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Research Article

Assessment of Anti-Zinc Transporter Protein-8 Function in Adults with Type 1 Diabetes

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Abstract

Background: Anti-glutamic acid decarboxylase antibodies (GAD65A), anti-tyrosine phosphatase antibodies (IA-2), insulinoma-associated 2 autoantibodies (IA-2A), and anti-zinc transporter antibodies (Zn-T8A) are some of the most important blood tests used to diagnose type 1 diabetes mellitus (T1DM). Integrating zinc transporter 8 autoantibody (ZnT8A) into the conventional diagnostic protocol for T1DM may enhance the overall sensitivity of autoantibody detection and may predict early complications. ***Objectives***: To analyze the presence of type 1 diabetes-related autoantibodies (GAD65A, IA-2A, and ZnT8A) and to determine any link with age, gender, and duration of the illness in adult populations. ***Methods***: This case-control research was performed at four teaching hospitals in Basra city during a five-month period on 100 adult patients confirmed to have T1DM and on 50 apparently healthy controls. Serum concentrations of ZnT8A, GAD65A, and IA-2A were individually assessed utilizing ELISA in both cases and control groups. ***Results***: A statistically significant difference exists in the concentrations of ZnT8A, GAD65A, and IA-2A autoantibodies between the case and control groups. All three immunological markers had no correlation with sex and duration of disease. ZnT8A positivity was associated with an earlier age of onset (OR=2.50, p=0.039) and the presence of IA-2A (OR=2.65, p=0.015). No correlation was found between ZnT8A and GAD65A. ***Conclusions***: ZnT8A shows the highest concentration among the three studied autoantibodies in both the acute-onset and slowly progressive T1DM patient groups and is considered an important serological marker of T1DM with other immunological markers for this disease.

Keywords: Autoantibody, Type 1 diabetes mellitus, Zinc transporter-8 antibody.

تقييم وظيفة بروتين ناقل الزنك المضاد-8 لدى البالغين المصابين بمرض السكري من النوع الأول

الخلاصة

الخلفية: الأجسام المضادة لحمض الجلوتاميك ديكاربوكسيلاز (GAD65A) ، والأجسام المضادة لفوسفاتيز التيروزين (IA-2) ، والأجسام المضادة الذاتية المرتبطة بسرطان الأنسولين (IA-2A) ، والأجسام المضادة المضادة للزنك (Zn-T8A) هي بعض من أهم اختبارات الدم المستخدمة لتشخيص داء السكري من النوع 1 (T1DM). قد يؤدي دمج الجسم المضاد الذاتي لناقل الزنك 8 (ZnT8A) في بروتوكول التشخيص التقليدي ل T1DM إلى تعزيز الحساسية الإجمالية للكشف عن الأجسام المضادة الذاتية وقد يتنبأ بالمضاعفات المبكرة. **الأهداف**: تحليل وجود الأجسام المضادة الذاتية المرتبطة بمرض السكري من النوع 1 (GAD65A و IA-2A و ZnT8A) وتحديد أي صلة بالعمر والجنس ومدة المرض لدى السكان البالغين. **الطرائق**: تم إجراء هذا البحث في الحالات والشواهد في أربعة مستشفيات تعليمية في مدينة البصرة خلال فترة خمسة أشهر على 100 مريض بالغ تم تأكيد إصابتهم بمرض السكري من النوع 1 وعلى 50 ضابطا يبدو أنه يتمتعون بصحة جيدة. تم تقييم تركيزات مصل ZnT8A و GAD65A و IA-2A بشكل فردي باستخدام ELISA في كل من الحالات والمجموعات الضابطة. **النتائج**: يوجد فرق يعتد به إحصائيا في تركيزات الأجسام المضادة الذاتية ZnT8A و GAD65A و IA-2A بين مجموعات الحالة والشاهدة. لم يكن لجميع العلامات المناعية الثلاثة أي علاقة بالجنس ومدة المرض. ارتبطت إيجابية ZnT8A بعمر مبكر من البداية (OR = 2.50 ، p = 0.039) ووجود IA-2A (OR = 2.65 ، p = 0.015). *لم يتم العثور على ارتباط* بين ZnT8A و GAD65A. **الاستنتاجات**: يظهر ZnT8A أعلى تركيز بين الأجسام المضادة الذاتية الثلاثة المدروسة في كل من مجموعات مرضى T1DM الحادة والتقدم البطيء ويعتبر علامة مصلية مهمة ل T1DM مع علامات مناعية أخرى لهذا المرض.

