



Early Detection of T1DM Using Anti- GAD and Anti-Insulin Antibodies

OBAID Reem M.^{1*}, ALWACHI Sabah N.², MUSTAFA Munthir³

¹MSc in Endocrinology, Biology Department, College of Science, University of Baghdad.
Baghdad-Al-Jadiria, Iraq.

²Prof of Endocrinology, Head of the Biology Department, College of Science, University of Baghdad. Baghdad-Al-Jadiria, Iraq.

³Ph.D. in Endocrinology, Ministry of Health.

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***Corresponding Author**

Reem M. Obaid

E-mail: reem_m2478@yahoo.com

Phone: 009647902553814

ABSTRACT

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

This study was conducted to find the relationship between antibodies for this antigens and T1DM which can be used for the early detection of T1DM in normal Iraqi patients. The study was carried out on 80 samples (50 men and 30 women) with age ranged from (20– 60 years old), they are divided into three groups: Group 1 which have fasting plasma glucose (FPG) above 180 mg / dL. Group 2 which have FPG ranged from 120 – 180 mg/dL. Group 3 which have FPG below 120 mg /dL. The statistical analysis results showed no significant difference in the presence of antibodies between men and women. Significant ($p<0.05$) elevation in the level of (anti - insulin) in T1DM patients compared with control group. No significant elevation in the level of (anti- GAD). The results also showed that no positive results for (anti - insulin) present in the control group. One positive result for anti-GAD present in the control group.

Keywords:

T1DM, anti-GAD, anti-insulin

ABBREVIATIONS

GAD: Glutamic Acid Decarboxylase

IAA: Anti- Insulin Antibody

T1DM: Type 1 Diabetes Mellitus

1. INTRODUCTION

Diabetes is a metabolic disease that is diagnosed on the basis of sustained high concentration of glucose in the blood (Ismail *et al.*, 2004; Peyrot *et al.*, 2005). Diabetes occurs when the pancreas does not produce enough insulin, or when the body cannot effectively use the insulin it produces (Norris *et al.*, 2001).

Type 1 diabetes, 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 at all ages (Batstra *et al.*, 2001). During the preclinical phase, this autoimmune process is marked by circulating auto-antibodies to beta cell as antigens, these auto-antibodies, such as anti - insulin antibody (IAA) and anti – (GAD) are present years before the onset of type 1 diabetes and prior to clinical symptoms (Batstra *et al.*, 2001).

Insulin was the first diabetes – related auto-antigen was discovered since 1992 (Greenbaum *et al.*, 1992). Auto-antibodies to insulin are found in 50 % – 70 % of type 1 diabetic children and was the first sign of an ongoing autoimmune process (Ziegler *et al.*, 1999). (GAD) is found in nerves and islet cells as a doublet structure of proteins commonly referred to as GAD - 65 and GAD – 67 (i.e. molecular weight 65,000 and 67,000 KD). Both isoforms of GAD contain a pyridoxal phosphate binding site, a cofactor required for enzymatic activity (Ellis and Atkinson, 1996). GAD is a key antigen for the development of autoimmunity against beta – cells by the production of GAD Ab . This provokes other auto-antibodies such as anti- insulin antibody and anti-tyrosine phosphatase prior to the clinical onset of type 1 diabetes. At onset, GAD Ab is detected in 50 – 80 % of patients (Baekkeskov *et al.*, 1982). To find the relationship between anti –insulin, and anti – GAD for the early detection of diabetes in normal Iraqi patients is the aim of the current study.

2. MATERIALS AND METHODS

2.1. Study population

This study included 80 individuals (50 men and 30 women) age ranged from 20 - 60 years old, they are divided into three groups: group 1 (chronic diabetic group) whom have blood sugar above 180 mg/dL, group 2 (early diabetic group) whom have blood sugar ranged from 120-180 mg/dL and group 3 (control group) whom have blood sugar below 120 mg/dL. The study was conducted for a period from February 1st to June 13th 2013 in Baghdad city.

2.2. Laboratory investigations

2.2.1. Fasting Plasma Glucose (FPG)

Plasma glucose was measured by enzymatic colorimetric assay (Tietz , 1995) using kits supplied by (Spinreact S, A. Spain).

