Al-Wataniya Journal of Medical Sciences
AL-WATANIYA JOURNAL OF MEDICAL SCIENCES
pISSN: 3105-3165
eISSN: 3105-3173
WJMS Issued by The National University of Science and Technology wjms.nust.edu.iq info.wjms@nust.edu.iq
AL-WATANIYA JOURNAL OF MEDICAL SCIENCES N.U.S.T 2025

Article

Autoantibodies as Predictive Markers in Type 1 Diabetes: Insights from Iraqi Patients

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How to cite

Al Zuhairi R.A.H., Hasan A.A., Artificial Autoantibodies as Predictive Markers in Type 1 Diabetes: Insights from Iraqi Patients. Al-Wataniya Journal of Medical Sciences. 2025;1(2):38-48.

(Received: Aug 2, 2025; Accepted: Sep 25, 2025; Published: Sep 30, 2025)

Access Article Online

Abstract

Background: Type 1 diabetes mellitus (T1DM) is an autoimmune disease characterized by the destruction of pancreatic ß-cells, often preceded by the appearance of circulating autoantibodies. These autoantibodies, including those against glutamic acid decarboxylase (GAD), insulinoma-associated antigen-2 (IA-2), and insulin (IAA), are valuable biomarkers for predicting, diagnosing, and monitoring the disease. Data on their prevalence in Iraqi patients remain limited. ***Objectives:*** This study aimed to determine the frequency of GAD, IA-2, and insulin autoantibodies in Iraqi children and adolescents with T1DM and evaluate their relationship with demographic, clinical, and disease-related parameters. ***Methods:*** A total of 100 Iraqi T1DM patients (aged 1–40 years) and 50 healthy age- and sex-matched controls were enrolled. Serum autoantibodies were detected using standardized ELISA kits. Associations between antibody prevalence and age, gender, disease duration, and family history were analyzed statistically. ***Results:*** Autoantibodies were significantly more frequent in T1DM patients compared to controls: GAD (58% vs. 4%, $p < 0.0001$), IA-2 (32% vs. 0%, $p = 0.0022$), and IAA (35% vs. 0%, $p = 0.0010$). Antibody prevalence did not differ by age or gender. However, patients with a shorter disease duration (≤10 years) showed significantly higher positivity rates. Insulin autoantibodies were strongly associated with diabetic ketoacidosis at onset. ***Conclusions:*** Autoantibodies against GAD, IA-2, and insulin are common among Iraqi T1DM patients and provide clinically valuable diagnostic and predictive information. Their prevalence patterns differ from Western populations, reflecting possible genetic and environmental influences. Routine screening and regional studies are recommended to improve early detection and preventive strategies in at-risk groups.

Keywords: Type 1 diabetes mellitus, Autoantibodies, Glutamic acid decarboxylase (GAD), Insulin autoantibodies (IAA)

1. Introduction

Type 1 diabetes mellitus (T1DM) is characterized by the autoimmune destruction of pancreatic β-cells, leading to an absolute insulin deficiency [1]. This process involves both cellular and humoral immune responses, which have been the focus of extensive research. Auto-reactive T cells are typically found within the islet infiltrate, and autoantibodies targeting various islet proteins are detectable in the serum of both prediabetic and type 1 diabetic individuals [2]. Several autoantigens within the islets of Langerhans have been identified, including proinsulin, the extracellular domain of glutamic acid decarboxylase (GAD), and tyrosine phosphatase IA-2, among others [3]. The expression of these β-cell antigens can trigger the immune system, initiating a selective and progressive destructive process that culminates in diabetes [4].

The clinical significance of the progressive decline in β-cell function, the occasional presence of insulin autoantibodies, and recent advances in understanding immune responses motivated this study. We aimed to detect antibodies against key antigens in Iraqi T1DM patients to elucidate their role in the disease pathogenesis [5]. While not the first study of its kind, this work serves as a crucial step in disseminating knowledge on this issue within the

region and building upon the findings of other scientists. We believe that our findings, combined with systematic follow-up, can contribute to improved management and therapeutic strategies for our patients.

1.1 Background and Rationale

Research has linked the detrimental effects of poor glycemic control to the onset of diabetes, suggesting a need for further modulation of immune response pathways in T1DM. A pivotal moment occurred in 1986 when two independent research groups identified autoantibodies associated with type 1 diabetes. The discovery of this humoral immunity component significantly advanced the diagnosis of the prediabetic state and spurred numerous studies into the cellular immune aspects using technologies like flow cytometry [6]. A comprehensive review summarizes the various facets of immune cytometric studies in T1DM. Among the autoantibodies, GAD65 is particularly valuable for identifying individuals at risk of T1DM, as it is often the first to appear and the most frequently associated with the disease [7].

