Original Article

Diagnostic value of Autoantibodies to GAD65 and IA-2 in Patients with Latent Autoimmune Diabetes in Adult (LADA)

Ahmed Bolad* and Razan Abdelmageed[1], Mustafa Khidir Elnimeiri[2]

Abstract

Background: Latent autoimmune diabetes in adults (LADA) accounts for11 % of all cases of diabetes and often misdiagnosed as type 2diabetes. LADA resembles type 1diabetes and shares common physiological characteristics of type 1 but it does not affect children and has been classified distinctly as being separate from juvenile diabetes. Autoantibodies against glutamic acid decarboxylase 65 (GADA) and tyrosine phosphatase (IA-2) are found frequently in patients with LADA. The presence of these autoantibodies in LADA predicts inevitable β cell failure and poor response to oral hypoglycemic therapy i.e., patients with LADA do not respond to oral hypoglycemic therapy.

Objective: To determine an immunological marker to diagnose patients not responding to oral hypoglycemic therapy.

Patients and methods: A facility-based cross sectional study was conducted in Jabbir Abu Eliz Diabetes Center, located at Khartoum 2. Venous blood samples were obtained from the study patients. They were divided into three groups, group1 included 27 diabetic patients treated with insulin, group2 included 15 diabetic patients of type 2 diabetes as controls, and group3 included 15 newly diagnosed patients older than 35 years at onset of diabetes. A standardized pre-tested administered questionnaire was used for data collection and the collected data were analyzed.

Results: Males encountered in the study were 28 (49.1%). On patient recently diagnosed to have type 2 diabetes mellitus (T2DM) was positive for autoantibodies to GDA/IA-2. These autoantibodies were also positive in 15 patients with diabetes mellitus type 1 (T1DM)

Conclusions: Autoimmune diagnostics is of particular importance in adults to discriminate between type 1 and type 2 diabetes and to assess the diagnosis of latent autoimmune diabetes in adults. The current study results revealed that autoantibodies to GAD/IA-2 are good marker for diagnosis of latent onset DM type 1. On the other hand, data indicate that the vast majority of cases of type 1 diabetes may be considered as immune-mediated, that multiple autoantibody to GAD/IA-2 analysis are of prognostic value to predict complications e.g., retinopathy. The current study recommends using of anti-GAD/IA-2 antibodies as marker for diagnosis of latent autoimmune diabetes in adults (LADA) who are not responding to oral hypoglycemic and may be at risk for getting complications. On the other hand, the study recommends using of anti-GAD/IA-2 antibodies for prognosis of the clinical progression of diabetes type 1 for prediction of insulin dependence.

Key words: juvenile diabetes, hypoglycemic, insulin, Diabetic Retinopathy.

In 1977 Irvine and others[1] reported that 11% of patients with type 2 diabetes were positive for Islet Cell Autoantibodies (ICA) and that this ICA subset of type 2 diabetic patients tended to fail sulfonylurea therapy and needed insulin treatment.

The type of diabetes in these patients was referred to as slowly progressive IDDM, latent type 1 diabetes, youth-onset diabetes of maturity, latent-onset type 1 diabetes, antibody-positive non-insulin-dependent diabetes and latent autoimmune diabetes in adults(LADA)[2,3,4]. The term LADA has been used to describe adult onset subjects who develop a phenotypic type 2 diabetes (T2DM), but with the presence of beta cell-specific autoantibodies (glutamic acid decarboxylase 65 (GADA), insulinoma-

1. Sudan Academy of Science, Council of Biological Science, New Technology and Environment
2. Al Neelain University, Faculty of Medicine, Department of Community Medicine
*Corresponding author: aaabolad@hotmail.com

antigen 2 (IA-2A) and with a slower progression to beta cell failure compared to classical type 1 diabetes[5-7].

