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Evaluation the Role of Certain Pancreatic Beta Cells Stress and/or Death Biomarkers in Patients with Diabetes Mellitus Type 1

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Abstract

Type 1diabetes (T1D) occurs due to immune system dysregulation and invading pancreatic beta (β)-cells by auto-reactive immune T cells, which leads to decreases of β-cells activity and viability in addition to prolonged therapy with exogenous insulin. The primary aim of this study was to delve into and explore the roles of adiponectin (ADP) and islet antigen-2 autoantibody (IA-2A) as potential predictors and/or diagnostic biomarkers for T1D and to determine the effects of them on pancreatic β-cells dysfunction. A case-control investigation was conducted between the months of August and December 2022, which included three study groups; the T1D group which included 35 newly diagnosed T1D patients, first degree relatives (FDRs) group which included a total of 35 FDRs of T1D patients and healthy control (HC) group with a total of 20 healthy individuals. Serum levels of connecting (C) peptide, ADP, and IA-2A were measured

for all study participants using an enzyme-linked immune sorbent assay (ELISA). The results showed significantly lower levels of C. peptide and ADP among T1D and FDRs subjects in comparison to HC group, whereas the IA-2A level was significantly higher in T1D and FDRs subjects than in HC subjects. The level IA-2A revealed a significant positive association with the levels of C. peptide and ADP within T1D and FDRs groups, whereas there exists a positive correlation between ADP levels and C. peptide levels across all research groups. The combination between lower C. peptide and ADP levels and higher IA-2A levels is the most robust and cost-effective predictive and diagnostic biomarker for T1D. The positive relationship between ADP and C. peptide indicated a possible utility of higher ADP levels as a marker of low β-cells stress and/or death, whereas the positive relationship between ADP and IA-2A indicated the vital anti-inflammatory role of ADP. High IA-2A level at the time of T1D diagnosis does not predict the degree of pancreatic β-cells stress and/or death.

Key Words: T1D, C. peptide, Adiponectin, IA-2A, β-cells, Stress.

Abbreviations

Abs, antibodies; **ADP,** adiponectin; **C,** connecting; **DM,** diabetes mellitus; **ELISA,** enzyme linked immune-sorbent assay; **HC,** healthy control, **FDRs,** first-degree relatives; **IA-2A,** islet antigen-2 autoantibody; **IR,** insulin resistance; **min,** minute; **ml,** milliliter; **ng,** nanogram; **pg,** picograms; **T1D**, type 1 diabetes; **β,** beta.

Introduction

Diabetes type 1 (T1D) is an autoimmune disease that occurs because of insulin-producing pancreatic beta (β) cells damaged by autoimmune reaction processes. It is characterized by a break in immune tolerance and the invasion of auto-reactive T cells that target the β-cells, resulting in the loss of its function and mass and permanent dependence on exogenous insulin (Lehuen *et al*., 2010). Many immunomodulatory drugs have been tested around the time of T1D clinical diagnosis to preserve β-cells function. The breakdown of β-cells plasma membranes during cell necrosis leads to the release of proteins and auto-antibodies (Abs) into the bloodstream, and measuring this mechanism could be used as a biomarker of β-cells stress and/or death (Costa *et al*., 2015). Prodromal and early clinical stages of T1D are manifested by the death and/or stress of the developmental and low generative pancreatic cells which are evaluated using many candidates intrinsic pathways. Of these are; the connecting (C) peptide, the auto-Abs, and adiponectin (ADP) (Sheet & Khudhair, 2019) .

The C-peptide plays an important part in the evaluation of insulin synthesis by pancreatic β-cells, which is used as an indicator of β-cells function, an alternative biomarker to monitor the progression of TID and T2D , and for demonstrating the impacts of therapeutic intervention to

improve and preserve the function of remaining β-cells (Wahren *et al.*, 2012). The examination of C. peptides is considered a method that directly evaluates attack of the immune system on pancreatic beta-cells (KHUDHAIR, 2019). Islet antigen-2 auto-Abs (IA-2A) is used as more specific cytoplasmic auto-Ab to diagnose autoimmune type IA pancreatic β-cells destruction (Lebastchi and Herold, 2012). Monitoring these systemic autoantibodies stands as the most dependable biomarker in the prodromal phase of Type 1 Diabetes (T1D), given that their emergence commonly predates the actual onset of overt T1D by several years or even decades (Brorsson *et al*., 2015). However, the predictive value of these biomarkers on the time occurrence of T1D and the degree of death and/or stress of pancreatic β-cells may go at the expense of diagnostic sensitivity as they detect only about 30% of pre-diabetic relatives (Shackelton *et al*., 2005). Adiponectin is a protein that is solely released by adipose tissue. Like other adipocytokines, it is responsible for the communication that occurs between the various organs in the body (Semple *et al*., 2007). Adiponectin is critical in glucose and fat metabolism, where it regulates glucose uptake, gluconeogenesis, and fatty acid oxidation in hepatocytes. This protein is important in the pathogenesis of insulin resistance and T2D which play a crucial part in development of pancreatic β-cells dysfunction that is considered as a positive pancreatic β-cell function regulator and may be a putative target for treatment of islet dysfunction (Wang *et al*., 2010). Thereof, the current study was designed to investigate the roles of ADP and IA-2A as potential predictors and/or diagnostic biomarkers for T1D and to find out the effects of them on pancreatic β-cells dysfunction (as represented by C. peptide concentration), it presumably could provide a new strategy for quicker diagnosis or inducing immunomodulatory medication for preservation of the residual β-cells in newly onset T1D and their first-degree relatives (FDRs).

