Evaluation of signs of immune or metabolic disturbances among diabetic patients with positive islet cell antibodies

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Summary:

Background: Autoantibodies to islet cell antigens are known predictors of type 1 diabetes and detected in latent autoimmune diabetes in adults.
Objectives: This study aimed to identify the metabolic and immunological disturbances in diabetic patients with positive and negative islet cell antibodies (ICAs)
Materials and methods: A total number of 235 known cases of diabetes mellitus type 1 (160) and type 2 diabetes (75) were admitted in the study. Serum ICA and immunoglobulins (IgA, IgM, IgG) as well lipid profile were measured.
Results: Positive ICAs was found in 40 out of 120 T1D (33.3%) and 28 out of 75 T2D (37.3%). All the patients were poorly controlled diabetes with the evidence of significant high HbA1c%. There were no significant differences in the lipid profile or immunoglobulin levels between positive and negative ICAs in T1D and T2D.
Conclusions: Autoimmunity in term of positive ICA does not play a role in metabolic or immunologic disturbances that associated with T1D and T2D.
Key words: Islet cell antibodies, lipid profile, immunoglobulins

Introduction:

Type 1 diabetes (T1D) is a chronic autoimmune disease with a long prodrome, which is characterized by dysfunction and ultimately destruction of pancreatic beta-cells. Autoantibodies to islet cell antigens are known predictors of type 1 diabetes and are commonly present at its diagnosis [1]. Autoantibodies specific to single tissue antigen like GAD 65, insulinoma-associated antigen-2, islet cell, and insulin have been identified in T1DM. [2-5]. Moreover, Islet reactive T cells and autoantibodies have been demonstrated in type 1 diabetes. Brooks-Worrell et al identified a group of adult autoimmune phenotypic type 2 diabetes patients who are 5 islet autoantibodies (ICA, GADA, IAA, IA-2A, ZNT8A) negative and positive islet reactive T cells [6]. Determination of these autoantibodies is usually used to screen the individuals at increased risk of developing diabetes [7]. The frequency of macrovascular complications was lower in islet cell antibody (ICA) positive diabetics than those with ICA negative [8]. In insulin-treated diabetics, the prevalence of humoral ICA was strongly dependent of the duration of the diabetes, being 60 per cent during the first year from diagnosis and falling to 20 per cent at two to five years and to 5 per cent at 10-20 years [9]. During prospective follow-up study of children with islet autoimmunity, Stene et al found that the increase in HbA1c predicted the increased risk of progression to T1D [10]. The metabolic profile was partially normalized after the seroconversion of autoantibodies. Autoimmunity may thus be a relatively late response to the early lipid profile disturbances. Recognition of these pre-autoimmune alterations may aid in studies of disease pathogenesis and may open a time window for novel type 1 diabetes prevention strategies [11]. Type 2 diabetes (T2D) characterized by insulin resistance coupled with a non immune-mediated β-cell failure and relative insulin deficiency [12]. Latent autoimmune diabetes in adults (LADA) shared biochemical markers of β-cell–directed autoimmunity with "classic" type 1diabetes i.e. documented by antibodies against GAD and treated with insulin earlier than in those with non-autoimmune diabetes mellitus [13]. Insulin sensitivity was severely impaired in autoantibodies negative (Ab^-) but not autoantibodies positive (Ab^+) patients and β-cell function was almost completely abolished in Ab^+ and not Ab^- type 2 diabetes [14]. Moreover, the Ab^- clinical diagnosis -type 2 diabetic patients had features consistent with the metabolic syndrome. Islet-cell Ab^- clinically diagnosed type 2 diabetic youth are characterized by severe insulin resistance and relative insulin deficiency, whereas Ab^+ youth have severe insulin deficiency and β-cell failure [14]. This comparative study is aimed to explore the differences in the biochemical and immunological indexes in poorly controlled diabetics with (Ab^-) and (Ab^+) type 1 and clinically diagnosed type 2 diabetes.

Materials and methods:

This study is conducted in Martyr Layla Qasm center for diabetes mellitus in Erbil, Iraq. Known cases of insulin dependent diabetes (T1D) and

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clinically diagnosed type2 diabetes (T2D) of both gender attended the diabetic center for clinical follow-up, were enrolled in the study. The criteria of exclusion include history of familial hyperlipidemia, ischemic heart disease, chronic renal failure and patients on the lipid lowering agents.

