The role of GaD65, ZNTS, IA-2, and IAA as predictive biomarkers for type 1 diabetes mellitus in children

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Background
Type 1 diabetes mellitus (T1DM) is a chronic disorder characterized by immune-mediated harm to the pancreatic β-cells that produce insulin. The four major autoantibodies implicated in the pathophysiology are insulin autoantibodies, glutamic acid decarboxylase antibodies, tyrosine phosphatase antibodies, and zinc transporter 8 antibodies.
Objective
We examined whether children with T1DM have particular antibodies related to T1DM and their association with clinical features.
Materials and methods
The study involved 60 Iraqi children who had been diagnosed with T1DM within the last 3 years, as well as a control group of 60 healthy individuals without diabetes or autoimmune diseases. Blood samples were collected from all participants to analyze the levels of serum autoantibodies, specifically insulin (IAA), glutamic acid decarboxylase (GADA), tyrosine phosphatase (IA-2A), and zinc transporter 8 (ZnT8A), using an enzyme-linked immunosorbent assay (Sandwich-ELISA).
Results and conclusion
The findings revealed that a significant number of patients with diabetes had elevated levels of antibodies against zinc transporter 8 ($P<0.001$), tyrosine phosphatase ($P<0.001$), insulin autoantibodies ($P<0.001$), and glutamic acid decarboxylase ($P<0.001$). Glutamic acid decarboxylase 65 antibodies were found to be the most prevalent. All four biomarkers showed remarkable effectiveness in distinguishing positive and negative cases. Logistic regression analysis revealed that glutamic acid decarboxylase 65 and insulin antibodies were significantly associated with the outcome, while tyrosine phosphatase and zinc transporter 8 did not show such a relationship. These findings indicate that measurements of anti-zinc transporter 8, tyrosine phosphatase, insulin autoantibodies, and glutamic acid decarboxylase could be important diagnostic markers for identifying patients with T1DM, aiding in early detection and understanding the disease process.

Keywords:
anti-Glutamic acid decarboxylase 65 antibody, anti-insulin autoantibodies antibody, anti-tyrosine phosphatase antibody, anti-zinc transporter 8 antibody, type 1 diabetes mellitus

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Introduction

Type 1 diabetes mellitus (T1DM) is a chronic disorder characterized by immune-mediated harm to the pancreatic β-cells that produce insulin. The loss of β-cells causes an insulin shortage, which could be life-threatening [1]. Autoantibodies against islet cell components are reliable indicators of the illness process, but evidence suggests that T cell cytotoxicity and cytokine release in conjunction with disease mechanisms within the islet cells are what cause damage to the cells [2]. Only 5–10% of people with diabetes are thought to have type 1 DM (T1DM) according to estimates [1]. The median age of diagnosis of hyperglycemia is 12 years, but it can develop at any age due to a progressive decrease in insulin-secretory capacity [3]. A turning point in medical history occurred on January 11, 1922, in Toronto, Canada, when 14-year-old Leonard Thompson became the first person to receive insulin as a replacement medication [4]. Diabetic ketoacidosis is a life-threatening condition caused by unregulated fatty acid mobilization and ketone body formation, and previously, it caused children with T1DM to die from insulin insufficiency [5]. Following the discovery of insulin, the lifespan of those with T1DM has increased. However, long-term consequences can persist despite insulin therapy, including blindness

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from retinopathy brought on by elevated glucose concentrations [6]. Islet autoantibodies are present in about 90% of people with childhood-onset T1DM at the time of diagnosis, whereas only about 70% of people with adult-onset T1DM that has been diagnosed by a clinician have autoantibody positivity [7]. The causes of this apparent drop are unknown. However, they could be related to the discovery of more unmeasured autoantibodies, the emergence of a brand-new and distinct kind of diabetes, or the unintentional misclassification of non-autoimmune diabetes. Autoantibodies have been demonstrated to be influenced by a person's genetic susceptibility to T1DM [8]. The interaction of hereditary and environmental factors has both been linked to an elevated risk of T1DM mellitus and other autoimmune diseases [9]. Years before the start of clinical symptoms, a pool of autoantibodies against proteins on β-cells develops in the blood, including antibodies against insulin (IAA), GAD (GADA), tyrosine phosphatase (IA-2A), and zinc transporter 8 (ZnT8A), which shows that the disorder is autoimmune [10]. It is currently unknown what causes the immunological tolerance to β-cell antigens to break down [11]. Therefore, a comprehensive immunogenetic study may help in understanding the pathophysiology and clinical importance of autoantibodies in children with T1DM, as well as the causes of the increased prevalence of autoantibody-positive in investigations. This study aims to detect the role of GaD65, ZNTS, IA-2, and IAA as predictive biomarkers for T1DM in children using the ELISA techniques.

Materials and methods

The study design

This study was carried out from the beginning of November 2022 to the end of March 2023. A total of 60 blood samples were collected from newly diagnosed T1DM children who attended the Dhi Qar Specialized Center for Diabetes and Endocrinology Hospital in Iraq.

Inclusion criteria

Type 1-diabetic patients' ages ranged between 3 and 15 years. They had a similar drug protocol that included insulin. In addition, they had a disease period of less than 3 years.

Exclusion criteria

Participants were not allowed to participate in the study if they had concurrent immune-mediated diseases, were receiving chemotherapy, had type 2 diabetes, or had the disease for more than 3 years. Participants with type 1 diabetes who were younger than 3 years old or older than 15 years were also not allowed to participate.

Ethical considerations

The Basra Health Institute's Ethics Research Committee granted authorization for the study to be carried out at the Thi-Qar Province and the Specialized Center for Diabetes and Endocrinology under permission number 2022/11/1. The parents or guardians gave informed consent before the potential subjects were included, indicating that they understood and agreed to take part in the study.