Materials and Method

Subjects and study design : A case-control paper was conducted in the Endocrinology and Diabetes Specialist Center in Thi-Qar Governorate, Iraq, between the time of August and December-2022. This study comprised three research groups: the T1D group which included thirty-five patients recently diagnosed with T1D (15 males and 20 females) ranging in age from (3-18) years, the FDRs group which include thirty-five FDRs of T1D patients (15 men and 20 females) ranging in age from 10 to 40 years, and a healthy control (HC) group that include twenty subjects (12 females and 8 males) with ages ranging from 5 to 20 years.

Exclusion and inclusion criteria:

The patients with the subsequent criteria had been eliminated from the current research; the existence of any chronic or autoimmune diseases, under biological agent therapy, under corticosteroid therapy for at least four weeks, recent surgery (during the last 6 months), the existence of any complications of diabetes mellitus (DM) such as retinopathy, neuropathy, or nephropathy, transfusions of blood coexistence within the previous six months, patients with uncontrolled hyperglycemia, and patients who had been diagnosed with T1D was more than one year, whereas the subjects who met the following criteria were included, recent T1D diagnosis (less than a year), absence of any form of diabetic complications, patients with well-controlled hyperglycemia, and not imposing any of the previously mentioned exclusion criteria.

For the FDRs group, the exclusion criteria were as follows; the existence of any chronic or autoimmune diseases, FDRs who have been on corticosteroid therapy for at least four weeks, FDRs under biological agent, and the presence of current surgery and blood transfusions (within the previous six months).

The HC exclusion criteria remained consistent with those applied to the FDRs group. Additionally, individuals with a family history of DM and with any simple infection were excluded.

Samples collection:

Disposable syringes (Medeco, Belgium) were used for five milliliters (ml) collection of venous blood from each individual enrolled in the current study. In gel vacuum tubes (China), the collected samples were coagulated at room temperature, then the samples were centrifuged for 10 minutes (min) at 3600 rounds/min to separate the serum. Each serum sample was separated into multiple aliquots in Eppendorf tubes and stored at ˉ80 degrees Celsius until the serological tests performance.

Serological assays: Detection and titration of C. peptide, ADP, and IA-2A in serum were performed using enzyme-linked immune sorbent assay (ELISA) kits for human C-peptide, human ADP, and human IA-2A (SunLong Biotech, China), respectively, which based on the sandwich-ELISA technique. The C. peptide and IA-2A were measured in picograms (pg)/ml, whereas ADP was measured in nanograms (ng)/ml. The cut-off values of serological assays were established using serum collected from the HC group described above, with a 95% confidence interval. The assays procedures were performed at Imam Al-Hussein Teaching Hospital, Thi-Qar Health Department according to manual manufacturer instructions.

Statistical analysis: For data presentation and analysis, a statistical package for social sciences (version 22) was used. Descriptive statistics include the use of frequencies, relative frequencies, and means. The degree of association between categorical variables was determined using the chi-square statistical test. When the p-values are less than 0.05, the results are regarded as statistically significant.

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Results

The current study was conducted during August and December-2022 and included three study groups which are; the T1D group included 35 newly onset T1D patients (20 females and 15 males) ranging in age from (3-18) years , the FDRs group that included a total of 35 FDRs of T1D patients (20 females and 15 males) ranging in age from (10-40) years , and HC group that included a total of 20 subjects with the percentage of females and males of (12/8) , and their ages ranged between (5-20) years .

The results in Figure 1-A show that most of the T1D subjects (57%) and FDRs (60%) were with a below-normal level of serum C-peptide with a significant difference ($p<0.05$) in comparison with the HC group (50%). Nevertheless, there were no significant differences ($p>0.05$) were noticed among the T1D group and FDRs group for this parameter. The average serum C-peptide titer displayed a significant difference ($p < 0.05$) when comparing the T1D group (264.3 pg/ml) and FDRs group (238.4 pg/ml) with the HC group (285.9 pg/ml). However, the variation in average titers between the FDRs and T1D groups was not found to be significant ($p > 0.05$), as shown in Figure 1-B.

