

Original Article

Glutamic Acid Decarboxylase (GAD65) and Thyroid Autoantibodies in Omani Patients with Type 2 Diabetes

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ABSTRACT

Introduction: Autoimmunity is a common feature in type 1 diabetes mellitus (DM). Little is known of the role of autoimmunity in type 2 diabetes. Antibodies to glutamic acid decarboxylase (GAD65) and thyroid antigens have been reported with various frequencies in diabetic patients. In this study, we investigated the prevalence and association of antibodies to GAD65, anti-thyroglobulin (A-TG-A), anti-thyroid microsomal (ATMA) antibodies, haemoglobin A_{1c} (HbA_{1c}) and fasting serum C-peptide in Omani patients with type 2 diabetes mellitus.

Material and Methods: We studied 100 Omani patients with type 2 diabetes for the presence of serum antibodies to GAD65, A-TG-A and ATMA by the use of commercially available kits. Results were compared with those from fifty patients with type 1 diabetes mellitus (DM) and fifty unaffected individuals who were used as controls.

Results: Results showed that type 2 DM had significantly high positivity levels (24%) of anti-GAD65 antibodies

than the control group (4% - $p < 0.05$) though less than that found in type 1 DM (38%). GAD65 antibodies were more commonly found in older females (> 40 years) with type 2 diabetes ($p < 0.05$). A higher prevalence of ATMA was noted in both type 2 and type 1 diabetes (20 & 26% respectively) compared to the levels in the control group (8%). There was a low prevalence and little difference in A-TG-A values among the three groups studied (9%, 14% & 4%). Both A-TG-A and ATMA were more often expressed in older females with type 2 diabetes. HbA_{1c} did not differ between groups with the duration of disease less or more than three years. When the same groups were tested for fasting serum C-peptide, those with disease of more than three years duration had significantly higher prevalence ($p < 0.05$) as compared to those less than three years.

Conclusion: This study confirms the presence and association of thyroid and GAD65 antibodies in some omani patients with clinical diagnosis of type 2 DM.

KEYWORDS: diabetes mellitus, GAD65, thyroid, type 1 diabetes, type 2 diabetes

INTRODUCTION

Type 2 diabetes mellitus (DM), is characterized by disorders in insulin secretion and, in many patients, relative insulin deficiency as well as the inability to effectively utilize insulin production (insulin resistance)^[1]. Many studies have shown that type 1 DM is caused by autoimmune destruction of the pancreatic β -cells, which is characterized by the presence of circulating islet autoantibodies, and has a strong association with endocrine autoimmunity^[2-4]. Little is known about the risk for autoimmunity in subjects with type 2 DM.

Autoantibodies to islet cell antigen Glutamic Acid Decarboxylase (GAD65) can be detected in 70-90% of new-onset type 1 DM. It has also been detected in 10-20% of type 2 DM and this has been suggested to reflect autoimmunity as well as being the most sensitive single marker for identifying persons at risk of developing the disease^[5, 6].

Type 2 DM with GAD65 autoantibodies has been classified as a latent autoimmune diabetes in adults (LADA)^[7] in different ethnic groups^[6, 8, 9]. Patients initially diagnosed with type 2 diabetes may, in many cases, suffer from LADA. Therefore, testing for GAD65 antibodies may be of assistance in diagnosis^[10].

Oman is a member of the Arabian Gulf Countries, who have a high prevalence (10-13%) of impaired glucose tolerance (IGT) and type 2 diabetes^[11]. The prevalence of GAD65, thyroid autoantibodies and LADA in the Omani diabetic population has not been studied previously.

The objective of this study was to determine the frequency of antibodies to GAD65 and the involvement of thyroid autoimmunity in Omani patients with type 2 DM. Also to compare these results with those from patients with type 1 diabetes and healthy controls.

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Table 1

Number of type 2 diabetes patients positive for glutamic acid decarboxylase (GAD65), anti-thyroglobulin (A-TG-A) and anti-thyroid microsomal (ATMA) antibodies in correlation to the duration of diabetes (less or more than 3 years) age range (less or over 40 years) and sex

Duration of Diabetes	Less than 3 years (n = 42)		Over 3 years (n = 58)	
	Under 40 years (n=17)	Over 40 years (n=25)	Under 40 years (n=22)	Over 40 years (n=36)
GAD (F:M)	2 (2:0)	8 (7:1)	4 (2:2)	10 (8:2)
A-Tg-A(F:M)	0	4 (3:1)	2 (1:1)	3 (2:1)
ATMA(F:M)	2 (1:1)	8 (7:1)	3 (1:2)	7 (3:4)

SUBJECTS AND METHODS

Subjects: One hundred adult subjects with a clinical diagnosis of type 2 DM and an age range from 17-73 years (average 48.6 years; female to male ratio 50:50) were included in the first group. A further 50 patients with type 1 DM and 50 control subjects were screened for the presence of GAD65 and thyroid antibodies. The three selected groups were Omani individuals and were matched in terms of age, sex and BMI. Members of the control group had no first degree relatives with a history of diabetes.

