The prevalence of autoantibodies among relatives for type 1 and 2 diabetic patients

Hadi Lazim Al-Abady, 1 Nadham Kadham Mahdi, 2 Lamia Mustafa Al-Naama, 3 Jawad Kadham Mahdi 4

Abstract
Objective: To estimate the prevalence of islet cells autoantibodies among non-diabetic relatives of type 1 and type 2 diabetic patients.
Methods: The study was conducted at the College of Medicinal Technology, Basra, Iraq, from December 2010 to December 2012, and comprised both diabetics and non-diabetics. The participants were first divided into two groups: type 1 diabetics; and type 2 diabetics. Both the groups had 2 sub-groups each; their non-diabetic relatives and non-relatives as controls. Laboratory investigations were done to estimate glutamic acid decarboxylase and islet cell antigen-2 autoantibodies by enzyme-linked immunosorbent assay for all groups. Significant differences were assessed with chi-square test.
Results: Of the 300 subjects, 100(33.3%) were diabetics and 200(66.6%) non-diabetics. Among diabetics, 40(40%) had type 1 while 60(60%) had type 2 diabetes. Among type 1 diabetics, 27(67.5%) tested positive for glutamic acid decarboxylase autoantibodies compared to 15(25%) in type 2 diabetics, 6(15%) among relatives of type 1, 7(11.7%) among relatives of type 2 diabetic patients, and none among controls. Besides, 16(40%) of type 1 diabetic patients tested positive for islet cell antigen-2 autoantibodies compared to 8(13.3%) in type 2, and none in the sub-groups. Conclusion: The relatives of both types of diabetic patients showed immunity to islet cell autoantibodies which confirmed the effect of genetic factors in disease pathogenesis and may be important in disease prediction.
Keywords: Glutamic Acid Decarboxylase, Islet cell antigen-2, Type 1 diabetes, Type 2 diabetes. (JPMA 66: 1064; 2016)

Introduction
Diabetes mellitus (DM) is a disorder characterised by hyperglycaemia in both the fasting and postprandial states. The two most common forms of DM are type 1 (T1DM) and type 2 (T2DM), with the former resulting from T-cell-mediated autoimmune destruction of β-cells of the pancreas, whereas the latter is characterised by insulin resistance with a non-autoimmune insulin secretory defect. 1 Autoimmune DM is characterised by the presence of one or more islet-specific autoantibodies, including islet cell autoantibodies (ICA), insulin (IAA) and autoantibodies directed against the three major islet autoantigens — glutamic acid decarboxylase 65 (GADA), protein tyrosine phosphatase IA-2A and its isoform IA-2b/phogrin (IA-2bA). 2, 3

DM-associated autoantibodies can be used as predictive markers of T1DM, and the risk assessment may be stratified by studying the insulin secretion and insulin sensitivity among pre-DM first-degree relatives. 4-6

Individuals with T2DM and their first-degree relatives can also be used as a predictor of late insulin dependency. High concentrations of glucose induce the production of GAD65 in β-cells which is associated with an increase in GAD65 gene expression. It has been shown that patients with T2DM may produce higher levels of GADA and after a short period of time develop T1DM. Thus, patients with T2DM having autoimmune aspects are increasingly recognised, on the basis of the existence of circulating antibodies against β-cells, 7 which leads to a reduction in the number of β-cells because autopsy studies of adults with DM suggest a greater than 50% reduction in β-cell mass compared with normoglycaemic adults. 8

In some patients, a clinical differentiation between T1DM and T2DM is not possible at manifestation and autoantibodies are used to define the type of DM. 9-11 The American Diabetes Association (ADA) recommends declaring children with DM and autoantibodies as T1DM regardless of their insulin dependency. 9 Some studies in the USA based on <50 children reported β-cell autoantibodies in 10-74% of children with T2DM, depending on ethnic background. 12, 13 The current study was planned to estimate the prevalence of ICA among non-DM relatives of T1DM and T2DM patients compared to non-DM, non-relatives.