2.2.2. Glycated hemoglobin HbA1c %

After collection of blood samples HbA1c was measured by boronate affinity assay.

2.2.3. Measuring of Anti - GAD Ab level in the serum

All samples were stored at -20c till tested. Anti - GAD Ab level was measured in the serum by using Enzyme – Linked Immunosorbent Assay (ELISA) test. The assay system uses the ability of GAD65 Abs acting divalently and forming a bridge between immobilized GAD65 and liquid – phase GAD65 - Biotin.

2.2.4. Measuring of Anti- insulin Ab in the serum

The level of anti- insulin was measured in the serum by using (ELISA) test.

2.2.5. Statistical analysis

The Statistical Analysis System (SAS, 2010) was used to effect the different factors in study parameters. Least significant difference -LSD or T-test was used to determine significant comparison between means in this study.

3. RESULTS

Mean $\pm$ SE values of FPG and HbA1C were higher in males than females, but there were no statistically significant differences in the Mean $\pm$ SE values between the two groups.

The results also reported that Mean $\pm$ SE of anti-insulin and anti- GAD was lower in males than females. But there were no significant differences in Mean $\pm$ SE values between them.

The Mean $\pm$ SE of FPG and HbA1c was higher in the diabetic patients compared to that of controls. Mean $\pm$ SE of anti- GAD was higher in the control group than in the early diabetic. While Mean $\pm$ SE of anti-GAD was lower in the early diabetic patients compared to that of chronic diabetic, no significant difference in the anti GAD was observed among the three groups (Table 1). It was found that the highest level of FPG was observed in chronic diabetic group while the lowest level of FPG was in control group. The highest level of HbA1C was in chronic diabetic group, and the lowest level of HbA1C was in control group. It was found that the highest level of anti – insulin was in chronic diabetic group while

the lowest level of anti – insulin was in control group (Figure 1). The highest level of anti – GAD was found in chronic diabetic group, but the lowest level was in early diabetic (Figure 2). In chronic diabetic group, it was found that 9 persons of them were positive for anti – insulin while 21 of them were negative for anti – insulin antibody. The results also reported that 11 person of early diabetic group were positive for anti – insulin while 19 of them were negative for anti – insulin antibody. The present study showed that no person of

control group was positive for anti - insulin while all were negative for anti – insulin antibody. In chronic diabetic group it was found that four persons of them were positive for Anti – GAD while 26 of them were negative for anti - GAD antibody. The results also reported that two persons of early diabetic group was positive for anti–GAD while 28 of them were negative for anti-GAD antibody. Our results also showed that one person of control group were positive for anti-GAD while 19 of them were negative for anti-GAD antibody.

Table 1: Comparison between different groups in parameters study

Parameters	Mean $\pm$ SE			LSD Value
	Chronic diabetic (No: 30)	Early diabetic (No: 30)	Control group (No: 20)	
FPG	310.67 $\pm$ 12.72	145.10 $\pm$ 2.15	96.75 $\pm$ 2.25	24.246 *
HbA1C	11.76 $\pm$ 0.28	7.06 $\pm$ 0.22	5.48 $\pm$ 0.13	0.639 *
Anti-insulin	17.49 $\pm$ 3.63	13.36 $\pm$ 1.50	6.24 $\pm$ 0.91	7.427 *
Anti-GAD	26.85 $\pm$ 13.76	8.87 $\pm$ 7.14	12.53 $\pm$ 10.39	31.663 NS

* (P<0.05), NS: Non-significant.

