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Autoantibodies to GAD and IA-2 in Saudi Arabian diabetic patients

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Abstract

Aims To determine the prevalence of autoantibodies in sera of Saudi diabetic patients including Type 1 and Type 2 diabetes mellitus (DM) and gestational diabetes mellitus (GDM) living in Jeddah, Saudi Arabia. Apart from data on the prevalence of islet-cell antibodies in patients in Ryhadh (Al-Attas *et al*. Freqency of islet cell antibodies in adult newly diagnosed diabetic patients. *Ann Saudi Med* 1990; **10**: 369–373) immunological markers of autoimmune diabetes have not been explored in Saudi Arabians.

Methods Autoantibodies to GAD65 (GADA) and IA-2 (IA-2A) were determined using radio-immunoprecipitation assays.

Results In Type 1 DM patients, 54% were GADA$^+$ and 27% were IA-2A$^+$. A greater negative effect of disease duration was noted for IA-2A than for GADA positivity. Autoantibodies were more prevalent with younger age of onset. GADA were slightly more common in female Type 1 DM patients. In Type 2 DM, 8/99 patients were GADA$^+$, and three of these patients with shorter disease duration were also IA-2A$^+$. GADA, and particularly IA-2A, were associated with a younger age of onset of Type 2 DM and all the autoantibody-positive Type 2 DM patients were insulin-treated. GADA were detected in 2.2% of GDM patients, but none of these patients possessed IA-2A.

Conclusions The prevalence and associations of autoantibodies in Saudi diabetic patients are very similar to those reported for diabetic patients in other ethnic groups.

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Keywords autoantibodies, diabetes, glutamic acid decarboxylase, IA-2

Abbreviations BSA, bovine serum albumin; DM, diabetes mellitus; GADA, glutamic acid decarboxylase antibodies; GDM, gestational diabetes mellitus; IA-2A, IA-2 antibodies; ICA, islet cell antibodies; LADA, latent autoimmune diabetes in adults; PBS, phosphate-buffered saline; RIP, radioimmunoprecipitation; SA, Saudi Arabia; T1DM, Type 1 diabetes mellitus; T2DM, Type 2 diabetes mellitus

Introduction

A characteristic of Type 1 diabetes mellitus (T1DM) is the occurrence of autoantibodies to glutamic acid decarboxylase (GADA) and IA-2 (IA-2A) [1]. Some Type 2 diabetic mellitus (T2DM) patients also have features of T1DM, termed latent autoimmune diabetes in adults (LADA) [2], and a small proportion of patients with gestational diabetes mellitus (GDM) develop T1DM [3].

There is a high prevalence of T1DM [4], T2DM [5] and GDM [6] in Saudi Arabia (SA). However, there is little information on markers of autoimmune diabetes in Arab populations,

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including SA [7]. These markers have proved to be valuable in other ethnic populations, particularly the numerous studies in western European and North American populations where GADA and IA-2A have been detected in > 80% and > 70% of newly diagnosed T1DM patients, respectively, using sensitive radio-immunoprecipitation (RIP) assays [1]. A lower prevalence of GADA has been reported in some racial/ethnic patient groups [8–10], but this may be related to variations in the assays employed, age of onset and disease duration, as well as possible influences of genetic or environmental factors. The prevalence of IA-2A in T1DM patients from non-Caucasian populations has not yet been thoroughly investigated. The aim of the present study was therefore to investigate the occurrence of GADA and IA-2A in Saudi diabetic patients (living in Jeddah) using established RIP assays to determine whether their prevalence is similar to that in other characterized ethnic patient groups.

Patients and methods

Subjects

Serum samples were collected at King Abdulaziz University Hospital, the Maternity and Children's Hospital, and the Diabetic Centre in Jeddah, SA with approval by respective ethics committees and with informed subject consent. Diabetes mellitus was diagnosed according to the WHO and American Diabetes Association criteria, where subjects had a fasting blood glucose concentration ≥ 7 mmol/l or 11.1 mmol/l 2 hours post-administration of a glucose load. Ninety serum samples were collected from T1DM patients: median age 11 years (range 1–25), 59% female. Ninety-nine serum samples were collected from T2DM patients whose initial diagnosis was based on their clinical presentation, age and absence of diabetic ketoacidosis: median age 49 years (range 25–70), 39% female. Twenty-five of these patients were on insulin therapy: the decision for insulin treatment was based on failure of therapy with diet and oral hypoglycaemic agents. Eighty serum samples were collected from healthy control individuals who tested negative for diabetes by WHO criteria, and who had no history of diabetes: median age 28.8 years (range 10–46), 28% female.

Ninety samples were collected from pregnant women with recently diagnosed GDM (most were Saudi Arabs, about 5% were from other Middle Eastern countries living in Jeddah). GDM was diagnosed according to the National Diabetes Data Group (NDDG) criteria. The median age of the GDM patients was 35 years (range 22–45). One hundred serum samples were collected from healthy pregnant women who were negative for GDM by NDDG criteria, and who had no family history of diabetes: median age 27.9 years (range 18–42).

