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Review

Study of the difficult glycemic control in relation to the presence of diabetes-autoantibodies in a sample of Egyptians with type 1 diabetes

Mohammed Saad Hamed [a], *Merhan Samy* [a], *Hanan Mahmoud* [a,*], *Noha Yehia* [b]

[a] *Internal Medicine & Endocrinology Department, Faculty of Medicine, Ain Shams University, Cairo, Egypt*
[b] *Endocrinology Department, Ain Shams University, Cairo, Egypt*

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ABSTRACT

Background: T1DM is divided into 1A (immune-mediated), 1B (virus-triggered, genetic and idiopathic). Presence of auto-antibodies may be correlated to glycemic control.
Aim: Assessment relation between the autoantibodies and the poor glycemic control in T1DM.
Methods: 60 patients T1DM 30 males, 30 females, subjected to full history, clinical, anthropometric assessment and laboratory assessment of fasting C-peptide, FBS, 2 h PP glucose, HbA1c, GADA, ICA and IAA level. Classified into two groups; Group I: negative auto-antibodies, Group II: positive auto-antibodies, Group II was further classified into 3 subgroups, Group II a:1 positive autoantibody, Group II b: 2 positive autoantibodies and Group II c: 3 positive autoantibodies.
Results: HbA1c was significantly higher in group II than group I (11.85 ± 1.61% vs. 8.52 ± 0.41%, p = 0.000). HbA1c was highest in group IIc followed by IIb then IIa (12.25 ± 1.48% vs. 11.57 ± 1.59% vs. 10.78 ± 1.73%, p = 0.038). Total insulin units per day was significantly higher in group II than group I (109.83 ± 7.77 U/day vs. 100.83 ± 1.83 U/day, p = 0.007). Duration of diabetes was significantly higher in group I than group II (10.17 ± 1.94 years vs. 8.11 ± 2.20 years, p = 0.033). HbA1c, total insulin units per day and duration of diabetes were independent predictive factors for presence of autoantibodies (p = 0.007, p = 0.033 and p = 0.043 respectively).
Conclusion: Autoantibodies affect the glycemic control presented by high HbA1c; also it causes increase in total insulin units needed by patients; the more autoantibodies, the higher HbA1c, the more insulin units required to control glycemic state.

Abbreviations: ADA, American Diabetes Association; ANOVA, analysis of variance; BMI, body mass index; DM, diabetes mellitus; ELISA, enzyme linked immuno-sorbent assay; GABA, gamma-amino-butyric acid; GADA, glutamic acid decarboxylase autoantibodies; HbA1c, hemoglobin A1c; IAA, insulin autoantibodies; IA2A, insulinoma antigen 2 antibodies; ICA, islet cell cytoplasmic autoantibodies; LADA, latent autoimmune diabetes of adults; SD, standard deviation; SPSS, statistical package for the social sciences

* Corresponding author.
E-mail address: hanan.mahmoud29@yahoo.com (H. Mahmoud).
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Contents

1. Introduction

Diabetes mellitus is a clinico-laboratory syndrome characterized by chronic hyperglycemia due to insulin deficiency or insulin resistance or both leading to disturbance of metabolism of carbohydrate, protein, fat, water and electrolytes [1]. There are three main types of Diabetes mellitus: type 1 DM, type 2 DM and Gestational diabetes [2]. An expert committee of the American Diabetes Association has recommended dividing type 1 DM into type 1A (immune-mediated [3]) and type 1B (other forms of type 1 DM that include virus-triggered autoimmune response[4] in which the immune system attacks virus-infected cells along with the beta cells in the pancreas (Coxsackie virus family or Rubella), genetic factors [5] and idiopathic [6,7].

Autoantibodies are strongly associated with the development of type 1 diabetes. The appearance of autoantibodies to one or more of the auto-antigens (Glutamic acid decarboxylase 65, Islet cell cytoplasm, or Insulin) signals an autoimmune pathogenesis of β-cell killing [8]. Although their appearance does not follow a distinct pattern, the presence of multiple autoantibodies has the highest positive predictive value for type 1 diabetes mellitus [9]. It is widely recognized that the presence of two or more auto-antibodies has a high sensitivity and specificity for rapid progression to insulin dependency within 5 years and may help clarify the diagnosis in some patients [8], In cases in which no evidence of autoimmunity can be detected, the classification used is idiopathic type 1 DM [6].

The autoantibodies that are associated with type 1 diabetes include (GAD65, ICA (also known as IA2A) and IAA) [10]. GADA65 is the most commonly detected autoantibodies in newly diagnosed type 1 diabetics, it is positive in 75% of patients at time of diagnosis [11].

