Detection of GAD_{65} Antibodies in Newly Onset Type 1 Diabetic Children

Eman M. Saleh* Ph.D

Nidhal abdul Mohymen** Ph.D

Summary:

Back ground: Glutamic acid decarboxylase (GAD) has been defined as a major target antigen in type 1 diabetes mellitus (T1DM).

Aim of the study: Assessment of GAD_{65} autoantibodies in the serum of T1DM children at onset of the disease.

Patients & Methods: Serum GAD_{65} autoantibodies has been estimated in 60 sera samples of newly diagnosed T1DM children (diagnosed less than 5 months) in comparison with 50 healthy control children using IRMA method.

Results & conclusion: A higher significant proportion of the patients were positive to GAD65 autoantibodies (50%) in comparisons with healthy controls (4.76%) in age group ≤10 years old and (6.90%) in > 10years old ($p<0.0001$), whereas a significant proportion of girls tested positive for GADA were in age group >10years old ($p<0.05$). We concluded that GADA are an excellent diagnostic marker for T1DM.

Key wards: GAD autoantibodies, T1DM.

Introduction:

Type 1 Diabetes Mellitus (T1DM) is an organ-specific autoimmune disease characterized by lymphocyte infiltration of pancreatic islets of langerhans, and by complete destruction of pancreatic β-cells. The disease process occurs over long periods of time, often years eventually reaching a stage where insufficient β-cells are available with insufficient insulin production [1].

The disease generally shows a peak for clinical onset between 10-14 years of age with a sharp drop in late teens. It may be that children susceptible to the disease are exposed to the predisposing factor(s) during their first 14 years of the life and that either exposure subsequently decreases or a large proportion of the susceptible individuals has already developed the disease by the time they reach 20 years of age [2].

The autoimmune mediated destruction of pancreatic β- cells is reflected by the presence of autoantibodies against prominent antigens in the pancreatic β-cells often long periods of time before the disease become clinically manifested [3]. The HLA type of the individual may control the recognition of certain autoantigens including insulin, glutamic acid decarboxylase (GAD_{65} and GAD_{67}), membrane proteins that are homologous to tyrosin phosphatase (ICA512 and IA-2), and islet neuroendocrine ganglioside [4], that may occur individually or in combination [5, 6].

The frequency of GAD autoantibodies (GADA) has been reported to vary from 0.5 – 3% among children from background population [7], from 6.4 - 13% among siblings of children with T1DM (5), and from 62 - 84% among patients with newly diagnosed disease [8]. More than 80% of prerdiabetics and most recent onset diabetics have autoantibodies directed against GAD [9]. The data of T-cell reactivity to various diabetes associated antigens in diabetic patients and non-diabetic controls have remained inconsistent. An increased proportion of activated T-lymphocytes in the peripheral circulation was reported in association with the presence of insulin autoantibodies (IAA) [10]. Increased T-cells responses to diabetes associated autoantigens were detected in newly diagnosed patients with T1DM and in antibody positive first degree relatives as compared with healthy controls [11]. Peripheral blood mononuclear cells (PBMs) from approximately one- half of new-onset T1DM patients were found to respond to GAD [12]. GADAs may play a significant role in the processing and presentation of T- cell epitope from the human GAD_{65} autoantigen to T-cells through increasing the efficiency of antigen capture by antigen presenting cells (APCs) [13]. GADAs are also more frequent in girls than in boys [14], and in individuals more than 10 years of age [15].

In Iraqi patients, we have no available data on the presence of GAD_{65} autoantibodies with the age at onset of the T1DM in children.

Subjects, Materials & Methods:

Subjects:

Sixty Iraqi Type 1 diabetic patients (28 males and 32 females) were subjected to this study. The patients were attending to National Diabetes Center at Al-Mustansirya University/ College of Medicine

during the period May 2004 to October 2005. Their ages range from 3 -17 years, and they were new onset of the disease (diagnosis was from one week up to five months). All the patients were treated with daily replacement doses of insulin at the time of blood sampling. The patients were divided into two groups according to their ages: 36 child equal or less than 10 years and 24 children more than 10 years. For the purpose of comparisons, 50 healthy subjects matched for age (4-17 years old) and sex, and were selected who have no family history or clinical evidence of type 1 diabetes or any chronic diseases and obvious abnormalities as a control group.