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Subject and Methods

The study was conducted from December 2010 to December 2012 at the College of Medicinal Technology, Basra, Iraq, and comprised both DM and non-DM individuals. The participants were first divided into 2 groups: those with T1DM (disease duration: 0.5-19 years); and those with T2DM (disease duration: 1-24 years). Both the groups had 2 sub-groups each; their non-diabetic relatives and non-relatives as controls.

Patients with hypertension or hormonal disorders like thyroid diseases were excluded. The control group included 60 healthy non diabetic subjects and they were not first degree relatives for type2 diabetic patient (age ranged 32-66 years, 26 males and 34 females). In addition to above groups 100 healthy non diabetic volunteers were included.

Informed written consent was obtained from all participants after approval from the institutional ethical committee.

After an overnight fasting, 3ml of venous blood was collected in ethylenediaminetetraacetic acid (EDTA)-containing tubes from the relatives and control groups. Samples were centrifuged at 3,000 revolutions per minute (rpm) for 10 minutes and stored in separated plain tubes at -20°C before testing.

The GADA and IA-2A were estimated by enzyme-linked immunosorbent assay (ELISA). The ELISA test kit provides a quantitative in vitro assay for human autoantibodies against glutamic acid decarboxylase (GAD) and tyrosine phosphatase (IA2) in serum (EUROIMMUN, Germany). Both immunoassays were done according to the instructions of the manufacturer.

Statistically significant differences were assessed with chi-square test at two levels of probability; \( \leq 0.05 \) and \( \leq 0.001 \).

Results

Of the 300 subjects, 40(13.3%) were T1DM and 60(20%) were T2DM patients. Of the 200(66.6%) non-diabetics, 40(13.3%) and 60(20%) were first-degree relatives of T1DM and T2DM patients, respectively; each of the two groups were matched with the same number of controls. T1DM patients had higher frequency of GADA as 27(67.5%) of them showed GADA positive in comparison to 15(25%) in T2DM, 6(15%) in relatives of T1DM, 7(11.6%) in relatives of T2DM and none in both control groups. The difference was highly significant (p<0.001) (Table-1).

<table>
<thead>
<tr>
<th>Groups</th>
<th>Positive for IA-2A</th>
<th>Positive for GADA</th>
</tr>
</thead>
<tbody>
<tr>
<td>Diabetic patients T1</td>
<td>16</td>
<td>27</td>
</tr>
<tr>
<td>Relatives</td>
<td>0</td>
<td>6</td>
</tr>
<tr>
<td>Control</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Exact Fisher test</td>
<td>0.168 (NS)</td>
<td>0.159 (NS)</td>
</tr>
</tbody>
</table>

T1DM and T2DM patients, respectively; each of the two groups were matched with the same number of controls. T1DM patients had higher frequency of GADA as 27(67.5%) of them showed GADA positive in comparison to 15(25%) in T2DM, 6(15%) in relatives of T1DM, 7(11.6%) in relatives of T2DM and none in both control groups. The difference was highly significant (p<0.001) (Table-1).

Besides, 16(40%) of T1DM patients were IA-2A positive compared to 8(13.3%) in T2DM, and none in relatives of both types and in both control groups. Statistical analysis showed a highly significant difference (p<0.001) (Table-2).

Discussion

GAD is found in a variety of tissues, with highest concentrations in the nervous system. GAD is an intracellular enzyme that catalyses the conversion of glutamic acid to the inhibitory neurotransmitter GABA (aminobutyric acid) and thus it is not normally expressed on the surface of the β-cell. There are two major isoforms of GAD, i.e. GAD65 (65 000-Da molecular weight) and GAD67 (67 000-Da molecular weight). IA-2 is a member of the protein tyrosine phosphatase family and is a transmembrane protein. The predominant autoreactive epitopes are in its C-terminal region and are oriented intracellularly in a way that is consistent with a sequestered autoantigen. Autoreactivity to the C-terminal construct of IA-2A (ICA512) is known as ICA512 autoantibodies (ICA512A).

