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Prevalence and clinical characteristics of insulin-treated, anti-GAD-positive, type 2 diabetic subjects in an outpatient clinical department of a Dutch teaching hospital

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ABSTRACT

Background: In clinical practice, type 1 and type 2 diabetic patients are sometimes difficult to distinguish. Type 1 diabetes has an immune-mediated pathogenesis, resulting in a loss of insulin-secreting β -cells. Type 2 diabetes mellitus is characterised by a relative insulin insufficiency, without the presence of an autoimmune aetiology, initially due to insulin resistance and later also accompanied by defective insulin release. Latent autoimmune diabetes of the adult (LADA) is a subgroup of diabetes, somewhere on the borderland between type 1 and type 2 diabetes. LADA is characterised by a late-age onset and relatively mild progression, but with unmistakable signs of autoimmunity, such as the presence of the autoimmune antibodies anti-GAD65, anti-insulin antibodies, or anti-Ia-2ab.

Objective: To establish the prevalence of anti-GAD in a diabetic outpatient clinic of a Dutch, non-university, teaching hospital and to describe these patients clinical and laboratory features, especially of the metabolic syndrome.

Methods: We evaluated GAD65 antibodies and other parameters in 244 selected diabetic patients, who had been on oral therapy for at least three months before becoming insulin-dependent.

Results: Twenty-six patients (11.6%) were positive for GAD65 antibodies. These patients had a significantly lower BMI (27.8 ± 4.5 vs 31.1 ± 4.9 ; $p < 0.01$); less often cerebrovascular accidents (19.2 vs 34.9% ; $p < 0.01$) and a higher HDL cholesterol (1.73 ± 0.53 vs 1.21 ± 0.38 ; $p < 0.05$). In contrast, anti-GAD patients had a significantly higher prevalence of hypothyroidism (23.0 vs 6.6% ; $p < 0.05$).

Conclusion: Anti-GAD-positive patients represent a sizable proportion of type 2 diabetes in a second-line outpatient clinic, and they are characterised by lower parameters of

the metabolic syndrome, but higher prevalence of other autoimmune phenomena such as hypothyroidism.

KEYWORDS

Anti-GAD, autoimmunity, LADA, type 2 diabetes

INTRODUCTION

Classification of adult-onset diabetes mellitus (DM) into type 1 or 2 based on the clinical presentation may be difficult.^{1,2} Diagnosis of the main forms of diabetes depends on clinical judgment based mainly on the age of the subject and the severity of insulin deficiency at presentation, as well as the presence or absence of features of the metabolic syndrome. In the WHO classification of 1998, an aetiological classification was chosen to subgroup the different types of diabetes.³

Type 1 DM refers to a loss of insulin-secreting β -cells of the islets of Langerhans, in most cases by immune-mediated pathogenic mechanisms highlighted by the presence of islet cell autoantibodies and by an altered frequency of immune-regulated genes in the HLA region. In European patients with type 1 DM 95% have positive glutamic acid decarboxylase (GAD65) and/or IA2 antibodies to antigens of the islets of Langerhans; especially the finding of GAD65 antibodies seems a quite stable finding after the age of 10 to 15 years in autoimmune diabetes.^{4,5} Type 2 DM is characterised by a relative insulin insufficiency, without the presence of an autoimmune aetiology, initially due to

insulin resistance and later also accompanied by defective insulin release due to amyloid deposition in the pancreas. Both of these major diabetes types include different stages ranging from not requiring insulin to diabetes that does require insulin for control or survival. It is recognised that the type 2 DM process does not invariably lead to insulin dependency nor, on the other hand, is total insulin deficiency exclusively classified as type 1 DM.

Furthermore a subgroup of diabetic patients can be distinguished with evidence of autoimmunity but who clinically resemble type 2 DM at diagnosis. Autoantibody positivity together with subsequent development of insulin deficiency led to the introduction of the eponym latent autoimmune diabetes in adults (LADA) for this subgroup,⁶ type 1.5 diabetes,⁷ or latent type 1 DM.⁸ However, there is still an ongoing debate as to whether these names cover the same subgroup of diabetes.⁹ LADA exists somewhere on the borderland between type 1 and type 2 diabetes, and exemplifies the difficulties we have in telling type 1 from type 2.¹⁰ Because of its slow progression to insulin therapy, early identification of anti-GAD-positive patients could lead to implementation of preventive measures to protect residual β -cell function.