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INTRODUCTION

Type 1 diabetes mellitus (T1DM) is a long-lasting condition characterized by the body's inability to produce insulin due to the autoimmune destruction of pancreatic beta cells. As a result, T1DM manifests as a systemic illness with hyperglycemia as its primary manifestation [1]. Iraq is classified as having an intermediate incidence rate of T1DM, with an average yearly incidence rate of 7.4 per 100,000. The mean age at diagnosis for type 1 diabetes in Iraq was 15.3 ± 9 years old, with a prevalence of 87 cases per 100,000 people [2]. Although several studies suggested that genetic factors play a major role in the development of T1DM [3], there is still a difficulty in differentiating T1DM from latent autoimmune diabetes in adults (LADA), also

known as slowly progressive T1D (SPIDDM), if certain autoimmune markers are not investigated [4,5]. Although insulin therapy is the cornerstone for controlling type 1 diabetes, early diagnosis is crucial for timely intervention and potential disease prevention (6). The ability to detect people who are at risk for T1DM offers great potential for early intervention strategies that aim to prevent or delay the death of beta cells and maintain insulin production. Anti-islet autoantibodies serve as effective humoral immune indicators for predicting and diagnosing type 1 diabetes because it is known that they generate prior to the disease's development [6]. Various autoantibodies were found, including glutamic acid decarboxylase (GAD65A), tyrosine phosphatase-like protein IA-2 autoantibodies, and zinc transporter 8 autoantibodies (ZnT8A), in addition to insulin autoantibody (IAA), and are now used for the diagnosis, pathological investigation, and prediction of T1DM [7,8,9]. One of the promising biomarkers for the diagnosis of T1DM is zinc transporter 8 (ZnT8) antibodies. ZnT8 is a transmembrane protein predominantly expressed in pancreatic beta cells and plays a crucial role in regulating intracellular zinc homeostasis [11]. ZnT8 plays a crucial role in supplying the necessary zinc for the maturation and/or storage functions of insulin in pancreatic beta cells responsible for insulin release. Consequently, antibodies targeting ZnT8 could interfere with insulin synthesis, secretion, and storage, as well as affect islet cell paracrine and autocrine communication [11]. Autoantibodies that specifically target intracellular ZnT8 are thought to be a defining feature of T1DM and can often be found years before symptoms show up [12]. Furthermore, it has been demonstrated that ZnT8 autoantibody levels correlate with an increase in beta cell loss, suggesting a possible biomarker for assessing disease activity [13]. ZnT8A alone or in combination with other autoantibodies, including glutamic acid decarboxylase autoantibody (GAD65A), islet antigen 2 autoantibody (IA2A), and insulin autoantibody (IAA), can help to assess the frequency, concentrations, and the relation with T1DM in people [14,15]. In spite of the expected role of ZnT8 antibodies in the diagnosis of T1DM, a thorough evaluation of their diagnostic accuracy is still required. Thus, we aim in this study to clarify the relationship between positive ZnT8A and other autoantibodies and to assess the ZnT8 antibody's accuracy for diagnosis and management of adult-onset T1DM.

METHODS

Study design and setting

This case-control study was performed on 50 apparently healthy controls and 100 patients with adult-onset T1DM who attended the outpatient units of four teaching hospitals in Basrah City, Iraq. The duration of sample collection was between May 2024 and December 2024. Of the 100 patients, 53 were males and 47 were females, ages more than 16 years, with a mean age of 42±26.5 years and a median duration of diabetes of 8.2±6.4 years. The patients were classified by physician according to the Japan Diabetes Society criteria into two groups. Acute-onset type 1 diabetes (n=78) and slowly progressive type 1 diabetes SPIDDM (n=22) [17,18]. The healthy control group (n=50) comprised the first-degree relatives of the patients who were matched in age and sex to the patients, and they are free of autoimmune diseases.

Inclusion criteria

Include all adult patients aged more than 16 years old who had been confirmed to have type 1 D.M according to International Society for Paediatrics and Adolescent (ISPAD) 2018 guideline [16].