Blood collection:

Two milliliter of blood was collected into plain test tubes, then the serum was separated by centrifugation at 2500 rpm for 10 min. and kept at-20°C until used.

Assessment of serum anti- GAD_{65} autoantibodies:

Serum anti-GAD_{65} autoantibodies were measured by Immunoradiometric assay (IRMA) using anti-GAD IRMA kit (Immunotech Beckman Coulter, France). Values are calculated by interpolation from the standard curve. Values below 1U/ ml were considered normal, whereas values above 1U/ ml should therefore consider as pathological.

Statistical analysis was performed by using Chi Square test and P value equal or below 0.05 is considered as significant.

Results:

The proportion of index cases positive for the both age groups in comparison with controls was shown in table (1). A higher significant proportion of the patients was positive to GADA in both age groups (18/36, 50% and 12/24, 50% respectively) as compared to control groups (1/21; 4.76% and 2/29; 6.9% respectively). This differences were highly significant P_1=0.0001.

GADA were detected in 30 of Iraqi children with newly diagnosed T1DM (50%). A higher significant proportion of the girls tested positive for GADA (5/9; 55.6%) were observed in age group >10 years old than of girls ≤10 years old (11/23; 47.8%), (P_2= 0.049); while the proportion of boys tested positive for GADA was higher in age group ≤10 years old than >10 years old (7/13; 53.8% vs 7/15; 46.7%), but this difference was not significant (P_2= 0.804), as shown in table (2). No statistical differences were observed between males and females in each age group (P_1= 0.729 and 0.673 respectively). Significantly higher proportion of the girls tested positive for GADA (16/30; 53.34%) in comparison to males (14/30; 46.66%) in both age groups.

Table 1: Differences of sero positive / negative of GADA between control and T1DM patient groups.

Age	Groups	No.	Sero positive		Sero negative		P_1	P_2
			No.	%	No.	%		
≤10 years	Controls	21	1	4.76	20	95.24	Chi 0.0001 (HS)	Chi 1.00 (NS)
	T1DM	36	18	50.00	18	50.00		
>10 years	Controls	29	2	6.90	27	93.10	Chi 0.0001(HS)	
	T1DM	24	12	50.00	12	50.00		

Table 2: Differences of sero positive / negative of GADA between T1DM males and females patients.

Parameter	≤10 years (n=36)					>10 years (n=24)					
	GADA+		GADA–		P_1	GADA+		GADA–		P_1	P_2
	No.	%	No.	%		No.	%	No.	%		
Males	7	53.8	6	46.1	Chi 0.729 (NS)	7	46.7	8	53.3	Chi 0.673 (NS)	Chi 0.804 (NS)
Females	11	47.8	12	52.2		5	55.6	4	44.4		Chi 0.049 (S)

Discussion:

GAD autoantigen is neither beta-cell nor islet specific and is expressed predominantly in the nervous system and other tissues, including the testes, ovary, adrenal, pituitary, thyroid and kidney [16].

The present results indicated that older children were more often tested positive than younger ones in females (55.6 vs 47.8%). This difference seems to be not significant between males in both age groups (table 2). This result is in disagreement with Sabbah, (2000), but in agreement with Graham *et al.* (2002) which indicated that GADA was less affected by age at clinical onset in patients than other autoantibodies marker. Islet cells reactivity as judged by the presence of antibodies to the GAD_{65} were observed in 50% of the patients studied in both age groups (table 1), 55.6% in >10 years old group were females. Our observation is in consistent with other studies and supports the notion that autoimmunity is more common among females more than 10 years old [14, 15, 17].

