

Prevalence of immunological marker (Anti-GAD) in patients with type 1 diabetes: hospital based study

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Summary:

Background: type 1 diabetes (T1DM) is a form of diabetes mellitus that results from autoimmune destruction of insulin-producing beta cells of the pancreas, leading to permanent insulin deficiency, categorized as either being positive or negative for various auto antibodies related to pancreatic function. An anti glutamic acid decarboxylase autoantibody (Anti-GAD) is recognized as one of the major serological markers for type 1 diabetes mellitus.

Objectives: to determine the prevalence of the immunological marker (Anti-GAD) among a sample of type 1 diabetes mellitus patients and to identify some factors that might be associated with its seropositivity.

Method: A cross-sectional retrospective study was carried out during February to May, 2011 in specialized diabetic clinic of Children Welfare Teaching Hospital, Baghdad. The study sample included 120 type 1 diabetes patients for whom (Anti-GAD) had been determined, patients reports were studied and analyzed statistically.

Results: The demographic characteristics of this studied sample with higher percentage 57.5% of females, 46.7% in the age group 10-14 years, 12.5% had family history of other autoimmune diseases and the majority 69.2% had healthy Body Mass Index (BMI). While the clinical characteristics revealed higher percentage 47% with onset of the disease (5-9) years, 64.2% having a duration of disease (1-4) years, 86.7% presented with classical type of presentation and 52.5% had good control of diabetes. The prevalence of Anti-GAD in this studied sample was 66.7%. Seropositivity of Anti-GAD was significantly associated with delayed age of patients at diagnosis with mean age (7.5± 3.25) years, short duration of the diseases with mean (3.85±2.57) years, female gender (65%) compared to (35%) of male gender had positive Anti-GAD test, while seropositivity of Anti-GAD was not related to other study variables (age of patients, control of diabetes mellitus, BMI, and family history of other autoimmune disease).

Conclusions: High prevalence of Anti-GAD and its titer in this studied sample, seropositivity was related mainly to female gender, delayed age at diagnosis and short duration of diabetes.

Keywords: type 1 diabetes mellitus, immunological marker (Anti-GAD).

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Introduction:

Diabetes mellitus type 1 (Type 1 diabetes, IDDM, or, formerly, juvenile diabetes) is a form of diabetes mellitus that results from autoimmune destruction of insulin-producing beta cells of the pancreas, leading to permanent insulin deficiency (1). The subsequent lack of insulin leads to increased blood and urine glucose. The classical symptoms are polyuria (frequent urination), polydipsia (increased thirst), polyphagia (increased hunger), and weight loss (2). Type 1 diabetes causes an estimated 5%–10% of all diabetes cases (11–22 million worldwide) (3, 4). The incidence of type 1 diabetes has been increasing by about 3% per year (5).

The broad concept of polyendocrinopathies takes into consideration those patients affected by at least one endocrine disease may have another autoimmune disorder specific auto antibodies (6), Autoimmune diseases such as Grave's

disease, Hashimoto's thyroiditis, Celiac disease, Multiple Sclerosis and Addison's disease may be associated with type 1 diabetes mellitus (7). Antibodies to islet cell antigens may be seen months to years before the onset of beta cells dysfunction, An anti-glutamic acid decarboxylase Anti-GAD auto antibody is recognized as one of the major serological markers for type 1 diabetes and has been reported to be higher in type 1 diabetes patients (6). Positivity varies based on age, duration of diabetes and ethnicity (8).