Participants and blood collection

This case–control study involved a total of 60 Iraqi children (27 males and 33 females), who were diagnosed with type 1 diabetes (T1D) at the Thi-Qar Specialized Center for Diabetes and Endocrinology. The children included in the study were aged from 3 to 15 years, and they were all diagnosed with T1D before reaching 15 years of age. They were also dependent on insulin to manage their condition. The diagnosis of T1D was based on the World Health Organization's (WHO) diagnostic criteria, which involved evaluating blood glucose levels. The participants provided information regarding their demographic details, clinical symptoms, presence of other autoimmune disorders, and hemoglobin A1C (HbA1c) levels. For the control group, 60 healthy individuals without T1D, autoimmune diseases, or a family history of T1D were included. Blood samples (5 ml) were collected from all participants to analyze the levels of serum autoantibodies, specifically insulin (IAA), glutamic acid decarboxylase (GADA), tyrosine phosphatase (IA-2A), and zinc transporter 8 (ZnT8A) using an enzyme-linked immunosorbent assay (Sandwich-ELISA).

Measurement method of biomarkers

The target group included children with new-onset T1DM, aged from 3 to 15 years. Whole blood was taken in 5 ml. After an overnight fast of between 8 and 12 hours, blood samples were taken in the morning. Right away, two portions of the taken blood were separated: the initial portion (3 ml) was put into gel tubes, and it was centrifuged for 5 minutes at a speed of 4000 rpm. The resultant serum was divided into two plain tubes, one for determining the amount of glucose and the other for determining the levels of anti-GAD65, IA-2, ZnT8A, and IAA. For assessing HbA1c levels, the second portion was collected into an anticoagulated EDTA tube. The samples were

taken based on clinical signs such as increased urination, intense hunger, intense thirst, and abrupt weight loss, as well as a fasting blood glucose test (above 126 mg/dl) and a random blood glucose test (above 200 mg/dl). During the questionnaire, the patient's parents or the partner provided information on the patient, such as age, gender, and any family members who had T1DM or other chronic illnesses. Anti-GAD65, IA-2, ZnT8A, and IAA were detected as biomarkers in the serum of the patients using the assays performed using a Synergy 2 multimode reader (Biotek, Winooski, VT, USA) and an enzyme-linked immunosorbent assay (ELISA) quantitative kit (BT LAB, China). according to the manufacturer's instructions.

Statistical analysis

Statistical analyses were performed using GraphPad Prism® (version 9.5.1) and SPSS. normally distributed independent t-test (two-tailed) was conducted for nonnormally distributed data, whereas the Mann-Whitney U test and Kolmogorov-Smirnov test were used for nonnormally distributed. Statistical significance was indicated by *$P<0.05$, $P<0.01$, and *$P<0.001$). Pearson's correlation coefficients were used when calculating correlations. $P\leq0.05$ was considered statistically significant.

Results

Table 1 and Fig. 1 shows that age and gender of the patient group are not significantly different from those of the control group. However, when it comes to other metabolic parameters, the patient group has significantly higher levels than the control group. For instance, the patient group has a significantly higher HbA1C level *($P<0.001$)* compared with the control group. Similarly, the patient group has significantly higher fasting blood sugar levels *($P<0.001$)*. Compared with the control group, in the present study, children with diabetes were evaluated for the presence of circulating antibodies to GAD65 (GADA), IA-2 (IA-2A), ZnT8A, and IAA at the time of diagnosis and were observed for the initial 3 years to explore whether these diabetes-related autoantibodies are associated with the clinical features at diagnosis and the natural history of the disease thereafter.

The data from the current research is consolidated and presented in (Table 2, which reveals significant

Table 1 Clinical and phenotypic characteristics of study participants

Characteristics	Patient group *n*=60	Control group *n*=60	*P* value
Age (years) mean±SD	9.97±3.56	8.61±3.18	0.078
HbA1C mean±SD	10.2±2.30	5.21±0.258	<0.001***
FBS (mg\dl) mean±SD	237±140	95.2±12.7	<0.001***
Sex			
Male, *n* (%)	27 (45%)	30 (50%)	0.583
Female, *n* (%)	33 (55%)	30 (50%)	
IA-2 (ng/ml) mean±SD	32.4±29.82	12.11±6.42	<0.001***
GAD65 (ng/ml) mean±SD	1.92±0.95	0.66±0.20	<0.001***
Insulin Abs (ng/ml) mean±SD	38.39±24.9	12.44±4.84	<0.001***
ZnT8 (ng/ml) mean±SD	432.7±553.8	113.6±52.75	<0.001***

*Parametric variables Independent Samples t-Test.

Table 2 Statistical analysis for immunological variables in the patient group compared with the control group as a sex (male and female)

	Male			Female		
Variables	Patient group *n*=27 mean±SD	Control group *n*=30 mean±SD	*P* value	Patient group *n*=33 mean±SD	Control group *n*=30 mean±SD	*P* value
IA-2 (ng/ml)	26.0±18.2	12.0±5.59	<0.001	37.7±36.2	12.2±7.26	<0.001
	KS *P* value (0.0018)	KS *P* value (0.038)		KS *P* value (0.001)	KS *P* value (0.036)	
GAD65 (ng/ml)	1.72±0.871	0.689±0.211	<0.001	2.09±0.991	0.633±0.191	<0.001
	KS *P* value (0.0021)	KS *P* value (0.014)		KS *P* value (0.034)	KS *P* value (0.037)	
Insulin Abs (ng/ml)	34.2±22.3	12.8±4.05	<0.001	41.8±26.8	12.1±5.58	<0.001
	KS *P* value (0.007)	KS *P* value (0.0028)		KS *P* value (0.001)	KS *P* value (0.013)	
ZnT8 (ng/ml)	413±604	121±54.3	<0.001	449±518	106±51.0	<0.001
	KS *P* value (0.001)	KS *P* value (0.041)		KS *P* value (0.001)	KS *P* value (0.006)	

*Mann–Whitney U test.

disparities in immunological markers between males and females. Differences were observed across all measures: insulinoma-associated-2 (IA-2), glutamic acid decarboxylase 65 (GAD65), insulin antibodies (Insulin Abs), and zinc transporter 8 (ZnT8). These low *P* values support the argument that the disparities noticed are not only coincidental but are indicative of a substantive divergence in immunological responses of males and females.