Detection of autoantibodies to GAD65 and IA-2 by radio-immunoprecipitation (RIP) assays

Both GADA and IA-2A in the serum samples from the T1DM and T2DM patients and the non-diabetic controls were determined by ^{125}I RIP assay using commercial kits (RSR Ltd, Cardiff, UK) [11,12] according to the manufacturer's instructions (except that, in the GADA assay, sera were incubated overnight at 4°C). The intra-assay and interassay variations were reported as 3.1 and 5.1%, respectively, for the GADA assay, and 4.3 and 3.4%, respectively, for the IA-2A assay [11,12]. In the Diabetes Antibody Standardization Program (DASP) first assay proficiency evaluation [13], the sensitivity and specificity were 84 and 90%, respectively, for the GADA assay, and 58 and 100%, respectively, for the IA-2A assay.

Autoantibodies to GAD65 and IA-2 in the GDM and non-diabetic pregnant subjects were measured by ^{35}S RIP assay as described previously [14]. The interassay variations were reported as 9.9 and 17%, respectively, for the GADA and IA-2A assays [14]. In the DASP first assay proficiency evaluation [13], the sensitivity and specificity were 78 and 94%, respectively, for the GADA assay, and 56 and 98%, respectively, for the IA-2A assay.

Sera with GADA or IA-2A values above the mean plus three times the standard deviation of the 80 or 100 appropriate control subjects were regarded as positive. The two RIP assays for GADA and IA-2A give highly comparable results [11,12] and we confirmed the good correlation between these assays by testing in the ^{35}S RIP assay [14] 37 of the Type 1 diabetic patients' serum samples analysed in the ^{125}I RIP assay in the current study. The *P*-values by Spearman's correlation analysis for the detection in the two assays of GADA and IA-2A were < 0.0001 and 0.0001, respectively.

Statistical analyses

Mann–Whitney *U*-test was employed to compare single parameters between groups. Multiple logistic regression was used to assess the dependence of autoantibody positivity in the T1DM patients on other variables (SPSS for Windows 11.0; SPSS Inc., Chicago, IL, USA). Groups of patients distinguished by paired criteria were compared by Fisher's exact test. *P*-values ≤ 0.05 were considered statistically significant.

Results

Prevalence of GADA and IA-2A in Type 1 DM patients

Serum autoantibodies (GADA and/or IA-2A) were detected in 67% of T1DM patients, of whom 54% were positive for GADA (40% for GADA alone, 14% for GADA + IA-2A), and 27% were positive for IA-2A (12% for IA-2A alone). In Table 1, the autoantibody status of the T1DM patients is categorized in terms of their disease duration. As expected, the highest prevalence of autoantibodies occurred in patients with a relatively recent disease onset (76% of those with diabetes duration of ≤ 1 years). However, Table 1 indicates a marked difference in the prevalence of IA-2A and GADA in relation to disease duration. Most patients with disease duration of ≤ 2 years were IA-2A$^+$ (23/34, 68%), but only 1/56 with disease duration ≥ 3 years was IA-2A$^+$. In multiple logistic regression analysis, there was a significant negative relationship between the serum positivity for IA-2A and diabetes duration ($P < 0.004$). By contrast, GADA occurred in a proportion of

Table 1 Prevalence of GADA and IA-2A in Type 1 DM patients in relation to duration of diabetes

Disease duration (years)	Number of patients	Ab^+ patients *n* (%)	Ab^- patients *n* (%)	All $IA\text{-}2A^+$ *n* (%)	All $GADA^+$ *n* (%)	Only $IA\text{-}2A^+$ *n* (%)	Only $GADA^+$ *n* (%)	Both Ab^+ *n* (%)
≤ 1	21	16 (76)	5 (24)	16 (76)	6 (29)	10 (48)	0	6 (29)
2	13	7 (54)	6 (46)	7 (54)	6 (46)	1 (8)	0	6 (46)
3	21	15 (71)	6 (29)	1 (5)	15 (71)	0	14 (67)	1 (5)
4	16	11 (69)	5 (31)	0	11 (69)	0	11 (69)	0
≥ 5	19	11 (58)	8 (42)	0	11 (58)	0	11 (58)	0
All	90	60 (67)	30 (33)	24 (27)	49 (54)	11 (12)	36 (40)	13 (14)

all T1DM patients, regardless of disease duration (Table 1). There appears to be a lower prevalence of GADA in patients with diabetes duration ≤ 2 years (12/34, 35%) than those with duration ≥ 3 years (37/56, 66%). It should be noted, however, that the average age of onset of T1DM in the group with disease duration ≥ 3 years was significantly higher than in those with duration ≤ 2 years (median age of onset 8 years and 4.5 years, respectively, $P = 0.0059$ by Mann–Whitney test). This may account for the apparently higher prevalence of GADA in the group with duration ≥ 3 years, as GADA have been reported by others to show higher prevalence with older age of disease onset [15–20].