Patients and Methods:

Cross-sectional retrospective study is carried out during the period extending from 1st February to 31st May, 2011. In the diabetic clinic in Children Welfare Teaching Hospital, in medical city. The children and adolescents with type 1 diabetes were diagnosed and registered on the basis of the presentation of either the classical symptoms of Type 1 diabetes or ketoacidosis (3). The study sample consisted (120) patients with Type 1 diabetes for whom An anti glutamic acid decarboxylase

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autoantibody(Anti-GAD) were determined(registered in Patient's files) by ELISA method test. Data collection methods: Patient's reports were reviewed and data collected from the files of the patients including the following: Demographic variables (age, and age at time of diagnosis, gender, family history of diabetes or other autoimmune diseases, weight and height). Clinical variables (age at diagnosis of diabetes, duration of disease, and pattern of disease presentation at diagnosis). Estimated variables 1st: the HbA1c (measuring the control of blood sugar), in nondiabetic individuals, the HbA1c fraction is usually less than 6%; in diabetics, values of 6-7.9% represent good metabolic control, values of 8.0-9.9% fair control, and values of 10% or higher, poor control (10) , 2nd An anti glutamic acid decarboxylase autoantibody(Anti-GAD) test results (positivity or negativity and titre) by ELISA (Enzyme Linked Immunosorbant Assay) method. According to the standard laboratory protocol, normal values <10 IU/ ml is considered negative ≥ 10 IU/ml is considered positive (11) , 3rd Body Mass Index (BMI): This is calculated in the same way for both children and adolescents based on the following formulas (12) ,

Formulas: $\text{weight(kg)/[height(m)]}^2$.

Statistical analysis:

All the statistical analysis was done by using Excel application and through the SPSS program (Statistical Package for Social Sciences), version 19.

Statistical analysis was performed using Pearson chi-square test a(P value)

The comparison of significance (p- value) in any test

S=significant difference (p<0.05)

HS=highly significant difference (p<0.01)

NS=no significant difference (p>0.05)

Results:

total of 120 patients with type I Diabetes Mellitus were included in the study, 51(42.5%) males and 69(57.5%) females. The age ranges from (1-18) years with a mean of 10.5 years \pm 3.6 SD, and mean duration of diabetes 4.4 \pm 2.63.

Table (1)Comparison of selected parameters between groups of type1 diabetes Anti-GAD +ve and Anti-GAD-ve

GAD antibody	+ve	-ve	P value
NO. of cases	80	40	
Age of patients(yr)	11 \pm 3.6	10 \pm 3.75	0.258(NS)
Age at diagnosis(yr)	7.5 \pm 3.25	5.4 \pm 2.6	0.001(HS)
Duration of DM(yr)	3.85 \pm 2.57	5 \pm 2.7	0.029(S)
HbA1C	9.47 \pm 3.03mg/dl (fair control)	9.26 \pm 2.7mg/dl (fair control)	0.702(NS)
BMI	17.86 \pm 3.45 (healthy weight)	17.4 \pm 3.62 (healthy weight)	0.501(NS)

Table 1 Show that AntiGAD seropositivity gave statistically highly significant differences (P value<0.01) with higher mean (7.5 \pm 3.25yr) age of patients at diagnosis .Also seropositivity

of AntiGAD gave statistically significant differences (P value <0.05) with low mean (3.85 \pm 2.57 yr) duration of disease in years; while seropositivity of was not (AntiGAD) related to the other variables; age of patients (yr), concentration of HbA1C and BMI.

Table 2 Relation of seropositivity of Anti-GAD to the gender:

AGAD	Gender		Total	P value (0.019) [S]
	F	M		
-ve	42.5%))17	57.5%))23	100%))40	
+ve	65.0%))52	35.0%))28	100%))80	
Total	57.5%))69	42.5%))51	100%))120	

Table 2 Show that seropositivity(Anti-GAD) was higher in females (65.0%) compared to males (35.0%).The difference is statistically significant (P value <0.05).

The relation of seropositivity of (Anti-GAD) according to family history of other autoimmune diseases was considerably low (15.0%) in those with positive history of other associated autoimmune diseases. The results were statistically not significant (P value >0.05).

Table (3) The relation of seropositivity of(Anti-GAD) according to family history of other autoimmune diseases.

AGAD	FHAI*		TOTAL	P value (0.242%) [NS]
	-VE	+VE		
-VE	37(92.5%)	3(7.5%)	40(100%)	
+VE	68(85.0%)	12(15%)	80(100%)	
TOTAL	105(87.5%)	15(12.5%)	120(100%)	

* family history of other autoimmune diseases.