Table 3 describes the relationship between age at diabetes onset and the duration of the disease and the prevalence of positive autoantibodies in patients with type 1 diabetes. Autoantibodies measured in this study were GAD65, ZNT8, IA-2, and IAA. The table shows that there was no significant difference *(P>0.05)* between the age at diabetes onset and the duration of the disease concerning the prevalence of positive autoantibodies.

The aggregated findings from the study are displayed in Table 4; the examination revealed substantial connections among various biomarker pairs in individuals diagnosed with type 1 diabetes.

The data encapsulated in (Table 5 and Fig. 2) offers an assessment of the efficacy of diverse biomarkers (IA-2, GAD65, insulin Abs, and ZnT8) in accurately classifying positive and negative instances. This efficacy is gauged through sensitivity, specificity, and

Table 3 Relationship between Age at diabetes onset with duration of disease and the prevalence of positive value of autoantibodies (GaD65, ZNT8, IA-2, and IAA) in patients with type 1 diabetes

			GADA best cut-off >0.92			ZNT8 best cut-off >167.6		
Age at diabetes onset	Duration of disease	*n.*	N.POS (%)	OR	CI	N.POS (%)	OR	CI
3–9 years	<1 Years	14	13 (92.86)	0.929	0.0459 to 18.9	11 (78.57)	0.917	0.183 to 4.60
	≥1 Years	1**5**	1**4** (93.33)			1**2** (80)		
≥10 years	<1 Years	**17**	**17** (100)	00	00	**12** (70.59)	1.33	0.275 to 6.58
	≥1 Years	**14**	**14** (100)			**9** (64.29)		
Age at diabetes onset	Duration of disease	*n.*	IA-2 best cut-off >14.8			Insulin Abs best cut-off >19.91		
			N.POS (%)	OR	CI	N.POS (%)	OR	CI
3-9 Years	<1 Years	14	11 (78.57)	0.917	0.183 to 4.60	12 (85.71)	0.00	0.00 to 1.97
	≥1 Years	1**5**	1**2** (80)			**15** (100)		
≥10 Years	<1 Years	**17**	**14** (82.35)	0.359	0.0259 to 2.76	**16** (94.12)	1.23	0.0605 to 24.7
	≥1 Years	**14**	13 (92.86)			13 (92.86)		

Table 4 Correlation between autoantibodies (GaD65, ZNT8, IA-2, and IAA) and other variables in patients with type 1 diabetes and healthy controls

	IA-2		GAD65		Insulin Abs		ZnT8	
Variables	Patient	Control	Patient	Control	Patient	Control	Patient	Control
Age								
r	−0.102	−0.052	−0.067	0.236	−0.171	0.201	−0.156	−0.150
P	0.437	0.689	0.608	0.071	0.191	0.123	0.233	0.251
HbA1C								
r	0.010	−0.219	0.160	−0.086	−0.033	0.025	−0.094	−0.155
P	0.935	0.092	0.221	0.511	0.800	0.847	0.473	0.163
FBS								
r	0.057	0.221	0.043	−0.060	0.089	0.221	0.018	−0.138
P	0.663	0.078	0.744	0.645	0.499	0.089	0.939	0.171
IA-2								
r			0.451	0.150	0.634	0.134	0.780	0.176
P			0.001**	0.249	0.001**	0.304	0.001**	0.178
GAD65								
r	0.451	0.150			0.649	0.189	0.444	−0.046
P	0.001**	0.249			0.001**	0.148	0.001**	0.722
Insulin Abs								
r	0.634	0.134	0.649	0.189			0.674	0.149
P	0.001**	0.304	0.001**	0.148			0.001**	0.253
ZnT8	0.780	0.176	0.444	−0.046	0.674	0.149		
	0.001**	0.178	0.001**	0.722	0.001**	0.253		

*Spearman correlation test.

Table 5 Receiver-operating characteristic (ROC) curve analysis of biomarkers for the patient group

Variables	Area under the curve	*P- value* (AUC=0.05)	Sens** %	Spec %	PPV**	NPV
IA-2	0.841	0.000	83.33	80.00	89.29	70.59
GAD65	0.989	0.000	96.67	90.00	95.08	93.10
Insulin Abs	0.967	0.000	90.00	100.00	100.00	83.33
ZnT8	0.839	0.000	78.33	80.00	88.68	64.86

*Sens: Sensitivity, spec.: Specificity, PPV: positive predictive value, NPV: negative predictive value.

the area under the receiver-operating characteristic (AUC) curve.

The logistic regression study elucidates a number of important relationships concerning the effects on results in pediatric patients diagnosed with type 1 diabetes (Table 6).

Discussion

This study showed that both male and female patients with T1DM had a significant prevalence of autoantibodies, which has also been concluded by other studies [12,13]. A potential cause of this high rate is genetic susceptibility to T1DM [14].