There is no consensus regarding diagnostic criteria of LADA. The grounds for designating LADA as a distinct aetiological entity are insubstantial. Several studies have used different criteria especially concerning the age of onset of diabetes and the duration of the insulin-free period.¹¹⁻¹³ Mainly because of these different criteria the prevalence of LADA patients varies in published studies, from 2.8%¹⁴ to 22.3%.¹⁵ In a large study, not restricted to hospital outpatients as the United Kingdom Prospective Diabetes Study (UKPDS) is, a prevalence of 10% was found.¹⁶

The aim of the present study was to establish the prevalence of anti-GAD in a diabetic outpatient clinic of a Dutch, non-university, teaching hospital and to describe these patients clinical and laboratory features of the metabolic syndrome.

PATIENTS AND METHODS

Patients

During a four-month period, we studied consecutive patients with DM type 2 who visited the outpatient clinic of the Jeroen Bosch Hospital, where 1600 patients with diabetes are monitored annually. Each year we see 200 new diabetic patients, 70% of whom start on insulin therapy. Inclusion criteria were DM type 2 (nonketotic diabetes without insulin treatment over at least three months of observation), who were treated with insulin at the start of the study or treated with maximal oral therapy and supposed to start on insulin in the next month.

Exclusion criteria were malignancy, autoimmune diseases, known abnormal thyroid function at the time of the study, use of NSAIDs or acetylsalicylic acid, or infections in the previous two weeks before the start of the study. These exclusion criteria were adopted because of further cytokine studies in the LADA population. From all the patients, blood tests were taken, including fasting glucose and lipids, HbA_{1c}, thyroid function and microalbuminuria (abnormal: >30 mg albumin/24 hours). They all underwent a standard physical examination for late diabetic complications and cardiovascular risk factors and diseases. Hypertension was defined as use of antihypertensive drugs or blood pressure >140/80 mmHg. Peripheral neuropathy was scored as positive when vibration sense by a 128 Hz vibration fork at both hallux was absent, combined with a disturbed 10 g Semmes-Weinstein monofilament test and absent ankle reflexes. Macroangiopathy was present when a patient had coronary disease, defined as one or more coronary events, PTCA or CABG, or when a patient had proven cerebrovascular damage. Peripheral vascular disease was defined as symptoms of claudication and absence of foot pulses and/or a toe pressure <30 mmHg or proven peripheral vascular disease, either by radiography or vascular intervention. The diagnosis of retinopathy was based on fundoscopy after pharmacological mydriasis by an ophthalmologist. Hypothyroidism was defined as treatment with thyroxine or a TSH concentration >6 mU/l and an fT₄ level <13 pmol/l.

Determination of autoantibodies

Ia-2a antibodies and GAD65 antibodies were determined by radiobinding assays with *in vitro* translated recombinant human ³⁵S-GAD65. GAD65-ab were expressed as relative indices, using one positive standard serum from one type 1 diabetic subject and two negative control sera from healthy subjects in each assay, and an upper level of normal of 0.035 U (mean +3SD from the indices observed in healthy individuals). The diagnostic sensitivity of the GAD65-antibody assays was 85%, the analytic specificity 100%.¹⁷ Informed consent was obtained from all patients.

STATISTIC ANALYSIS

Data will be given as mean \pm standard deviation (SD) or as median and range. Student t-test was used for continuous variables, χ^2 test for dichotomous variables. Level of significance was p value <0.05.

RESULTS

A total of 407 type 2 DM patients visited the outpatient clinic during the study period. Of these patients, 183 were

excluded from the study because of refusal (1 patient), missing variables (6) or because of the following exclusion criteria: malignancy (10), autoimmune disease (31), acetylsalicylic acid use (84), NSAID use (49), and known abnormal thyroid function (2).