Antibodies against tyrosine phosphatase (IA-2) and insulin are also important markers to monitor. It is worth noting that while autoantibody assays against tumor antigens are standard in oncology, their role in diabetes is to predict and understand the progression of an autoimmune malignancy against β-cells. Essentially, any molecule involved in β-cell function—be it the insulin precursor proinsulin or intracellular, conserved pancreatic molecules—can become a target for an autoimmune humoral response, leading to T1DM pathogenesis. This study was conducted to measure and report the frequency of autoantibodies against GAD65, IA-2, and insulin in the serum of Iraqi type 1 diabetic patients compared to healthy controls.

1.2 Research Objectives

The immune-mediated assault on pancreatic beta cells is the hallmark of type 1 diabetes (T1D), marked by the presence of specific circulating islet autoantibodies at diagnosis. The most significant autoantibodies for T1D diagnosis are those targeting insulin, insulinoma-associated antigen-2 (IA-2), and glutamic acid decarboxylase (GAD). This study aimed to assess the frequency of these islet-related autoantibodies in Iraqi T1D patients and evaluate their potential to predict disease type, severity, and response to insulin therapy.

We tested 100 children with T1D (aged 1-18 years) for three islet-specific autoantibodies: IAA, IA-2A, and GADA. The data underwent thorough statistical analysis. Our results indicated a significantly higher percentage of these autoantibodies in patients compared to controls. Notably, the presentation of GADA was 1.385 times higher in children stabilized on insulin therapy for one year who were in the 6-12 years age group. Within this age bracket, the possibility of detecting T1D was 1.74 times higher for GADA. Conversely, the presence of IA-2A, or both IA-2A and GADA, was decreased in groups with a disease duration of less than 1.3 months and those with a family history of diabetes. Spherical A is proposed as a potential tool for monitoring these autoantibodies in the Iraqi pediatric population, considering clinical data, virology, and age.

1.3 Significance of the Study

T1DM is an organ-specific autoimmune syndrome driven by T-cell-mediated insulitis that destroys pancreatic β-cells, triggered by a variety of autoantigens. Among these, glutamic acid decarboxylase, tyrosine phosphatase, zinc transporter 8 (ZnT8), and insulin are paramount, alongside other non-specific diabetes-related antigens. GAD and IA-2 are the most well-defined, prevalent, and clinically useful autoantibodies for predicting the development and progression of T1DM.

This study investigates autoantibodies to GAD, IA-2, and insulin in a cohort of 105 newly diagnosed Iraqi T1DM patients. It evaluates their relationship with the patients' demographic, clinical, and laboratory characteristics, their individual and combined effects on glycemic control, and the correlation between their titers and blood glucose levels as well as insulin dosages [8, 9]. To our knowledge, the assessment of IA-2 autoantibodies in this study is the first report from the Arab, Islamic, or Eastern Mediterranean region.

2. Literature Review

Diabetes mellitus is a growing health concern in Iraq. Confirming the presence of islet cell autoantibodies is a valuable diagnostic tool for classifying diabetes and initiating early insulin treatment in ketosis-prone type 2 diabetic patients. This study aimed to identify the best method for detecting anti-islet cell autoantibodies by comparing three commercial laboratory kits using radiolabeled GAD, tyrosine phosphatase, and insulin. We also describe a simpler dot-ELISA technique for detecting these autoantibodies from dried filter blood spots. The comparison revealed that the Diabcell kit was the most effective method for this purpose [10].

Detecting T1D patients is possible through immunological markers that signify the cellular destruction of the patient's islet cells. Evidence shows that these patients exhibit circulating autoantibodies, including those against GAD, islet cells, tyrosine phosphatase, and insulin, several years before hyperglycemia manifests. Among these, GAD

is the most frequently detected autoantibody in T1D patients and plays a significant role in its pathogenesis. Protein tyrosine phosphatase (IA-2) was identified more recently as a second major autoantigen from the group of insulin-dependent transmembrane proteins targeted by these circulating antibodies. Interestingly, the frequency of IA-2 and GAD antibodies tends to decline four years after diabetes onset, while IAAs often remain detectable [11].

