Frequency of islet cell autoantibodies (IA-2 and GAD) in young Brazilian type 1 diabetes patients

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Abstract

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Type 1 diabetes, as an autoimmune disease, presents several islet cell-specific autoantibodies such as islet cell antibody (ICA), anti-insulin, anti-glutamic acid decarboxylase (GAD) and the antibody (Ab) against tyrosine phosphatase (PTP)-like protein known as ICA-512 (IA-2). In order to determine the frequency of the anti-GAD and anti-IA-2 autoantibodies in Brazilian type 1 diabetes patients we studied 35 diabetes mellitus (DM) type 1 patients with recent-onset disease (≤12 months) and 37 type 1 diabetes patients with long-duration diabetes (>12 months) who were compared to 12 children with normal fasting glucose. Anti-GAD$_{65}$ and anti-IA-2 autoantibodies were detected with commercial immunoprecipitation assays. The frequency of positive results in recent-onset DM type 1 patients was 80.0% for GADAb, 62.9% for IA-2Ab and 82.9% for GADAb and/or IA-2Ab. The long-duration type 1 diabetes subjects presented frequencies of 54.1% for GADAb and IA-2Ab, and 67.5% for GAD and/or IA-2 antibodies. The control group showed no positive cases. Anti-GAD and IA-2 assays showed a high frequency of positivity in these Brazilian type 1 diabetes patients, who presented the same prevalence as a Caucasian population.

Type 1 diabetes is a chronic autoimmune disease caused by the destruction of insulin-secreting islet cells of the pancreas (1,2) by several islet cell-specific autoantibodies. These autoantibodies are valuable markers to predict type 1 diabetes and can be detected many months or years before the onset of diabetes (3).

Many autoantibodies against ß-cells have been identified. The most important ones are: islet cell antibody (ICA) (1), anti-insulin (3), anti-glutamic acid decarboxylase (GAD) (4) and the antibody (Ab) against the tyrosine phosphatase (PTP)-like protein known as ICA-512 (IA-2) (5,6). Antibodies against GAD and IA-2 can be detected in type 1 diabetes patients using radioimmunoassays. These autoantibodies have been useful in differentiating late-onset type 1 from type 2 diabetes occurring in young adulthood (7). In addition, they have been used to confirm the autoimmune process in gestational diabetes (8) and to separate type 1 diabetes from other types of non-autoimmune diabetes like maturity-onset diabetes of the young (9) or diabetes related to mitochondrial DNA mutation (10). Knowing the frequency of these autoantibodies in a population is an impor-

tant step for a better understanding and diagnosis of type 1 diabetes.

This study was conducted using commercial assays to determine the frequency of anti-GAD and anti-IA-2 autoantibodies in serum of young Brazilian type 1 diabetes patients with recent-onset and long-duration diabetes.

We studied 35 type 1 diabetes patients (16 females, 13.2 ± 5.7 years) who had been diagnosed with diabetes within one year prior to the study. These subjects were called recent-onset diabetes patients. Thirty-seven type 1 diabetes patients (19 females, 13.9 ± 3.3 years) who had had diabetes for more than one year were also studied and were called long-duration diabetes patients. These groups were compared to 12 children (11.8 ± 4.4 years) with normal fasting glucose (<100 mg/dl) and no family history of type 1 diabetes (Table 1). The type 1 diabetes patients were selected while attending a diabetes vacation camp and all patients' mothers gave informed consent to collect blood samples to perform this study. Diabetes was diagnosed according to World Health Organization cri-

teria (11).

Anti-GAD$_{65}$ and anti-IA-2 autoantibodies were detected with commercial immuno-precipitation assays using ^{125}I-labeled human recombinant GAD$_{65}$ and IA-2, respectively (RSR Ltda., Cardiff, UK). The lower detection limit was 0.1 U/ml for both assays. The reference values used, based on our controls, for anti-GAD$_{65}$ and anti-IA-2 were <1.0 and ≤0.5 U/ml, respectively. These cut-off levels for positivity were established using the 99th percentile of the control groups. The test serum samples were first incubated with ^{125}I-labeled human recombinant protein (GAD$_{65}$ and IA-2), followed by the addition of solid phase protein A to precipitate the labeled protein and antibody complex. After centrifugation, ^{125}I was counted in the precipitates. The amount of radioactivity in the precipitates was proportional to the concentration of IA-2 antibody in the test sample. The intra-assay and the inter-assay coefficients of variation (CV) for anti-GAD$_{65}$ were 3.1 and 3.5%, respectively, for normal reference values. The intra-assay CV for IA-2 was 4.3% and the inter-assay CV was 3.4% for normal reference values.

Data are reported as mean $\pm$ SEM, unless otherwise stated. The Mann-Whitney U-test was used when comparing two groups. Statistical analyses were performed using a Fisher's exact probability test. The correlation between two variables was determined using Spearman's test. Data were analyzed with the Statistical Package for Social Science for Windows, version 7.0 (SPSS Inc., Chicago, IL, USA). A P value of less than 0.05 was considered statistically significant.

Among the 35 recent-onset type 1 diabetes patients, 28 (80.0%) were positive for GADAb, 22 (62.9%) were positive for IA-2Ab, and 29 (82.9%) were positive for GADAb and/or IA-2Ab. Among the 37 long-duration type 1 diabetes subjects, 20 (54.1%) were positive for GADAb and IA-2Ab, and 25 (67.5%) were positive for GAD and/or IA-2 antibodies (Table 2). All individuals in

Table 1 - Clinical characteristics of type 1 diabetes patients and control subjects.

F/M, Female/male; ns, nonsignificant (Mann-Whitney U-test).

	Type 1 diabetes		Control group	P
	Recent-onset	Long-duration		
N (F/M)	35 (16/19)	37 (19/18)	12 (3/9)	
Age (years)	13.2 ± 5.7	13.9 ± 3.3	11.8 ± 4.4	ns
Duration of diabetes (months)	6.5 ± 4.0	50.7 ± 30.0	-	<0.005
Dose of insulin (U/day)	27.6 ± 17.1	44.7 ± 14.4	-	<0.005

Table 2 - Prevalence of anti-glutamic acid decarboxylase (GAD) and ICA-512 (IA-2) antibodies in type 1 diabetes patients with recent-onset and long-duration diabetes (Fisher's exact test).

	GAD	IA-2	GAD and/or IA-2
Recent-onset diabetes	80.0% (28/35)	62.9% (22/35)	82.9% (29/35)
Long-duration diabetes	54.1% (20/37)	54.1% (20/37)	67.5% (25/37)
Control group	0.0% (0/12)	0.0% (0/12)	0.0% (0/12)

the control group had results within the reference values. Duration of type 1 diabetes showed a significant negative correlation with autoantibody levels (GADAb $r = -0.23$, P<0.05; IA-2Ab $r = -0.25$, P<0.04).

In the recent-onset diabetic population, GAD frequency was higher than IA-2 frequency and their combination did not increase the prevalence of positive assays. However, in long-duration type 1 diabetes patients, combining these antibodies improved the probability of detection of an autoimmune reaction. The Brazilian population studied here had the same GADAb prevalence as that reported in Caucasian patients (65-84%) and Asian type 1 diabetes patients (5-83.3%) (12-14).

IA-2 autoantibody is present in approximately 60% of recent-onset type 1 diabetes patients (15,16), which is consistent with our findings. Also, testing for both antibodies increases positive results in type 1 diabetes patients, even in long-duration ones.

Some studies have also shown that the combined analysis of GADAb and IA-2Ab is more effective in detecting type 1 diabetes than the histochemical ICA test, which is an important advantage because the ICA test is technically difficult to perform, requires human pancreatic tissue and has low reproducibility (17,18). Anti-GAD and IA-2 assays showed a high frequency of positivity in these Brazilian type 1 diabetes patients and these patients presented the same prevalence as a Caucasian population reported by others (12).

Acknowledgments

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