in HOMA-β and QUICKI when compared with GIII.

In addition, the results showed that there was a highly significant increase in both GAD-65 and ICA levels in both GI and GII groups compared to the control group GIII, table (3).

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Means ± SD</th>
<th>Comparison of GI and GIII</th>
<th>Comparison of GI and GIII</th>
</tr>
</thead>
<tbody>
<tr>
<td>FSG (mg/dl)</td>
<td>204.3±63.335</td>
<td>289.776±122.13</td>
<td>95.666±5.409</td>
</tr>
<tr>
<td>HbA1c (%)</td>
<td>9.663±1.779</td>
<td>10.903±2.165</td>
<td>5.2±0.387</td>
</tr>
<tr>
<td>Insulin (µIU/mL)</td>
<td>9.157±4.226</td>
<td>17.040±5.276</td>
<td>2.931±0.727</td>
</tr>
<tr>
<td>C-Peptide (ng/mL)</td>
<td>0.889±0.873</td>
<td>2.085±0.811</td>
<td>1.112±0.724</td>
</tr>
<tr>
<td>HOMA-IR</td>
<td>5.633±3.362</td>
<td>2.814±1.169</td>
<td>1.317±0.824</td>
</tr>
<tr>
<td>HOMA-β</td>
<td>36.884±27.284</td>
<td>137.926±74.144</td>
<td>13.826±9.870</td>
</tr>
<tr>
<td>QUICKI</td>
<td>0.308±0.027</td>
<td>0.329±0.019</td>
<td>0.358±0.025</td>
</tr>
</tbody>
</table>

Table (3): Metabolic profile of LADA and control group

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Means ± SD</th>
<th>Comparison of GI and GIII</th>
<th>Comparison of GI and GIII</th>
</tr>
</thead>
<tbody>
<tr>
<td>GAD-65</td>
<td>2.6±0.944</td>
<td>1.795±0.678</td>
<td>0.437±0.238</td>
</tr>
<tr>
<td>ICA</td>
<td>1.653±0.826</td>
<td>1.687±0.553</td>
<td>0.511±0.287</td>
</tr>
</tbody>
</table>

There was no significant difference in serum TC and TAG among the three groups. While, there was a substantial difference in serum HDL-C and LDL-C in GI and GII as compared to GIII. Moreover, there was a substantial difference in serum VLDL in GII as paralleled to GIII, table (4).

Table (4): Serum lipid profile of LADA and control group

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Means ± SD</th>
<th>Comparison of GI and GIII</th>
<th>Comparison of GI and GIII</th>
</tr>
</thead>
<tbody>
<tr>
<td>TC (mg/dl)</td>
<td>187.533±2.515</td>
<td>186.973±7.323</td>
<td>185.633±5.561</td>
</tr>
<tr>
<td>TAG (mg/dl)</td>
<td>108.8±4.490</td>
<td>101.533±7.094</td>
<td>106.566±7.276</td>
</tr>
<tr>
<td>HDL-C (mg/dl)</td>
<td>41.233±1.406</td>
<td>38.0±4.110</td>
<td>46.333±2.294</td>
</tr>
</tbody>
</table>
### Table 1: Lipid Profiles in LADA Patients and Controls

<table>
<thead>
<tr>
<th>LDL-C (mg/dl)</th>
<th>LADA Patients</th>
<th>Control</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>124.56±2.756</td>
<td>12.27±7.976</td>
<td>118.35±5.809</td>
<td>1.932x10^-6</td>
</tr>
<tr>
<td>VLDL-C (mg/dl)</td>
<td>LADA Patients</td>
<td>Control</td>
<td>p-value</td>
</tr>
<tr>
<td>20.69±1.503</td>
<td>19.70±1.348</td>
<td>21.43±1.491</td>
<td>0.061</td>
</tr>
</tbody>
</table>