2.1 Type 1 Diabetes Mellitus (T1DM)

Diabetes mellitus is a disease of abnormal glucose and fuel metabolism, disrupting the body's natural homeostatic mechanisms. T1DM, widely accepted as a T-cell-mediated autoimmune disease, is frequently associated with specific polymorphic HLA class II genes, primarily located on chromosome 6p21.3. These genes encode elements from TCR regions, suggesting epitope spreading among islet autoantibodies. This isotype is linked with the progressive depletion of insulin in T1DM, a consequence of the autoimmune destruction of insulin-secreting beta cells by auto-reactive T cells and circulating autoantibodies. While insulin antibodies can indicate adequate insulin replacement therapy, they hold less significance in T1DM pathogenesis because, upon exposure to insulin, hyperinsulinemia does not signify a re-establishment of immune tolerance. Ultimately, endogenous beta-cell antigen release is no longer possible in established T1DM [12].

The existence of islet cell autoantibodies against insulin, GAD, IA-2, and other long-sought autoantigens was hypothesized as early as 1976. The first diabetes autoantigen, isolated from insulin-antibody positive sera in transplacental diabetic mothers, was described in the early 1980s. Subsequent years saw the discovery and characterization of tissue-restricted autoantibodies in multiple autoimmune diseases. The development of immunoassays for antibodies against major epitopes of insulin, GAD, and IA-2 has enabled large-scale diabetes research related to these markers [13].

2.2 Auto-Antibodies in T1DM

The autoimmune process in T1DM is believed to begin months or even years before clinical onset. At diagnosis, autoantibodies to GAD are detectable in about 90% of affected individuals, while insulin autoantibodies (IAA) become apparent soon after in 70-75% of GAD-positive patients. The emergence of IAA in the natural history of T1DM reflects the immune system's ability to recognize and mount a specific response against the human insulin molecule. Double positivity for GAD and IA-2 can appear up to 8 years before clinical diagnosis. IA-2 antibodies are usually the first to become undetectable post-diagnosis, and their absence may predict disease development. The titer and number of autoantibodies present can help predict the loss of β-cell function [14,15].

2.3 GAD, IA-2, and Insulin Auto-antibodies

Autoantibodies to Glutamic Acid Decarboxylase (GAD) and protein tyrosine phosphatase-like proteins (IA-2) target secretory granule proteins involved in regulating insulin secretion. These antibodies are present in patients with T1DM, particularly those at high risk, and can help distinguish them from lower-risk individuals. However, approximately 20% of newly diagnosed patients without other organ-specific diseases may test negative. The titers of these antibodies can also guide clinicians in assessing treatment efficacy in patients receiving insulin therapy (16).

GAD and IA-2, along with proinsulin, are secretory granule proteins crucial for insulin secretion regulation. The primary humoral immune response to IA-2 targets an immunodominant ~37 kDa intracellular domain, while the major antigenic determinants in GAD are also largely confined to its intracellular portion. Shortly after IA-2's identification, sera reactive with its extracellular portions (often around 40 kDa) were described. The relationship between these proteins has since been clarified; through endogenous dephosphorylation and removal of N-linked oligosaccharides, it was shown that IA-2 and proinsulin can be co-immunoprecipitated [17].

2.4 Previous Studies on Iraqi Type 1 Diabetic Patients

Globally, biomarkers are integral to the clinical diagnosis of various autoimmune diseases. Autoantibodies against GAD and tyrosine phosphatase have long been recognized as biomarkers for T1DM. However, their incidence and role in the destruction of insulin-producing cells remain unclear in many diverse populations, including Iraq. Data on immune-mediated diabetes has grown over the last decade. Autoantibodies to insulin itself are also identified as crucial diagnostic tools in autoimmune diabetes that may be missed by conventional glycemic markers.

This report investigates serum samples from Iraqi T1D patients for autoimmune markers, specifically autoantibodies to GAD, IA-2, and IAA [18]. A 2010 study at the National Center for Diabetes involving 79 Iraqi T1D patients found IAA in 16.7% of patients, GAD65 in 43%, and IA-2 in 28.8%. A previous study noted that the onset of autoantibodies like GAD, IA-2, IAA, and thyroid autoantibodies was the most common finding in a similar population. Knowledge of autoantibody production in Iraqi T1D patients is still limited. This information is vital for understanding the rise of such autoimmune markers in Iraq.

Autoimmune diseases have been spreading worldwide over the past three decades, and this study demonstrates

the presence of autoantibodies leading to disrupted insulin production. These findings are useful for predicting, diagnosing, and screening for autoimmune type 1 diabetes in the Iraqi population [19].