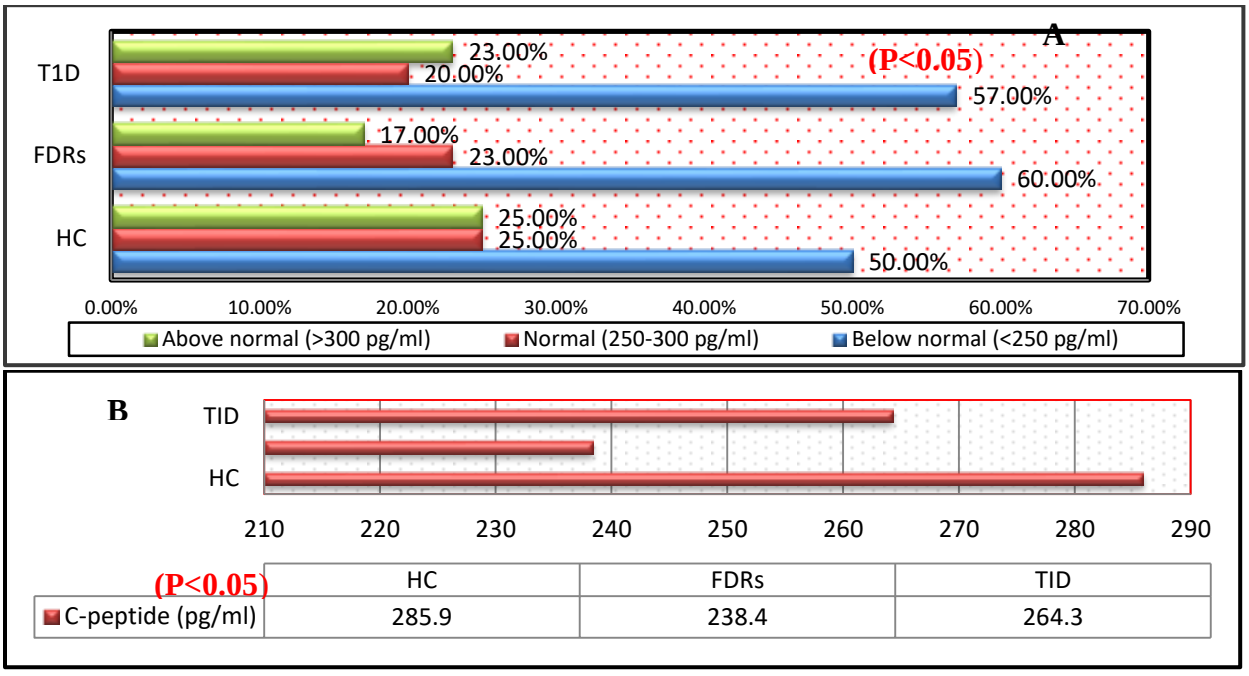

Figure (1): The results of connecting peptide in all study groups, (A): frequency (%), (B): mean titer, (**FDRs,** first degree relatives; **TID,** type 1 diabetes; **HC,** health control; **pg,** picograms; **ml,** milliliter; **C,** connecting).

The results in Figure 2-A show that the frequency percent of high levels of IA-2A was higher among the T1D group (8.58%) and FDRs group (8.57%) compared to the HC group (0%) but without significant differences (p>0.05) as well as there were no significant different (p>0.05) was noticed among T1D group and FDRs group for this parameter. In comparison to the HC group (6.6 pg/ml), the mean titer of serum IA-2A was substantially (p <0.05) higher in the T1D group and the FDRs group (7.1 pg/ml and 7.3 pg/ml, respectively). There was no statistically significant difference (p>0.05) between the T1D and FDRs groups in terms of mean titer (Figure 2-B).