Exclusion criteria

Include all patients confirmed to be type 1 DM aged less than 16 years old, all patients with type 2 DM, and all patients with other auto immune disease and other chronic illness or tumors.

Sample selection, analysis, and outcome measurements

Patients' demographic characteristics and results were obtained from routine laboratory tests that were carried out at the central laboratory of the attained hospitals. These include complete blood count (CBC), lipid profile, insulin level, glycated hemoglobin (HbA1c), urea and creatinine levels, and body mass index (BMI). A full urine analysis using a dipstick to detect ketone bodies was also obtained. Blood samples were taken from patients, separated into aliquots, and kept at -20°C until diabetes-associated autoantibodies were examined. Serum levels of ZnT8A, GAD65A, and IA-2A were determined separately using sandwich ELISA kits purchased from the U.K. (RSR Limited, Cardiff). All the procedures were carried out according to the kit's manual suggested by the manufacturer. Then start the procedure as described in the kit's manuals. The results were derived from a calibration curve established during the same run as the calibrators and were expressed in U/mL. ZnT8A levels were assessed with great accuracy (CV 7.5-9.3% and CV 3.5-6.2%, respectively) using the ELISA RSR ™ZnT8 Ab ™ ELISA kit. GAD56A was identified using the ELISA RSR TM GAD Ab kit (RSR Ltd., Cardiff, UK) at a cutoff value of 5 U/mL, demonstrating inter- and intra-assay precision with coefficients of variation of 5.2-6.4% and 3.5-8.5%, respectively. The ELISA RSR TM IA-2 Ab Version 2 kit evaluated IA-2A using a cutoff value of 7.5 U/mL, demonstrating inter- and intra-assay precision with coefficients of variation (CV) of 4.2-4.5% and 1.3-3.1%, respectively. The cut-off values for anti-ZnT8 were set at ≥ 15 U/mL, for GAD65A at ≥ 5 U/mL, and for IA-2A at ≥ 7.5 U/mL, respectively.

Ethical considerations

Every patient provided informed consent for laboratory research in accordance with the standards of the Committee on Medical Ethics of Al-Zahraa College of Medicine (certificate No. E/T/50/2024).

Statistical analysis

The Statistical Package for the Social Sciences (SPSS), version 24, developed by IBM, which is based in Armonk, New York, was used to analyze the data. For parametric data, the findings of numerical variables were expressed as mean ± standard deviation; for non-parametric data, they were expressed as median and range. Frequency and percentage were used to express the categorical data. Whereas the Fisher exact test or the chi-square test was used to compare frequency differences. The independent t-test was employed to assess variations in parametric data. Statistics were considered significant if the *p*-value was less than 0.05.

RESULTS

Table 1 presents the clinical and demographic features of individuals with type 1 diabetes.

Table 1: Clinical and demographic characteristics

Characteristics	T1DM (n=100)	Controls (n=50)	*p-value*
Sex			
Male	43(43)	24(48)	0.5
Female	47(47)	26(52)	
[a]Age	32±8.5	-	<0.001
[b]Disease duration (year)	8.05± 5.22	-	
BMI (kg/m^2)	23.1±0.3	21.8±3.45	0.426
HbA1c (%)	9.5±1.3	5.01± 0.25	<0.001
T Chole (mg/dL)	140.3±51.02	97.5 ±41.21	<0.001
Urea (mmol/L)	5.95±1.88	3.99±1.65	<0.001
Creatinine (µmol/L)	50±15.42	36.21±12.2	0.03
Positive urinary ketones	20(40)	-	

Data are expressed as the numbers, percentages, and mean±SD. BMI: body mass index; HbA1c: glycated haemoglobin; T chole: total cholesterol; [a] Age at diagnosis of T1DM, [b] Duration from diagnosis. *p*-value <0.0.5 is considered statistically significant.

The current study showed that out of 100 diabetic cases, 53% were males and 47% were females, with the mean age of 32±8.5 years at diagnosis of T1DM patients. In the control group, 48% were males and 52% were females, with a mean age of 46.12±12.1 years. Diabetes duration was 8.05±5.22 years. Compared to the sibling group, the diabetic group exhibited significantly higher levels of glycated hemoglobin, urea, creatinine, total cholesterol, and positive urine ketones (p<0.001, 0.01, <0.001, <0.001, and <0.001, respectively). The concentration of ZnT8, GAD65A, and IA-2A autoantibodies shows a notable difference between the patient and control groups (18.7±5.9, 7.35±2.4, and 8.12±3.5, respectively), with *p*-values of 0.002, 0.0001, and 0.0001, respectively, as shown in Table 2.