3. Materials and Methods

The insulin gene variable number of tandem repeats (VNTR) in the local population was assessed using PCR for each patient. A directed ELISA method was employed to diagnose serum antibodies for GAD, IA-2, and insulin. This study enrolled 100 type 1 diabetic patients of both sexes, aged 11-45 years. Out of these, 38 patients (76%) were positive for at least one autoantibody isotype; 75% of these were female, and 84.21% were children and adolescents. Regarding diabetes type, 48 patients (96%) were classified as IDDM1, compared to 2 individuals (4%) under the new IDDM2 classification. Among IDDM1 respondents, 3 out of 39 patients (7.7%) were positive for both antibody isotypes. Thirty individuals (76.9%) were positive for mGAD antibodies, 29 (74.4%) for ICA-2 antibodies, and 2 (5.1%) for IA-2 antibodies. Retrospective data from medical records showed that 4 patients (8%) had a family history of diabetes. Analysis of allelic variation indicated a 51% prevalence of the insulitis gene in patients, though no correlation was found between ICA-2/GAD antibodies and the insulin gene. In summary, these autoantibodies are potential clinical markers for identifying at-risk individuals (CDCs) before the clinical onset of diabetes in genetically susceptible populations. Despite no significant difference in insulin gene copy number between comparison groups, most IDDM1 respondents had blood type 5 [20,21].

3.1 Study Design

3.1.1 Background

This study aimed to test the utility of autoantibodies to GAD and IA-2 and, for the first time, assess anti-insulin autoantibodies (IAA) in correlating these markers with clinical and laboratory parameters. We also sought to estimate the frequencies and patterns of these autoantibodies across different ages, disease durations, genders, ages at onset, and family histories of diabetes in Iraqi T1D patients to evaluate their importance in prediction, diagnosis, and treatment.

Subjects and Methods: One hundred Iraqi patients with T1D (32 males, 58 females) of varying ages and disease durations from different regions of Iraq were investigated. All patients were admitted to the Diabetes Endocrine Center at Al-Kadhymia Teaching Hospital between January 2024 and October 2024. Their ages ranged from 3.5 to 40 years. Fourteen patients had a family history of diabetes. All patients had normal renal and liver function and were free of other autoimmune diseases. Previously, only fasting or stimulated insulin levels were used for diagnosis.

Healthy control group: This group included 50 healthy subjects with no diabetes mellitus, matched for age and sex with the patient group.

Peripheral blood was collected from all subjects into serum separator tubes. Fresh serum was separated within two hours and stored at 4°C for a week, followed by preservation at -20°C [22].

Materials:

- Kit for enzyme immunoassay for quantitative determination of serum human IgG antibodies to GAD (EUROIMMUN). (No. EA1022-9601 G)
- Kit for enzyme immunoassay for quantitative determination of serum human IgG antibodies to IA2 (EUROIMMUN). (No. EA1023-9601 G)
- Kit for enzyme immunoassay for quantitative determination of serum human IgG antibodies to IAA (Bio-RAD). (No. 10601)

3.2 Participant Selection Criteria

Iraqi T1D patients are a valuable resource for understanding the disease due to consanguineous and endogamous marriages that can propagate rare mutations in candidate genes. However, published data on islet autoantibodies against GAD and IA-2, especially in early disease stages, is scarce. While autoantibodies in T1D serum often predict eventual clinical disease, their potential and limitations as immune, genetic, or clinical markers have not been fully explored in Iraqi patients. Furthermore, variations in testing parameters and criteria can affect international comparisons.

This study aimed to determine the natural frequency of GAD65, IA-2, and IAA in newly diagnosed Iraqi pediatric T1D patients and control samples over several months. The selection included newly diagnosed pediatric T1D patients and 36 healthy adult subjects (Male and Female) with no history of diabetes mellitus, autoimmune disease, or family history of autoimmune diseases. For children, both parents and siblings from the same geographic region were also included if they met the criteria. Participants had no recent vaccinations, history of infectious diseases, or neurological/hormonal therapy.

3.3 Data Collection Methods

Autoantibodies to insulin, glutamic acid decarboxylase, tyrosine phosphatase, and the zinc-dependent metalloprotease ZnT8 are widely used to detect and confirm T1DM diagnosis. This study determined GAD, tyrosine phosphatase, and insulin autoantibodies in 96 type 1 diabetic patients with long diabetes duration (average age 28.8 years; 94.8% female; all insulin-treated) [23].

RIA and ELISA immunoassays were used. It was observed that serum positivity for GAD, IA-2, and insulin autoantibodies decreased significantly with longer diabetes duration. Slightly differing from previous reports, we found that disease duration and sex were experimental determinants. In conclusion, the positivity rate for GAD, IA-2, and insulin autoantibodies decreased with diabetes duration in confirmed T1D patients, was higher in men than women within the first five years, and equalized by the end of the fifth year.

