

Relationship between Type 1 Diabetes Mellitus and Insulinoma-Associated Antigen-2 Autoantibodies in Kerbala Province

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Abstract

Background: Case control study was conducted at Imam Al-Hussein Medical City and Imam Al-Hassan Al-Mujtaba Hospital in Karbala, and it included information about changes in some immunologic parameters in type 1 diabetes mellitus (T1DM) patients. The study comprised 30 healthy and 60 patients aged 5 to 20 years from female and male between January 2024 and October 2025. This study measured immunological alterations include antibodies levels such as Insulinoma Associated Antigen-2 autoantibodies (IA-2A), immunoglobulins (IgM), Proinflammatory cytokines (IFN- γ) and anti-inflammatory cytokines (TGF- β 1). The present study discovered that There was a significant increase ($P \leq 0.01$) in IFN- γ , TGF- β 1, IA-2A and IgM in patients group compared with healthy group. The goal of this research is to investigate the immunological profile of patients with T1DM in Kerbala Province by analysing the levels of IA-2 autoantibodies (IA-2A), interferon-gamma (IFN- γ), transforming growth factor- β 1 (TGF- β 1), and immunoglobulin M (IgM). The purpose of this study is to ascertain how these immunological markers relate to the pathophysiology of type 1 diabetes, ascertain their possible roles in the disease's progression, and find patterns that could help with early diagnosis, better monitoring, and a deeper comprehension of the autoimmune mechanisms underlying T1DM in the local population.

Keywords: Type 1 diabetes mellitus, IA-2A , IFN- γ , TGF- β 1 and IgM



1. Introduction

Type 1 diabetes mellitus (T1DM) is an autoimmune illness that causes gradual death of β -cells in pancreatic islets, resulting in loss of endogenous insulin secretion, hyper-glycaemia, and lifelong need on exogenous insulin. Over the recent period, There are now more people with this illness overall (Gregory *et al.*, 2022). This disease's pathophysiology is not well understood, and therapy options are limited, necessitating further research and innovation (Cudini & Fierabracci, 2023). T1DM is induced by an autoimmune attack on β -cells by CD4+ and CD8+ T lymphocytes that are drawn in by islet autoantigens. One predictive risk factor is the presence of islet-specific autoantibodies (Ilonen *et al.*, 2019). Approximately half of the known polymorphisms are found in the region of human leukocyte antigen (HLA) genes, particularly class II HLA on the 6p21 chromosome; additionally, about 60 loci other than HLA have been found to reliably affect the risk of disease progression. Currently, about 90 genetic loci have been linked to T1DM, containing risk factors that facilitate T1DM initiation and progression (Herold *et al.*, 2024). T1DM development involves a variety of immune cell populations, including CD8+ T-cells (CD8+ stem T-cells, affected CD8+ T-cells, effector memory T-cells, central memory T-cells, exhausted CD8+ T-cells, and regulatory CD8+ Treg), CD4+ T-lymphocytes (naïve and effector Th1, Th2, Th17, follicular T-helpers Tfh, and regulatory CD4+ Treg), and B-cells (CD20+ B-cells, Breg, etc.) (Abdelsamed *et al.*, 2020; Chen *et al.*, 2024). In spite of several research, the pathophysiology of T1DM, including fundamental causes and specific mechanisms of islet autoimmunity, remains unclear. In theory, Early childhood viral infections boost T-cell immunity to beta-cell antigens and induce viral mimicry (Wagner *et al.*, 2025). T1D makes up 5–10% of all diabetes diagnoses and typically affects children and teenagers, however it can strike at any age (Popoviciu *et al.*, 2023). Patients with type 1 diabetes have a higher risk of stroke, which is most likely brought on by vascular dysfunction brought on by hyperglycemia (Liu *et al.*, 2021). Through insulin insufficiency, lipolysis, and metabolic dysregulation, T1D results in non-alcoholic fatty liver disease and fibrosis (Addissouky *et al.*, 2021). One of the most common endocrine and metabolic conditions affecting children is type 1 diabetes. T1DM-related autoimmunity leads to the loss of β -cells in 70-90% of patients, accompanied by the development of T1DM-associated autoantibodies. These patients have autoimmune T1DM, also known as type 1a diabetes mellitus (Care, 2015). Triggers of β -cell-targeted autoimmunity may differ among nations because to differences in infections and herd immunity, as seen in a study comparing β -cell-targeted autoimmune and T1DM in Finland and Russia (Kondrashova *et al.*, 2013). There have been various environmental variables identified as significant in the development of T1DM. Some of the most commonly stated environmental variables are gut microbiota loss, obesity, early exposure to fruit or cow milk as a child, gluten, pollutants, vitamin deficiency, and viruses (Adamczak *et al.*, 2014). Weight gain is another environmental factor in diabetes that causes an increase in beta-cell burden and insulin resistance (Hindmarsh *et al.*, 1988). Infant weight increase has been identified as a risk factor for T1DM later in childhood (Wilkin, 2001).

