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Anti-Glutamic Acid Decarboxylase 65 as a Predictor of Progressive Insulin Deficiency in Type 2 Diabetes

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Abstract

Background: **For type 2 diabetes, there are few usual highly specific indicators, though the presence of risk factors such as obesity indicates the likelihood of developing type 2 diabetes.** ***Aim:*** **To assess the anti-glutamic acid decarboxylase 65 (anti-GAD65) level in type 2 Egyptian Diabetic patients and if it could be used as a marker for progression to insulin deficiency and treatment with insulin.** ***Patients and Methods:*** **a cross-sectional study was conducted on 100 patients with type 2 diabetes mellitus (T2DM) who were divided into group1 as 50 patients on oral antidiabetic drugs (OADs) for more than 5 years and another 50 patients as group 2 on insulin after being previously on OADs. Patients with type 1 diabetes, T2DM associated with any auto-immune disease, T2DM with chronic kidney disease (CKD), or chronic liver disease (CLD) were excluded. Full history taking, clinical examination, and liver and kidney functions were performed in addition to FBS, HbA1c, C- peptide, and anti-glutamic acid decarboxylase 65 (anti- GAD65) antibody.** ***Results:*** **statistically significant positive correlation between Anti-GAD 65 levels and age (r = 0.262, P-value =0.008) and negative correlation between Anti-GAD 65 levels and fasting C-peptide (r = -0.27, P-value =0.006) were found.** ***Conclusion:*** **Anti-glutamic acid decarboxylase antibody 65 is more positive in the OAD group than in the insulin group but it is not p-value significant. Anti-glutamic acid decarboxylase antibodies are positively correlated with age also, it is negatively correlated with fasting C-peptide. So, it can be used as a marker for pancreatic reserve and progressive Insulin Deficiency in type 2 Egyptian patients.**

Keywords: Anti-glutamic acid decarboxylase 65, type 2 diabetes, insulin, oral antidiabetic drugs.

Introduction

Diabetes mellitus is a complex, chronic illness requiring continuous medical care with Multifactorial risk-reduction strategies beyond glycemic control. Ongoing patient self-management education and support are critical to prevent acute complications and reduce the risk of long-term complications. Significant evidence exists that supports a range of interventions to improve diabetes outcomes[1]**.** For type 2 diabetes, there are few usual highly specific indicators, though the presence of risk factors such as obesity indicates the likelihood of developing type 2 diabetes. Hopefully, future research will reveal some specific markers of type 2 diabetic disease process[2]**.** For the most part, in type 2 diabetic patients, positivity for Glu

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tamic acid decarboxylase autoantibodies, as well as autoantibodies to other islet cell antigens, correlates with some of the phenotypic features consistent with those of type 1 diabetes, such as younger age at diagnosis, lower body mass index (BMI), and a loss of B-cell function. This form of the disease with initial type 2-like diabetes presentation and with serological evidence of islet cell autoimmunity has been termed latent autoimmune diabetes, or type 1.5 diabetes, and has been associated with progressive decline in B-cell function and future insulin requirement in some populations[3]. However, no study to date has assessed the progression to insulin deficiency in Glutamic acid decarboxylase antibody-positive Egyptian patients with Type 2 diabetes. This study will assess the rate of progression to insulin deficiency in Glutamic acid decarboxylase antibody-positive patients with Type 2 diabetes. This study aimed to assess Anti glutamic acid decarboxylase antibody levels in type 2 Egyptian Diabetic patients and if it could be used as a marker for progression of insulin deficiency and initiation of insulin therapy.

Patients and Methods

Study Design and Setting

The present study was a cross-sectional study that was carried out from March 2019 to July 2019 at the inpatient and outpatient clinic in the internal medicine department and Endocrinology unit at Ain Shams University hospital. The study included 100 patients with T2DM who met the study's criteria. We utilized a convenient sampling technique to choose the patients from the inpatient and outpatient clinics in the internal medicine department Endocrinology unit at Al Demardash hospital Ain Shams University.

Study Population

Patients were classified into the following groups: *Group I:* will include 50 patients with type 2 diabetes mellitus on oral antidiabetic drugs for more than 5 years duration. *Group II:* will include 50 patients with type 2 diabetes mellitus on insulin therapy with a previous history of oral antidiabetic drugs for more than 5 years duration with secondary failure.