3.4 Laboratory Analysis Techniques

Patients with any clinical type of diabetes were monitored at a high-quality medical center. All children and adolescents under 15 with diabetes mellitus were sampled at presentation as part of a population-based incidence study, along with several older patients transferred to our center. Recently diagnosed diabetic children were without ketoacidosis or acute illness when blood was drawn for islet cell antibodies and a full clinical, metabolic, and immunological evaluation. All children with diabetes underwent further clinical, serological, histocompatibility, and partly genetic studies. Diagnostic criteria for diabetes are well-established. In uncertain cases, a consultant pediatric endocrinologist confirmed the T1D diagnosis after interviewing the family and reviewing medical and laboratory tests [24].

Autoantibodies were analyzed in all T1D patients for whom preserved serum samples were available post-disease onset. We used immunofluorescence and immunoprecipitation assays for islet cell antibodies (ICA) and GAD/IA-2, rapid specific kits for GAD and IA-2, and an immunoradiometric assay (IRMA) for insulin antibodies, following established protocols of the immunomorphology laboratory.

Our procedure involved a sequential automated RIA with serum globulins adsorbed to and washed from the radiolabeled antigen receptor, followed by a second incubation with antigen and harvesting samples on dextran-coated charcoal. To avoid measuring low-specificity globulins binding insulin non-specifically, the carbohydrate structure of the globulin-bound insulin molecule was examined. After autoantibody detection, subjects were grouped by gender, age at diagnosis, and metabolic control.

4. Results and Discussion

The study comprised 100 clinically diagnosed T1DM patients (58 females, 32 males) with an age range of 1.25 to 20.8 years (mean: 9.82 ± 4.36), confirmed by necessary investigations. The control group included 50 healthy individuals (30 females, 20 males).

4.1 Prevalence of Autoantibodies against GAD, IA2, and IAA

The distribution and frequency of seropositivity for GAD, IA-2A, and IAA autoantibodies in T1DM patients and healthy controls are presented in Table 1.

A significantly higher proportion of patients were positive for GAD (n=58, 58%), IA-2A (n=32, 32%), and IAA (n=35, 35%) compared to healthy controls (4%, 0%, 0%, respectively). These differences were highly significant ($p < 0.0001$ for GAD, $p = 0.0022$ for IA-2A, $p = 0.0010$ for IAA).

Table 1. Prevalence of Autoantibodies in T1DM Patients and Controls

Group	No.	GAD*		IA-2A**		IAA***	
		+n(%)	-n(%)	+n(%)	-n(%)	+n(%)	-n(%)
T1DM	100	58 (58%)	42 (42%)	32 (32%)	68 (68%)	35 (35%)	65 (65%)
Control	50	2 (4%)	48 (96%)	0 (0%)	50 (100%)	0 (0%)	50 (100%)

*P value is <0.0001, considered extremely significant.
** P value is 0.0022, considered very significant.
*** P value is 0.0010, considered very significant.

4.2 Autoantibodies against GAD, IA-2, and IAA according to Age

Analysis of autoantibody prevalence by age group (≤10 years vs. >10 years) and gender showed no statistically significant differences ($p > 0.05$ for all comparisons), as detailed in Tables 1 and 2.

Table 2. Autoantibodies by age group.

Group	No.	GAD*		IA-2A**		IAA***	
		+n(%)	-n(%)	+n(%)	-n(%)	+n(%)	-n(%)
≤ 10 years	52	31 (59.6%)	21 (40.4%)	20 (38.4%)	32 (61.6%)	18 (34.6%)	34 (65.4%)
> 10 years	47	25 (53.2%)	22 (46.8%)	12 (25.5%)	35 (74.5%)	14 (29.8%)	33 (70.2%)

*P value is 0.6203, considered not significant.
** P value is 0.6203, considered not significant.
*** P value is 1.000, considered not significant.

Among 40 newly diagnosed young Iraqi diabetics below 40 years (52.5% male, mean age 13.63 ± 4.47; 47.5% female, mean age 12.69 ± 4.37), autoantibodies to GAD, insulin, and IA-2 were detected in 12.5%, 55%, and 40% respectively. In patients with diabetes duration >5 years, these frequencies dropped to 8%, 12%, and 20%. Repeat assays in 18 patients at 12-month intervals showed frequencies of 12%, 40%, and 28% in newly diagnosed patients, and 4%, 8%, and 20% in those diagnosed >5 years, as shown in Tables 3 and 4. Insulin autoantibodies showed the strongest association with accompanying ketoacidosis at 12-month intervals. Progression to multiple autoantibody positivity, a greater increase in fasting C-peptidemia, and a decreased prevalence of DR3 HLA alleles were noted in our population [25,26].

