Study of the prevalence of anti Glutamic Acid Decarboxylase antibody in Iraqi children and adolescent with type 1 Diabetes mellitus

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الخلاصة:

داء السكري من النوع الأول هو مرض سببه تحطم اختياري ومتقدم لخلايا بيتا في البنكرياس عن طريق ميكانيكية ذاتية المناعة, الدراسات الأخيرة تدعم هذه الصفة الذاتية المناعة. في داء السكري من النوع الأول تم التعرف على عدد من المستضدات النوعية وغير النوعية. المستضد الّذاتي الأساسي المشترك في عملية التحطم لخلايا بيتا المؤدي الى تطور داء السكري من النوع الأول هو(Glutamic Acid Decarboxylase) anti-GAD.

الهدف من الدراسة الحالية هو تقييم وجود anti-GAD في الأطفال والمراهقين العراقيين المصابين بداء السكري من النوع الأول .ذشملت هذة الدراسة 60 مريض من الأطفال والمراهقين المصابين بداء السكري من النوع الأول (26 من الذكور و34 من الإناث) و 20 من الأصحاء المطابقين بالعمر والجنس كمجموعة سيطرة. معدل العمر للمرضى كان 9,8 ±4,7 سنة ومعدل فترة المرض كان 2,7±1,5 سنة ومعدل منسب كتلة الجسم كان 17,8 ±3 كغم\م^2 . في مرضى السكري تم تحديد وجود anti-GAD في 45 (75%) مريض في حين لم يتم تحديد وجودanti-GAD في مجموعة السيطرة مع وجود فرق معنوي بينهما (P<0.001). من ضمن المرضى ال45 الذين أظهروا نتائج موجبة ل -anti GADكان 19(42,2%) من الذكور و 26(57,8%) من الإناث.

أظهرت النتائج عند مقارنة مجموعة الأطفال الذين أظهروا وجود anti-GAD مع مجموعة الأطفال الذين لم يظهروا وجود anti-GAD عدم وجود فرق معنوي في العمر, وهيموغلوبين HbA1C, وفحص السكر في الدم وفحص السكر في الدم خلال الصيام، في حين أظهرت النتائج وجود فرق معنوي بين المجموعة التي أظهرت وجودanti-GAD والمجوعة التي لم تظهر وجود anti-GAD في كل من فترة المرض ومنسب كتلة الجسم BMI.

Abstract:

Type 1 diabetes mellitus is a disease caused by the progressive and selective destruction, by autoimmune mechanisms, of pancreatic beta cells. Recent findings support this autoimmune character, and various autoimmune

markers have been described in type1 diabetes, a number of specific and non-specific antigens have been identified. The major autoantigen involved in the destructive process of beta-cells leading to the development of type 1 diabetes is glutamic acid decarboxylase (GAD).

The aim of the present study was to assess the occurrence of anti-glutamic acid decarboxylase (Anti-GAD) antibodies in Iraqi children and adolescents with type 1 diabetes mellitus. A total of 60 patients (34 males and 26 females) with type 1 DM and 20 of their age and gender matched control group was included in this study. The mean age for the patients, disease duration and body mass index was 9.8 ± 4.7year, 2.7 ± 1.5years and 17.8±3 kg/m2 In patients with type 1 DM, positive anti-GAD was detected in 45(75%), while non of the control group showed positive results for anti GAD antibody with a significant difference between them (p=0.001). Out of the 45 patients with positive anti GAD antibody, 19(42.2%) were males and 26(57.8%) were females.

Comparing the results of group of children with anti GAD antibody and the group without detectable autoantibodies showed that there were no significant differences in age, HbA1C, random blood sugar and fasting blood sugar. However the results showed a significant statistical difference ($P<0.05$) between the group with positive anti GAD and the group with negative anti GAD in disease duration and BMI.

Introduction:

Current classification of diabetes endorsed by both the American Diabetes Association and the World Health Organization is based on etiopathogenesis. The two major classifications of diabetes are type1 diabetes, characterized by a state of β-cell destruction, and type2 diabetes, characterized by a combination of resistance to insulin action and an inadequate compensatory response in insulin secretion [1]. The world-wide incidence of the disease varies, ranging from 1.7/100,000 person-years in Japan to 29.5/100,000 person-years in Finland. In Western industrialized countries, Type I diabetes is the second most common chronic childhood illness after asthma [2].