Various European studies have demonstrated that the prevalence of autoantibodies is higher in females than in males [13,15]. The prevalence of ZnT8A is correlated with the age of onset of diabetes. ZnT8A declined in the first years after the disease onset and was less persistent than IA-2A or GADA in the longer-term. ZnT8 is specifically expressed in pancreatic β-cells and is more restricted in its tissue distribution than other autoantigens such as glutamic acid decarboxylase 65 (GaD65) and insulinoma-associated antigen-2 (IA-2) [16]. Similar results were found in other studies, including European, American, and Chinese studies [17–20]. While no correlation was identified in a study on newly diagnosed Czech children, a British investigation on ZnT8-positive children discovered that it was connected with an older age of diagnosis [21]. Although it is unclear why ZnT8A is now showing up later, earlier research has shown that ZnT8A is typically found in patients who develop T1DM more slowly [22,23].

GADA had a higher prevalence than IA-2, ZnT8A, and IAA in all of the categories of insulin-dependent diabetes mellitus (IDDM) patients who were examined. This early presence and the relative ease of assaying for anti-GAD autoantibodies have made them the most commonly used screening method to assess the risk or progression to the insulin-requiring stage of the disease [24]. The results of this study agree with those of previous studies [14,25,26]. Hence, a study conducted in Iraq indicated that anti-GAD65 could be considered an important diagnostic marker for the identification of T1DM patients, and these autoantibodies are more common in earlier affected T1DM patients [11].

The determination of GAD autoantibodies has special characteristics in clinical application because of its age-related incidence in susceptible populations. It is generally known that other islet autoantibodies, such as insulin autoantibodies and phogrin, are significant indicators of the likelihood that a child will develop T1DM [27]. In contrast, GaD65 autoantibodies are thought to be associated with adult ages, and 15 years after the beginning of T1DM, high GaD65 antibody titers have been linked to longer-term diabetes problems, such as retinopathy [28]. The importance of GaD65 as a particular biomarker in predicting diabetes risk as well as a potential route for immunoregulatory medications has continued to grow in research. The prevalence of IA-2A positivity was 80% in this study with a disease duration of less than 3 years. IA-2A most often seems in combination

Table 6 Logistic regression analysis of biomarkers for predicting disease risk

					95% CI for odds ratio	
Variables	B (coef)	SE	*P. value*	Odds ratio	Lower	Upper
IA-2	0.062	0.066	0.366	1.062	0.932	1.209
GAD65	1.593	1.042	0.036	3.428	1.177	13.201
Insulin Abs	0.421	0.108	0.001	1.512	1.221	1.871
ZnT8	0.006	0.005	0.906	0.999	0.988	1.010

*B (coef): regression coefficient. CI, confidence Interval; SE, standard Error.

with other islet autoantibodies and seldom alone, which explains why it is often regarded as a marker of extensive beta-cell destruction. IA-2A is a marker of active β-cell lesions. Positivity for a single autoantibody usually reflects harmless nonprogressive β-cell autoimmunity, but isolated IA-2A positivity is more common among patients who are newly diagnosed with T1DM than isolated IAA or GADA [29]. The results of this study agree with previous studies [25,30,31]. Although the precise role of IA-2's in β-cell physiology is still unknown, various investigations have suggested that IA-2 and its homolog phogrin may be involved in the release of insulin granules [32–34]. A study hypothesized that cleaving IA-2's cytoplasmic region would cause the peptide fragment that it produces to be transported to the β-cell nucleus [35].

The prevalence of IAA in this study was 93%, showing that it is a particular marker for T1DM. IAAs are typically recognized as the first autoantibodies to occur in infants in birth cohort studies, with a peak between 6 months and 2 years. They are reasonably related to age and have a high frequency in children diagnosed with T1DM at a young age [10]. The results of this study were consistent with the results of other studies [36–38]. When insulin therapy is initiated, IAA antibodies are less important in the diagnosis of this condition because they can be obscured by the formation of antibodies against exogenous insulin [39]. Continuous beta-cell regeneration, protein mimicry, incomplete beta-cell death, release of autoantigen from other sources, and cross-reactivity have all been implicated in the persistence of autoantibodies despite the loss of beta cells as the antigen source after the disease has started [18]. The duration of the disease seems to have an impact on the prevalence of autoantibody positivity, particularly for GADA and ZnT8. A study on British patients with T1DM revealed that ZnT8 AAb positivity declined from 58% in those with an illness duration of less than 2 years to 10% in those with a disease duration of more than 9 years. In an Italian investigation, Fabris and colleagues discovered that during the first 4 years following a T1DM diagnosis, the prevalence of ZnT8 autoantibodies remained steady [40]. ZnT8 autoantibodies were more common in children than in adults in a Polish cohort of newly diagnosed patients with T1DM (81.1%, median age 9 years (interquartile range: 6–13 years)) [41]. In individuals with more advanced disease, there was a significant decline in GAD antibody and IA-2 antibody positivity. In particular, at least one antibody was found in those with GADA (67%) and IA-2 children with T1DM who had a disease duration of less than 5 years (59%) [42].