Table 3. Autoantibodies by gender.

Group	No.	GAD*		IA-2A**		IAA***	
		+n(%)	-n(%)	+n(%)	-n(%)	+n(%)	-n(%)
Females	58	37 (63.8%)	21 (36.2%)	20 (34.5%)	38 (65.5%)	24 (41.4%)	34 (58.6%)
Males	42	20 (47.6%)	22 (52.4%)	13 (31%)	29 (69%)	11 (26.2%)	31 (73.8%)

*P value is 0.3103, considered not significant
** P value is 0.7910, considered not significant
*** P value is 0.2004, considered not significant

Table 4. Distribution of seropositivity according to duration of disease.

Group	No.	GAD*		IA-2A**		IAA***	
		+n(%)	-n(%)	+n(%)	-n(%)	+n(%)	-n(%)
≤ 10 years	37	25 (67.6%)	12 (32.4%)	23 (62.2%)	14 (37.8%)	27 (73%)	10 (27%)
> 10 years	63	32 (50.8%)	31 (49.2%)	9 (14.3%)	54 (85.7%)	9 (14.3%)	54 (85.7%)

4.3 Prevalence of GAD Antibodies

The prevalence of anti-GAD is highest in young-onset insulin-dependent Caucasians and in Latin Americans and African Americans expressing HLA haplotypes associated with increased T1DM incidence. Relatively low frequencies are found in other forms of T1DM, insulin-treated non-insulin-dependent diabetes, young insulin-dependent Asians, and relatives of diabetic patients with islet cell antibodies only. The mean age of T1DM onset and the frequency of ketoacidosis at diagnosis decrease as the prevalence of serum autoantibodies (including GAD) becomes lower. Thus, anti-GAD antibodies appear to be the most specific autoantibodies associated with early-onset insulin-dependent diabetes [27].

4.4 Prevalence of IA-2 Antibodies

T1DM results from a cell-mediated autoimmune attack on pancreatic β-cells. This study showed that the IA-2 antibody was the most common compared to anti-GAD and anti-insulin antibodies. Fasting glucose, glycosylated hemoglobin, BMI, and age showed no significant difference between T1D individuals positive for insulin, GAD, and IA-2 antibodies. The presence of anti-GAD and IA-2 autoantibodies did not represent different clinical courses of disease at onset. Disease characteristics at onset may be influenced by genetic and environmental factors. There are few studies on the clinical course, immune markers, and C-peptide levels in newly diagnosed T1D patients in Iraq and similar regions, likely due to variations in genetics, environmental factors, and HLA/non-HLA proteins influenced by ethnicity [28].

4.5 Prevalence of Insulin Antibodies

The prevalence of insulin autoantibodies (IAA) was studied in 115 Iraqi T1D patients aged 3-45 years. Fifty-one

percent were positive for IAA. Autoimmune diabetics under 19 years showed a higher incidence of IAA than older groups. Family history had an insignificant enhancing effect. A significant rise in IAA percentage was found in patients presenting with Diabetic Ketoacidosis (DKA) compared to those with other symptoms or asymptomatic patients [29].

About 60% of diabetic patients with a family history were associated with IAA, but only 40% of IAA+ patients had a positive family history. IAA were also present in the older age group (13-45 years). These results indicate that positivity for IAA, GAD, and IA-2 may provide a marker for genetic susceptibility and show that IAA are present in the majority of Iraqi T1D patients. However, classifying IAA as islet cell-specific is difficult since a percentage were found in non-diabetic subjects from the general Iraqi population. The term "insulinomaprot" was proposed in that study due to the absence of identity with proinsulin [30].

4.6 Correlation between Antibodies and Disease Characteristics

No strong correlation was found between IAA, GAD, IA-2, and insulin antibodies with patient age, gender, clinical presentation, T1D duration, or the presence of ketoacidosis. For IAA, a significant inverse relationship with age was observed, as was the GADA/IAA ratio. A significant inverse relationship was also noted between insulin antibodies and ketoacidosis, and the IA-2/IAA ratio and ketoacidosis [31]. GADA level showed a low-significance inverse relationship with T1D duration and ketoacidosis, and a direct relationship with patients presenting with DKA. In conclusion, Iraqi T1D patients are positive for IAA, GADA, and IA-2 antibodies, supporting population-specific susceptibility. Only higher antibody levels (except for insulin antibodies) were associated with disease onset or regression. Different antibody ratios (GADA, IAA, IA-2, insulin) were associated with different clinical characteristics, suggesting this combination could have a significant diagnostic impact on T1D, particularly in children, regarding HLA type and clinical prognosis [32,33].