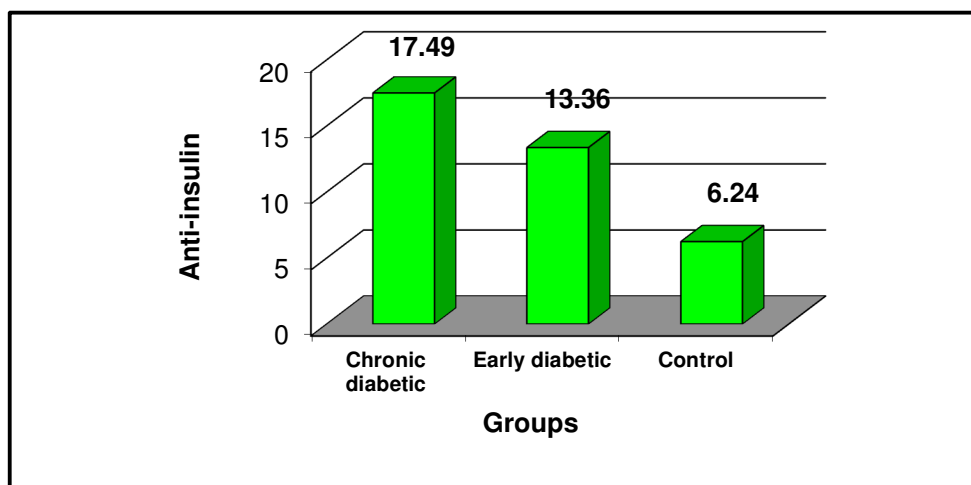


Figure 1: Comparison between differences in Anti-insulin of different groups

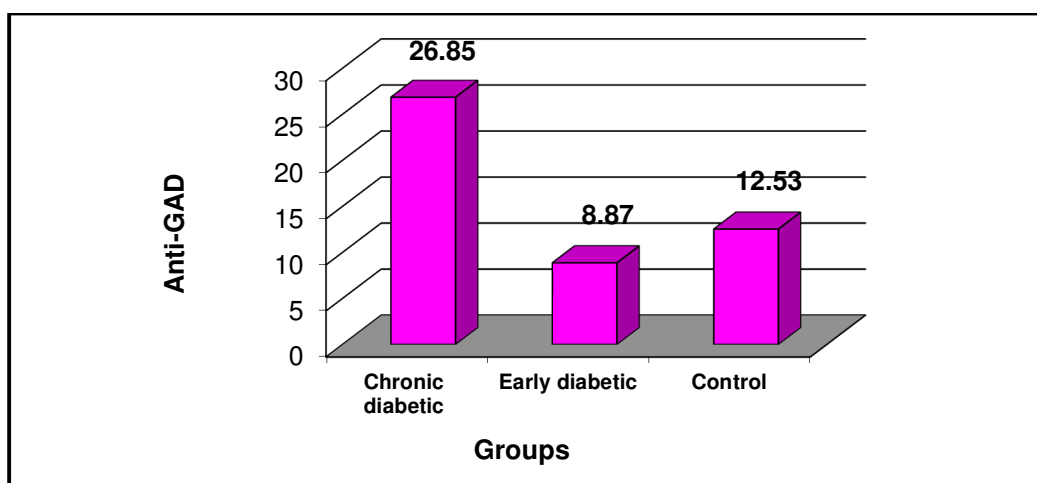


Figure 2: Comparison between differences in Anti- GAD of different groups

Here, the mean of Anti-GAD in control group was higher than that of early diabetic group which indicate the importance of Anti-GAD in the early detection of T1DM.

4: DISCUSSION

This study investigated the existence of anti-GAD and anti-insulin in T1DM patients and in normal individuals. Also the effect of gender variation. To achieve these objectives anti-GAD, anti-insulin, HbA1C and FPG levels were measured for 80 patients (50 males and 30 females) in Baghdad city. As T1DM is often associated with other endocrine autoimmune disorders, so circulating auto-antibodies could be hallmark of clinical or sub clinical autoimmune polyendocrine disease. The present study in general is important in order to identify all these antibodies which could be a good prognosis for T1DM. The frequency of these auto-antibodies in a population is an important step for a better understanding and diagnosis of type 1 diabetes. Among the effect of gender of patients on parameters study, our data reported that there is a non- significant difference between males and females in all parameters study related to T1DM with a little difference between their mean. This result is similar to the result of Berwary *et al.* (2013) who found that T1DM can be observed among males and females, and there is no significant difference in spread of T1DM between the genders. Also in agreement with the finding of Soltez e t al. (2007); found that T1DM is common amongst males and females with a very little difference. The present study showed that the mean FPG and HbA1C was higher in diabetic patients when compared with control group with significant difference between these two groups. This finding is similar to the findings of Izzat *et al.* (2011) found that mean fasting and random blood sugar and HbA1c were higher in patients than control group. Also in agreement with Lundgren *et al.*