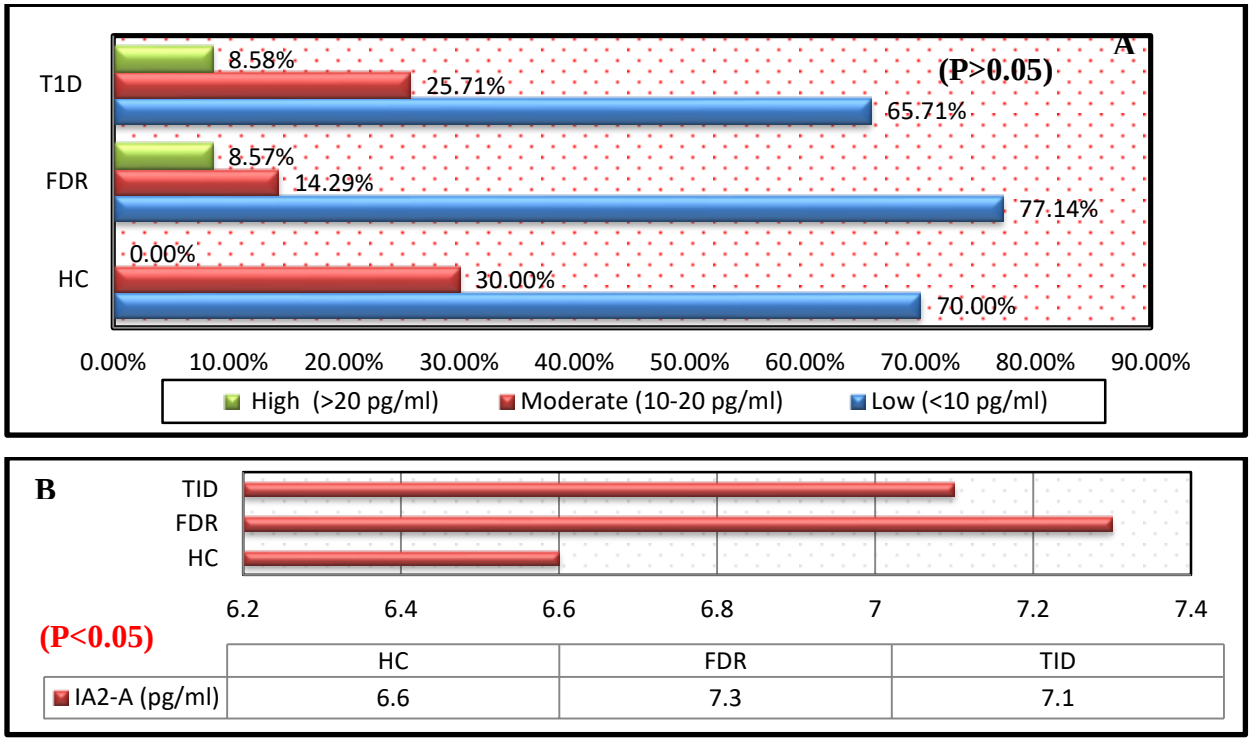

Figure (2) : The results of islet antigen-2 antibody in all study groups, (A): frequency (%), (B): mean titer, (**FDRs,** first degree relatives; **TID,** type 1 diabetes; **HC,** health control; **pg,** picograms; **ml,** milliliter; **IA-2A,** islet antigen-2 antibody).

Figure 3-A shows that the T1D and FDRs groups had a significantly (p<0.05) higher percentage of below-normal ADP levels (88.57% for both) in comparison with the HC group (50%). Nevertheless, there were no significant differences (p<0.05) in the frequency percent of ADP levels among T1D and FDRs groups. Concerning the mean titers of ADP (Figure 3-B), the lowest mean titers were reported in the T1D group (1.1 ng/ml) and FDRs (1.2 ng/ml) when compared to the HC group (2.2 ng/ml) with a significant difference (P<0.05) . The variation in mean titers among T1D and FDRs groups was not significant (p>0.05) .

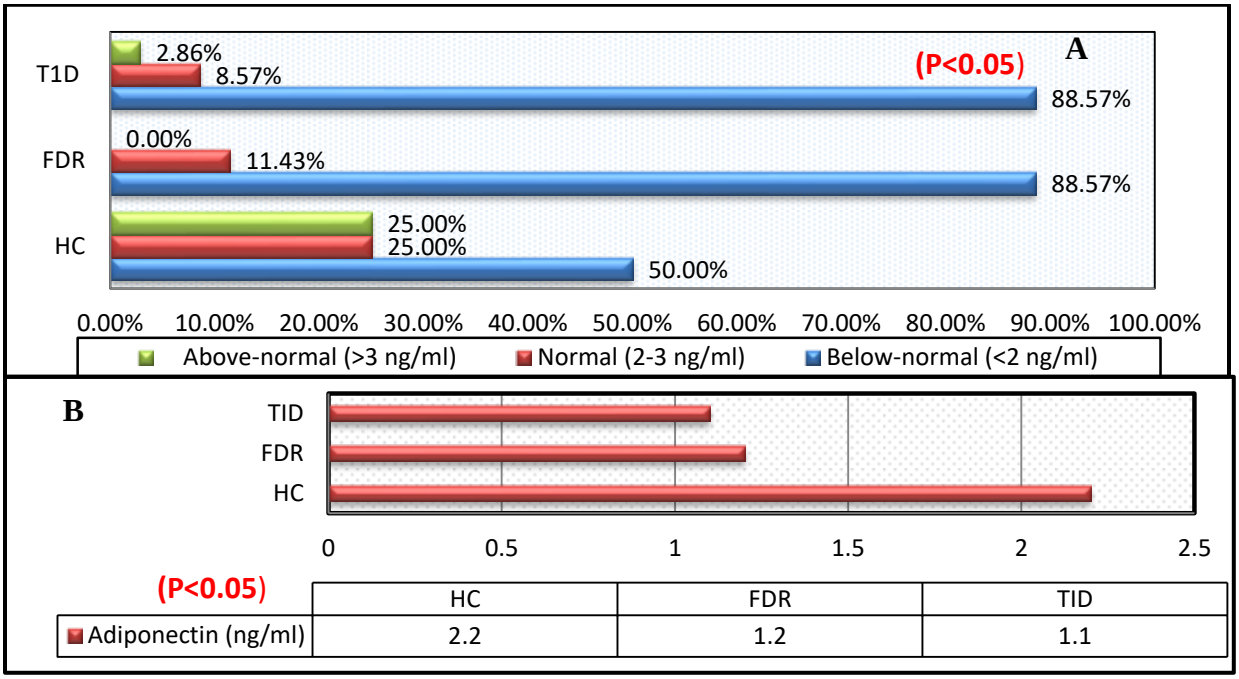

Figure (3): The results of adiponectin in all study groups, (A): frequency (%), (B): mean titer, (**FDRs,** first degree relatives; **TID,** type 1 diabetes; **HC,** health control; **ng,** nanogram; **ml,** milliliter).