Table 2: Titers of ZnT8, GADA65, IA-2A auto-antibodies in patients and control groups

Parameter	T1DM (n=100)	Control (n=50)	*p*-value
ZnT8 Ab titer IU/mL	18.7±5.9	3.72±1.5	0.002
GAD65Ab titer IU/mL	7.35±2.4	1.75±0.53	0.0001
AI-2A Ab titer IU/mL	8.12±3.5	1.01±0.27	0.0001

Values were presented as mean±SD. Chi square test was used to analyze significance at $p<0.05$

Figure 1 shows that the study subjects were split into three groups: those with acute-onset T1DM, those with SPIDDM, and healthy controls.

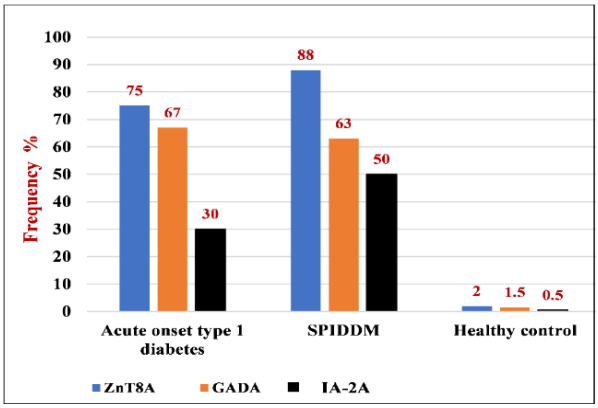

Figure 1: A comparison of GAD65A, IA-2A, and ZnT8A frequencies in patients with acute onset type 1 diabetes, SPIDDM, and healthy controls.

This was done so that the rates of certain autoantibodies in each group could be compared. The results show that the frequency of all three screened autoantibodies was higher in both diabetes groups than in healthy controls. We found that ZnT8A and IA-2A were more common in SPIDDM than in acute-onset T1DM. On the other hand, GAD65A was more common in acute-onset T1DM than in SPIDDM (67% vs. 63%, respectively). Table 3 demonstrated the connection between age at sampling and the prevalence of GAD65A and/or ZnT8 antibodies in 63 patients with acute-onset and slowly progressing type 1 diabetes who had been diagnosed with T1DM for 10 years.

Table 3: The relationship between age and the occurrence of glutamic acid decarboxylase and zinc transporter 8 autoantibodies in 63 patients with acute-onset and slowly progressing type 1 diabetes with a duration of ≥10 years

Age	n	ZnT8			GADA65		
		n(%)	OR	95% CI	n(%)	OR	95% CI
16–30	15	8(53.3)	1.0	-	7(60)	1.0	-
30–50	21	6(28.5)	0.37	0.14-0.9	15(71.4)	1.8	0.70-4.1
≥ 50	27	6(25.9)	0.31	0.11-0.85	21(77.8)	2.33	0.79-6.1

OR: odd ratio, CI: confidence interval.

ZnT8 antibody prevalence was substantially greater in the patient age group (16-30 years) (53.3%) than in other age groups. Furthermore, there were no relationships found between the presence of ZnT8 antibody and sex, disease duration, or GAD65A antibody. Table 4 shows that having ZnT8A positivity was linked to both an earlier age of onset (OR = 2.50, 95% confidence interval = 1.02 - 7.00, p= 0.039) and the presence of IA-2A (OR = 2.65, 95% confidence interval = 1.00 - 6.98, p= 0.015).

Table 4: Logistic regression analysis of the relationship between clinical and immunological factors and zinc transporter-8 auto-antibody positivity in patients with acute-onset and slowly progressive type 1 diabetes, with a duration ≥ 10 years

Variable	OR	95% CI	*p*-value
Sex (female)	1.1	0.40-2.62	0.785
Duration (year)	1.0	0.71-1.2	0.321
Age at sampling (≥ 16 years)	2.5	1.02-7.0	0.039
GAD65A positive	3.01	0.81-6.87	0.214
AI-2A positive	2.65	1.0-6.98	0.015

CI: confidence interval; GADA: glutamic acid decarboxylase autoantibodies'; IA-2A: insulinoma-associated antigen-2 autoantibodies; OR: odds ratio

The assay sensitivities and specificities for GAD65A (69.0% and 97.8%), for IA-2A (73.0% and 97.9%), and for ZnT8A (77.0% and 98.7%) are shown in Figure 2.