A strong correlation between insulin antibodies and ZnT8 may suggest a considerable coexistence or co-expression of these biomarkers in patients with T1DM, which indicates robust positive correlations between IA-2 and insulin antibodies. These findings suggest that an increase in IA-2 levels may be directly associated with a rise in both insulin autoantibodies and ZnT8 levels, indicating that IA-2, insulin autoantibodies, and ZnT8 may play key roles in an autoimmune reaction that occurs with or is connected to T1DM [28,43]. This may be because ZnT8 and IA-2 are both proteins that are found on the secretory granule membrane of β-cells and are both discharged as particulate matter when β-cells are damaged. Similar results were found in other studies [26,44]. In addition, the pronounced positive correlation between GaD65 and insulin antibodies might represent a mutual autoimmune response against these antigens [45]. The moderately positive correlation between GaD65 and ZnT8 suggests a similar, albeit marginally less potent, relationship. This result agrees with those of previous studies [41]. This correlation implies that higher IA-2 levels are likely to coincide with an elevation in GaD65 levels and vice versa. Given that IA-2 and GaD65 are autoantibodies typically found in cases of T1DM, this correlation could imply a synchronized or simultaneous immune response against these antigens in individuals with T1DM. This finding agrees with the Vaziri-Sani *et al.* results [43]. Collectively, these discoveries hint at the potential for a significant degree of interrelationship or co-regulation among these biomarkers in the autoimmune response of T1DM patients. In this study, all four biomarkers displayed high efficacy in distinguishing between positive and negative instances, as indicated by their elevated AUC values, sensitivity, and specificity. The results suggested that these biomarkers could be potentially invaluable instruments in diagnosing or monitoring T1DM. This finding supports earlier studies and demonstrates the value of measuring autoantibodies in the identification of autoimmune diabetes [17,26,46]. Among all the biomarkers assessed, only GaD65 and insulin antibodies displayed a noteworthy relationship with the outcome, which is consistent with earlier studies [47,48]. The data indicate that anti-GaD65 autoantibodies are potentially useful for the early detection of T1DM, which agrees with previous studies [24,37,49–51], which enhances the comprehension of potential biomarkers, and warrants further exploration.

Conclusion

The high associations show that the autoimmune response in type 1 diabetes is interrelated, which could be used to better understand the disease process, improve early identification, and guide treatment strategies. More research is needed, however, to confirm these findings and further investigate their relevance in the development and management of type 1 diabetes. Longitudinal studies could provide important insights into the course of the autoimmune response over time, as well as the factors that influence its advancement. Individual patient features and the unique causes leading to their disease could be considered in personalized therapy techniques.

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Consent for publication: Informed written consent was obtained from all the study participants.

Availability of data and material: The datasets used and analyzed during the current study are available from the corresponding author upon reasonable request.

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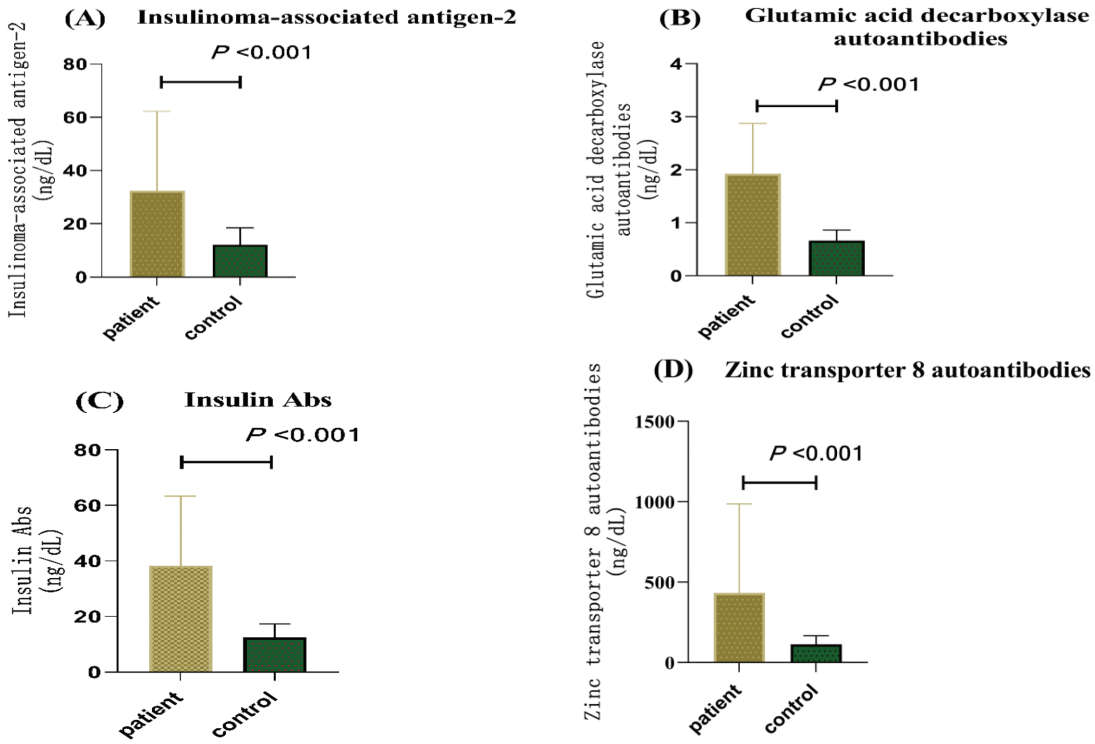
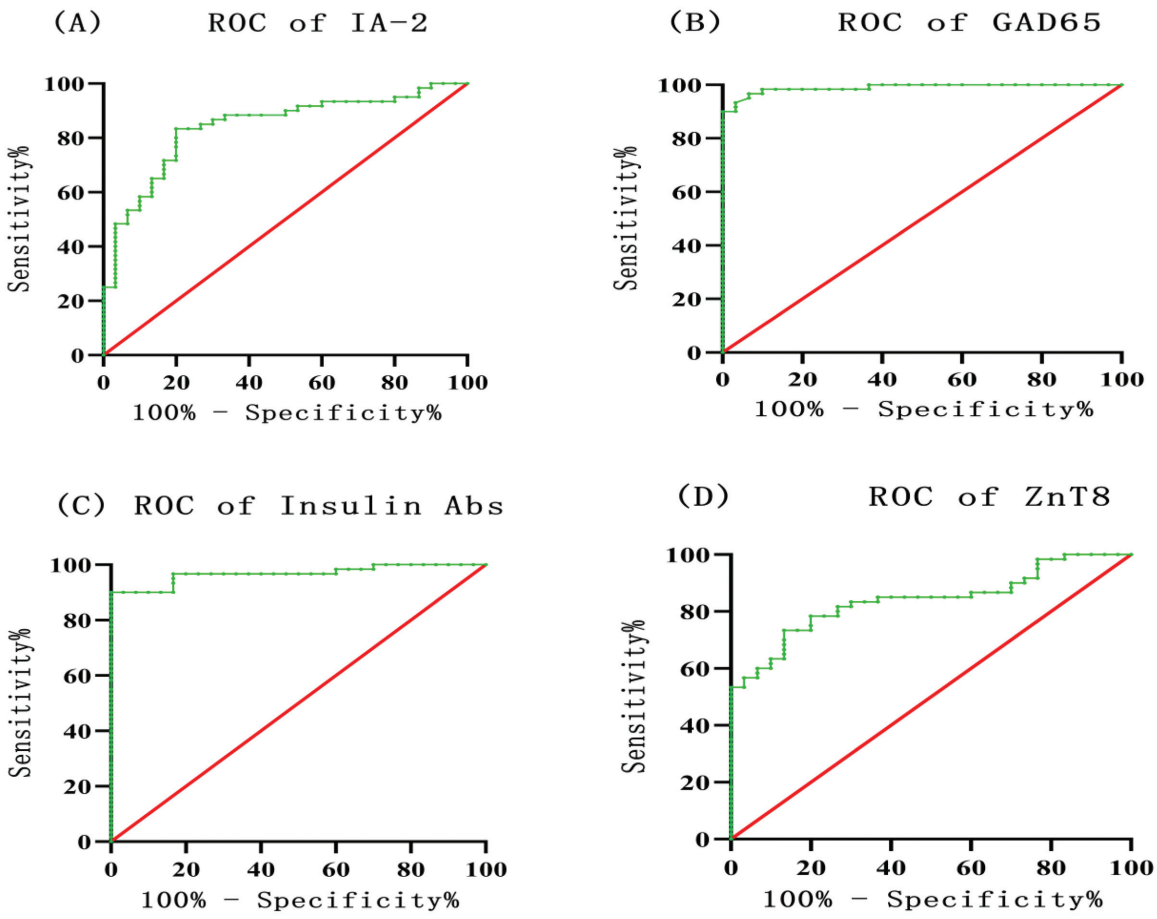
Figure 1