Table (1) shows an association between the C-peptide and IA-2A. The frequency % of low IA-2A levels among T1D and FDRs subjects with below-normal serum the C-peptide was high (70% and 90.4%, respectively) with significant differences ($p<0.05$) as compared with T1D and FDRs subjects with above-normal the C-peptide (37.5% and 0%, respectively), whereas the HC groups subjects revealed non-significant different ($p>0.05$) in frequency percent of IA-2A level among groups with below-normal , normal , and above-norma l C-peptide level . The average serum IA-2A titer was notably higher ($p<0.05$) in individuals with type 1 diabetes (T1D) and first-degree relatives (FDRs) who exhibited above-normal C-peptide levels (12.6 pg/ml and 19.7 pg/ml, respectively), in contrast to those with below-normal C-peptide levels (5.5 pg/ml and 5.1 pg/ml, respectively). In the case of the HC group, there were no significant differences ($p<0.05$) observed in the mean serum IA-2A titer among groups with below-normal, normal, and above-normal C-peptide levels.

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Table (1): The correlation between connecting peptide and islet antigen-2 antibody

Biomarkers			Islet antigen-2 antibody (pg/ml)								P. valu
			Low (<10)		Moderate (10-20)		High (>20)		Total		
			FR(%)	Mean	FR (%)	Mean	FR(%)	Mean	FR(%)	Mean	
Connecting peptide (pg/ml)	T1D	**Below N (n=20)**	**14(70)**	1.7	5(25)	12.7	1(5)	22.1	20(100)	**5.5**	**<0.05**
		Normal (n=7)	6(85.7)	4	1(14.3)	13	0(0)	0	7(100)	5.3	
		Above N (n=8)	**3(37.5)**	3.3	3(37.5)	14.6	2(25)	23.7	8(100)	**12.6**	
		Total (n=35)	23(65.7)	2.5	9(25.7)	13.3	3(8.6)	23.2	35(100)	7.1	
	FDRs	**Below N (n=21)**	**19(90.4)**	3.9	1(4.8)	12.1	1(4.8)	20.8	21(100	**5.1**	**<0.05**
		Normal (n=8)	8(100)	3.8	0 (0)	0	0(0)	0	8(100)	3.8	
		Above N(n=6)	**0 (0)**	0	4(66.7)	14.3	2(33.3)	30.4	6(100)	**19.7**	
		Total (n=35)	27(77.1)	3.9	5(14.3)	13.9	3(8.6)	27.2	35(100)	7.3	
	HC	**Below N (n=10)**	6(60)	3	4(40)	11.4	0(0)	0	10(100	6.7	**>0.05**
		Normal (n=5)	5(100)	1.6	0(0)	0	0(0)	0	5(100)	1.6	
		Above N(n=5)	3(60)	6.1	2(40)	16.3	0(0)	0	5(100)	10.2	
		Total (n=20)	14(70)	3.3	6(30)	13.1	0(0)	0	20(100)	6.6	

pg, picogram; **T1D,** type 1 diabetes; **HC**, healthy control; **FDRs,** first degree relatives; **ml,** milliliter; **N,** normal; **n**, number; **FR,** frequency; **%,** percent; **below N,** <250 pg/ml; **normal,** 250-300 pg/ml; **above normal,** >300 pg/ml.

Table (2) shows the correlation between the C. peptide and ADP levels. The percentage of ADP levels that were below normal was significantly higher ($p<0.05$) among T1D, FDRs, and HC subjects with below-normal C. peptide levels (95%, 100%, and 90%, respectively) in comparison with T1D, FDRs, and HC subjects with above-normal C. peptide level (62.5%, 33.3%, and (0%), respectively). For mean titer of ADP level, the findings within the same table illustrated a significantly ($p<0.05$) lower ADP mean value among T1D, FDRs, HC subjects with below-normal C. peptide level (0.9 ng/ml, 0.9 ng/ml, and 1.2 ng/ml, respectively) in comparison with T1D, FDRs, HC subjects with above-normal C. peptide level (1.9 ng/ml, 2.1 ng/ml, and 3.8 ng/ml, respectively).