Of the remaining 224 patients, 26 had positive GAD65 antibodies (11.6%). The median anti-GAD level was 0.55 U (range 0.12 to 2.3 U). Of the 26 GAD65-positive patients, five had positive Ia-2A antibodies (19.2%). In the other 198 (anti-GAD-negative) type 2 diabetic patients there were no positive Ia-2A antibodies. Demographic characteristics of the anti-GAD population in comparison with the anti-GAD-negative type 2 diabetic population are mentioned in *table 1*. There was a female predominance in the anti-GAD population (73%) compared with the non-anti-GAD population (57%). The body mass index in the non-anti-GAD population was significantly higher than in the anti-GAD population ($p < 0.01$). The prevalence of hypertension was similar in both groups, as were macroangiopathic complications, with the exception of cerebrovascular accidents, which were seen more frequently in the non-anti-GAD type 2 diabetic population ($p < 0.01$). The prevalence of microvascular diabetic complications did not differ between the two groups. In 23% of the anti-GAD patients there was a hypothyroidism, compared with 7% in the non-anti-GAD population ($p < 0.05$). The laboratory results of the two diabetic populations are shown in *table 2*. HbA_{1c} was similar in both groups. Total cholesterol results were comparable in the two groups, but in the anti-GAD patients the high-density lipoprotein (HDL) cholesterol value was significantly higher ($p < 0.05$): the triglycerides were lower

in the anti-GAD patients, but this was not statistically significant.

DISCUSSION

The main finding of our study is that the prevalence of anti-GAD-positive patients in a Dutch non-university, teaching hospital was 11.6 % of the total type 2 diabetic population and that in the anti-GAD population less features of the metabolic syndrome were present. The prevalence found is comparable with other studies, for instance the UKPDS.¹⁶ If only type 2 diabetic patients were included with at least a six-month insulin-free period at diagnosis, recommended by Fourlanos *et al.* for diagnosing LADA, the prevalence would have been almost the same: 12.7% (23/181).¹³ However, our study results have some limitations concerning the selection of the study population. No type 2 diabetic patients on only oral diabetic medication were included. It is possible that we overestimated the prevalence of anti-GAD-positive patients in our population because of the exclusion criteria, especially the exclusion of patients who were on acetylsalicylic acid: 84 of the 183 excluded patients (45.9%). Patients usually take acetylsalicylic acid because of macrovascular complications, a result of the metabolic syndrome. In this subgroup the prevalence of anti-GAD is supposed to be small. On the other hand, if LADA is a slow-developing autoimmune diabetes, by excluding patients with autoimmune diseases, we may have underestimated the prevalence. In line with this hypothesis is the high prevalence (23%) of hypothyroidism found

Table 1. Demographic characteristics of the anti-GAD-positive population in comparison with the anti-GAD-negative type 2 diabetic population

Variable	Anti-GAD positive (n=26)	Anti-GAD negative (n=198)	P
Sex (% female)	73	57	ns
Age (years) (mean ± SD)	59.3 ± 16.3	63.6 ± 12.8	ns
BMI (kg/m ²) (mean ± SD)	27.8 ± 4.5	31.1 ± 4.9	0.006
Time to starting insulin after DM diagnosis (mean ± SD) (months)	13.2 ± 9.1	14.7 ± 8.0	ns
Smoker (%)	23.1 (6/26)	24.7 (47/190)	ns
Neuropathy (%)	42.3 (11/26)	59.2 (103/174)	ns
Retinopathy (%)	26.9 (7/26)	34.7 (65/188)	ns
Microalbuminuria (%)	32.0 (8/25)	29.4 (40/136)	ns
Hypertension (%)	46.2 (12/26)	43.5 (84/193)	ns
Cerebrovascular incident (%)	19.2 (5/26)	34.9 (55/166)	<0.01
Peripheral vascular disease (%)	30.8 (8/26)	27.1 (52/192)	ns
Coronary damage (%)	11.5 (3/26)	19.7 (38/193)	ns
Hypothyroidism (%)	23 (6/26)	6.6 (13/198)	<0.05

GAD = glutamic acid decarboxylase; BMI = body mass index; DM = diabetes mellitus; ns = not significant.

Table 2. Laboratory results of the anti-GAD-positive population in comparison with the anti-GAD negative type 2 diabetic population

Variable	Anti-GAD positive (n=26)	Anti-GAD negative (n=198)	P
Positive GAD65 antibodies (N)	26	0	-
Anti-GAD65 level (U) (median/range)	0.55 (0.12 - 2.3)	-	-
Positive IA-2Ab (%)	19.2%	0	-
IA-2Ab level (U) (median/range)	0.001 (-0.6 - 2.62)	-	-
HbA _{1c} (mmol/l) (mean ± SD)	8.3 ± 1.6	8.5 ± 1.4	ns
Total cholesterol (mmol/l) (mean ± SD)	5.4 ± 1.06	5.2 ± 0.9	ns
HDL cholesterol (mmol/l) (mean ± SD)	1.73 ± 0.53	1.21 ± 0.38	<0.05
LDL cholesterol (mmol/l) (mean ± SD)	2.88 ± 1.16	3.08 ± 0.8	ns
Triglycerides (mmol/l)	1.63 ± 1.88	2.07 ± 1.52	ns

