Glutamic acid decarboxylase autoantibodies in Sudanese diabetic children and their siblings

Mariam Zaki[1], Salah Ahmed Ibrahim[1], Murtada .N.Elbagir[2], Mohamed .A .Eltom[2], Berne C[3].

[1]*Department of Pediatrics and Child Health ,Faculty of Medicine ,University of Khartoum.*

[2]*Endocrinology and Diabetes Research Centre , Omdurman ,Sudan.*

[3]*Department of Internal Medicine, Uppsala University,Sweden.*

***Correspondening author:** Mariam Zaki, Dept. of Pediatrics and child Health, Faculty of Medicine, University of Khartoum, P .Box 102 Khartoum ,Sudan. E-mail : mariam 555za @gmail .com*

Abstract

Background: Type -1 diabetes mellitus(T1DM)is known to be an autoimmune disease . Glutamic acid decarboxylase (GAD) is the enzyme responsible for the conversion of glutamic acid into the inhibitory neurotransmitter gamma amino butyric acid (GABA).GAD is a major auto antigen in type 1 diabetes ,being recognized by auto antibodies present in sera of the majority of patients at onset of the disease . It is also one of the important predictive immunological markers in developing the disease among first degree relatives.

Objectives: (a) To determine the prevalence of anti- GAD antibodies in Sudanese diabetic children , their healthy younger siblings and the control group. (b) To evaluate the presence of GAD antibodiesas an indicator of autoimmunity in T1DM in Sudanese diabetic Children .

Methods: This is a hospital- based, prospective, case- controlled study.The patients were randomly selected from diabetic children attending two hospitals in Khartoum State **.**Sixty –five diabetic children , 25 of their healthy siblings and 31 healthy controls were enrolled . A precoded questionnaire was completed . The presence of GAD antibodies was investigated for the study subjects,their siblings and control subjects using Diamyd Anti GAD65 Radioimmunoassay.

Results: The majority of diabetic patients were between 11 – 15 years representing 37(57.1%). Antibodies to glutamic acid decarboxylase { considered by the lab to be positive if >10.2 U/ml } .GAD antibodies were found to be significantly positive in 30 (46.1%) of the diabetic children compared to only one (3.2 %) of the controls , P- value was highly significant { $P < 0.0001$ } . Highest titers were detected in diabetics with disease duration over one year . GAD antibodies were tested in 25 of siblings.Significant titers were detected in only 2 of them representing 8% .

Conclusions: It was concluded that GAD antibodies is an important immunological marker in Sudanese children with IDDM and similar to Asian and European populations , However, further research using other tests like antibodies against insulin(IAA) , islet cells (ICA) and zink transporter 8 (ZnT8Ab) as well as insulinoma- associated -2 autoantibodies (IA-2A) would be more specific .The role of GAD in disease prediction among siblings needs further research

Introduction

Diabetes mellitus is a common chronic metabolic syndrome characterized by hyperglycemia as a cardinal biochemical feature. The major forms of diabetes are classified according to those caused by deficiency of insulin secretion due to pancreatic beta cell damage (T1DM) and those that are a consequence of insulin resistance.(1) Type 1 diabetes mellitus, the commonest endocrinological disorder occurring in childhood ,is the end point of many disease processes resulting in progressive loss of beta cell function and insulin deficiency .

The disease is quite distinct by its association with certain histocompatibility antigens (HLA) ;the association with circulating antibodies for cytoplasmic and cell surface components of islet cells; antibodies for insulin and glutamic acid decaroxylase (GAD) antibodies.