The study is approved by the local scientific committee of college of Pharmacy, Hawler Medical University. A consent form was obtained from each participant prior to the study. A total number of 160 T1D and 75 T2D patients as well as 60 healthy subjects served as control were enrolled in the study. The body mass index (BMI) was (kg/m^2) calculated according to the Quettlet's equation: BMI = weight (kg)/ $height^2$ (m). A fasting venous blood samples were obtained from participants and the sera were separated for determination of glucose, glycosylated hemoglobin (HbA_{1c} %) and lipid profile including serum total cholesterol (TC), triglycerides (TG) and high density lipoprotein-cholesterol (HDL-c). Very low density lipoprotein is equal to 1/5 TG level and the LDL-c is calculated according to the Friedewald equation {LDL=TC-(HDL+VLDL)}. Immunoglobulins IgG, IgM and IgA and islet cell antibodies (cut off absorbance point was 0.05 at wavelength 405 nm) were determined in serum using single radial immune-diffusion (SRID) and indirect enzyme linked immunosorbent assay (ELISA) technique.

Statistical analysis: The results are expressed as number, percent and mean ± SD. The data were analyzed using two tailed unpaired student's "t" test, difference between percentage test and simple correlation test taking $p \leq 0.05$ as the lowest limit of significance

Results:

Table 1 shows that there is non significant difference between diabetic patients with Ab^+ and Ab^- regarding the age and the number of female cases is higher than corresponding males cases in T1D and T2D. The body mass index of T2D is significantly higher than T1D and there is no significant difference between Ab^+ and Ab^- in each type of diabetes. The duration of diabetes in Ab-T2D is significantly longer than corresponding Ab^+ while in T1D the duration of illness is significantly longer in Ab^+ compared to Ab^- patients [Table 1]. Family history of diabetes is reported in higher number in T2D than T1D. The results of biochemical testing revealed non significant differences between Ab^+ and Ab^- in serum glucose, glycated hemoglobin and lipid profile [Table 2]. Significant high serum glucose and glycacated hemoglobin and low levels of lipid profile are observed in Ab^+ T1D compared with Ab^+ T2D [Table 2]. The same findings are observed with Ab^- T1D compared with Ab^- T2D. Table 3 revealed non significant difference immunoglobulin levels between Ab^+ and Ab^- in T1D and T2D. Ab^+. The poor glycemic control represented by glycated hemoglobin is significantly correlated with body mass index and triglycerides level in T2D whether Ab^+or Ab^- [Table 4]. Moreover, glycated hemoglobin in Ab^- T2D is significantly correlated with duration of illness. Significant inverse correlation between glycated hemoglobin and high density lipoprotein and positive correlation with triglycerides in Ab^- are found in T1D.

Table 1 Characteristics of the study

	Control (N=20)	T2D Positive ICA (N=28)	T2D Negative ICA (N=47)	Control (N=40)	T1D Positive ICA (N=40)	T1D Negative ICA (N=120)
Age	49.6 ± 5.6	40.7±4.04	41.1±4.37	27.1±7.8	21±9.4	22.5±9.3
Sex (M/F)	9/11	6/22	19/28	20/20	12/28	58/62
Residency	8/12	14/14	20/27		34/6	76/44
(urban/rural)	7/13	14/14	21/26		11/29	20/100
Socio-economical	29.47 ±	27.73±2.04	27.81±1.32		23.71±4.84	23.71±4.4
(2/3)	3.789	7.75±1.11	8.489±1.52*		9.722	6.211±2.96**
Body mass index					±1.042	
Duration of disease		9	22	-		30
Family history	-	11	18	-	18	20
First	-				6	
Second						

* $p < 0.05$, * $p < 0.001$ compare with corresponding positive ICA antibodies.

Table 2 Biochemical indexes

	Control (N=20)	T2D Positive ICA (N=28)	T2D Negative ICA (N=47)	Control (N=40)	T1D Positive ICA (N=40)	T1D Negative ICA (N=120)
Fasting serum glucose	90.2±6.3	202.5 ±17.7	216.4±28.9	91.4±5.9	394.2±61.1†	317.1±73.2§
HbA1c%	5.421±0.54	8.460±0.695*	8.757±0.727*	5.282±0.484	9.722±1.042*†	9.406±1.234*§
Total cholesterol	208.8 ± 36.9	187.3±12.5***	191.7±15.4***	136.3±23.2	177±15.5*††	174.1±15.7*§
Total triglycerides	161.2 ± 47.8	174±16.8	180.2±19.5	133.6±23.2	148.4±20.3**†	152.9±16.9*§
HDL	45.4 ± 8.9	43±3.2	41.2±2.4	56.6±7.5	41.8±5.2*	41.7±5.6*
LDL	128.9 ± 33.7	143.9±10	147.4±12.8	98.2±8.5	138.9±13.4*	135.2±14.2*§
VLDL						
Atherogenic index (TG/HDL)						