Measurements and assays: Circulating autoantibodies to GAD65 antigen in serum was detected using a quantitative ELISA assay (Isletest TM-GAD) from DRG INTERNATIONAL, Inc. USA. A positive result (> 1.05) indicates the presence of autoantibodies in serum samples. A negative result (< 1.00) indicates the absence of GAD65 autoantibodies or levels below the limit of resolution of the test.

For detection of A-TG-A and ATMA in serum, a commercial assay for a passive haemagglutination test (Murex Thymune T & Thymune M; Murex Biotech Limited, Kent-England) was employed.

HbA_{1c} concentrations were tested by immunoturbidimetry (HbA_{1c} Unimates 3, Boehringer Mannheim, Mannheim, Germany). The reference values for this assay were 3-6%. Fasting serum C-peptide levels were measured by radioimmunoassay (BRAHMS Diagnostica, Berlin, Germany).

Statistical analysis: Statistical analysis was performed using χ^2 test from SPSS for windows program. A p value < 0.05 was considered as significant.

RESULTS

The overall prevalence of GAD65-antibody was 24% in type 2 diabetic patients and 38% in patients with type 1 DM, each of which was significantly higher (p < 0.05) than that found in the control

Table 2

Number of type 2 diabetes patients with positive results for glutamic acid decarboxylase (GAD65), anti-thyroglobulin (A-TG-A) and anti-thyroid microsomal (ATMA) antibodies in correlation to haemoglobin A1c (HbA1c) values (less or over 8%) and the duration of diabetes (less or more than 3 years)

Duration of Diabetes	Less than 3 years (n = 42)		Over 3 years (n = 58)	
	< 8% (n=22)	> 8% (n=20)	< 8% (n=32)	> 8% (n=26)
GAD	4	7	10	3
A-Tg-A	4	5	4	3
ATMA	8	2	2	1

group (4%) (Fig. 1). The duration of diabetes in patients with type 2 DM fell into two groups; those less than three years (42%), and those more than three years (58%). Anti-GAD65 positivity was 10% for those with DM less than three years duration and 14% for those with DM for more than three years. Similarly, the values for A-Tg-A and ATMA for subgroups of less than three years duration were four and 10% respectively. For the subgroup with DM for more than three years, these were five and 10%. Table 1 shows these findings together with their correlation to the age and sex of patients. A higher prevalence of GAD65 antibody was noted in older females (> 40 years), type 2 diabetes (18% Vs 6% in males, p < 0.05).

Similarly, the prevalence of ATMA was 20%, 26% and 8% in type 2, type 1 DM and control group respectively, while the prevalence of A-TG-A was 9%, 14% and 4% in type 2, 1 and control group respectively (Fig. 1). Higher expression of ATMA and A-TG-A was noted in older females (> 40 years old) with type 2 diabetes (ATMA: 10 Vs 5 in males; p < 0.05) and (A-TG-A: 5 Vs 2 in males; p < 0.05). There were no significant differences in thyroid autoantibodies (ATMA and A-TG-A) between type 2 and 1 DM, but both groups showed a significant higher prevalence (p < 0.05) of ATMA when compared to the control group.

Three treatment regimes were employed on the patients (insulin, oral hypoglycemic agents and "diet and exercise"). Of the 18% receiving insulin, 12% were diabetic for more than three years while 6% were patients for less than three years. Those receiving oral hypoglycemic agents comprised 77% (31% were less than three years duration and 46% were more than three years). The remaining 5% who were undergoing treatment with "diet and exercise" were all diabetics for less than three years. Four of those receiving insulin had a very high anti-GAD65 titre.