The bar chart labeled A, B, C, and D showcases the median values of IA-2, GAD65, insulin Abs, and ZnT8, respectively. The graphs provide an overview of the variation in variable levels in patient children and the control group. The statistical significance was calculated using the Mann–Whitney U test.

Figure 2



The receiver-operating characteristic (ROC) curve, which showcases the efficacy of immunological biomarkers in detecting and diagnosing patients with type 1 diabetes. The chart depicts the performance of different parameters, such as insulinoma-associated antigen-2 autoantibodies (A), glutamic acid decarboxylase autoantibodies (B), insulin Ab (C), and zinc transporter 8 autoantibodies (D).

Conflicts of interest

The authors declare that there are no conflicts of interest.

References

1 Calabrese CM, Valentini A, Calabrese G. Gut Microbiota and Type 1 Diabetes Mellitus: the effect of mediterranean diet. Front Nutr 2021; 7:612773.

2 Bauer W, Gyenesei A, Krętowski A. The multifactorial progression from the islet autoimmunity to type 1 diabetes in children. Int J Mol Sci 2021; 22:1–22.

3 Roep BO, Thomaidou S, van Tienhoven R, Zaldumbide A. Type 1 diabetes mellitus as a disease of the β-cell (do not blame the immune system?). Nat Rev Endocrinol 2021; 17:150–161.

4 Gerstein HC, Rutty CJ. Insulin therapy: the discovery that shaped a century. Can J Diabetes 2021; 45:798–803.

5 Dayan CM, Besser REJ, Oram RA, Hagopian W, Vatish M, Bendor-Samuel O, *et al.* Preventing type 1 diabetes in childhood. Science 2021; 373:506–510.

6 Pasquel FJ, Lansang MC, Dhatariya K, Umpierrez GE. Management of diabetes and hyperglycaemia in the hospital. Lancet Diabetes Endocrinol 2021; 9:174–188. Available from: http://dx.doi.org/10.1016/S2213-8587(20)30381-8

7 Thomas NJ, Walkey HC, Kaur A, Misra S, Oliver NS, Colclough K, *et al.* The relationship between islet autoantibody status and the genetic risk of type 1 diabetes in adult-onset type 1 diabetes. Diabetologia 2023; 66:310–320.

8 Lernmark Å. Etiology of autoimmune islet disease: timing is everything. Diabetes 2021; 70:1431–1439.

9 Awchi DW, Rasool SN. Evaluation of Anti-GAD65 and HbA1c Prevalence among newly diagnosed Type 1 diabetes of some iraqi children. Iraqi J Ind Res 2022; 9:125–130.

10 Jónsdóttir B. Childhood Thyroid and Islet Autoimmunity Immunogenetics, Risk Factors and Prediction. [Doctoral Thesis (compilation), Paediatric Endocrinology]. Lund University: Faculty of Medicine. 2017

11 Strollo R, Vinci C, Napoli N, Pozzilli P, Ludvigsson J, Nissim A. Antibodies to post-translationally modified insulin as a novel biomarker for prediction of type 1 diabetes in children. Diabetologia 2017; 60:1467–1474.