Type 1 diabetes, also known as insulin-dependent diabetes mellitus (IDDM), results from a chronic autoimmune destruction of the insulin secreting pancreatic beta cells, probably initiated by exposure of genetically susceptible host to environmental agents. Autoimmune destruction of beta cells is thought to be completely asymptomatic until 80-90% of the cells are lost. This process may take years to complete and may occur at any time in all ages. During the preclinical phase, this autoimmune process is marked by circulating autoantibodies to beta cell antigens. These autoantibodies, such as anti-insulin antibody (IAA), anti-glutamic acid decarboxylase (GAD) and anti-tyrosine phosphatase ICA 512 (IA2), are present years before the onset of type1 diabetes and prior to clinical symptoms [3]. Glutamic acid decarboxylase is found in nerves and islet cells as a doublet of proteins commonly referred to as GAD65

and GAD67 (i. e. molecular weight65,000 and 67,000 KD). Both isoforms of GAD contain a pyridoxal phosphate binding site, a cofactor required for enzymatic activity [4].

Apart from its presence in the central and peripheral nervous systems, GAD is observed only within pancreatic islet cells, epithelial cells of the fallopian tube, and spermatozoa of the testes. In terms of function, GAD is the rate-limiting enzyme in the pathway involving the conversion of glutamic acid to GABA, a major inhibitory neurotransmitter of both the central and peripheral nervous system [5].

The function of GAD within tissues other than neurons is not clear. The presence of both GAD and GABA (γ-aminobutyric acid) within islet beta cells and the presence of GABA receptors on these cells suggests that GABA is involved in paracrine signalling in the islet. The identification of GAD as a target autoantigen of Type I diabetes dates back to a report in 1982 of a 64,000 KD that was immunoprecipitated from human islets with sera fromnewly diagnosed Type I diabetic children. Glutamic acid decarboxylase (GAD) catalyzes the formation of gamma-aminobutyric acid (GABA), which is a major transmitter in the central nervous system. Two forms of GAD (GAD65 and GAD67) are known to be expressed in human tissues and GAD65 is predominantly expressed in pancreatic beta-cells. Recent findings revealed that GAD functions as an autoantigen in human autoimmunity, especially in insulin-dependent diabetes mellitus (IDDM). GAD is a key antigen for the development of autoimmunity against beta-cells and the production of GADAb precedes other autoantibodies such as anti-insulin antibody (IAA) and anti-tyrosine phosphatase (ICA512/IA-2Ab) prior to the clinical onset of IDDM. At onset, GADAb is detected in 50-80% of patients [6].

In European patients with type 1 DM 95% have positive glutamic acid decarboxylase (GAD65) and/or IA2 antibodies to antigens of the islets of Langerhans; especially the finding of GAD65 antibodies seems a quite stable finding after the age of10 to 15 years in autoimmune diabetes [7].

Materials and Methods:

The study population included 60 children and adolescents (male/female: 26/34) with clinical diagnosis of type1 diabetes mellitus, followed up in national diabetic center of al-Mustansiriya university and 20 unrelated apparently healthy, age and gender matched subjects as controls. The mean age (± SD) of patients was 9.8 (± 4.7) years and mean diabetes duration was 2.7 (± 1.5) years.

The criteria for the diagnosis of type1 diabetes mellitus were: fasting plasma glucose levels of 126 mg/dL or symptoms of hyperglycemia (polyuria, polydipsia, and unexplained weight loss) with a random plasma glucose 200 mg/dL or2-hour plasma glucose 200 mg/dL during an oral glucose tolerance test. Body mass index was calculated as weight in Kg per height (m) squared [8].

Blood glucose and HbA1c measurements

Venous blood glucose was measured using an enzymatic method (SPINREACT, Spain). The accuracy range was 0.04mg/dL to 500mg/dL.
Glycosylated hemoglobin (HbA1c) was measured by using the variant hemoglobine A1C programme developed by BIO-RAD.