(2012). The results reveal a significant difference between the serum glucose in T1DM patients and non-diabetes individuals, and agreed with Berwary *et al.* (2013) who showed that the result of statistical analysis shows there is a significant difference in the mean value of serum glucose between T1DM patients and control group. This fact may be explained by the decreased production of insulin by pancreatic beta cells in T1DM patients that leads to accumulation of glucose in the blood. When blood glucose enters the erythrocytes, it glycates the amino terminals of hemoglobin. The fraction of hemoglobin glycated, normally about 5%, is proportionate to blood glucose concentration. Since the half- life of an erythrocyte is typically 60 days, the level of glycated hemoglobin reflects the mean blood glucose concentration over the preceding 6-8 weeks. Measurement of HbA1c therefore provides valuable information for management of diabetes mellitus. (Murray *et al.*, 2012).

The results also demonstrated that mean anti-insulin antibodies were significantly ($P < 0.05$) higher in diabetic patients than in the controls. This result is in agreement with Murray *et al.* (2012) who found that mean $\pm$ SE anti-insulin of T1DM patients was (0.033 ± 0.023), while mean $\pm$ SE anti- insulin of Healthy controls was (0.0037 ± 0.0019). It was also found in the present study that mean anti- GAD antibodies were higher in chronic diabetic patients than in the early diabetic patients and control group. This result also in agreement with the result of Farhan *et al.* (2013) in which mean $\pm$ SE anti- GAD in T1DM patients was (0.024 ± 0.003) while mean $\pm$ SE anti- GAD in healthy controls was (0.004 ± 0.0020). Our results demonstrated that 9 persons of chronic diabetic group were positive for anti- insulin antibody, 11 person of early diabetic group were positive for anti- insulin antibody. While the total number of T1DM patients who are positive for anti- insulin antibody was 20. Among the control group there is no one of them that was positive

for anti-insulin antibody. These results similar to the results of Bilbao *et al.* (2000) who found that insulin antibody found in 70% of diagnosed T1DM patients. Our result is in agreement with Valentina *et al.* (2010) that insulin antibodies were found in 3 of 686 (0.43%) children. In the current study four persons of chronic diabetic group were positive for anti-GAD antibody, two persons of early diabetic group were positive for anti- GAD. The total number of T1DM patients who are positive for anti- GAD antibody was six (10%). In the control group, one person of them (5%) was positive for anti-GAD. This disagreed with the result of Pardini *et al.* (1999), found that the frequency of positive results in recent-onset DM type1 patients was 80.0% for GAD Ab. The control group showed no positive cases. Anti- GAD assays showed a high frequency of positivity in these Brazilian type 1 diabetes patients, who presented the same prevalence as a Caucasian population. Our results also disagreed with Pardini *et al.* (1999) who found that the control group showed no positive cases for anti- GAD. The differences between our results and the results of these research may be due to the differences in the environmental factors, genetic factors and ethnic factors. Our results are in agreement with Borg *et al.* (1997) who showed that in the 100 control children GADA were found in 3 persons. The research of Mezher *et al.* (2011) about the prevalence of anti Glutamic Acid Decarboxylase antibody in Iraqi children and adolescent with type 1 Diabetes mellitus showed that 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 ($p=0.001$). The difference between this result and our result may be related to genetic factor or due to age difference.

CONCLUSIONS

Anti- AD antibodies were found in diabetic patients and in one case of control group, which indicate the importance of anti-GAD assay in normal population. The level of anti- GAD antibodies in control group was higher than that of early diabetic group, this means the possibility of this parameter could be a hidden factor for future diabetes for our control. We recommended to study the effect of other antibodies on T1DM such as anti tyrosine phosphatase antibody and study the relationship between T1DM and other autoimmune diseases as autoimmune thyroiditis.

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