ICAs were thought to be of IgG class only and were shown to be directed against the cytoplasmic antigen present in cells of the islet of Langerhans. The pathogenic role of ICA is uncertain in view of the distribution of the auto-antigen in the cytoplasm of all four types of endocrine cells within the islets, while the immune damage is focused entirely upon beta cells [12]. Insulin autoantibody IAA is the first marker to appear and the first to disappear in children [13]. These autoantibodies appear prior to insulin treatment and are present in approximately 70% of children and adolescents at the diagnosis of type 1 diabetes [14].

Diabetes autoantibodies don't persist long-life [15]; IAA is the first marker to appear in children, and the first to disappear during early adulthood, followed by ICA, then GADA which is the most important of them because it disappears the last almost around the end of the third decade [16].

2. Aim of work

This study aimed to assess the relation between the presence of diabetes autoantibodies and the poor glycemic control in a sample of Egyptians with type 1 diabetes.

3. Patients and methods

A cross sectional study that included 60 subjects of type 1 diabetes mellitus and taking more than 100 units of insulin per day, 30 males and 30 females enrolled from the diabetes clinic of the medical center of Ain Shams University students' affairs over 2 months duration from December 2016 till January 2017. Before inclusion, an oral consent was obtained from each patient after full explanation of the study protocol. They were classified according to the absence or presence of diabetes autoantibodies into two groups: Group I included 6 patients with negative auto-antibodies and Group II included 54 patients with positive auto-antibodies, Group II was further classified according to the number of positive autoantibodies into 3 sub-groups, Group II a: 9 patients with 1 positive autoantibody, Group II b: 12 patients with 2 positive autoantibodies and Group II c: 33 patients with 3 positive autoantibodies. Exclusion criteria included Obese patients (BMI more than 25), patients who are subjected to any kind of stress, infection, surgery or any other comorbidity as these factors increase HbA1c level and the insulin dose. Full medical history was taken from all subjects, emphasizing on the duration of diabetes mellitus, diabetic complications (regular fundus examination, puffiness of eye lids, tingling, and claudication pain), co-morbid conditions and total insulin dose per day (multiple daily insulin injections) and smoking. Thorough clinical examination was undertaken including vital data. BMI (kg/m^2) and examination of sites of injection.

Table 1 – Descriptive analysis for all studied patients as regarding demographic, anthropometric and clinical data.

		(No = 60)
Age (years)	Mean ± SD	19.15 ± 1.3
	Range	17 – 22
Sex	Female	30 (50%)
	Male	30 (50%)
Height	Mean ± SD	1.73 ± 0.06
	Range	1.6 – 1.86
Weight	Mean ± SD	67.55 ± 6.82
	Range	50 – 80
BMI	Mean ± SD	22.38 ± 1.09
	Range	19.5 – 24.6
SBP (mm Hg)	Mean ± SD	112.50 ± 9.85
	Range	90–130
DBP (mm Hg)	Mean ± SD	71.50 ± 8.20
	Range	60–90

4. Laboratory studies

Laboratory tests included fasting blood glucose, 2 h postprandial blood glucose, HbA1c, fasting C peptide level in blood, anti-glutamic acid decarboxylase antibodies GADA, anti-islet cells antibodies ICA and anti-insulin antibodies IAA. Fasting and 2 h postprandial blood glucose were assayed by hexokinase method using Dimension RXL max provided by Simens. HbA1c was measured by Stanbio Procedure No.0350 "Quantitative colorimetric determination of Glycohemoglobin in blood". Fasting C peptide level was measured by C-Peptide KA1259ELISA Kit provided by Abnova. GADA was measured by the RSR GAD65 autoantibody (GAD Ab) ELISA Kit. ICA was measured by ELISA Kit provided by ALPCO diagnostics. IAA was measured by ELISA KIT Cat. # 3600-HIG provided by Alpha Diagnostic International.

5. Results

This study was conducted on 60 patients 30 males (50%) and 30 females (50%), with a mean age of (19.15 ± 1.31) years. They were age and sex matched. There was no statistical significant difference among all studied groups as regarding age, weight, height, body mass index and systolic and diastolic blood pressure (p = 0.389, P = 0.694, P = 0.446, P = 0.872, P = 0.829, P = 0.568) respectively. However, HbA1c level, total insulin units per day and the duration of diabetes mellitus showed significant difference (P < 0.000, P < 0.007, P < 0.033) respectively. (Table 1).