12 Krischer JP, Liu X, Lernmark Å, Hagopian WA, RRewers MJ, She J-X, *et al.* on behalf of the TSG. Page 1 of 33 Diabetes. 2017; 5332:1–33

13 Bilbao JR, Rica I, Vazquez JA, Busturia MA, Castano L. Influence of sex and age at onset on autoantibodies against insulin, GAD65 and IA2 in recent onset type 1 diabetic patients. Horm Res Paediatr 2000; 54:181–185.

14 Al Alwan I, Bin Dajim N, Jawdat D, Tamimi W, Al Ahmdi R, Albuhairan F. Prevalence of autoantibodies in children newly diagnosed with type 1 diabetes mellitus. Br J Biomed Sci 2012; 69:31–33.

15 Hagopian WA, Sanjeevi CB, Kockum I, Landin-Olsson M, Karlsen AE, Sundkvist G, *et al.* Glutamate decarboxylase-, insulin-, and islet cell-antibodies and HLA typing to detect diabetes in a general population-based study of Swedish children. J Clin Invest 1995; 95:1505–1511.

16 Hussein H, Ibrahim F, Sobngwi E, Gautier JF, Boudou P. Zinc transporter 8 autoantibodies assessment in daily practice. Clin Biochem [Internet] 2017; 50:94–96. Available from: http://dx.doi.org/10.1016/j.clinbiochem.2016.06.008

17 Wenzlau JM, Juhl K, Yu L, Moua O, Sarkar SA, Gottlieb P, *et al.* The cation efflux transporter ZnT8 (Slc30A8) is a major autoantigen in human type 1 diabetes. Proc Natl Acad Sci U S A 2007; 104:17040–17045.

18 Vaziri-Sani F, Oak S, Radtke J, Lernmark Å, Lynch K, Agardh CD, *et al.* ZnT8 autoantibody titers in type 1 diabetes patients decline rapidly after clinical onset. Autoimmunity 2010; 43:598–606.

19 Kong YH, Kim MS, Lee D-Y. Comparison of the prevalence of islet autoantibodies according to age and disease duration in patients with type 1 diabetes mellitus. Ann Pediatr Endocrinol Metab 2013; 18:65–70.

20 Niechciał E, Rogowicz-Frontczak A, Piłaciński S, Fichna M, Skowrońska B, Fichna P, *et al.* Autoantibodies against zinc transporter 8 are related to age and metabolic state in patients with newly diagnosed autoimmune diabetes. Acta Diabetol 2018; 55:287–294. Available from: https://doi.org/10.1007/s00592-017-1091-x

21 Howson JMM, Krause S, Stevens H, Smyth DJ, Wenzlau JM, Bonifacio E, *et al.* Genetic association of zinc transporter 8 (ZnT8) Autoantibodies in type 1 diabetes Cases. Diabetologia 2012; 55:1978–1984.

22 Long AE, Wilson IV, Becker DJ, Libman IM, Arena VC, Wong FS, *et al.* Characteristics of slow progression to diabetes in multiple islet autoantibody-positive individuals from five longitudinal cohorts: the SNAIL study. Diabetologia 2018; 61:1484–1490.

23 Long AE, Gooneratne AT, Rokni S, Williams AJK, Bingley PJ. The role of autoantibodies to zinc transporter 8 in prediction of type 1 diabetes in relatives: lessons from the European Nicotinamide Diabetes Intervention Trial (ENDIT) cohort. J Clin Endocrinol Metab 2012; 97:632–637.

24 Pietropaolo M, Becker DJ, LaPorte RE, Dorman JS, Riboni S, Rudert WA, *et al.* Progression to insulin-requiring diabetes in seronegative prediabetic subjects: the role of two HLA-DQ high-risk haplotypes. Diabetologia 2002; 45:66–76.

25 Delic-Sarac M, Mutevelic S, Karamehic J, Saracevic S, Subasic D, Jukic T, *et al.* ELISA test for analyzing of incidence of type 1 diabetes autoantibodies (GAD and IA2) in children and adolescents. Acta Inform Medica 2016; 24:61–65.

26 Petruzelkova L, Ananieva-Jordanova R, Vcelakova J, Vesely Z, Stechova K, Lebl J, *et al.* The dynamic changes of zinc transporter 8 autoantibodies in Czech children from the onset of Type 1 diabetes mellitus. Diabet Med 2014; 31:165–171.

27 Williams AJK, Aitken RJ, Chandler MA-M., Gillespie KM, Lampasona V, Bingley PJ. Autoantibodies to islet antigen-2 are associated with HLA-DRB1* 07 and DRB1* 09 haplotypes as well as DRB1* 04 at onset of type 1 diabetes: the possible role of HLA-DQA in autoimmunity to IA-2. Diabetologia 2008; 51:1444–1448.

28 Jensen RA, Agardh E, Lernmark Å, Gudbjörnsdottir S, Smith NL, Siscovick DS, *et al.* HLA genes, islet autoantibodies and residual C-peptide at the clinical onset of type 1 diabetes mellitus and the risk of retinopathy 15 years later. PLoS ONE 2011; 6:e17569.

29 Andrade Lima Gabbay M, Sato MN, Duarte AJS, Dib SA. Serum titres of anti-glutamic acid decarboxylase-65 and anti-IA-2 autoantibodies are associated with different immunoregulatory milieu in newly diagnosed type 1 diabetes patients. Clin Exp Immunol 2012; 168:60–67.

30 Lounici Boudiaf A, Bouziane D, Smara M, Meddour Y, Haffaf EM, Oudjit B, *et al.* Could ZnT8 antibodies replace ICA, GAD, IA2 and insulin antibodies in the diagnosis of type 1 diabetes? Curr Res Transl Med 2018; 66:1–7. Available from: https://doi.org/10.1016/j.retram.2018.01.003

31 Kawasaki E. Type 1 diabetes and autoimmunity. Clin Pediatr Endocrinol 2014; 23:99–105.

32 Harashima S, Clark A, Christie MR, Notkins AL. The dense core transmembrane vesicle protein IA-2 is a regulator of vesicle number and insulin secretion. Proc Natl Acad Sci 2005; 102:8704–8709.