GAD is highly expressed in the central nervous system. It is also found at lower concentrations in several other tissues.But ,after the CNS ,the Islets of Langerhans, are the tissues where highest GAD activity is expressed.[2]

In patients positive for anti- GAD antibodies, there was a strong association with other organ-specific autoimmune diseases, such as insulin-dependent DM, hypothyroidism, Grave's disease and pernicious anemia.[3]

Antibodies against the 65-kDa isoform of glutamic acid decarboxylase ($GAD^{5,6}$) can be applied as a predictive tool for childhood type-1 diabetes and to facilitate the differential diagnosis of diabetes in adults.[4]

Anti-GAD antibodies has recently been recognized as a reliable immunological marker for type 1 diabetes.[5,6] Recently, researchers became more interested to evaluate risks of autoimmunity.[5] In UK one study was concerned with determinants of risk in HLA DR3 –DQ2/ DR4 –DQ8 siblings .GAD auto antibodies affinity is being tested to identify specific epitopes profiles in children at risk for T1DM.[7,8] In the Diabetes Prevention Trial-Type 1 Diabetes (DPT-1), subjects at high risk for developing diabetes were followed with serial IVGTTs and oral glucose tolerance tests (OGTTs), and in a subsequent study, the metabolic factors associated with progression to diabetes were evaluated.[9] In another study, an autoantibody response directed to the extracellular domain of IA-2 was associated with very high risk of type 1 diabetes progression, suggesting the presence of new antigenic determinants within the extracellular domain of IA-2 .[10] Type 1 diabetes is closely related to both cellular and humoral immune responses to insulin producing beta cells. Antibodies to glutamic acid decarboxylase(GADA) and islet cell antibodies (ICA) have been observed to persist after the diagnosis of diabetes mellitus and with highly fluctuating concentrations for GADA.[11 ,12]

Patients and Methods

This prospective, cross-sectional, case-controlled, hospital-based study was conducted in Khartoum State during the period from Aug1998 - Aug1999.

The study population consisted of 3 groups:

Group A: 65 diabetic children aged 1-1 6 years attending two diabetic centers in Omdurman and Khartoum . Sample size was calculated according to the formula:

Group B: 25 healthy younger siblings of group A.

Group C: 31 healthy children matched with group A for age and sex with no family history of type 1 diabetes, thyroid disease or autoimmune diseases and were selected from surgery and orthopedic referred clinics in Omdurman Hospital .

An informed consent was taken from patients , controls and parents or care takers.

Group A patients were individually interviewed by the author. The research objectives were fully explained; a pre-coded questionnaire including: socio-demographic characteristics, clinical features and insulin requirements was completed .

Evidence of autoimmune diseases and complications of diabetes mellitus were also noted .

The other groups (B&C) were interviewed and an informed verbal consent was obtained. Finger prick blood testing was done using Glucometer (Sensor ,Boeringer M). Urine was tested for glucose , ketones and albumin using dipsticks .

5 ml of venous blood was taken from all 3 groups using vacutainer needles : 2 mls were put in the EDTA container , 3mls were separated and serum was put in 2ml containers .Samples were kept frozen till being sent abroad for testing .

Group B were interviewed by the author and 3 mls of venous blood were taken using vacutainer needles. Two mls were put in the EDTA container

and the rest was centrifuged

The sera of all the 3 groups were stored in _20 degrees C to be shipped in dry ice to the laboratories of the Dept. of Internal Medicine & Endocrinology – Uppsala University – Sweden .

Anti-GAD antibodies were measured in the sera of the 3 groups using Diamyd antiGAD 65 Radioimmunoassay, which is a solid phase direct radioligand assay (manufactured by Diamyd Diagnostics AB Stockholm, Sweden) .During incubation antiGAD antibodies in the samples and I -125 labeled GAD 65 formed a complex.Then, the formed antigen- antibody complex was separated by centrifugation.Then, the centrifugation was measured by measurement of radioactivity which was directly proportional to the anti-GAD titer in the sample .

Data were entered in the computer using Epinfo version 6 .Simple tabulation was done .Chi Square test was used to the 95% significance level. Linear regression analysis was also used to determine the relation and to calculate the correlation coefficient (r) between GAD antibodies and various parameters

The task of patients selection, interviewing,questionnaire completion and blood sampling were all conducted by the author.

Ethical Considerations

Medical problems of diabetic children were dealt with either by the author herself, or else, by referral to hospital.

The study was approved by The Ethical and Research Committee, Faculty of Medicine, University of Khartoum as well as relevant authorities in Uppsala University, Sweden . Permission from the health authorities and consultants in Omdurman and Khartoum hospitals was obtained

Funds & Grants

The study was self-funded and partially funded by the Department of Internal Medicine, Uppsala University, Sweden who performed the laboratory work .