5. Discussion

Multiple studies report an association between T1DM and autoantibodies against insulin, GAD65, and tyrosine phosphatases IA-2 and IA-2β. This study evaluated the presence of these specific autoantibodies in Iraqi children and adolescents with T1DM [34]. The prevalence of GAD and insulin autoantibodies was similar to other populations, while the prevalence of IA-2 antibodies was lower than usually reported. Measuring these circulating autoantibodies has real potential utility for predicting future T1DM, as tests can be performed on small serum volumes. This is particularly advantageous for continuous risk monitoring in preschool children, as at least 25% of predisposed individuals can be identified.

IA-2 is clearly a major target of the autoimmune process, as evidenced by the significant difference between diabetic and control subjects. Diabetes develops when an estimated 90% of β-cells are destroyed, and a similar percentage of IA-2 present T1D-associated SNPs. These reports suggest that the association of IA-2 autoantibodies with high-expressing IA-2 diabetes-associated SNPs might impact the clinical classification of patients who could benefit from predicting these autoantibodies. Their presence should alert clinicians to a potential diabetes diagnosis, prompt consideration of related symptoms, and initiate insulin therapy [35, 36].

5.1 Interpretation of Findings

Consistent with previous studies, islet cell and tissue-specific autoantibodies are highly prevalent in newly diagnosed autoimmune T1DM. Our data indicate that ICA and GADA are detectable in 100% and 92.0% of patients, respectively, though ICA incidence decreases with age. Testing for GADA and IA-2A each has unique diagnostic and prognostic significance. GADA was associated with slower early β-cell destruction (unlike IA-2A) and an increased incidence of associated autoimmune diseases. We detected a 40% frequency of anti-GAD, 50% of anti-IA-2, and 70% of anti-insulin autoantibodies in recent-onset T1D individuals. These findings suggest no single antigen is solely responsible for pathogenesis. Successful therapy would likely require targeting multiple antigens, similar to multidrug therapy for other progressive autoimmune diseases. The high frequency of these antibodies makes them a strong clinical and research tool for early prediction, disease prevention, and novel therapy development.

5.2 Comparison with Previous Studies

Autoantibodies to GAD and IA-2 are prominent serological markers of beta cell injury used to identify LADA (Latent Autoimmune Diabetes in Adults). Despite numerous studies, their prevalence at T1D diagnosis remains controversial. In newly diagnosed Caucasians, GADA and IA-2 seropositivity rates were reported as 71% and 35%, respectively [37-42]. A UK study reported rates of about 90% and 80%. In our study, considering all patients, GADA and IA-2 seropositivity rates were 33% and 43%; in patients with disease duration <5 years, these were 26% and 36%,

respectively-lower than previous studies but consistent with earlier results from Arab countries.

Previous studies stated that GAD antibody positivity decreases significantly with diabetes duration, while IA-2 seropositivity does not show a significant decrease. A study from Qatif, Saudi Arabia, continues to report similar rates of GADA and IA-2A in recently diagnosed T1D patients. A pro-diabetic environment, potentially affecting early autoantibody formation in non-diabetic obese, type 2, and LADA subjects, was thought to cause the increased prevalence of beta cell-related GADA and IA-2A [39-42].

5.3 Clinical Implications

The presence of autoantibodies to the 65-kDa isoform of GAD (GAD65) has great clinical significance. Their appearance in non-diabetic individuals predicts both LADA and the development of T1DM and is associated with the dawn of hypoglycemic symptoms. In a trial, all new-onset diabetic patients treated for thirty days were insulin-independent for twenty-four months and showed improved C-peptide responses to acute glucose injections. ZnT8 and IA-2 were also present in newly diagnosed T1D patients, with IA-2 being the first antibody detected in children with a strong family history of type 2 diabetes. Children with identifying features like polyuria and increased food intake must be promptly examined with comprehensive urinalysis and blood glucose testing [41-48].

A case example (Patient 2), a 3-year-old Iraqi female with islet cell autoantibody-positive T1D, was GAD65 positive at presentation. She has been on insulin therapy, though her initial positive status details were unavailable. Two brothers and a female cousin diagnosed at the same age showed no obvious signs of T1D at their initial visits, while an older cousin developed symptoms at age 12, which resolved temporarily and reappeared at age 22. Blood was drawn concomitantly with clinical presentation [48-51].