GAD = glutamic acid decarboxylase; HDL = high-density lipoprotein; LDL = low-density lipoprotein; ns = not significant.

in our anti-GAD population. The association between autoimmune thyroid disease and type 1 diabetes has been reported in the past.^{18,19} Recent studies have also found a higher prevalence of thyroid autoantibodies in adult diabetic patients with positive GAD65 antibodies.^{17,20} The fact that 73% of the anti-GAD population is female is in accordance with an autoimmune pathogenesis of LADA. In older studies, the presence of islet autoantibodies was also associated with female sex.^{8,21} In the study by Gambelunghie *et al.* the same high female prevalence in the anti-GAD patient group was found.¹⁷

The patients with anti-GAD in this study also had a better lipid profile and lower body mass index (BMI), compared with the type 2 diabetic patients without anti-GAD.²² There was a tendency of less cardiovascular events in the anti-GAD population, which only reached statistical significance in the prevalence of cerebrovascular accidents.²² The prevalence of hypertension did not differ between the two groups, as was found in several other studies.^{22,23} Concerning the long-term diabetic complications there was no difference in prevalence of retinopathy, neuropathy and microalbuminuria.

These phenotypic differences of anti-GAD-positive patients compared with type 2 DM are more subtle and there seems to be less evidence of the metabolic syndrome. Probably this anti-GAD population of diabetic patients are type 1 diabetics diagnosed at an earlier phase or there is a true difference in the progression rate of insulin deficiency.

Hosszúfalusi showed similar clinical characteristics and a high prevalence of predisposing risk alleles (HLA-DQB1*0302, -DR4, -DR3, -DR3/DR4) and risk haplotypes (DR4-DQB1*0302) in patients with LADA and adult-onset type 1 diabetes with rapid progression.²⁴ But he also showed that patients with LADA often had single positive islet cell-specific autoantibodies, in contrast to those with adult-onset type 1 diabetes with rapid progression. Others

found a decreased frequency of the protective HLA type 0602 in the type 1 DM population.²⁵ Preliminary data of a prospective study on 22 newly diagnosed diabetic patients showed after a median follow-up of 2.3 years an unchanged fasting and glucagon stimulated C-peptide concentration in 11 LADA patients compared with 11 type 1 diabetic patients.²⁶ These facts pointed to a difference in the rate of deterioration of β-cell function between the two groups.

Assuming that LADA is a slow progressive type 1 form of diabetes, prevention of β-cell destruction should be attempted.²⁷ A pilot trial in 1996 comparing insulin and sulphonylureas in LADA patients showed that insulin-treated patients maintained higher B-cell function than those treated with sulphonylureas.²⁸ Early use of insulin could preserve endogenous insulin secretion and probably delay or prevent the decline of B-cell function. Cabrera-Rode *et al.* showed in 2002 that exclusion of sulphonylureas in treatment of slowly progressing type 1 DM patients partially decreases specific autoimmunity against endocrine pancreatic cells and improves metabolic control.²⁹ This suggests that initial insulin monotherapy is a good choice for the treatment of LADA patients, but this hypothesis has, beside the study by Kobayashi,²⁸ never been proved. The UKPDS confirmed that patients with LADA are more likely to progress to insulin, but it also showed that after ten years, or indeed at any point in between, those initially randomised to diet or sulphonylurea therapy did not differ in any respect from those initially randomised to insulin.³⁰ Another possibility is medication with anti-inflammatory properties, as shown by the reduction of cytokines such as tumour necrosis factor. Rosiglitazone has been shown to possess such effects *in vitro* and reduced the incidence of autoimmune diabetes in NOD mice.³¹ More and prospective studies are needed to confirm the superiority of this suggested treatment regime and to implement it in daily care.

In daily practice this means that we could measure GAD antibodies in early type 2 diabetic patients (lower BMI, better lipid profile, hypothyroidism, women). In case of positive antibodies, an earlier start of insulin is often needed. This anti-GAD-positive diabetic population with slow progression to insulin therapy compared with type 1 diabetes gives the opportunity of testing therapeutic modalities to protect the β -cell.

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