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Table (2): The correlation between connecting peptide and adiponectin

Biomarkers			Adiponectin (Ng/Ml)								P. Value	
			Below N (<2)		Normal (2-3)		Above N (>3)		Total			
			FR(%)	Mean	FR(%	Mean	FR(%)	Mean	FR(%)	Mean		
Connecting Peptide (Pg/Ml)	T1D	**Below N (N=20)**	**19(95)**		0.9	1(5)	2.1	0(0)	0	20(100)	**0.9**	**<0.05**
		Normal (N=7)	7(100)		0.8	0(0)	0	0(0)	0	7(100)	0.8	
		Above N (N=8)	**5(62.5)**		0.9	2(25)	2.2	1(12.5)	6.4	8(100)	**1.9**	
		Total (N=35)	31(88.6)		0.8	3(8.6)	2.1	1(2.8)	6.4	35(100)	1.1	
	Fdrs	**Below N (N=21)**	**21(100)**		0.9	0 (0)	0	0 (0)	0	21(100)	**0.9**	**<0.05**
		Normal (N=8)	8(100)		1.2	0(0)	0	0(0)	0	8(100)	1.2	
		Above N (N=6)	**2(33.3)**		1.4	4(66.7)	2.4	0(0)	0	6(100)	**2.1**	
		Total (N=35)	31(88.6)		1	4(11.4)	2.4	0(0)	0	35(100)	1.2	
	HC	**Below N (N=10)**	**9(90)**		1.1	1(10)	2.2	0(0)	0	10(100)	**1.2**	**<0.05**
		Normal (N=5)	1(20)		1.4	3(60)	2.4	1(20)	3.5	5(100)	2.4	
		Above N (N=5)	**0(0)**		0	1(20)	2	4(80)	4.2	5(100)	**3.8**	
		Total (N=20)	10(50)		1.2	5(25)	2.3	5(25)	4.1	20(100)	2.2	

Pg, Picogram; **T1D,** Type 1 Diabetes; **HC**, Healthy Control; **Fdrs,** First Degree Relatives; **Ml,** Milliliter; **N,** Normal; **N**, Number; **Ng,** Nanogram; **FR,** Frequency; **%,** Percent; **Below N,** <250 Pg/Ml; **Normal,** 250-300 Pg/Ml; **Above Normal,** >300 Pg/Ml.

Table (3) shows the association between the IA-2A and ADP levels. The frequency % of below-normal ADP levels was significantly higher ($p < 0.05$) among T1D and FDRs subjects with low IA-2A levels (95.7% and 100%, respectively) in comparison with T1D and FDRs subjects with high IA-2A levels (33.3% for both), whereas there were no statistical significant ($p > 0.05$) in ADP serum level among subjects of HC group with low, moderate, or high IA-2A levels. For mean titer of ADP, the findings within the same table illustrated a significantly *(*$p < 0.05$) lower ADP mean titer among T1D and FDRs subjects with low IA-2A level (0.9 ng/ml, and 1 ng/ml, respectively) in comparison with T1D and FDRs subjects with high IA-2A level (1.9 ng/ml and 2.1 ng/ml, respectively), while the HC subjects revealed no-significant differences ($p > 0.05$) in ADP mean value among subject with low, moderate or high IA-2A level.

Table (3): The correlation between islet antigen-2 antibody and adiponectin levels

Biomarkers			Adiponectin (Ng/Ml)								P. Valu
			Below N (<2)		Normal (2-3)		Above N (>3)		Total		
			FR(%)	Mean	FR(%)	Mean	FR(%)	Mean	FR(%)	Mean	
Islet Antigen-2 Antibody (Pg/Ml)	T1D	**Low (N=23)**	**22(95.7)**	0.8	1(4.3)	2.1	0(0)	0	23(100	**0.9**	**<0.05**
		Moderate (N=9)	8(88.9)	0.9	0(0)	0	1(11.1)	6.4	9(100)	1.5	
		High (N=3)	**1(33.3)**	1.2	2(66.7)	2.2	0(0)	0	3(100)	**1.9**	
		Total (N=35)	31(88.6)	0.8	3(8.6)	2.1	1(2.8)	6.4	35(100)	1.1	
	Fdrs	**Low (N=27)**	**27(100)**	1	0(0)	0	0(0)	0	27(100	**1**	**<0.05**
		Moderate (N=5)	3(60)	1.4	2 (40)	2.4	0(0)	0	5(100)	1.8	
		High (N=3)	**1(33.3)**	1.3	2 (66.7)	2.5	0(0)	0	3(100)	**2.1**	
		Total (N=35)	31(88.6)	1	4 (11.4)	2.4	0(0)	0	35(100	1.2	
	HC	**Low (N=14)**	6(42.9)	1.3	5(35.7)	2.3	3(21.4)	3.8	14(100	2.2	**>0.05**
		Moderate (N=6)	4(66.7)	0.9	0(0)	0	2(33.3)	4.5	6(100)	2.1	
		High (N=0)	0(0)	0	0(0)	0	0(0)	0	0(0)	0	
		Total (N=20)	10(50)	1.2	5(25)	2.3	5(25)	4.1	20(100)	2.2	

Pg, Picogram; **T1D,** Type 1 Diabetes; **HC**, Healthy Control; **Fdrs,** First Degree Relatives; **Ml,** Milliliter; **N,** Normal; **N**, Number; **Ng,** Nanogram; **FR,** Frequency; **%,** Percent; **Low,** <10 Pg/Ml; **Moderate,** 10-20 Pg/Ml; **High,** >20 Pg/Ml.