Glutamic acid decarboxylase autoantibodies (GAD-Abs), which is present in the cytoplasm of the human β-cell, is an enzyme required for γ-aminobutyric acid (GABA) synthesis that acts as neurotransmitter in neurons of central nervous system, in pancreatic islets[8] and probably involved in controlling the release of insulin from secretary granules. Autoantibodies to Glutamic Acid Decarboxylase 65 antibodies (GAD65) show no correlation with age at onset and are therefore particularly attractive markers for autoimmune diabetes in the adult population[9]. Moreover, GAD65Ab can be detected years after the clinical onset of the disease, indicating that these autoantibodies may be permanent markers for the autoimmune response[10]. However, it has been suggested that antibodies to antigens other than GAD and IA-2 are more prevalent in LADA[11] and raise the intriguing possibility that some unidentified antigens are more commonly involved in LADA than type 1 diabetes.

Patients with type 1 DM retinopathy have shown inverse correlation to GAD65Ab[12]. GADA may have some effect on the development of diabetic retinopathy since GAD65 is expressed in the neural retina as well as the pancreas and the central nervous system[13,14,15] and were associated with an increased risk of diabetic retinopathy 15 years later[16].

The aim of this study was to determine an immunological marker to diagnose missed cases of LADA among Sudanese patients not responding to oral hypoglycemic therapy. On the other hand, the study measured levels of autoantibodies to GAD 65 and IA-2 in patients with diabetes mellitus type 1 to predict patients at risk of diabetic retinopathy.

Materials and methods:

Ethical consideration: An ethical clearance was obtained from the authorities of Jabbir Abu Eliz Diabetes Center. Informed consents were obtained from the study patients before interviewing and the aims and procedures of the study were explained clearly to all patients.

Study design: facility-based cross sectional study.

Study setting:

Jabbir Abu Eliz Diabetes Center is composed of many departments to provide care of diabetic patients in all ages.

Study population:

Diabetic patients of both genders of various age groups and different ethnic groups who were regularly attending the referral diabetic clinics, were enrolled in the study. Patients were divided into 3 groups; group1 included 27 diabetic patients treated with insulin, group2 included 15 diabetic patients of type 2 diabetes as controls, and group3 included 15 newly diagnosed patients older than 35 years at onset of diabetes.

Data collection:

A standardized pre-tested administered questionnaire was used for data collection. The questionnaire was composed of close-ended questions covering personal characteristics, history of diabetes, duration of diabetes mellitus, family history of diabetes, history of other illnesses, treatment modulation, duration of treatment and result of fasting blood glucose.

Sample processing: Five ml of blood collected from each patient was left to clot for 2-3 hour at room temperature and then centrifuged at 3000 rpm for 10 minutes and serum was stored at minus 20^{o}C until use.

Measurement of fasting blood glucose level: Glucose level was measured immediately before blood clotting by using Trinder's glucose oxidase method. In brief, glucose was essentially oxidized to gluconic acid and hydrogen peroxide by glucose oxidase. The hydrogen peroxide produced was reacted in the presence of peroxidase with 4–aminoantipyrine and p.hydroxybenzene sulphonate to form aquinonimine dye. The intensity of the colour produced was measured at 505 nm. Glucose concentration was calculated according to the following formula:

Glucose concentration of samples in mg/dl =

$$\frac{\text{A sample} - \text{A blank}}{\text{A standard} - \text{A blank}} \times \text{concentration of standard in mg / dl}$$

Detection of autoantibodies using Enzyme Linked Immuno-Sorbent Assay (sandwich ELISA):