* $p < 0.001$, ** $p < 0.01$,***$p < 0.02$ compared with corresponding control
† $p < 0.001$, †† $p < 0.01$ compared with corresponding T2D positive ICA
§ $p < 0.001$ compared with corresponding T2D negative ICA

Table 3 Immunological indexes

	Control (N=20)	T2D Positive ICA (N=28)	T2D Negative ICA (N=47)	Control (N=40)	T1D Positive ICA (N=40)	T1D Negative ICA (N=120)
ICA	0.0±0.0	0.1418±0.1260*	0.0±0.00	0.02±0.013	0.096±0.035*†	0.0±0.0*
IgA	374±99.3	364.5±104.9	362±150.6	370.6±114.4	354.8±96.7	372.2±148.5
IgM	224.4±45.2	190.7±51.7***	179.6±55.7	219±44	192±49.7**	177.5±61.3*
IgG	1380±319.2	1097±326.8*	1097±394*	1364.5±285.3	1109.1±326.8*	1075.5±355.7*

* $p < 0.001$, ** $p < 0.02$,***$p < 0.05$ compared with corresponding control
† $p < 0.001$ compared with corresponding T2D positive ICA

Table 4 The correlation between glycemic control expressed as HbA 1c (%) and other indexes.

HbA_{1c}(%)	T2Dpositive N=28	T2D negative N=47	T1Dpositive N= 40	T1D negative N=120
Body mass index	0.387*	0.390*	-0.064	-0.079
High density lipoprotein	-0.242	-0.267	-0.144	-0.437***
triglycerides	0.497**	0.791***	-0.110	0.213*
duration of diabetes	0.126	0.446**	-0.092	-0.153
IgG	-0.047	-0.040	-0.062	0.056
IgM	0.040	-0.020	-0.051	0.031
IgA	0.021	-0.230	0.117	-0.033

The results presented as correlation coefficient (r).
* $p < 0.05$, ** $p < 0.01$,***$p < 0.001$

Discussion:

The results of this study pointed out that the significant differences of biochemical and immunological tests between T1D and T2D are not related to the autoimmunity represented by positive islet cell antibodies. Regarding the age and type of diabetes, this study explores higher percent of Ab^+ patients in T2D and older patients. This finding does not agree the others. Several reports showed that higher percent of positive islet cell antibobodies were found among young T2D [15-17]. The presence of positive islet cell antibodies among T2D indicated that those patients are belonged to the latent autoimmine diabetes in adults [LADA]. There is evidence that the glycated hemoglobin in T2D Ab^+ patients is significantly higher than those with T2D Ab^- patients [18]. In this study, T1D Ab^+ patients show significant difference in high glycated hemoglobin compared with T1D Ab^-. The lipid profile that pointed the presence of risk of cardiovascular events showed that both types of diabetes whether they have positive or negative ICA are similar. Recent study shows that high serum triglycerides and low HDL level are observed in

T1D pregnant women with pregnancy compared with T2D [19] and the macrovascular complications of diabetes are lower in T2D Ab^+ compared with Ab^- [20].

Glycated hemoglobin percent does not significantly differ between Ab^+ and Ab^- in T1D or T2D which in agreement with Ekholm et al study who found that HbA1c did not differ between the Ab^+ and Ab^- groups after 20 years follow-up [21]. Moreover, the present study adds another finding that HbA1c in T1D Ab^+ is significantly higher than Ab^- while in T2D is significantly lower among Ab^+. Oresic et al demonstrated that the autoimmunity is relatively late response to the early metabolic disturbances in T1D [22]. In the present study, metabolic disturbances in term of lipid profile do not show significant differences between Ab^+ and Ab^- in T1D or T2D. T-cell autoimmunity can be detected in latent autoimmune diabetes in adult patients and the inverse correlation between humoral and cellular beta-cell autoimmunities was reported [23]. In this study, the immunoglobulins levels in Ab^+ T1D or T2D do not differ from those with Ab^- . It concludes that the autoimmunity in term of positive ICA not does not play a role in metabolic and immunological disturbances that associated with T1D and T2D.

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