Results

We studied 65 diabetic children , 25 siblings and 31 healthy children as controls. Ages of diabetics varied from 1.5 -16 years .The mean (+or – S.D.) age was 8.75 (3.4)years, compared to an age range of 1.5 -15 ,the mean (+or-S.D.) .The predominant age group among diabetic children was 11 – 15years representing 37(57.1%) (**Table 1)**. Males predominated in both groups. M/F ratio was 1.3 : 1 and 1.27 :1for diabetics and controls respectively.

Twenty nine (44.6%) of the diabetics were from Gaaliyeen Tribe. Poverty and Illiteracy predominated in parents of diabetics and controls .

Newly discovered diabetics were 23 (35.3%). The same number existed for duration of 13 – 36 month. Eleven patients (17%) had a disease for 37-60 month. Only 8 patients (12%) had the disease for more than 5 years **.**

Antibodies to glutamic acid decarboxylase (considered by the lab to be positive if >10.2 U/ml) were detected in significant levels in the sera of 30 (46.1%) of the diabetics compared to only one (3.2 %) of the controls ,P- value was highly significant($P < 0.0001$) .

GAD positive patients with levels of 8-20% were 8 (12.3%) compared to one (3.2%) of the controls .Levels of 21 -60% of GAD were found in 14(21.5%) of the diabetic children **(Table 2)**.

Very high levels of more than 60% were detected in sera of 8 (12.3%), the only child from healthy controls who was GAD positive had a level of 21 U/ml ,considered as moderately high .

Nine patients (39.1%) of the newly discovered (duration < than 12 month) were found to be GAD positive .

GAD in the Siblings of diabetic children

Twenty five healthy siblings of the diabetic children were included in the study , majority of them were in the age group of 6-10 years and comprising 10 (40.0%) . Eleven (44%) of the siblings aged more than 10 years **(Table 3)**.

GAD antibodies were tested in 25 of the siblings. Significant titers were detected in only 2 of siblings representing 8% while it was detected in one (3.2 %) of the study group.

The first sibling had moderately elevated levels. She was a female of 15 years with no abnormality on clinical examination. The second one was a sibling of 2 diabetics.He was 12 year boy with significantly high levels(> 450) U /ml **(Table4)**.

Table 1.Age distribution of the diabetic children (Group A, n = 65) and the controls (Group C, n = 31)

Age (years	Diabetics n (%)	Control n (%)
1.5 – 2	2(3.1%)	1(3.2%)
3 – 5	8(12.3 %)	3(9.6 %)
6 – 10	10(15.3 %)	8(25 .8%)
11 – 15	37(57 %)	19(61.4 %)
16	8(12.3 %)	0 (0.0%)

P value = 0. 58

Table 2.Antibiodies to glutamic acid decarboxylase "GAD" in the diabetic children (Group A, n = 65) and the controls (Group C, n = 31)

GAD Ab U/mL	GAD Ab %	Diabetics n = (%)	Control n (%)
0	0	26(40.0)	28(90.3)
< 10.2	< 7	9(13.9)	2(6.5)
10.45 – 28.85	8-20	8(12.3)	1(3.2)
• 29.55 – 450	21-60	14(21.5)	0(0.0%)
• 450	> 60	8(12.3)	0(0.0)

GAD levels are expressed in units per ml with the corresponding percentage from the nomogram

(df = 1, X^2 = 15.78, p < 0.0001)

Table 3. Age distribution of the siblings of diabetic children (Group A, n = 25) and the controls (Group C, n = 31)

Age (years)	Siblings n (%)	Control n (%)
< 1.5	1 (4.0%)	0 (0.0%)
1.5 – 2	2 (8.0%)	1 (3.2%)
3-5	1 (4.0%)	3 (9.6%)
6-10	10 (40.0%)	8 (25.8%)
> 10	11 (44.0%)	19 (61.4%)
Total	**25 (100%)**	**31 (100%)**

P value = 0.24

Table 4. GAD antibodies among siblings of the diabetic children (Group A, n = 25) and the controls (Group C, n = 31)