**Discussion:**

In the present study, the age of patients with LADA was above 40 years. It was well-known that the age of patients with LADA starts from 30 years and above, as confirmed by various earlier studies (9,10). Furthermore, the BMI of LADA patients was< 25 Kg/m², which is indicated by previous study (11). The results showed that there is an increase in FSG in LADA patientsas compared to the control and this is due to a defect in the secretion or action of insulin or both and this is confirmed by the earlier study (12). The high level of HbA1c in diabetics is due to the exposure of hemoglobin to a high level of blood glucose concentration, which binds glucose to free amino acid units in the hemoglobin chain without the need for enzyme help and this is compatible with the present study (13). The low level of insulin in the GI group compared with the GII due to insufficient production of insulin or damage to β-cells in the pancreas, while the group GII maintained the level of insulin within the normal level and this is because patients are given insulin by injection and this is consistent with the previous study (14). The pathophysiology of LADA is that of gradual β-cell damage and absolute insulin deficiency rather than IR seen in T2DM patients. However, some studies have reported that LADA patients also exhibit some degree of IR state as in T2DM patients. These studies showed that high intake of sweetened beverages and low birth weight increase the risk of LADA of the same strength as for T2DM, suggesting a common pathway possibly involving IR (15, 16). The current study reported that LADA patients weremore insulin resistant than control subjects. Parallel results were obtained from assessing IR using the QUICKI model, which was reduced in those patients. These observations propose that both diabetic state and obesity are essential factors to IR of T2DM and LADA. And, since many patients with LADA are obese, clinical outcome in LADA patients is determined by the interaction of IR as in T2DM and an autoimmune β-cell lesion as in T1DM (17, 18).

Higher levels of c-peptide in LADA patients have reliably been shown to be related with cardiovascular and all-cause mortality in individuals with DM (19). This is probably because elevated c-peptide levels are a marker of IR and MS phenotype. Similar outcomes have been detected in some, but not all observational studies in T2DM (20). The LADA has been characterized for the presence of specific autoantibodies for the islet cells. It includes GADA and IA-2 antibodies (21). Relative to LADA patients with high GAD65 titers were, had lower BMI, and lower β-cell function, which is reflected by HOMA-IR and QUICKI values. Thus, consistent with previous studies (22,23). Although the LADA patients had hyperglycemia and dyslipidemia, but the rate of metabolic syndrome (MS) in those patients were low due to low TAG, despite having higher incidence of IR as compared to those who taken tablets only (24). The lower TAG values supported the low incidence of MS. Also, serum HDL-C values were very low, contributing majorly in all the documented cases of MS seen in this study. This is in accordance with another study in subgroup pattern among type 2 Diabetic patients attending tertiary health facility in

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Northern Nigeria (25). This supports the fact that LADA has more of defective $\beta$-cell function than IR and closer to T1DM. The continual autoimmune attack on small $\beta$-cell mass in LADA will accelerate the defective secretory process and lead to absolute insulin deficiency. The HDL-C and TAG levels were low in both the LADA and T2DM groups. The same scenario was reported in some community surveys (26, 27). However, low HDL-C values were recorded in the current study, in contrast to earlier studies (28, 29). The low normal TAG values recorded in this study might also be explained by this racial difference, lifestyle, and nutrition. Also, it may warrant redefining the limits of the values used in the diagnosis of MS among those populations.

**Conclusions:**

Therefore, it is concluded among the population studied in this region of the country that LADA subset of patients are lean and have low TAG, greatly reduced HDL-C values, low blood pressure readings, no sex preference, and poor beta cell secretory function, with consequent poor blood glucose control. The prevalence of MS in LADA group was significantly lower than in T2DM and much higher than in normal population.

**Correlation relation:**

The correlation coefficient ($r$) test is used to describe the association between the different studied parameters with $P \leq 0.05$ was considered statistically significant.