Determination of three major islet autoantigens - insulin, GAD65, and ICA512 (IA-2) — has become central component of studies of the natural history of DM. Cytoplasmic ICA represents antibodies reacting with GAD65, ICA512, and other unknown antigens, but not insulin autoantibody. High-titer cytoplasmic ICA is most often associated with the presence of multiple anti-islet autoantibodies (of GAD65, ICA512, or insulin) and therefore is associated with a high risk of progression to DM.

In this study, 6(15%) T1DM relatives were positive for GADA while no one of them showed IA-2A reactivity. The
control group showed negative results for GADA and IA-2A (Table-1). These results indicate a good presumptive predictive value of this type of antibodies for a possible future development of T1DM in the first-degree relatives. This conclusion was confirmed by others.\textsuperscript{17-21} This could be explained that autoantibodies against IA-2 (IA-2A) and zinc transporter-8 (ZnT8A) generally appear later than autoantibodies against insulin (IAA) or GAD (GADA) in pre-T1DM and have been associated with more rapid disease progression in first-degree relatives and IAA, anti-ICA512, and anti-GAD appear to be stable over time. The presence of more than one of these autoantibodies allows the identification of a subset of relatives with sufficiently high risk for T1DM to begin preventive trials.\textsuperscript{22,23}

Autoantibodies that are reactive to islet antigens are present at the time of diagnosis in most patients with T1DM. Additionally, approximately 10% of phenotypic T2DM patients are positive for at least one of the islet autoantibodies, and this group is often referred to as latent autoimmune DM in adults (LADA).\textsuperscript{24} GADA and cytoplasmic ICAs play a key role in distinguishing LADA from T2DM in clinical practice.\textsuperscript{25}

According to findings of this study, 7(11.6%) of the relatives of T2DM patients were positive for GADA and no one of them showed IA-2A reactivity while control group showed negative results for GADA and IA-2A (Table-2). Although this result was statistically not significant (p>0.05), it revealed that islet cell antibodies were more prevalent in the relatives than those with no family history of DM.

Some other researchers\textsuperscript{25-27} registered nearly the same percentage of these antibodies in first-degree relatives of T2DM patients, while others reported higher percentages.\textsuperscript{28}

GAD65 gene is located on chromosome 10 and expressed in β-cells of pancreas. The presence of high concentrations of anti-GADA in serum of patients with insulin-dependent DM (IDDM) is evident. Evaluation of GADA levels in individuals with non-insulin-dependent DM (NIDDM) and their first-degree relatives can also be used as a predictor of late insulin dependency. High concentrations of glucose induce the production of GAD65 in β-cells which is associated with an increase in GAD65 gene expression. It has been shown that patients with NIDDM may produce higher levels of GADA and after a short period of time develop IDDM.

However, these are broad and prospective studies in which a large number of relatives were included for different islet autoantibodies screening and followed up to predict DM development as the presence of these antibodies is highly predictive for future DM; 3-5 years risk may reach 28-66%.\textsuperscript{29} The positive predictive value within five years had been proved by Verge et al.\textsuperscript{30} since they found 50/763 islet cell-positive first-degree relatives who developed clinical disease within this period.

Others found that the development of persistent islet antibodies is associated with human leukocyte antigen (HLA) DR3-DQ2 / DR4-DQ8 genotype, both in relatives of T1DM and in children from general population, whereas transient islet autoantibodies were not correlated with known genetic factors. So the combination of islet cell autoantibodies and HLA typing is much useful in predicting disease even in general population.\textsuperscript{31,32} Madha et al.\textsuperscript{33} reported that GADA, C3 and C4 levels (collectively or separately) may be used as an earlier marker rather than fasting blood sugar for the prediction of developing of T1DM in the first-degree relatives of DM patients.

**Conclusion**

The relatives of T1DM and T2DM patients showed islet cell immunity which confirmed the effect of genetic factors in disease pathogenesis in T1DM and LADA group of T2DM patients.

**Disclosure:** No

**Conflict of Interest:** No

**Funding Sources:** No.

**References**