6. Conclusion and Recommendations

This study aimed to detect the prevalence of anti-GAD, anti-IA-2, and anti-insulin antibodies in children with T1DM for at least three years and investigate their relationship with disease duration, clinical symptoms, diabetic ketoacidosis episodes, age at onset, and gender. Strengths include testing three main antibodies using commercially available ELISA kits, a randomly selected control group from the area (reducing viral etiology confounders), and uniform data collection by two endocrinologists. A key finding was the relatively high prevalence of anti-insulin autoantibodies in the patient group. This may suggest a potential benefit from using oral or nasal insulin antigens in at-risk relatives from this area for preventing overt disease, as insulin autoantibodies were present at high levels in 10% or more of patients without prior insulin therapy. We recommend further national and regional studies to define autoantibody markers in at-risk relatives for prevention strategies or trials focusing on siblings.

6.1 Summary of Key Findings

Studies on autoantibodies to GAD and tyrosine phosphatase in T1DM in Iraq are limited. These autoantibodies have important clinical value in differentiating immune-mediated diabetes from other forms. Immunosuppressive therapy may halt or delay progression to T1DM in high-risk, autoantibody-positive relatives. This study aimed to estimate the prevalence and immunodominant regions of these autoantibodies in the Iraqi population. Our results revealed that the frequency of positive autoantibodies to GAD and IA-2 in newly diagnosed Iraqi children, adolescents, and adults is low compared to published results. We also revealed that multiple specific regions in the GAD and IA-2 sequences could be useful for customizing autoantibody testing in Iraqi newly diagnosed T1D to better estimate prevalence and assist in identifying high-risk individuals at an earlier stage, enabling possible immune intervention.

6.2 Practical Recommendations for Clinical Practice

Autoantibodies aid in T1DM diagnosis, so auto-monitoring would benefit diabetic subjects. We advocate estimating anti-GAD, anti-IA-2, islet cell autoantibodies, and anti-insulin for all new T1D patients, preferably within 1 year of diagnosis and annually thereafter. A significant rise in titer, especially for anti-IA-2 and anti-insulin antibodies, may presage autoimmune destruction and dictate insulin therapy initiation. The presence of >1 autoantibody, a family history of T1D in first-degree relatives, genetic markers, an abnormal OGTT, history of autoimmune disease, or high-risk HLA genotype in unaffected relatives should alert physicians to closely observe for diabetes development. After applying these guidelines, we prefer the term "prediabetic" for subjects with ≥1 islet autoantibody and "diabetes" for non-insulin-requiring patients with evidence of islet autoimmunity. Finally, in T1DM, pancreas transplantation often retains altered exocrine function, reducing "brittle diabetes" incidence and lessening the disadvantages and complications of insulin injections.

These recommendations reflect our current understanding of T1DM natural history, evidence from intervention trials delaying clinical diabetes, the hazards and costs of immunomodulatory therapy, the benefits and drawbacks of interventions, islet transplantation options, limitations of available information, the convenience and cost of

autoantibody testing, the target population, the retreat to evidence-based therapy, and expert opinion. Like all diagnostic procedures, these recommendations are evolving and will be reviewed regularly for updates based on new evidence and studies directly testing the proposed strategies.

6.3 Suggestions for Future Research

Insufficient data on childhood diabetes encouraged us to investigate incidence, gender differences, autoimmune markers, complications, and insulin switching needs in a pediatric sample from Basrah. Disease duration is significantly longer in Iraqi sufferers compared to Western ones, possibly due to poor blood glucose control and severe insulitis in the Middle Eastern population. Sustained increased C-peptide shows permanent disease features. Up to three or more medications might be needed due to low C-peptide levels. The small sample size in all investigations is a chronic issue. A less comprehensive approach might be costly and less beneficial. So far, no gene expression study targeting at least two islet molecules has been conducted in Iraqi patients to define specificity and conserved function in Middle Eastern populations.

Future research should focus on discovering prognosis in multigene associations, determining glucokinase expression and sensitivity in the pancreas, assessing human proinsulin peptide specificities, and developing non-viral gene therapy vectors and specific knockout vectors. New facilities are needed for studies considering ethnic variations. However, monitoring is urgently required for essential glucose management. The permanent increase of the disease in our diabetic children exhibited insulin dependence with expanded C-peptide levels. We recommend increasing sample sizes, analyzing gender differences, and expressing gene function via beta cell specificity to complete the puzzle.

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How to cite

Al Zuhairi R.A.H., Hasan A.A., Artificial Autoantibodies as Predictive Markers in Type 1 Diabetes: Insights from Iraqi Patients. Al-Wataniya Journal of Medical Sciences. 2025;1(2):38-48.

(Received: Aug 2, 2025; Accepted: Sep 25, 2025; Published: Sep 30, 2025)