HbA1c level was significantly higher in Group II than in Group I (P = 0.000) (Table 2). HgbA1c showed a non- significant difference among group II subgroups being highest in Group IIc followed by group IIb then group IIa. (P = 0.038) (Table 3). On studying the relation between HbA1c level and each autoantibody individually we found that it was significantly higher in patients with positive GADA than those with negative GADA (P = 0.000), higher in patients with positive ICA than those with negative ICA (P = 0.000) and higher in patients with positive IAA than those with negative IAA (P = 0.012).

As regarding the number of insulin units used, it was significantly higher in Group II than in Group I (P = 0.007) (Table 2) but it showed no statistical difference among Group II subgroups.

The current study showed that the duration of developing diabetes mellitus was significantly higher in Group I than in Group II (P = 0.033) (Table 3), The duration of diabetes was significantly higher in patients with negative GADA and ICA than in patients with positive GADA and ICA, (P = 0.001, p = 0.019 respectively). The current study also revealed that the most common auto-antibody found in those patients was the GADA, it was found in 94.4% of patients, while ICA and IAA were found in 83.3% and 66.7% respectively. By doing the multivariate regression analysis we found that HbA1c level, total number of insulin units per day and the duration of developing diabetes mellitus were independent predictive factors for the presence of diabetes autoantibodies; (P = 0.007), (P = 0.033) and (P = 0.043) respectively.

6. Discussion

The current study showed that Group II (patients with positive auto-antibodies) had a significantly higher HbA1c level than Group I (patients with negative antibodies) this was in agreement with *Zaharieva et al. 2017* who studied 180 patients, 14 of which were LADA patients, 128 were type 2 diabetic patients and 38 were control subjects. All patients and control subjects were assayed for GADA, ICA and HbA1c level. By comparing LADA positive autoantibodies patients and

Table 2 – Comparison between Group I and Group II as regarding the number of insulin units per day, HbA1c level and C.PEP.

		Group I Negative Abs	Group II Positive Abs	Test value•	P-value	Sig.
		No. = 6	No. = 54			
Insulin units (U/day)	Mean ± SD	100.83 ± 1.83	109.83 ± 7.77	−2.808	0.007	HS
	Range	99 – 104	100 – 130			
FBS (mg/dl)	Mean ± SD	100.17 ± 11.36	112.56 ± 26.91	−1.110	0.272	NS
	Range	84 – 116	69 – 185			
2HRPP (mg/dl)	Mean ± SD	165.50 ± 27.79	195.41 ± 48.51	−1.476	0.145	NS
	Range	117 – 192	120 – 350			
HBA1C (%)	Mean ± SD	8.52 ± 0.41	11.85 ± 1.61	5.011	0.000	HS
	Range	8 – 9	8.9 – 14.2			
C.PEP (ng/ml)	Mean ± SD	0.14 ± 0.05	0.18 ± 0.07	−1.303	0.198	NS
	Range	0.1 – 0.24	0.09 – 0.36			

Table 3 – Comparison between Group II a, II b and II c as regarding the number of insulin units per day, HbA1c level and duration of diabetes mellitus.

		No. of +ve Abs			Test value•	P-value	Sig.
		Group II a I +ve antibody	Group II b 2 +ve antibodies	Group IIc 3 +ve antibodies			
		No. = 9	No. = 12	No. = 33			
Insulin units (U/day)	Mean ± SD Range	110.67 ± 6.38 100 – 120	110.00 ± 10.40 100 – 130	109.55 ± 7.24 100 – 125	0.074	0.928	NS
HBA1C (%)	Mean ± SD Range	10.78 ± 1.73 8.9 – 13.4	11.57 ± 1.59 9.4 – 13.9	12.25 ± 1.48 8.9 – 14.2	3.492	0.038	S
Duration of DM (years)	Mean ± SD Range	9.67 ± 2.00 7 – 13	7.33 ± 1.61 5– 10	7.97 ± 2.28 3– 13	3.327	0.044	S