There was a significant negative relationship between autoantibody positivity and age of onset of diabetes ($P < 0.0004$ by multiple logistic regression), although this was not seen for the positivity for each autoantibody tested separately.

GADA tended to be more prevalent in female than in male T1DM patients: 33/53 females were $GADA^+$ (62%) compared with 16/37 males (43%), although this difference did not reach statistical significance ($P = 0.097$ by multiple logistic regression). There was no significant difference between the female and male patients in age of onset or duration of disease. Moreover, there was no significant difference in the prevalence of IA-2A between females and males.

Prevalence of GADA and IA-2A in Type 2 DM patients

Autoantibodies to GAD were detected in eight of the 99 T2DM patients studied (8.1%) (Table 2). Three of these $GADA^+$ individuals were also positive for IA-2A, but no T2DM patients expressed IA-2A in the absence of GADA (Table 2).

Table 2 Characteristics of Type 2 DM (LADA) patients positive for GADA and IA-2A

Sex	Age at onset	Duration (years)	Insulin treatment	GADA	IA-2A
M	29	1	+	+	+
M	27	2	+	+	+
F	23	2	+	+	+
M	40	2	+	+	–
F	37	3	+	+	–
F	62	3	+	+	–
F	34	5	+	+	–
F	29	6	+	+	–

Twenty-six T2DM patients had disease duration ≤ 2 years and 73 had disease duration ≥ 3 years. In each of these groups, four patients were positive for GADA. It was significant that all three $IA\text{-}2A^+$ patients had disease duration ≤ 2 years ($P = 0.0166$ by Fisher's exact test).

The occurrence of GADA was significantly associated with a younger age of disease onset: 16% (7/43) of T2DM patients with an age of disease onset of ≤ 40 years were $GADA^+$ compared with 2% (1/56) of those with an age of onset > 40 years ($P = 0.0197$ by Fisher's exact test). All three $IA\text{-}2A^+$ patients were amongst the seven with an age of onset ≤ 30 years, which was highly significant ($P = 0.0002$ by Fisher's exact test). (There was no significant difference in disease duration between the groups based on age of onset.) Equal numbers of the eight $GADA^+$ patients had an age of onset ≤ 30 or > 30 years.

All of the $GADA^+ \pm IA\text{-}2A^+$ individuals were also insulin treated, which was significantly different from the $GADA^-/IA\text{-}2A^-$ patients, of whom 17/91 (19%) were insulin-treated ($P < 0.0001$ by Fisher's exact test).

Prevalence of GADA and IA-2A in gestational DM patients

Autoantibodies to GAD were detected in two of 90 GDM patients studied (2.2%) and none of 100 healthy pregnant control subjects. Autoantibodies to IA-2 were not detected in the GDM patients or pregnant controls.

Discussion

Like studies in other ethnic groups, our results show a highly significant association between T1DM and the occurrence of GADA and IA-2A in Saudi patients. The prevalence of GADA and IA-2A in these individuals was 54 and 27%, respectively. At first sight, this appears lower than the prevalences reported in some other studies that also employed RIP assays [1]. However, the levels of these autoantibodies are influenced by duration of diabetes, age of onset and sex. We therefore analysed the Saudi patients in relation to these parameters.

IA-2A were present only in the Saudi T1DM patients with relatively recent onset of disease (≤ 2 years), in agreement with

studies in other ethnic groups [15,21]. By contrast, expression of GADA was maintained for much longer after onset. This is also in accord with other reports [15,21,22]. Overall, autoantibodies were more prevalent in the Saudi T1DM patients with younger age of onset. Females showed a higher prevalence of autoantibodies than males, as also reported in European patients [23,24]. Thus, most features of the occurrence of GADA and IA-2A in Saudi Type 1 diabetic individuals are consistent with those in other populations.

The prevalence of GADA amongst Saudi T2DM patients in our study was about 8%. This is consistent with previous reports of a lower, but significant, prevalence of GADA and IA-2A in those with T2DM compared with T1DM, and helps to define those with LADA [2]. In agreement with other studies of LADA, we found that GADA were more common than IA-2A in the Saudi patients and the latter occurred only in conjunction with the former [2,25–27]. Features of the occurrence of autoantibodies in the Saudi LADA patients, were consistent with our findings in the younger Saudi T1DM patients discussed above. Thus, IA-2A were detected only in LADA patients with short disease duration whereas GADA were also present in those with longer disease duration. Autoantibodies occurred more frequently in T2DM patients with a younger age of onset as reported by others [2,25,28], and we found this to be particularly the case for IA-2A (also reported by Grasso *et al.* [27]). Also in line with other studies [2,27–29], the presence of autoantibodies was significantly associated with insulin treatment in those with T2DM.

The low prevalence of GADA and IA-2A in GDM patients in our study (2.2 and 0%, respectively) is consistent with findings in most previous studies [30], although a somewhat higher prevalence of autoantibodies has been reported [3].

In conclusion, our data indicate that the prevalence and associations of GADA and IA-2A in Saudi diabetic patients are very similar to those reported in other ethnic groups.

Competing interests

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