Discussion:

Glutamic acid decarboxylase (GAD) is considered to be one of the strongest candidate auto antigens involved in triggering β -cell-specific autoimmunity. The majority of recent onset type 1 diabetes patients and prediabetic subjects have(Anti-GAD) in their sera (13).

The main finding from this study was higher prevalence of detecting seropositivity of (66.7%) (Anti-GAD) among patients with type 1diabetes ,this is nearly compatible to the finding of Park et al. in Korea (6) .Our result is higher than that documented by Barova et al. (14) in Czech Republic who found elevated(Anti-GAD) in 46%(97/210) in patients with type 1 DM .While Elkadhi A.in Tunis found that the prevalence of was 51.2%(Anti-GAD) and decreased as function of increasing duration of the disease(15) . Damanhouri conducted a similar study in Saudi Arabia, the prevalence of was 54 %(Anti-GAD) (16) while the overall(Anti-GAD) detected by Rodacki , et al. (Brazilian) in 45.8%of diabetic children(17). Sabbah E et al.GAD Antibody was detected at diagnosis in (53) % of children and adolescents with type 1diabetes (18) .Explanation

of these controversial observations might be due to different cut-off value set among laboratories or to other environmental factors that affect the diseases pathogenesis since the dietary habits and living stages are quite diversion the areas mentioned above even within the same ethnic group (19).

It was surprisingly to find that seropositivity and (Anti-GAD) was significantly correlated with delayed onset (7.5 ± 3.25 y) and shorter duration (3.85 ± 2.57 y) of the disease.

Rodacki et al. (16) and Hosszuzfalusi et al. (20), found that the duration of the disease did not influence GAD antibodies prevalence or its titers in individuals with T1DM for one year or more, but Elkadhi A (14) detected that the prevalence of GAD antibodies was 84.4% in children with newly diagnosed diabetes (within 6 months of diagnosis) and only 24.4% in those with longer duration of the disease (more than 5 years). Shiau M-Y et al. (19) in Taiwan found the frequency of (Anti-GAD) is quite constant with longer disease duration but it is sharply decreased when the duration of the disease is longer than 11 years.

Seropositivity of (Anti-GAD) and its titre was highly related to gender difference. Block et al. (21) found no statistically significant differences in mean concentrations of (Anti-GAD) between men and women with T1DM, although there was a slight trend towards higher values in females. Shiau MY et al. (19) found that male/female ratio 29/63 in positive patients. Sabbah E et al. (18) also found that GADA was more frequent in girls. Explanations to support this view that organ specific endocrine autoimmunity occurs more frequently in females regardless of racial difference.

The age difference to seropositivity of GADA was not statistically significant and this in agreement with data reported by Pardini et al. (22) study in Brazilian patients. Sabbah E et al. (18) noted that GADA was more frequent in those older than 10 years of age at clinical disease manifestation. Anti-GAD discretonal may be related to other different mechanisms of pathogenesis among the ethnic groups, which resulted in the age related Anti-GAD.

The associated other autoimmune diseases may influence the control of diabetes by impairing function of the respective organs (23); yet in our study autoantibody positivity had no influence on the control of diabetes.

Hickey et al. in Newzeland (24) found no association between antibody status and disease control which agree with this study as both groups had fair HBA1C, while Zanone et al. found inverse relationship between autoantibody levels and HbA1C (25).

In this study the results showed that BMI had no significant relation to the seropositivity. The finding of Cambuli et al. (26) was a single auto antibody was present in 2.18% of over weight /obese subjects and 1.86% in normal weight subjects.

Conclusions:

The prevalence of anti GAD antibodies in type1 diabetes mellitus children and adolescent included in this study was 66.7%.- There is a higher frequency of anti GAD antibodies in

females than males.3- Seropositivity was associated with older age at diagnosis and short duration time of the disease.

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