When fasting serum C-peptide levels were measured on type 2 patients by radioimmunoassay, results showed that those with DM for more than

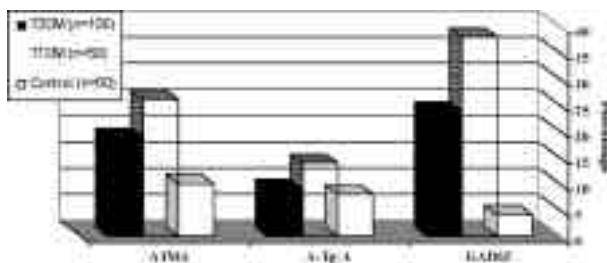


Fig. 1: Percentage of Omani diabetes patients with serum antibodies to glutamic acid decarboxylase (GAD65), anti-thyroglobulin (A-TG-A) and anti-thyroid microsomal (ATMA) antibodies

three years had a higher mean of 18 ± 8.0 ug/L as compared to that of 7 ± 5.6 ug/L in those with DM for less than three years duration ($p < 0.05$). No significant difference in antibody levels (GAD65 and thyroid) between the groups was observed.

When long and short-term subgroups of type 2 patients were tested for haemoglobin A1c (HbA1c), results showed that values as high as $> 8\%$ (a poor glycemic control marker) were seen in 26/58 (45%) patients in the long-term group as compared to 20/42 (48%) patients in the short-term group, with no statistical differences between the two groups. Levels of antibodies in these subgroups are illustrated in Table 2.

DISCUSSION

Autoantibodies to GAD65 are an important marker of the autoimmune-mediated β -cell destruction in type 1 (insulin-dependent) diabetes. However, these autoantibodies have recently been found in some patients with type 2 (non-insulin dependent) diabetes^[6,12,13].

Some populations of type 2 diabetes around the world have been screened for GAD65 antibodies, with frequencies varying from 5% or less, to 30% in some Caucasian populations^[14]. A previous study on Chinese diabetic patients showed that 39.6% type 1 and 16% type 2 diabetics had anti-GAD65 antibodies^[8]. In another study carried out on Latvian diabetic patients^[15], the prevalence of GAD65 autoantibodies was found in as high as 30% of patients with type 2 DM. Although these frequencies differ from those we obtained in the current study, nevertheless, this study confirms the presence of GAD65 antibody in some Omani patients with type 2 DM. The variations in the GAD65 values observed between our study and others could be due to differences in age, number of the subjects studied, variable duration of disease, mode of therapy and/or ethnicity^[4,8,10,13,14,16].

The present study demonstrated that type 2 (as in type 1) diabetic patients have an association with the presence of autoantibodies directed against thyroid antigens. This association was more often

expressed in older females with type 2 DM. These findings are in agreement with previous studies which have established a high prevalence of thyroid autoantibodies in diabetic patients^[13,17,18]. Several other studies have reported that the prevalence of thyroid disorders such as Hashimoto's thyroiditis among type 1 diabetic individuals is very high (38.7%)^[19,20,21] compared to that in the general population ($< 1-7\%$)^[22,23]. Another study^[25], performed on a large population ($n = 1310$) of diabetic patients to determine the frequency of thyroid diseases, showed that the overall prevalence of thyroid diseases was 13.4%, and was highest (31.4%) in type 1 DM, and lowest in type 2 DM (6.9%). Autoantibodies to thyroid antigens (thyroglobulin and thyroid peroxidase) were, also, reported among patients with type 2 DM^[25]. The association of thyroid antibodies with type 1 is to be expected due to the autoimmune nature of disease; the relationship with type 2 DM is unexplained.

Measurement of C-peptide is considered to be a good parameter for evaluating pancreatic β -cell function^[27]. Data presented in this study showed that patients with short-term diabetes had lower serum C-peptide levels compared with the long-term diabetes. Unlike serum C-peptide, haemoglobin A_{1c} measurements did not show any significant differences when used to compare between the different groups of type 2 patients studied. In fact, the usefulness of HbA_{1c} in the screening and diagnosis of diabetes has been widely debated^[27,28].

The presence of GAD65 as well as thyroid autoantibodies in patients with type 2 DM, demonstrated in this study indicates that there is an autoimmune mechanism in the pathogenesis of this disease and a possible autoimmune process responsible for their insulin secretory deficiency.

In conclusion, there is a significant prevalence of anti-GAD65 antibody among type 2 diabetic patients studied, in particular among females over 40 years of age. Omani type 2 diabetic patients show similar prevalence of GAD65 autoimmunity and LADAAs in other population groups. They also express thyroid autoimmunity. The significance of GAD65 and thyroid autoimmunity among these patient and the role of early insulin therapy needs to be evaluated.

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