Inclusion and Exclusion Criteria

We included patients who were above 40 years old with type 2 diabetes mellitus of more than 5 years duration on oral hypoglycemic drugs or insulin therapy with a previous history of oral hypoglycemic drugs for more than 5 years duration with secondary failure who agreed to sign the written informed consent. We excluded patients with Type 1 diabetes mellitus, Type 2 diabetes mellitus with any autoimmune diseases, Type 2 diabetes mellitus with chronic kidney disease or liver cell failure, and Type 2 diabetes patients less than 40 years old.

Study Procedure and Data Collection

In the present study, each eligible patient underwent the following: Full history taking including the age of onset, family history, and the pattern of presentation. Full clinical examination, Laboratory investigations: (Liver function tests (SGOT, SGPT, ALP), (Kidney function tests (Blood Urea, Nitrogen, and Serum creatinine), (HbA1c levels that were measured using an ADAMS A1c HA-8180 automatic glycohemoglobin analyzer (Arkray Inc., Kyoto, Japan), (Fasting and 2 hours postprandial blood sugar), Fasting C-peptide, and (Anti-Glutamic acid decarboxylase 65.

Assessment of anti-GAD65

Serum samples were obtained and frozen at −80°C. Anti-GAD antibody levels were measured using RIA and ELISA at the LSI

Medience Corporation (Tokyo, Japan) using commercial kits (manufacturer: RSR, Cardiff, UK; distributer: Cosmic Corporation). The cut-off value for the kit using RIA is 1.5 U/mL, and that of ELISA is 5.0 U/mL. The coefficient of variation for the kit using RIA is <19%, and that of ELISA is <15%.

Ethical Consideration

We confirm that the present study runs in concordance with international ethical standards and applicable local regulatory guidelines. The study does not have any physical, psychological, social, legal, economic, or other anticipated risks to study participants. The study conserves participants' privacy. Investigators are responsible for keeping the security of the data. We also confirm that the participants' data were not used for any other purpose outside this study. Personal data (e.g., Name, Contact info) were not entered in our data entry software to conserve the participants' privacy, however, each subject got a unique identifier code. Written informed consent was obtained from every patient before study enrollment. The study's protocol was reviewed and approved by the IRB, ethics committee, or audit department of the Faculty of Medicine, Ain-Shams University.

Statistical Analysis

An Excel spreadsheet was established for the entry of data. We used validation checks on numerical variables and the option-based data entry method for categorical variables to reduce potential errors. The analyses were carried out with SPSS software (Statistical Package for the Social Sciences, version 24, SSPS Inc, Chicago, IL, USA). Frequency tables with percentages were used for categorical variables and descriptive statistics (mean and standard deviation) were used for numerical variables. Either Independent Student-t or Mann-Whitney tests were used to compare quantitative variables, while the Chi-square test was used to analyze categorical variables. A p-value < 0.05 is considered statistically significant.

Results

Table 1 shows the comparison of studied groups in terms of DM characteristics. There was no statistically significant difference between both types of therapy and number of hypoglycemic attacks (P-value =0.128), HbA1c (%) (P-value =0.075), neuropathy (P-value =0.28), foot ulcers (P-value 0.14), and hydration status (P-value =0.29). On contrary, there was a statistically significant difference between both types of therapy and the duration of diabetes(years) (P-value =0.001), and peripheral pulsation (P-value = 0.029). Table 2 shows the comparison between both groups on average Anti-GAD 65. Our study showed that the mean of anti-glutamic acid decarboxylase antibodies was 43.52 (±23.6) (u/ml) (12 % positive) in the oral hypoglycemic drugs group and 36.8±17.8u/ml) (10% positive) in the insulin group, with no significant difference between the two groups (P-value = 0.112). Table 3 shows the correlations between the levels of Anti-GAD 65 of all patients included in the study and other parameters. There was statistically significant positive correlation between Anti-GAD 65 levels and age(years) (r = 0.262, P-value =0.008) and negative correlation between Anti-GAD 65 levels and fasting C-peptide (ng/ml) (r = -0.27, P-value =0.006). Table 4 shows the correlations between the levels of Anti-GAD 65 of the OAD group and other parameters. There was statistically significant positive correlation between Anti-GAD 65 levels and age (r = 0.301, P-value =0.011) and negative correlation between

Anti-GAD 65 levels and fasting C-peptide (ng/ml) (r = -0.402, P-value =0.003). Table 5 shows the correlations between the levels of Anti-GAD 65 of the insulin group and other parameters. There was no statistically significant positive correlation between Anti-GAD 65 levels and any of the parameters.