Discussion

Diabetes type 1 is distinguished by the autoimmune damage of pancreatic β -cells by macrophages and T lymphocytes. The disease is usually identified when the invading immune system has destroyed more than 80-90% of the β –cells . Because diabetes type 1 develops slowly, there is a potentially large window of time during which those at risk can be identified and theoretically treated (Assmann et al., 2017). Connecting peptides is well-known for its essential function in insulin production. Connecting peptides is a useful biomarker of the function of pancreatic β-cells, and has been utilized as a surrogate biomarker to monitor the progression of T1D and T2D as well as reveal the effects of therapeutic strategies for preserving and improving the role of residual β-cells (Wahren et al., 2012) . The C-peptide levels are considered an effective marker to both the amount of serum insulin and the role of the pancreatic β-cells (Shetty et al., 2017). Consistent with these findings, the present study reported a significant decrease in the level of C-peptide in the T1D group in comparison to the HC group (**Figure 1**). The current study observations were in agreement with another previous study (Kuhtreiber et al., 2015). Low levels of C-peptide may be a biomarker to determine which individuals are at risk for T1D(Leslie et al., 2021) . In line with the former study, recent study findings revealed a significantly lower C. peptide level in the FDRs group in comparison with the HC group (**Figure 1**). Because C-peptide levels rise with age, this indicates that children may have a greater capacity for interpreting C-peptide level fluctuations, a level decline may be

equivalent to the absence of an increase (Leslie *et al.*, 2021). Type 1 Diabetes is characterized by the presence of specific circulating auto-Abs, including glutamic acid decarboxylase auto-Abs, IA-2A, insulin auto-Abs, zinc transporter 8 auto-Abs. Measuring of such auto-Abs is highly important in the diagnosis of T1D and prediction of T1D in patient's relatives, especially in first-degree family ties (Delic-Sarac et al., 2016). Auto-Abs are widely accepted by the scientific community as a hallmark of T1D (Mathieu et al., 2018) . In line with these findings, the current study reported a higher IA-2A level among T1D and FDRs groups in comparison with the HC group (**Figure 2**). This study's results are analogous to another study (Damanhouri et al., 2005) carried out in Saudi Arabia , which showed that the frequency, of positive cases for IA-2A was 27%. Another study revealed an IA-2A positivity was 23% among T1D patients in Taiwan (Chang et al., 2004). The differences in frequency percentages of auto-Abs in various studies may be related to a combination of genetic and environmental factors, in addition to the test sensitivity and specificity. The existence or absence of auto-Abs in the sera of relatives of T1D patients would suggest an important possibility for the development of T1D in the near or distant future (Knip et al., 2010). Based on the strong correlation between high levels of islets auto-Abs and β-cell death and /or stress through the disease prodromal, stages , this study finding concluded that FDR individuals are more likely to develop T1D in the future. Adipose tissue is a powerful endocrine organ that secretes a variety of adipokines with important roles in the regulation of metabolic homeostasis. This type of hormone plays a critical role in the pathogenesis of resistance to insulin (IR) and T2D (Kettaneh et al., 2006). In marching with these findings, the current investigation showed a lower ADP level among T1D patients and their FDRs compared to the HC group (**Figure 3**). Inhibiting ADP secretion results in the loss of a number of processes that, under typical circumstances of fat cell homeostasis, protect against IR, diabetes, and atherosclerotic . Adiponectin secretion can be inhibited by adipose factors , which are activated as fat cell mass increases, such as cytokines, the adipose renin-angiotensin system, and increased oxidative stress (Stern et al., 2007). Other previous studies reported the same findings of recent research (Zou et al., 2007); (Karamifar et al., 2013).

According to the findings of several studies, the presence of IA-2A, could be associated with an, obvious decrease in residual mass and activity of β-cells (Cai et al., 2011) . On the contrary, a recent study revealed a significant positive association between C. peptide level and IA-2A levels among T1D and FDRs groups , whereas the HC group exhibited a minor non-significant positive association (**Table 1**) . Anti-islet auto-Abs do not directly induce the killing of pancreatic -cells; rather, they develop as a byproduct of -cell death mediated by T-cells . Individuals who test positive for two or more anti-islet auto-Abs in stages 1 and 2 experience the progression of -cell destruction, which results in glucose intolerance or dysglycemia . At this stage, the probability of developing T1D over the next five years is almost 75%, and the risk over the course of a lifetime is over 100% . In stage 3, there are only 20–30% of the usual number of remaining pancreatic –cells . Anti-islet auto-Abs are utilised as a technique for