The frozen samples were prepared by transferred to room temperature (RT). Sixty-five wells plate (six wells for calibrators from 1 to 6, one well for negative controls, and one for positive control and 57 wells for samples) from ELISA plate (Euroimmun) coated with anti-GAD/IA2 pool was used. Fifty µl of each sample, calibrators and control were added to the wells, and then 25 µl of sample buffer was added to each well. The plate was covered and shacked for five seconds at 500 rpm, and incubated for 18 hours at 4°C to 8°C and then washed 3X using washing buffer. Following that, 100µl of GAD/IA2 (biotin-labelled GAD and IA2) was added and the plate incubated for 1 hour at RT on microplate shaker set at 500 rpm. After the second wash, 100 µl of enzyme conjugate (peroxidise-labelled avidin) was added to each well, covered and then incubated for 20 minutes at RT on shaker set at 500 rpm. Then washed 3X by washing buffer. In the final step, 100 µl of chromgen/substrate solution was added to each well, incubated for 20 minutes at RT, away from direct sunlight contact, and then 100 µl of stop solution was added to each well. The intensity of the colour was read at wave length of 450 nm. A value of 4 IU/ml was considered positive.

Statistical analysis:

The samples were analyzed using SPSS (statistical package for social sciences) programme.

Results

The 57 participants consisted of 28 (49.1%) men and 29 (50.9%) females. Among these, 27 of type 1 DM, 15 diabetic patients of type 2 diabetes (control group) and 15 newly diagnosed patients older than 35 years at onset of diabetes (type 2 DM).

As shown by table 1 that the age of onset of diabetes in controls and in newly diagnosed patients was between 25 and 35 years of age. As shown in table 2, autoantibodies to GAD/IA-2 were detected in 26.3% (15/27) of type 1 diabetes, on the other hand only one of 15 (1.8%) who had been diagnosed recently as type 2 DM (>35 years old) was positive for anti GAD/IA-2 (i.e., LADA), and that patient was on oral hypoglycemic therapy (table 3)

Table1-: Age distribution of the study population

Age (in years)	T1 DM No. (%)	T2DM* No. (%)	T2DM$^{\Psi}$ No. (%)	Total
< 15	3 (5.3%)	-	-	3 (5.3%)
15 – 24	16 (28.7%)	-	-	16 (28.1%)
25 – 34	8 (14.0%)	8 (14.0%)	-	16 (28.1%)
> 35	-	7 (12.3%)	15 (26.3%)	22 (38.6%)
Total	27 (47.4%)	15 (26.3%)	15 (26.3%)	57 (100%)

* T2DM* (control group). T2DMΨ (newly diagnosed)

Table 2-: Autoantibodies to GAD/IA-2 pool in study population

ELISA test for GAD/IA-2	T1 DM No. (%)	T2DM* No. (%)	T2DM$^{\Psi}$ No. (%)	Total
Negative	12 (21.1%)	15 (26.3%)	14 (24.6%)	41 (71.9%)
Positive	15 (26.3%)	-	1 (1.8%)	16 (28.1%)
Total	27 (47.4%)	15 (26.3%)	15 (26.3%)	57 (100%)

Table 3-: Relation between the type of treatment and the positive result of GAD/IA-2 pool

Groups	Type of treatment	ELISA test for GAD/IA-2 Negative	Positive	Total
Type I	Diet	3 (11.1%)	-	3 (11.1%)
	Oral hypoglycaemia	1 (3.7%)	1 (3.7%)	2 (7.4%)
	Insulin	7 (25.9%)	13 (48.1%)	20 (74.1%)
	Combination	1 (3.7%)	1 (3.7%)	2 (7.4%)
Total		12 (44.4%)	15 (55.6%)	27 (100.0%)
T2DM$^{\Psi}$	Oral hypoglycaemia	11 (73.3%)	1 (6.7%) LADA	12 (80.0%)
	Insulin	2 (13.3%)	-	2 (13.3%)
	Combination	1 (6.7%)	-	1 (6.7%)
Total		14 (93.3%)	1 (6.7%)	15 (100.0%)

Table 4-: GAD/IA-2 concentration ± SD* in positive patients in relation with DKA history

Diabetic population	No. (%) of positive patients for GAD/IA-2	Mean ± SD FBS result	Mean ± SD for GAD/IA-2 Concentration	No. (%) with DKA history	
				Yes	No
Total	16 (28%)	160.38± 81.47	49.256± 58.288	11 (68.8%)	5 (31.3%)
P. value		0.44	0.64		

* Stander deviation (± SD)

In addition, we found no significant correlation between GAD/IA-2 concentrations and the complication e.g., diabetic ketoacidosis history (DKA) ($p < 0.64$) (table 4).