Discussion

The current study included 100 patients with type 2 diabetes of more than 5 years duration aged < 40 yrs. old. The patients were assigned insulin or oral hypoglycaemic drugs. The study aimed to assess the anti-GAD65 level in type 2 Egyptian diabetic patients, and whether it could be used as a marker for the progression of insulin deficiency and treatment with insulin. Comparison between the two studied groups regarding baseline characteristics revealed that there was no significant difference between them regarding age, family history of DM, number of previous operations, and vaccination.

Table 1: Comparison of Studied groups in terms of DM characteristics

Variables	**OHD (N =50)**	**Insulin (N =50)**	**P-value**
Duration of DM in years			
Mean ±SD	6.43 ±1.5	7.36 ±1.43	0.002
Median (Range)	6 (5 -11)	7 (5 -10)	
HbA1c (%)			
Mean ±SD	8.98 ±1.14	8.57 ±1.2	0.075
Median (Range)	9 (7 -11)	8.5 (7 -10)	
Number of Hypoglycemic attacks, No (%)			
One attack	12 (24%)	19 (38%)	0.128
Two attacks	38 (76%)	31 (62%)	
Neuropathy, No (%)			
Yes	22 (44%)	18 (36%)	0.289
No	28 (56%)	32 (64%)	
Foot Ulcers, No (%)			
Yes	7 (14%)	12 (24%)	0.143
No	43 (86%)	38 (76%)	
Peripheral Pulsation, No (%)			
Normal	31 (62%)	40 (80%)	0.029
Weak	19 (38%)	10 (20%)	
Hydration Status, No (%)			
Hydrated	43 (86%)	45 (90%)	0.290
Dehydrated	7 (14%)	5 (10%)	

*****Data are presented as mean ±SD, median (IQR), or number (%)

While body mass index was significantly larger in patients on insulin than in patients on oral hypoglycaemic drugs (P<0.001). Our study showed that the mean of anti-GAD antibodies was 43.52 (±23.6) (u/ml) (12 % positive) in the oral hypoglycemic drugs group and 36.8 (±17.8) (u/ml) (10% positive) in the insulin group, with no significant difference between the two groups (P= 0.11).

Table 2: Comparison between group 1 and group 2 as regard anti-GAD 65 levels					
Variables	**OHDs group (N -50)**		**Insulin group (N -50)**		**P-value**
	Mean ±SD	*Median (Range)*	*Mean ±SD*	*Median (Range)*	
ANTI GAD 65 (U/ml)	43.52 ±23.6	38 (15 – 99)	36.8 ±17.8	34.5 (12 -98)	0.112

**Data presented as mean ±SD, median (IQR)*

A previous cross-sectional conducted on 100 patients with type 2 diabetes found that 14% of participants were positive for glutamic acid decarboxylase antibodies. This finding accorded with a previous study that included 256 diabetic patients during a 2-year follow-up and reported a proportion of 10% positive for glutamic acid decarboxylase antibodies[4]. In a study of 256 diabetic patients with more than 25 years, GAD antibodies were positive in 10.2% of the patients[5].