predicting the start of T1D since they manifest in the peripheral blood throughout stages 1 and 2 (Kawasaki, 2023) . The current study cohort was a newly onset T1D (recently diagnose), and The severity of T1D presentation was not highly correlated with the auto-Abs level at diagnosis, but it may be related to -cell function at follow-up (Grace et al., 2022) . In line with the present study findings, (Grace et al., 2022), did not observe an association of auto-Abs levels with C-peptide at diagnosis. Another previous study showed a fasting plasma C-peptide levels did not decrease after diagnosis in T1D patients with only IA-2A or patients without Abs, indicating that patients with isolated IA-2A positivity at the diagnosis of diabetes was revealed slowly progressive β-cells dysfunction (Borg et al., 2001), whereas (Törn, 2003), reported a positivity only for islets cells auto-Abs indicates a more preserved β-cells function for the first three years compared to positivity for other autoimmune markers. These findings indicated that IA-2A in human serum are important T1D diagnostic markers and prognostic predictor, but its level does not rely on it as a marker for the degree of pancreatic β-cells destruction.

The role of adipokines is known to have a role in insulin sensitivity and/or resistance, with relation to ketosis-prone diabetic mellitus subtypes and T1D. Adiponectin is an anti-inflammatory mediator that also increases insulin sensitivity (Gupta et al., 2015). Several molecules involved in the signaling cascade of C-peptide are also involved in the regulation of different adipocytokines in adipose tissue (Garcia Serrano et al., 2015). Analogous to these observations, this study results reported a significant positive correlation between serum C. peptide and serum ADP levels among all study groups (**Table 2**). Adiponectin levels may physiologically contribute to improvements in sensitivity to insulin and β-cells function, where the ability to discontinue insulin medication was proof of improved β-cells activity (Kim & Hyun, 2020). Human studies have also revealed that levels of ADP are positively associated with insulin sensitivity. On the other hand, ADP may stimulate insulin production by improving the exocytosis of insulin granules and upregulating insulin gene expression, which increases insulin sensitivity (Lee et al., 2011). Earlier studies indicated that individuals exhibiting a higher count of positive auto-Abs had elevated ADP levels, diminished leptin levels, and increased ADP/leptin ratios compared to those with a lower count of positive auto-Abs. Our research aligns with this trend, as we observed elevated ADP levels with rising IA-2A levels among individuals with T1D and their FDRs (**Table 3**). In line with current study findings, (Kaas et al., 2010), It was noted that individuals demonstrating three positive auto-antibodies were predominantly associated with the rapid progression group, which exhibited the highest serum ADP level. Another study (Hecht Baldauff et al., 2016) The data demonstrated a rise in ADP levels proportionate to the increase in positive auto-Abs. These findings indicate a correlation between higher IA-2A levels and elevated ADP levels, reinforcing the idea of ADP's protective (anti-inflammatory) response during the progression of T1D. To gain a deeper understanding of

ADP's role in T1D patients, additional studies focusing on the intricate biological and metabolic functions of ADP are essential for the future.

The current research possesses certain potential constraints. Initially, the unavailability of serum samples for all patient FDRs stemmed from the fact that samples were exclusively collected from FDRs who accompanied their patients to the Endocrinology and Diabetes Specialist Center. Secondly, our evaluations relied on a solitary measurement of total C. peptide and ADP, potentially insufficient to portray their relationship over an extended period. To substantiate our discoveries, a more extensive prospective study will be essential, encompassing an assessment of serum ADP and C. peptide alongside their connection to other islet auto-Abs.

Conclusion

The combination between lower C. peptide and ADP levels and higher IA-2A level is the most robust and cost-effective predictive and diagnostic biomarkers for T1D. There is a positive relationship between ADP and C. peptide in subjects with new-onset T1D and their FDRs which indicated a possible utility of higher ADP levels as a marker of low β-cells stress and/or death, whereas the positive relationship between ADP and IA-2A indicated the vital anti-inflammatory role of ADP which support the concept of the protective response of ADP during T1D progression. High IA-2A level at the diagnosis does not predict the degree of pancreatic β-cells damage, whereas its level was considered a good biomarker for T1D diagnosis and prediction.

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Conflict of interest: The authors declare that there are no conflicts of interest.

Ethics approval: The present research has been approved by the ethical consideration committee, Thi-Qar Health Directorate, Ministry of Health, Iraq, based on the research committee decision number Thi-Qar/2022-182 on 19/7/2022.

Consent to participate: To fulfill international research ethical criteria, each patient (or his guardian) written consent to participate in the study was obtained.

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Tables Legend

- **Table (1):** The correlation between connecting peptide and islet antigen-2 antibody.
- **Table (2):** The correlation between connecting peptide and adiponectin**.**
- **Table (3):** The correlation between islet antigen-2 antibody and adiponectin levels.

Figures Legend

- **Figure (1):** The results of connecting peptide in all study groups, (A): frequency (%), (B): mean titer.
- **Figure (2) :** The findings of the islet antigen-2 antibody in all research groups, (A): frequency (%), (B): mean titer.
- **Figure (3):** The results of adiponectin in all study groups, (A): frequency (%), (B): mean titer.

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