Anti glutamic acid decarboxylase (GAD) antibody estimation:

Enzyme-linked immunosorbent assay (ELISA) was used to detect anti-GAD antibodies (Bio-Rad). Isoform GAD65 from human recombinant glutamic acid decarboxylase was used. The assay system uses the ability of GAD65 Abs acting divalently and forming a bridge between immobilized GAD65 and liquid-phase GAD65- Biotin. In the first step GAD65 antibody from the sample bind to GAD65 coated on the microtiter plate. In a second step GAD65-Biotin binds to this complex. The bound GAD65-Biotin correlates with the amount of GAD65 Abs in patient's serum. Unbound GAD65-Biotin is removed by washing. The bound GAD65-Biotin could be quantified by addition of Streptavidinperoxidase and a colorogenic substrate Tetramethylbenzidin (TMB) and reading the optical density (OD) at 450 nm. For the anti- GAD antibodies, the upper limit of the normal range was set at 10 IU/mL, and any greater value was considered as positive.

Statistical analysis:

Results were expressed as mean values (±SD.).The data were analyzed using the program SPSS for Windows. All *P* values were two-tailed, with statistical significance indicated by a value of $P < 0.05$.

Results:

Data demonstrated by table-1 shows the characteristics of children and adolescents patients with type1 diabetes mellitus which revealed that the number of male and female patients was 26 and 34 patients respectively. The mean age of males was 9.5±4.5 years and mean age of females was 9.7±4.8 years, while the mean age of the total number of patients was 9.8 ± 4.7 years. The mean diabetic duration was 2.5±1.7 years in males, 2.8±1.6 years in females and 2.7 ± 1.5 years in the total number of patients. The same table also shows that the body mass index (BMI) was 18.9±3.5 kg.m-2 in males, 17.9 ±3.3 kg.m-2 in females and 17.8±3 kg.m-2 in total number of patients.

variable	Age (mean ± SD) years	Diabetes duration years	BMI (kg.m-2)
Male (26)	9.5±4.5	2.5±1.7	18.9±3.5
Female (34)	9.7±4.8	2.8±1.6	17.9±3.3
Total (60)	9.8 ± 4.7	2.7 ± 1.5	17.8±3

Table-1: The characteristics of type 1 diabetic patients included in the study.

Table-2 shows the prevalence of anti GAD antibodies which revealed that 45(75%) of patients with type1 diabetes were anti GAD positive while only

15(25%) were anti GAD negative. On the other hand the control group showed no prevalence of the antibody with a highly significant difference when compared with the patients group (P<0.001).

Case	GAD Ab positive Number (%)	GAD Ab negative Number (%)	Statistical significance (P value)
Type1 diabetes (n=60)	45(75%)	15(25%)	< 0.001
Control (n=20)	0(0%)	20(100%)	

Table-2: Prevalence of Anti-GAD antibody among patients with type1 diabetes mellitus and control group.

Table -3 demonstrates a significant difference between type1 diabetic patients with anti GAD antibody and control group regarding HbA1C, BMI, random blood sugar and fasting plasma glucose. The same table also shows a significant difference between type1 diabetic patients with no anti GAD antibodies regarding HbA1C, random blood sugar and fasting blood sugar.

parameter	Patients with +ve anti GAD Ab	Patients with -ve anti GAD Ab	Control group
HbA1C (%) (mean±SD)	10.2±2*	9.2±1.9*	5.3 ± 0.4
BMI(kg/m2) (mean±SD)	17.2±2.6*	20±3.6	21.1 ± 2.1
Random blood sugar(g/dl) (mean±SD)	210.5±58.9*	229.2±66.7*	129.1 ± 20.4
Fasting blood sugar (g/dl) (mean±SD)	178.6±52.3*	197±66.2*	89.1 ± 9.2

Table-3: Comparison of selected parameters between groups of type1 diabetes (anti GAD +ve AND anti GAD -ve) and control group

* $P < 0.05$

Discussion:

Anti GAD antibodies have been studied repeatedly in population samples during the last number of years. They predict insulin requirement even before the clinical onset of diabetes. They also predict insulin requirement in type 2 diabetes mellitus. Moreover, they have also been used to characterize a subset of diabetic patients called latent-onset auto-immune diabetes mellitus in adults (LADA). It is known that anti-GAD is positive in more than70 % of children with recent onset of type 1 diabetes and its level seems to decrease with the duration of the disease and decreasing number of residual beta cells. Knowing

the frequency of these autoantibodies in a population is an important step for a better understanding and diagnosis of type1 diabetes [9].