1. **Correlation relation between GAD-65 and BMI**
   
   A significant negative correlation relation between GAD-65 and BMI P-value ($5.8 \times 10^{-60}$), ($8.8 \times 10^{-48}$), ($6.01 \times 10^{-67}$) for control and patients groups (GI) and (GII) respectively with correlation coefficient value $r$ (-0.357), (-0.205), (-0.081) respectively.

2. **Correlation relation between GAD-65 and HbA1C**

   A significant positive correlation relation between GAD-65 and HbA1C with P-value ($1.4 \times 10^{-18}$), ($8.14 \times 10^{-27}$) and ($8.22 \times 10^{-30}$) for control and patients groups (GI) and (GII) respectively with correlation coefficient value $r$ (0.057), (0.239), (0.134) respectively.

3. **Correlation relation between GAD-65 and HOMA-IR**

   A significant positive correlation relation between GAD-65 and HOMA-IR with P-value ($1.11 \times 10^{-15}$), ($6.07 \times 10^{-7}$) for control and (GI) group respectively with correlation coefficient $r$ value (0.063), (0.188) respectively while showing a significant negative correlation relation between GAD-65 and HOMA-IR with P-value ($3.2 \times 10^{-6}$) for (GII) group with $r$ value (-0.033).

4. **Correlation relation between GAD-65 and HOMA-$\beta$**

   A significant negative correlation relation between GAD-65 and HOMA-$\beta$ with p-value ($1.74 \times 10^{-14}$) for control with correlation coefficient value (-0.129) while showing a significant positive correlation relation between GAD-65 and HOMA-$\beta$ with $P$-value ($6.32 \times 10^{-5}$), ($2.49 \times 10^{-9}$) for (GI) and (GII) groups respectively with correlation coefficient $r$ value (0.070), (0.074) respectively.

5. **Correlation relation between GAD-65 and cholesterol**

   A significant negative correlation relation between GAD-65 and cholesterol with P-value ($1.05 \times 10^{-31}$), ($6.01 \times 10^{-74}$) for control and (GII) group respectively with correlation coefficient $r$ value (-0.018), (-0.007).
respectively while showing a significant positive correlation relation between GAD-65 and cholesterol with P-value \((5.32\times10^{-100})\) for (GI) group with r value (0.285).

6. Correlation relation between GAD-65 and triglyceride
A significant negative correlation relation between GAD-65 and triglyceride with P-value \((5.19\times10^{-61})\), \((1.38\times10^{-72})\) and \((5.4\times10^{-60})\) for control and patients groups (GI) and (GII) respectively with correlation coefficient value \(r\) (-0.053), (-0.036), (-0.062) respectively.

7. Correlation relation between GAD-65 and HDL-C
A significant positive correlation relation between GAD-65 and HDL-C with P-value \((8.55\times10^{-69})\), \((3.51\times10^{-48})\) for control and (GII) group respectively with correlation coefficient r value (0.234), (0.052) respectively while showing a significant negative correlation between GAD-65 and HDL-C with P-value \((3.24\times10^{-72})\) for (GI) group with r value (-0.073).

8. Correlation relation between GAD-65 and LDL-C
A significant negative correlation relation between GAD-65 and LDL-C with P-value \((2.83\times10^{-69})\), \((3.22\times10^{-63})\) for control and (GII) group respectively with correlation coefficient r value (-0.036), (-0.135) respectively while showing a significant positive correlation between GAD-65 and LDL-C with P-value \((1.81\times10^{-81})\) for (GI) group with r value (0.123).

9. Correlation relation between GAD-65 and VLDL-C
A significant positive correlation relation between GAD-65 and VLDL-C with P-value \((7.89\times10^{-60})\) for control with correlation coefficient r value = (0.008) while showing a significant negative correlation relation between GAD-65 and VLDL-C with P-value \((4.08\times10^{-52})\), \((7.2\times10^{-57})\) for (GI) group and (GII) respectively group with correlation coefficient r value (-0.355), (-0.193) respectively.

References:


