CLINICAL STUDY

Clinical phenotype and β -cell autoimmunity in Italian patients with adult-onset diabetes

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Abstract

Objective: To characterize the phenotype of a large population of Italian patients with adult onset (≥ 40 years) diabetes who were attending outpatient clinics and who were screened for glutamic acid decarboxylase 65 autoantibodies (GADA), protein tyrosine phosphatase IA-2 (IA-2A) and IA-2 β /phogrin (IA-2 β A).

Design and methods: This was a cross-sectional study comprising a total of 881 patients, aged ≤ 70 years, diagnosed with type 2 diabetes after the age of 40 years, and consecutively recruited in five clinics located in different geographic areas of Italy (Milan, Florence, Rome, Naples and Catania). Their mean disease duration was 8.1 (6.9; s.d.) years. GADA, IA-2A and IA-2 β A were measured with radiobinding assays with *in vitro* translated S-methionine-labelled glutamic acid decarboxylase 65 (GAD65) or IA-2 or IA-2 β . Anthropometric and clinical data were collected and compared amongst patients with or without autoantibodies.

Results: Sixty-three (7.1%) patients had one or more autoantibodies, 58 (6.6%) had GADA, 22 (2.5%) had IA-2A, six (0.7%) had IA-2 β A and 19 (2.15%) had two or more autoantibodies. IA-2A or IA-2 β A, in the absence of GADA, were found in only five patients. Autoantibody-positive patients were more often female (63.5 vs 36.5%; $P < 0.009$), had higher glycated haemoglobin (Hb A1c) ($P < 0.001$), lower body mass index (BMI; $P < 0.0005$) and waist/hip ratio (WHR; $P < 0.01$); female gender being the main contributor to BMI and WHR. We did not observe any differences in age at diagnosis or duration of disease with respect to the presence or absence of islet autoantibodies. The proportion of patients on insulin therapy was higher in patients with two or more antibodies, compared with those with one antibody only, and no antibodies (P for trend < 0.001), and among patients with GADA, in those with higher antibody titre (73.9% in those with > 10 units vs 42.0% in those with ≤ 10 units; $P < 0.007$).

Conclusions: Patients with adult onset diabetes characterized by autoimmunity to β -cells showed a clinical phenotype with anthropometric features that differed from those classically observed in patients with type 2 diabetes. The number and titre of autoantibodies, which reflect the severity of autoimmunity and β -cell impairment, amplified this difference. The usefulness of autoantibody screening in adult-onset diabetes is further emphasized by these findings.

European Journal of Endocrinology 154 441–447

Introduction

A proportion of adult patients diagnosed with type 2 diabetes develops insulin-requiring diabetes during follow-up and many of these can be identified by the presence of circulating islet autoantibodies. This form of diabetes is also referred to as latent autoimmune

diabetes in adults (LADA) (1–3) and according to the last classification criteria is now considered a form of type 1 diabetes (4).

Autoimmune diabetes is characterized 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-2 (IA-2A) and its isoform IA-2 β /phogrin (IA-2 β A) (5–8).

The detection of either ICA or GADA at the time of diagnosis of adult-onset diabetes identifies a subgroup of patients with clinical characteristics more similar to those of type 1 diabetes and is predictive of a more rapid progression to insulin requirement (9).

Several studies have been performed in order to evaluate the prevalence of autoimmunity to β -cells in patients with adult onset diabetes and have been based on screening for ICA and/or GADA. In these studies, the prevalence of LADA ranged from 8 to 45%, according to different populations and selection criteria (10–24). Most of these studies assessed patients attending a single diabetes clinic and few of them evaluated the correlation between the presence of GADA and the clinical features of affected patients. While GADA is recognized as the marker with the highest sensitivity for LADA, fewer data are available on IA-2A in adult-onset diabetes (25), while IAAs are unlikely to be useful since they are a feature of childhood diabetes (26).

The prevalence of LADA in Italy was estimated in a population-based study carried out in one province in the region of Lombardy, the prevalence of which was 2% of all cases of adult diabetes and 2.8% of those diagnosed (15). The proportion of LADA among those patients attending diabetes clinics has been investigated in two previous studies in Italy; one performed in a subset of lean diabetic patients (13) and the other at a single institution (14).

The aim of our study was to evaluate the prevalence of islet autoimmunity and its distinctive clinical phenotype in patients with adult-onset, clinically diagnosed type 2 diabetes, in a large sample representative of patients attending diabetes clinics in Italy.

Patients and methods

GADA, IA-2A and IA-2 β A have been measured in patients with adult-onset, clinically diagnosed type 2 diabetes attending five outpatient clinics located in all Italian geographical macro areas representing the north, centre, south and the islands, except Sardinia, in order to identify possible regional differences. Patients with islet autoantibodies were compared with those without autoantibodies; assessing gender, age, duration of disease, family history of diabetes, metabolic control, body mass index (BMI), waist circumference, hip circumference, waist/hip ration (WHR), presence of hypertension and insulin therapy requirement.

Patients

A total of 881 Italian patients clinically diagnosed as affected by type 2 diabetes was studied. Participating

patients were consecutively recruited from five outpatient clinics located in different cities excluding Sardinia (Milan, Florence, Rome, Naples and Catania). Each centre was requested to collect at least 200 patients. To avoid selection bias, once the starting date of the investigation was decided, each centre recruited patients strictly in a consecutive series. Inclusion criteria were (a) diagnosis of type 2 diabetes, according to WHO criteria (4) at the age of 40 years or above and (b) age at recruitment $\geq$ 70 years. Duration of diabetes was not an inclusion criterion. Of the 1017 patients initially recruited, 136 patients were excluded from the study because, on further analysis, they did not fulfil the inclusion criteria or data/samples were not available.

All patients signed an informed consent form. A clinical file containing personal, historical and clinical data was filled in. Blood pressure was taken after the patients had been sitting down for 10 min; the first measurement was discarded and the mean of the next two measurements was recorded in the file. Systolic and diastolic pressures were determined by Korotkoff phase I and V respectively. Presence of hypertension was defined as blood pressure values $>$ 130/85 mmHg (27) and/or current treatment with anti-hypertensive drugs.

Body weight and height were measured with subjects wearing light clothing without shoes. Waist circumference was measured with a soft tape on standing subjects midway between the lowest rib and the iliac crest. Hip circumference was measured over the widest part of the gluteus region and WHR as a measure of central obesity was calculated.

Whether the patient had fasted or not, blood samples were taken at the end of the visit. A sample with sodium EDTA was used for glycated haemoglobin (HbA1c) measurement, serum was obtained from another sample serum and stored at -20°C until shipment in a frozen condition for autoantibody measurement.

Glycated haemoglobin

HbA1c concentrations were measured by HPLC in each centre's laboratory. A quality control assay had been performed previously at participating centres to standardize HbA1c results, with possible accuracy differences in measurements being considered. On two different days, the centre in Naples sent, at 4°C , five blood samples for measuring HbA1c in duplicate. Each assay was performed on the same day, under blind conditions in all laboratories, 4 days after the blood sample had been obtained. The samples were stored at 4°C until the test was carried out. Results were transmitted to Naples, where a coefficient of correction was calculated for each centre on the basis of the mean differences of values obtained from those that were theoretical (means of results of all centres).

This coefficient of correction was used to correct HbA1c values obtained in each centre for enrolled patients.

Autoantibody determination

GADA, IA-2A and IA-2 β A measurements were performed by radiobinding assay with *in vitro* translated 35 S-methionine-labelled glutamic acid decarboxylase 65 (GAD65), IA-2 and IA-2 β as previously described (5–7). Results were converted into arbitrary units, by extrapolation from a standard curve with a local standard, designated as 100 units. The thresholds for positivity were determined from the 99th centile of control subjects, and corresponded to 3 units for GADA and 1 unit for IA-2A and IA-2 β A. In the Diabetes Autoantibody Standardization Proficiency workshop these assays had the following sensitivities and specificities: GADA 84% and 97%, IA-2A 62% and 100% respectively (8).

Statistical analysis

Data are expressed as frequency and percentage or as means $\pm$ S.D.. Frequency differences were tested using χ^2 test with Yates correction when appropriate. Statistical differences between groups with HbA1c, BMI, waist circumference, hip circumference, WHR, years of disease and arterial blood pressure were evaluated by using the Student's *t*-test. All variables with a significance level ≤ 0.1 were candidates for a multivariate logistic analysis. Waist and hip measurement were excluded from multivariate analysis because of high correlation and lack of independence with other variables such as BMI and WHR. All analyses were performed with STATA 6 (www.stata.com).

Results

The clinical features of patients enrolled in this study are shown in Table 1; females had a higher BMI,

more frequently a first-degree relative affected by diabetes, a worse metabolic control and were more often on insulin therapy than males. Out of a total of 881 patients, GADA were found in 58 (6.6%), IA-2A in 22 (2.5%) and IA-2 β A in six (0.7%) patients. Sixty-three (7.1%) patients had $\geq$ one autoantibody and 19 (2.1%) $\geq$ two autoantibodies; in 818 patients no autoantibodies were detected (Table 2). The Venn diagram in Fig. 1 represents the distribution of autoantibodies; the majority of patients had GADA alone ($n = 40$; 63% of those with any autoantibody) while three (4.7%) patients had IA-2A alone and only one patient was positive for IA-2 β A alone. Of the patients with $\geq$ two autoantibodies, 14 were positive for GADA and IA-2A (73.6%), one had IA-2A and IA-2 β A and four were positive for all three autoantibody specificities (21%). No significant differences were observed in the prevalence of autoantibodies between the five regions in which the patients were recruited.

Personal, anthropometric and clinical features of patients were investigated in relation to the presence or absence of autoantibodies (Table 3). Age at diagnosis, family history for diabetes and disease duration were not different between the two groups. Among autoantibody-positive patients, there was a significantly higher number of females than males (63.5 vs 36.5%; $P < 0.009$). BMI was significantly lower in patients with autoantibodies (26.88 ± 4.90 vs 29.20 ± 5.00 kg/m 2 ; $P < 0.0005$) and, interestingly, this significant result was due to the female gender. Furthermore, the presence of $\geq$ two autoantibodies identified patients even more lean than those positive for one autoantibody only and those without autoantibodies (24.9 ± 3.2 vs 27.8 ± 5.3 vs 29.2 ± 5 kg/m 2 respectively; $P < 0.01$) (Fig. 2).

Waist circumference and hip circumference were lower in patients with autoantibodies compared with those without autoantibodies both in female (90.17 ± 14.10 vs 98.81 ± 13.17 ; $P = 0.001$ and 101.57 ± 9.14 vs 106.88 ± 11.74 ; $P = 0.020$) and male patients (95.35 ± 10.41 vs 100.23 ± 9.79 ;

Table 1 Clinical features of patients enrolled in the study. Values are means $\pm$ S.D.

	All	Female	Male	P value
Number	881	420	461	NS
Age (years)	60.16 $\pm$ 6.89	60.38 $\pm$ 6.85	59.97 $\pm$ 6.93	0.373
Age at diagnosis (years)	52.08 $\pm$ 7.34	52.07 $\pm$ 7.27	52.09 $\pm$ 7.43	0.976
Family history of diabetes (%)	56.4	61.17	53.7	0.027
Duration of diabetes (years)	8.11 $\pm$ 6.98	8.33 $\pm$ 6.99	7.92 $\pm$ 6.97	0.374
BMI (kg/m 2)	29.04 $\pm$ 5.03	29.85 $\pm$ 5.64	28.29 $\pm$ 4.28	<0.0001
Waist circumference (cm)	99.11 $\pm$ 11.68	98.07 $\pm$ 13.45	100.01 $\pm$ 9.86	
Hip circumference (cm)	104.62 $\pm$ 10.18	103.03 $\pm$ 8.47	106.42 $\pm$ 11.62	
WHR	0.95 $\pm$ 0.08	0.92 $\pm$ 0.08	0.97 $\pm$ 0.06	<0.0001
HbA1c (%)	7.51 $\pm$ 1.80	7.64 $\pm$ 1.77	7.39 $\pm$ 1.83	0.042
Insulin therapy (%)	23.27	27.62	19.31	0.004
Hypertension (%)	78.66	81.43	76.14	0.056

NS, not significant.

Table 2 Prevalence of islet-related autoantibodies.

	Number	Prevalence (%)
GADA	58	6.58
IA-2A	22	2.50
IA-2βA	6	0.68
≥ One autoantibody	63	7.15
≥ Two autoantibodies	19	2.15
No autoantibodies	818	92.85

$P = 0.046$ and 98.88 ± 13.72 vs 103.23 ± 8.11 ; $P = 0.038$) respectively. WHR which, together with waist circumference, is a marker of visceral adiposity and is an independent risk factor for cardiovascular diseases, remained significantly lower in patients with autoantibodies (0.92 ± 0.12 vs 0.95 ± 0.07 ; $P < 0.05$) (Table 3). As for BMI, the latter result was due to female gender. Finally, hypertension was significantly more present in patients without autoantibodies (80.1%) than in those with autoantibodies (60.3%; $P < 0.0001$).

Metabolic control assessed by HbA1c was worse in autoantibody-positive patients (8.23 ± 1.95 vs 7.45 ± 1.78 ; $P < 0.001$) (Table 3). Among the 63 patients positive for $\geq$ one autoantibody, 40 (63.5%) were treated with insulin versus 165 (20.2%) of the 818 patients without autoantibodies. Furthermore, patients with $\geq$ two autoantibodies compared with those with one autoantibody only and with those who were autoantibody negative, were more frequently already on insulin therapy (78.9% vs 56.8% vs 20.2%; $P < 0.001$) (Fig. 3). Moreover, among the 58 patients positive for GADA, those with a higher antibody titre

(> 10 units) were more frequently on insulin therapy than those with lower antibody titre (≤ 10 units) (73.9% vs 42.0%; $P < 0.034$) (Fig. 4). The duration of diet or oral anti-diabetic treatment before the introduction of insulin therapy was shorter in patients with autoantibody versus those without autoantibodies (3.1 ± 3.3 vs 9.0 ± 6.3 years respectively; $P < 0.001$). The significant differences for gender, BMI, hypertension and insulin requirement observed with the univariate analysis between patients with and without islet autoantibodies were confirmed by a multivariate logistic analysis, whereas the difference in WHR was not (Table 3).

Discussion

This is the first study that has evaluated the prevalence of autoimmune diabetes in a large setting of consecutive patients with a clinical diagnosis of type 2 diabetes, attending diabetes clinics and representative of the whole Italian population (except Sardinia) with this form of diabetes. Indeed, in a recent study conducted in Italy on over 10 000 patients with type 2 diabetes attending outpatient clinics, the patients' phenotype was not different from that reported in the present study (28).

We have shown that in this multi-centre hospital-based series of patients the prevalence of autoantibodies directed against β -cell antigens was 7.1%. This result was slightly lower than that obtained in other studies (10, 12, 24) and higher than that observed in an Italian population-based study (15), reflecting an expected enrichment of LADA cases among those attending diabetes clinics. We did not find a significant variation in the prevalence of GADA and IA-2A in relation to the region of origin (north, centre or south Italy), a finding that was different from that which has been reported for childhood type 1 diabetes, which showed significant differences in antibody prevalence within Italy (29). These data suggest that type 1 diabetes in the young and autoimmune diabetes in adults may reflect different genetic and immunological backgrounds.

We also found that GADA was the most prevalent and, in most cases, the only detectable autoantibody in patients with a clinical diagnosis of type 2 diabetes, and that IA-2A and IA-2βA were less frequent and almost invariably associated with GADA. In general, the number of autoantibodies and, in the case of GADA, the antibody titre are assumed to reflect the severity of the autoimmune process and β -cell damage. Accordingly, patients with two or more autoantibodies and with a high titre of GADA were nearly all on insulin therapy and showed a clinical phenotype more close to that of juvenile-onset type 1 diabetes.

The phenotype of these adult-onset autoimmune diabetes patients confirms the ability of islet-related autoantibodies to identify, within patients with a clinical

Figure 1 Distribution of islet-related autoantibodies represented by a Venn diagram. The majority of patients (40) had GADA alone, while three patients had IA-2A alone and only one patient was positive for IA-2βA alone. Of the patients with $\geq$ two autoantibodies, 14 were positive for GADA and IA-2A, one had IA-2A and IA-2βA and four were positive for all three autoantibody specificities.

Table 3 Personal, anthropometric and clinical features of patients according to the presence or absence of islet autoantibodies. Values are means $\pm$ S.D.

	Antibody positive	Antibody negative	P value	P value*
Number	63	818		
Age (years)	60.76 $\pm$ 6.89	60.12 $\pm$ 6.89	0.47	—
Age at diagnosis (years)	52.01 $\pm$ 6.79	52.01 $\pm$ 7.39	0.94	—
Gender; M/F (%)	36.5/63.5	53.6/46.4	0.009	0.018
Family history; yes/no (%)	46.8/53.2	58.1/41.9	0.083	—
Duration of diabetes (years)	8.74 $\pm$ 6.68	8.06 $\pm$ 7.00	0.45	—
BMI (kg/m ²)	26.88 $\pm$ 4.90	29.20 $\pm$ 5.00	0.0005	0.002
BMI females (kg/m ²)	26.88 $\pm$ 5.34	30.16 $\pm$ 5.59	0.0005	—
BMI males (kg/m ²)	26.86 $\pm$ 4.11	28.36 $\pm$ 4.28	0.10	—
Waist females (cm)	90.17 $\pm$ 14.10	98.81 $\pm$ 13.17	0.001	—
Waist males (cm)	95.35 $\pm$ 10.41	100.23 $\pm$ 9.79	0.046	—
Hip females (cm)	101.57 $\pm$ 9.14	106.88 $\pm$ 11.74	0.020	—
Hip males (cm)	98.88 $\pm$ 13.72	103.23 $\pm$ 8.11	0.038	—
WHR	0.92 $\pm$ 0.12	0.95 $\pm$ 0.07	0.013	—
WHR females	0.88 $\pm$ 0.09	0.92 $\pm$ 0.08	0.017	—
WHR males	0.97 $\pm$ 0.14	0.97 $\pm$ 0.05	0.68	—
Hypertension; yes/no (%)	60.3/39.7	80.1/19.9	0.0001	0.002
HbA1c (%)	8.23 $\pm$ 1.95	7.45 $\pm$ 1.78	0.001	—
Insulin; yes/no (%)	63.5/36.5	20.2/79.8	0.0001	<0.001

*Multivariate logistic analysis.

Figure 2 BMI values in relation to the number of islet autoantibodies. Patients without autoantibodies had a significantly higher BMI (29.2 $\pm$ 5.0 kg/m²) compared with those with one autoantibody (27.8 $\pm$ 5.3) and those with $\geq$ two autoantibodies (24.9 $\pm$ 3.2; $P < 0.01$). **Figure 3** Prevalence of patients treated with insulin in relation to the number of islet autoantibodies. There was a significantly higher prevalence in those with $\geq$ two autoantibodies (78.9%) than those with one autoantibody (56.8%) and those without autoantibodies (20.2%; $P < 0.001$). **Figure 4** Prevalence of patients treated with insulin in relation to the GADA titre. Among the 58 patients positive for GADA, those with a higher antibody titre (> 10 units) were more frequently on insulin therapy (73.9%) than those with lower antibody titre (≤ 10 units) (42.0%; $P < 0.034$).

diagnosis of type 2 diabetes, a subgroup with a clinical phenotype similar to type 1, or at least intermediate between type 1 and type 2 diabetes. Such a phenotype in our cohort is characterized by female gender, reduced BMI, earlier and more frequent need for insulin therapy and lower prevalence of arterial hypertension.

Metabolic syndrome is present in more than 75% of type 2 diabetic patients and identifies subjects with increased cardiovascular morbidity and mortality (30). In a recent study performed in a large cohort of patients with type 2 diabetes, the prevalence of the metabolic syndrome was lower in GADA-positive patients in comparison with those without autoantibodies (31). In the Botnia study (32), indicators of metabolic syndrome such as increased body weight, central obesity (by means of WHR), hypertension and dyslipidaemia were

less represented in LADA than in type 2 diabetes. In the same study, there was no difference in the prevalence of microvascular and macrovascular diabetic complications between LADA and type 2 diabetes. In a multiple logistic regression analysis, glycaemic control was associated with coronary heart disease in LADA patients but not in type 2 diabetic patients and the authors concluded that this result could be related to the lower prevalence of the metabolic syndrome seen in LADA (32). In our cohort, indeed, we found, on the one hand, a worse metabolic control and, on the other, a lower prevalence of markers of metabolic syndrome in autoantibody-positive type 2 diabetic patients. These phenotypic differences might be predictive of differences in the clinical outcome of patients with an adult onset of diabetes (31, 33).

A higher HbA1c level found in patients with autoimmune diabetes is a finding indicating that therapy in these patients is probably inappropriate. Under normal circumstances, patients with type 2 diabetes are not treated with insulin unless some special conditions occur (e.g. infection, surgery, secondary failure). Autoantibody screening in patients initially diagnosed as having type 2 diabetes would help to better define the disease process as well as pointing towards the most appropriate therapy which, in the case of autoimmune diabetes, should be the timely introduction of insulin therapy.

In conclusion, this study carried out in Italian patients with an adult-onset clinically diagnosed type 2 diabetes has indicated that the proportion of autoimmune diabetes among them is slightly lower than that observed in northern European countries. Although the clinical phenotype may help to characterize patients with adult-onset autoimmune diabetes, the determination of GADA represents the best tool for a correct classification and a necessary prerequisite for a correct therapeutic appraisal.

Acknowledgements

This study was supported by an unrestricted grant from Roche Diagnostics – Italy. The authors thank Ms Tanya Szendeffy for editorial help.

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Received 24 June 2005

Accepted 28 December 2005