autoantibodies negative control subjects, HbA1c level was significantly higher in autoantibodies positive patients (P = 0.001) [17]. Also this came in agreement with *Lohmann et al., 2001* who studied 51 antibodies positive LADA patients, and 51 autoantibodies negative diabetic patients who were well matched for age, race, and the degree of hyperglycemia. They found that HbA1c level was significantly higher in the autoantibodies positive patients than in autoantibodies negative patients and that was statistically significant (P = 0.025) [18]. On the other hand this was not in agreement with *Verkauskiene et al., 2016* who studied 1166 known type 1 diabetic patients and 164 newly diagnosed type 1 diabetic patients, they found that among the 1166 known type 1 diabetic patients 87 were antibodies negative and 1079 patients were at least 1 antibody positive and among the 164 newly diagnosed patients 20 were antibodies negative and 144 patients were at least 1 antibody positive. They concluded that HbA1c level didn't show any statistical significant difference between antibody negative and positive patients (P > 0.05) [19]. Also according to *Arslan et al. 2014* who studied 52 type 1 diabetic patient who had been tested for GADA, ICA and HbA1c. they found that in auto-antibody positive patients, the mean HbA1C level was slightly higher than that in autoantibody negative patients, this difference was not considered statistically of significant value (P >0.05) [20].

Our results also revealed that by dividing group II study population into 3 sub-groups; Group IIa (patients with 1positive antibody), IIb (patients with 2 positive antibodies) and IIc (patients with 3positive antibodies) HbA1c was significantly higher in Group IIc followed by group IIb then group IIa which means that as the number of autoantibodies increase, HbA1c level increases, this was in agreement with *Lohmann et al., 2001* who studied 51 antibodies positive LADA patients, of those 51 patients 16 were ICA positive, 12 were GADA positive and 23 were positive for both ICA and GADA. They found that mean HbA1c level was significantly higher in patients with two positive antibodies than in patients with single positive autoantibody (p = 0.05) [18].

The results also showed that Group II receive higher number of total insulin units per day than Group I with high statistical significance (P = 0.007), this was in agreement with *Verkauskiene et al., 2016* who studied 1166 known type 1 diabetic patients they found that the mean insulin dose among the autoantibody-positive patients was significantly higher than in those with antibody-negative patients (P = 0.001) [19].

But after dividing group II study population into three subgroups Group IIa, IIb and IIc the relation between them as regarding the number of received insulin units per day was statistically non-significant (P = 0.928), this means that the total number of insulin units used by patients per day is independent to the number of diabetes autoantibodies present but dependent to the presence of autoantibodies even one of them. This finding is also consistent with *Verkauskiene et al., 2016* who didn't find any statistical significant difference as regarding total insulin dose per day between patients with 1 positive autoantibody, those with 2 positive autoantibodies and those with 3 positive autoantibodies (P = 0.39) [19].

The present study showed a statistically significant inverse relation between the duration of diabetes mellitus and the presence of diabetes autoantibodies (P = 0.033) specially with Glutamic acid decarboxylase autoantibodies GADA (P = 0.001) and islet cell cytoplasmic autoantibody ICA (P = 0.019); this was In agreement with *Kong et al. 2013* who investigated the prevalence of diabetes autoantibodies in children and adults with T1DM according to their age and the duration of disease, they examined 137 patients and they found that 66.3% of patients showed positive autoantibodies at the time of diagnosis falling to 39.2% 1 year later. Thus they reported that the titer of GADA as well as ICA decreases as type 1 DM progresses [21].

Finally from the results of the current study we found that GADA was the most common autoantibody found in type 1diabetic patients (94.4% of the study population) this was in compliance with *Anna and Waytt 2017* who reported that GADA is the most persistent autoantibody found in type 1diabetic patients [11].

Among Eastern Mediterranean and Middle Eastern countries, the largest contribution to the total number of estimated T1DM cases comes from Egypt which accounts for about a quarter of the region's total. The incidence varies between 1/100,000 per year (Pakistan) and 8/100,000 per year (Egypt) in children under the age of 15 years. The prevalence of diabetes in Egypt is high, and the gradient in risk factors and disease from rural to urban areas and in urban areas from lower to higher socioeconomic status (SES) suggest that diabetes is a major, emerging clinical and public health problem in Egypt [22].

The high socio-economic level is associated with the improvement of level of HbA1c but the low socio-economic level interferes with the patient's ability to follow the rules associated with the diabetes regimen such as administering insulin at the right time and following the diet which in turn can lead to poor metabolic and glycemic control Obstacles for good glycemic control in Egypt are lack of self- monitoring of blood glucose (SMBG), also non adherence to the diet modification, lack of exercises, low socioeconomic status and low level of education [23].

7. Conclusion

In summary, we found that the presence of diabetes autoantibodies affect the glycemic control represented by HbA1c level, also it affects the total number of insulin units per day used by the patients; the more the presence of diabetes autoantibodies, the higher the HbA1c level, the more insulin units required by patients to control their glycemic state.

Conflict of interest

The authors declare no conflict of interests.

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