Figure 2: The sensitivity and specificity of GAD65, IA-2A, and ZnT8 autoantibodies.

DISCUSSION

Type 1 diabetes mellitus is one of the immunopathological disorders that passes into multiple stages silently over a period of months to years to become a clinically apparent condition. Assessing the concentration of specific autoantibodies in T1DM is critical for diagnosis and clinical prognosis [19]. There are few studies about the prevalence of ZnT8 antibodies among adult T1DM patients in Iraq/the Middle East, and even worldwide, most studies investigate the prevalence of autoantibodies, including ZnT8 antibodies, in children [20-22]. Therefore, this study assessed the frequencies of different autoantibodies among adults with T1DM. The current study reveals that ZnT8, IA-2A, and GAD65 antibodies were present in all patients with T1DM, whereas the prevalence of autoantibodies was only ≤ 2% in the control group, and there was a significant difference between their concentration in the T1DM patients' group and the control group. This was in alignment with other studies done in adults in different parts of the world [22,23]. In 2024, a systematic review and meta-analysis study about evaluating the role of the ZnT8 antibody in T1DM was done in Brazil and showed that there is a connection between ZnT8 antibodies and T1DM occurrence and progression [24,25]. ZnT8 autoantibodies are useful tools for early diagnosis and risk stratification because they can be seen in the serum of people with T1DM, frequently years before clinical symptoms appear [26]. It has been demonstrated that these antibodies have a high specificity for T1DM and are barely detectable in other autoimmune diseases [27]. Furthermore, it has been demonstrated that ZnT8 autoantibody levels correlate with the advancement of beta cell loss, offering a possible biomarker for tracking disease activity [28]. The prevalence of ZnT8 antibody was higher in SPIDDM (88%) compared to patients with acute-onset T1DM (75%). This finding contrasts with another study conducted in Japan, which revealed that ZnT8 prevalence was significantly higher in acute-onset T1DM than in SPIDDM. This variation could be linked to differences in age groups, the length of diabetes, the proportion of T1DM subtypes, or even the differing cut-off values of the ELISA kits utilized in both studies [23]. In this study, ZnT8A frequency was substantially greater in the patient age group (16-30 years) (53.3%) than in other age groups with disease duration more than 10 years (p< 0.05). Some studies showed that serum concentration of ZnT8 increased more with increased duration of the disease, while others did not. There are controversial results that require more research to be done to evaluate the presence of ZnT8 antibody and T1DM duration and occurrence of complication [23,29]. In the present study, no correlation had been found between the presence of autoantibodies, gender, duration of disease, and GAD65A antibodies (p > 0.05). This was inconsistent with another study done in 2018 in Poland on children and adults with T1DM [30]. On the other hand, some studies showed a negative correlation between ZnT8 antibody and T1DM disease duration (OR = -0.350; p < 0.05) [23]. The current study indicates an interaction between the age at sampling and the positivity for ZnT8 autoantibody in patients with T1DM who have had the condition for more than 10 years. Gomes *et al*. reported a negative correlation between ZnT8A levels and age at sampling; however, no correlation was found between age and the onset of diabetes [29]. These findings suggest that in long-term type 1 diabetic patients, the age at which samples are taken may be prioritized over the age of onset when assessing ZnT8 antibody levels.

Study limitations

There were few limitations in this study, like difficulties during sample collection when taking enough blood for the three studies of autoantibodies; some patients refused to give blood for the research. Also, it was better

to collect patients diagnosed with T1DM from diabetes centers than from outpatients' clinics in the hospitals.

Conclusions

This study revealed that measuring the ZnT8 antibody may offer a useful extra marker for Iraqi people suffering from type 1 diabetes mellitus, potentially increasing the number of cases that can be diagnosed and differentiating clinical presentations.

Conflict of interests

The authors declared no conflict of interest.

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Data sharing statement

Supplementary data can be shared with the corresponding author upon reasonable request.

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