33 Trajkovski M, Mziaut H, Schubert S, Kalaidzidis Y, Altkruger A, Solimena M. Regulation of insulin granule turnover in pancreatic β-cells by cleaved ICA512. J Biol Chem 2008; 283:33719–33729.

34 Henquin J-C., Nenquin M, Szollosi A, Kubosaki A, Notkins AL. Insulin secretion in islets from mice with a double knockout for the dense core vesicle proteins islet antigen-2 (IA-2) and IA-2β. J Endocrinol 2008; 196:573–581.

35 Mziaut H, Trajkovski M, Kersting S, Ehninger A, Altkrüger A, Lemaitre RP, *et al.* Synergy of glucose and growth hormone signalling in islet cells through ICA512 and ST AT5. Nat Cell Biol 2006; 8:435–445.

36 Ates D, Akinci A, Dundar I. The relationship between autoimmunity and HbA1c in type 1 diabetes mellitus patients. Med Sci | Int Med J 2021; 10:1505.

37 Jacobsen LM, Larsson HE, Tamura RN, Vehik K, Clasen J, Sosenko J, *et al.* Predicting progression to type 1 diabetes from ages 3 to 6 in islet autoantibody positive TEDDY children. Pediatr Diabetes 2019; 20:263–270.

38 Mortensen HB, Swift PG, Holl RW, Hougaard P, Hansen L, Bjoerndalen H, *et al.* Multinational study in children and adolescents with newly diagnosed type 1 diabetes: Association of age, ketoacidosis, HLA status, and autoantibodies on residual beta-cell function and glycemic control 12 months after diagnosis. Pediatr Diabetes 2010; 11:218–226.

39 Achenbach P, Warncke K, Reiter J, Naserke HE, Williams AJK, Bingley PJ, *et al.* Stratification of type 1 diabetes risk on the basis of islet autoantibody characteristics. Diabetes 2004; 53:384–392.

40 Fabris M, Zago S, Liguori M, Trevisan MT, Zanatta M, Comici A, *et al.* Anti-zinc transporter protein 8 autoantibodies significantly improve the diagnostic approach to type 1 diabetes: an Italian multicentre study on paediatric patients. Auto Immun Highlights 2015; 6:17–22.

41 Bhola S, Cave EM, Bhana S, Crowther NJ, Padoa CJ. Zinc transporter 8 (ZnT8) autoantibody prevalence in black South African participants with type 1 diabetes. BMC Endocr Disord 2021; 21:1–8.

42 Głowińska-olszewska B, Michalak J, Łuczyński W, Larosa P, Chen S, Furmaniak J, *et al.* Organ-specific autoimmunity in relation to clinical characteristics in children with long-lasting type 1 diabetes. 2016

43 Masuda M, Powell M, Chen S, Beer C, Fichna P, Rees Smith B, *et al.* Autoantibodies to IA-2 in insulin-dependent diabetes mellitus. Measurements with a new immunoprecipitation assay. Clin Chim Acta 2000; 291:53–66.

44 Balducci S, Sacchetti M, Haxhi J, Orlando G, D'Errico V, Fallucca S, *et al.* Physical Exercise as therapy for type II diabetes. Diabetes Metab Res Rev 2014; 32:13–23. Available from: http://libweb.anglia.ac.uk/

45 Holmberg H, Vaarala O, Sadauskaite – Kuehne V, Ilonen J, Padaiga Ž, Ludvigsson J. Higher prevalence of autoantibodies to insulin and GAD65 in Swedish compared to Lithuanian children with type 1 diabetes. Diabetes Res Clin Pract 2006; 72:308–314.

46 Vaziri-Sani F, Delli AJ, Elding-Larsson H, Lindblad B, Carlsson A, Forsander G, *et al.* A novel triple mix radiobinding assay for the three ZnT8 (ZnT8-RWQ) autoantibody variants in children with newly diagnosed diabetes. J Immunol Methods 2011; 371:25–37.

47 Wuragil DK, Susanto H, Herawati A, Nugroho YM, Fajri WNL, Putri PF, *et al.* The early detection of type 1 diabetes mellitus and latent autoimmune diabetes in adults (LADA) through rapid test reverse-flow immunochromatography for glutamic acid decarboxylase 65kDa (GAD65). Heliyon 2022; 8:e08695.

48 Yosef T. Knowledge and attitude on insulin self-administration among type 1 diabetic patients at Metu Karl referral hospital, Ethiopia. J Diabetes Res 2019; 2019:7801367.

49 Tiberti C, Verrienti A, Fiore B, Yu L, Eisenbarth GS, Dotta F, *et al.* IA-2 combined epitope assay: a new, highly sensitive approach to evaluate IA-2 humoral autoimmunity in type 1 diabetes. Clin Immunol 2005; 115:260–267.

50 Andersson C, Larsson K, Vaziri-Sani F, Lynch K, Carlsson A, Cedervall E, *et al.* The three ZNT8 autoantibody variants together improve the diagnostic sensitivity of childhood and adolescent type 1 diabetes. Autoimmunity 2011; 44:394–405.

51 Lynam A, McDonald T, Hill A, Dennis J, Oram R, Pearson E, *et al.* Development and validation of multivariable clinical diagnostic models to identify type 1 diabetes requiring rapid insulin therapy in adults aged 18–50 years. BMJ Open 2019; 9:e031586.