Type 1 diabetic when compared with control group (type2), there was significant difference ($P<0.007$) in the concentration of GAD/IA-2 positive subject, likewise when compared with newly diagnosed type 2 diabetic patients a significant difference ($P<0.008$) was also found.

Discussion:

This is the first study to describe the presence of autoantibodies to GAD/IA-2 in patients with diabetes mellitus type 2 who are not responding to oral hypoglycaemic therapy. On the other hand the current study is the first to measure levels of these autoantibodies in patients with diabetes mellitus type 1 to predict patients at risk of diabetic retinopathy.

Males encountered in the study were 28 (49.1%) cases while females were 29 (50.9%) cases. 27 of them were suffering from type 1DM and 15 were diagnosed as type 2 DM (control group). The remaining 15 individuals were newly diagnosed as having type 2 DM and were above 35 years of age at onset of diabetes. The presence of autoantibodies to GAD/IA-2 in one individual (1.8%) with late onset DM and in 15 out of 27 (26%) patients with classic childhood type 1 diabetes supports the evidence that the underlying disease process in both patient groups is autoimmune. The findings by the current study were in agreement with study conducted by Torn and others[17] who reported that a higher percentage [80% (135/169)] of patients positive for anti GAD65/IA-2 were found in patients with type 1 DM in South Indian and Australia. In addition, it was in accordance with a finding by Abigail and others[18] who found that around 2.6% (13/500) of individuals were LADA patients.

The Immunology Diabetes Society has recently proposed the following criteria for diagnosis of patients with LADA: patients should be at least 30 years of age, positive for at least one of the four antibodies commonly

found in type 1 diabetic patients (ICAs and autoantibodies to GAD65, IA-2, and insulin), and not treated with insulin within the first 6 months after diagnosis. Accordingly, the current study results indicate that autoantibodies to GAD65 and IA-2 are good marker for diagnosis of LADA and this has also been shown by Engl and others in U.S.A who stated that GAD65/IA-2 test has clinical significance for diagnosis of LADA[19].

The results show that there was no significant correlation between anti GDA/IA-2 concentration and history of diabetic ketoacidosis ($p < 0.64$). This finding is in agreement with a recent study in adult European diabetes patients that has shown that metabolic syndrome is not more prevalent in patients with autoimmune diabetes than in control subjects i.e., metabolic syndrome is not a characteristic of autoimmune diabetes[20].

Fifteen patients with type 1 DM were positive for GAD65/IA-2 pool. Since GAD65 autoantibodies are detected against glutamic acid decarboxylase (GAD), which is mainly expressed in islets and nervous tissue in type 1 diabetic patients, GAD65A autoantibodies might be associated with retinopathy in these patients[12]. Accordingly, testing for these autoantibodies has a prognostic value for the severity of the disease in patients with type 1 DM. Thus; the current study recommends measuring autoantibodies to GAD 65 in all individuals with suspected retinopathy, and should be a part of routine investigations to follow the severity and various stages of the disease.

Conclusions

Data indicate that the vast majority of cases of type 1diabetes may be considered as immune-mediated, that multiple autoantibody analysis improves identification of the disease, and that screening is provided by the combined detection of autoantibodies to GAD/IA-2. Autoimmune diagnostics is of particular importance in adults to discriminate between type 1 and type 2 diabetes and to assess the diagnosis of latent autoimmune diabetes in adults. Using of autoantibodies to GAD/IA-2 as diagnostic and prognostic marker for diagnosis of latent autoimmune diabetes in adult (LADA) who are not responding to oral hypoglycemic and may be at risk for getting complications and on the other hand for prognosis of the clinical progression of diabetes type 1 for prediction of insulin dependence and complication namely retinopathy.

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