The prevalence of anti GAD antibodies in type1 diabetes mellitus patients included in our study was 75%, which was similar to that found in Caucasian patients, however it differs from the prevalence found in Tunisian and Japanese children which was 54% and 34% respectively The results obtained from those studies showed that the prevalence of anti GAD antibodies in normal subjects was 2.2% which also differs from our results [10, 11]. Our results also differs from the results of a study conducted in Saudi Arabia which concluded that the prevalence of anti GAD antibodies was 54% in type 1 diabetes mellitus patients[12].

Glutamic acid decarboxylase 65 autoantibodies (GAD65 Abs) are present in 70-80% of newly diagnosed patients with type 1 diabetes. GAD65 Abs also occurs in a subset of adults with type 2 diabetes. These patients can have pronounced hyperglycemia, and after therapy with oral hypoglycemic agents for several months to years they may become insulin dependent [3]. According to the literature, anti-GAD prevalence among Asian groups was relatively low compared with that of Caucasians [13, 14]. Anti-GAD prevalence rates are reported to be only 5–29% in Japanese, Koreans, Thais, and Chinese residents of Hong Kong [15, 16]. However, the frequency of anti-GAD in our patients is higher compared with those results. High rates for anti- GAD, similar to the Caucasians, have also been reported [17, 18]. These controversial observations might be due to the different cut-off values set among laboratories or to other environmental factors that affect the disease pathogenesis, since the dietary habits and living styles are quite diverse in the areas mentioned above even within the same ethnic group.

Recent studies have concluded that the diagnostic sensitivity of GAD65, IA-2, and insulin autoantibodies varies with age at onset and sex.GAD65 antibodies are less frequent among boys developing diabetes before the age of 10 years, but in older children, teenagers, and young adults, the diagnostic sensitivity is 80% in both males and females. GAD65 antibody titers are higher and more prevalent in patients with other associated autoimmune diseases, such as thyroiditis [19].

The differences in the prevalence rates reported in various studies are probably due to a different genetic background associated with differences in the selection of patients and/or also antibody determination. The prevalence of anti-GAD antibody was higher in females than males, however Our result of gender-related anti-GAD positivity is at variance with other studies where no gender difference of GAD antibody prevalence was seen This discrepancy might be due to the racial difference of type 1 DM pathogenesis [20].

Although not statistically significant, another interesting observation is that there is a higher frequency (57.8% vs 42.2%) of anti-GAD in females than in males. Our observation is in accordance with three other studies and supports

the view that organ-specific endocrine autoimmunity occurs more frequently in females regardless of racial difference [21, 22]. However, anti-GAD was reported to be independent of sex in Caucasian type 1 DM patients [23]. The finding that anti-GAD in type 1 DM is gender related, being more frequent in females than in males, further supports the theory that the autoimmune responses may be operating differently in different ethnic groups and may be gender related. Patients who did not have antibodies to GAD were more obese (higher BMI) than those who had antibodies to GAD, these findings suggest that diabetes mellitus in the population is often part of a multifaceted syndrome, commonly known as the ‘metabolic syndrome. These results were in agreement with the results obtained by a study in china which concluded that patients who had antibodies to GAD had lower BMI , a higher blood pressure, higher triglyceride levels, lower HDL-cholesterol levels, and increased albuminuria [24].

According to the results obtained by the present study both groups of type1 diabetes (anti GAD positive and antiGAD negative) were significantly different as far as the duration of the disease is concerned (1.3±0.8 vs. 3.5±1.8years). It is known that anti-GAD is positive in more than70 % of children with recent onset of type 1 diabetes and its level seems to decrease with the duration of the disease and decreasing number of residual beta cells [25].

There was no significant difference between patients with positive anti GAD antibody and those with negative anti GAD antibody regarding age, HbA1C, random blood sugar and fasting blood sugar level. These results are in agreement with other studies [26].

Conclusions:

1- The prevalence of anti GAD antibodies in type1 diabetes mellitus children and adolescent included in this study was 75%.
2- There is a higher frequency of anti GAD antibodies in females than males.
3- Patients with negative antiGAD antibodies had higher BMI ratio than those with positive anti GAD antibodies.

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