Table 3: Correlations between the ANTI GAD 65 of all patients included in the study to other parameters (n=100)		
Variables	ANTI GAD 65	
	R	P-value
Age at onset	0.262	0.008
Duration of treatment(years)	0.122	0.226
HbA1c (%)	0.190	0.059
FBS (mg/dl)	-0.023	0.868
2 hours PPBS (mg/dl)	-0.049	0.626
Fasting C-peptide(ng/ml)	-0.270	0.006

Table 4: Correlations between the ANTI GAD 65 of OAD group to other parameters (n=51).		
Variables	ANTI GAD 65	
	r	P-value
Age (years)	0.301	0.011
Duration of treatment (years)	0.122	0.226
HbA1c (%)	0.22	0.126
FBS (mg/dl)	-0.004	0.97
2 hours PPBS (mg/dl)	-0.12	0.36
Fasting C-peptide (ng/ml)	-0.402	0.003

Kim et al. assessed the prevalence of glutamic acid decarboxylase autoantibody among type 2 diabetic Korean patients and mentioned that 10.1% were positive for glutamic acid decarboxylase antibodies[5]. In Saudi Arabia, a study reported 8% of type 2 diabetic patients were glutamic acid decarboxylase antibodies positive[6]. On the other hand, a lower prevalence of glutamic acid decarboxylase antibodies

positive (2.5%) was reported in a study conducted in South Africa including patients with type 2 diabetes[7]. The variations in the proportions of glutamic acid decarboxylase antibodies may be due to the variations in the population studied[8]. Our results showed that anti-glutamic acid decarboxylase antibodies positively correlated with age at onset (r = 0.262, P-value = 0.008). This finding came from the results of Lindholm et al. who screened GAD antibodies in 4974 diabetic patients and found that patients aged 40 to 59 years had higher levels of glutamic acid decarboxylase antibodies[9]. In contrast, our results showed that anti-GAD negatively correlated with fasting C-peptide (r=-0.270, P-value = 0.006). Similar results were reported by Lindholm et al. who reported that diabetic females aged 40 to 59 years had lower fasting plasma C-peptide levels[9]. The results of the current study did not show a significant correlation between anti-glutamic acid decarboxylase and duration of treatment, HbA1c, fasting blood glucose, and 2 hours postprandial blood sugar. Khudhair found no significant association between latent autoimmune diabetes in adults with age, sex, and body mass index[10].

Table 5: Correlations between the ANTI GAD 65 of insulin group to other parameters (n=50)

Variables	ANTI GAD 65	
	r	P-value
Age (years)	0.19	0.16
Duration of treatment(years)	0.122	0.226
HbA1c (%)	0.09	0.55
FBS (mg/dl)	-0.025	0.86
2 hours PPBS (mg/dl)	-0.12	0.36
Fasting C-peptide (ng/ml)	-0.16	0.25

Similar results were reported by Juneja et al.[11]. However, Lindholm et al. reported that diabetic females aged 40 to 59 years had higher glutamic acid decarboxylase antibodies and higher frequency of other autoimmune endocrine diseases compared to males. The study showed that the duration of diabetes, levels of glutamic acid decarboxylase antibodies, and low body mass index were related to total beta-cell failure[9]. In addition, Zimmet et al. found that females were associated with a higher prevalence of glutamic acid decarboxylase antibodies than males[12]. In terms of diabetes mellitus characteristics, patients assigned to insulin were associated with a longer duration of diabetes mellitus (P-value = 0.002) and a larger number of peripheral pulsation (P-value = 0.029) compared to patients with oral hypoglycemic drugs. While there was no significant difference in terms of HbA1c, several hypoglycaemic attacks, neuropathy, foot ulcers, and hydration status.

Conclusion

the current study showed that the levels of anti-glutamic acid decarboxylase antibodies were comparable in the oral hypoglycemic drugs and insulin groups. Anti-glutamic acid decarboxylase antibodies positively correlated with age. So it can be used as a marker for pancreatic reserve or progression of insulin deficiency; however, it is negatively correlated with fasting C-peptide. Anti-glutamic acid decarbox-

ylase antibody65 could be used as a marker for insulinopenia in type 2 Egyptian patients. Anti-glutamic acid decarboxylase antibody65 is more positive in the OAD group than insulin group but it is not p-value significant, adjustment of treatment will be indicated instead of current medications. Anti-glutamic acid decarboxylase did not correlate with duration of treatment, HbA1c, fasting blood glucose, and 2 hours postprandial blood sugar.

Study Limitations

We acknowledge that the present study has several limitations. The sample size of our cohort was relatively small which may affect the generalizability of our findings. Moreover, long-term patient-centered outcomes were not utilized in our study.

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