In conclusion GADA were present in 50% of diabetic children. Older children were tested positive for GADA more than younger ones, especially females.

References:

1- Boic B: Diabetes and Autoimmunity. The Journal of International Federation of Clinical Chemistry (JIFCC). 2004; 13(5): 1-9.

2- Gale EAM: The rise of childhood type 1 in the 20th century. Diabetes.2002; 51: 3353-3361.

3- Schatz DA.; Krischer J. and Skyler J. : Now is the time to prevent type 1 diabetes. The Journal of Clinical endocrinology and metabolism.2000; 85(2): 495-498.

4- Lernmark A.: Type 1 diabetes. Clinical chemistry.1999; 45: 8(B) 1331-1338.

5- Kimpimaki T; Kulmala P; Savola K *et al.,* and The Childhood Diabetes in Finland Study Group: Disease associated autoantibodies as surrogate markers of type 1 diabetes in young children at increased genetic risk. J. Clin. Endocrinol. Metab.2000; 85: 1126-1132.

6- Krischer JP; Cuthbertson DD; Yu L *et al.,* and The Diabetes Prevention Trial-type 1 Study Group: Screening strategies for the identification of multiple antibody-positive in relatives of individuals with type 1 diabetes. The Journal of Clinical Endocrinology and Metabolism.2003; 88(1): 103-108.

7- Kulmala P; Rahko J; Savola K *et al.*: β-cell autoimmunity, genetic susceptibility and progression to type 1 diabetes in unaffected schoolchildren. Diabetes Care.2001; 24: 171-173.

8- Sabbah E; Savola K; Kulmala P *et al.,* and The Childhood diabetes in Finland Study Group: Diabetes-associated autoantibodies in relation to clinical characteristics and natural course in children with newly diagnosed type 1 diabetes. J. Clin. Endocrinol. Metab.1999; 84: 1534-1539.

9- Baekkeskov S ; Landin M ; Kristensen J K *et al.* : Antibodies to a 64,000 Mr human islet cell antigen precede the clinical onset of insulin-

dependent diabetes. J. Clin. Invest.1987; 79:926-934.

10- Tun RYM; peakmann M; Alviggi L *et al.*: Importance of persistent cellular and humoral immune changes before diabetes develops: a prospective study on identical twins. BMJ.1994; 38: 1063-1068.

11- Durinovic-Bello I; Hummel M and Ziegler AG: Cellular and immune response to diverse islet cells antigens in IDDM. Diabetes. 1996; 45:795-800.

12- Honeyman MC; Cram DS and Harrison LC: Glutamic acid decarboxylase 67- reactive T- cells: a marker of insulin- dependent diabetes. J. Exp. Med.1993; 177: 535-540.

13- Reijonen H; Daniels TL; Lernmark A. and Nepom GT: GAD-65 specific autoantibodies enhance the presentation of an immunodominant T-cell epitope from GAD-65. Diabetes.2000; 49: 1621-1626.

14- Rais NM; Maclaren NK ; Makhija P and Majithia H: Gender differences in islet cell reactivity and autoimmunity in insuline dependent diabetes mellitus. Int. J. Diab. Dev. Countries. 1996; 16: 114-117.

15- Sabbah E : Role of antibodies to glutamic acid decarboxylase in type 1 diabetes. Relation to other autoantibodies, HLA risk markers and clinical characteristics. Ph.D. Thesis. 2000; University of Oulu. Finland.

16- Winter WE; Harris N and Schatz D: Immunological markers in the diagnosis and prediction of autoimmune type 1 diabetes. Clinical diabetes. 2002; 20(4): 183-191.

17- Graham J; Hagopian WA.; Kockum I *et al.,* for the Swedish childhood diabetes study group: Genetic effects on age-dependent onset and islet cell autoantibody markers in type I diabetes. Diabetes. 2002; 51: 1346-1355.

*Department of Microbiology, Al-Kindy College of Medicine, Baghdad University.

** Department of Microbiology, College of Medicine, Al-Nahrain University, Baghdad.