GAD Ab U/mL	GAD Ab %	Diabetics n = (%)	Control n (%)
0	0	23 (92.0)	28 (90.3)
< 10.2	< 7	0 (0.0)	2 (6.5)
10.45 – 28.85	8-20	1 (4.0)	1 (3.2)
• 29.55 – 450	21-60	0 (0.0)	0 (0.0%)
• 450	> 60	1 (4.0)	0 (0.0)
Total		25 (100.0)	31 (100.0)

- GAD levels are expressed in units per ml with the corresponding percentage from the nomogram. (Fisher exact test: P = 0.58)

Discussion

The presence of GAD auto-antibodies was detected in significant levels in the sera of nearly half of the diabetics compared to only one (3.2 %) of the controls ,P- value was highly significant(P < 0.0001). This frequency was higher than that reported in the European study where less than tenth of the patients were found to have significant titers for GADA[12] ;but the comparison could be rather difficult if we consider that in the latter, patients with longer disease duration were tested for presence of GADA. However, it wasn't the same

in a study among Spanish population of T1DM children where two third of them tested positive for GADA[13] .

Our findings were rather similar to reports from South Africa and Ethiopia reporting higher rates of auto antibodies in patients T1DM [14,15] , while it was not the same in Tanzania and Nigeria [16,17] , where lower levels of less than tenth of the patients had positive auto antibodies. On the other hand, in line with our study, significantly positive titers were detected in nearly half of the study population of Tunisian children.[18]

Compared to Asian populations, our results were higher than that obtained in the Chinese study which reported one quarter as positive for GADA [19]; while nearly half of the T1DM patients in the Saudi study were found to be positive for GADA[20]

These differences from European and Asian populations could be related to ethnic variations or probably to the rare DR4/ DQW2 haplotype previously reported by Almagzoub.[21]

In this study, children with longer disease duration had increased frequency of positive titers than those with disease duration less than one year. That is contradictory to the results of European and Saudi researchers who described younger patients with shorter disease duration.[12,20]

The same was obtained by Yokota et al.[22] On the other hand, titers were found to be lower in children with longer disease duration among Tunisian children.[18]

In agreement with the literature, no relationship of GADA with age and gender was found.[23] However, female predominance had been observed in European as well as Saudi patients.[12,20]

GADA was only found in 2 siblings of patients compared to one of the controls. Higher frequency was found among first degree relatives of Spanish population of patients with T1DM.[24] It is also lower than the incidence reported among 882 American first degree relatives of Type 1 diabetics where 90 % proved to be serologically positive and later developed T1DM.[25] Our findings may be explained by the small sample size of the siblings enrolled and lack of follow- up in our study.

Predictive characteristics of GAD auto-antibodies also depends on genetic markers. A study in Finland in an unselected population of 701 siblings of children with type 1 diabetes siblings carrying the protective DR2 and DQB1 *0602 -3 alleles were characterized by lower frequencies of ICA , IA and GADA.[26]

Risk of diabetes associated with HLA DR3-DQ2-DQ8 in U.K families was studied among 2,134 siblings and followed to a median duration of 22 years. In HLA identical DR3 –DQ2 / DR4 /DQ8 siblings, the cumulative risk of diabetes by age 15 was 17% versus 6% in those sharing one haplotype or none(P= 0.095) [27,28]. Relatively similar to our results, frequencies of GAD antibodies were detected in sera of siblings of Syrian and Jordanian diabetic children , 1(1.3%) and 2(2%) respectively. Those are considered among the highest reported in the world. This would ,more than ever, highlight the evidence of occurrence of a true autoimmune type of diabetes in Sudanese children.[29]

Recommendations

The study emphasizes the importance of GAD auto-antibodies as an immunological marker in children with T1DM and could add to the value of antibodies in disease monitoring and its complications . Because prevention of type 1 diabetes is still at the stage of research trials, the trials are often mentioned in the popular press. As a result, many patients with type 1 diabetes (or their parents) ask their doctors about screening of other family members (particularly children) and what could be done if the family member has a high risk for the development of type 1 diabetes?. The role of GADA in disease prediction among siblings of diabetic children needs to do further research using larger samples, additional predictive antibodies, genetic markers and follow- up.[29,30]

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