2. Materials and Methods

2.1 study groups and blood samples collection

The case-control study was conducted at Imam Al-Hussein Medical City and Imam Al-Hassan Al-Mujtaba Hospital in Karbala, and it included information about changes in some immunological markers in type 1 diabetes mellitus patients. The study comprised 30 healthy include female (13) and male (17) and 60 patients include female (42) and male (18) aged 5 to 20 years from female and male

between January 2024 and October 2025. Five millilitres of venous blood were collected. To achieve complete serum separation, 5 ml of samples were transferred immediately into plain plastic tubes. These tubes were then immersed in a 37 C° water bath for 10 minutes and centrifuged at 3000 rpm for 15 minutes for the assessment of immunological parameters such as IFN- γ , TGF- β 1, AntiIA-2 and IgM.

2.2 Parameters of study

2.2.1 Immunological parameters

2.2.1.1 Autoantibodies

2.2.1.1.1 Insulinoma Associated Antigen-2(IA-2A)

Insulinoma-associated antigen 2 antibodies were detected by ELISA, The upper limit of the typical range (cut-off value) suggested by (EUROIMMUNE/ Germany) is 20 international per milliliter (IU/ml), so outcomes upper 20 (IU/ml) is positive

2.2.1.2 Immunoglobulins

2.2.1.2.1 IgM

The single radial immunodiffusion method was used to measure the concentration of serum IgM in the gel, using the kit provided by LTA S. R. I via Milano, according to the supplier's instructions, and based on the principle of (Mancini et al., 1965), which described the linear relationship between the antigen concentration and the radius of the immune precipitation ring formed in the agar containing the specific antibodies for that antigen.

2.2.1.3 interleukins

2.2.1.3.1 IFN- γ and TGF- β 1

Serum levels of IFN- γ and TGF- β . This was accomplished using the enzyme-linked immunosorbent test (ELISA) technology, which is based on the principle of color distinction caused by the binding of particular antibodies to the antigen and the standard calibration by the British firm PepRotech.

2.3 Inclusion Criteria

- 1-Age from 5 to 20 years.
- 2-Clinically diagnosed with Type 1 Diabetes Mellitus using ADA criteria for patients.
- 3-Disease duration: at least 6 months (to rule out temporary or stress-induced hyperglycemia) for patients.
- 4-No history of diabetes or autoimmune/inflammatory illness to manage.

2.4 Exclusion Criteria

- 1-The presence of other autoimmune conditions (e.g., Hashimoto thyroiditis, rheumatoid arthritis, celiac disease, and SLE).
- 2-Acute or chronic infections within the last four weeks.

- 3- Chronic inflammatory illnesses (such as IBD and psoriasis).
- 4-Use of immunosuppressive medications or corticosteroids within the previous three months.
5. Pregnant or lactating ladies.
- 6-History of cancer or renal/hepatic dysfunction.
7. Recent significant surgery or trauma (within three months).

2.5 Ethical considerations

2.5.1 Ethical Approval:

The Ethical body / Institutional examine Board (IRB) of the College of Applied Medical Sciences, University of Kerbala (or the relevant local ethical body) (ethical No. PAAMSKU/3 on 7 July 2025) will examine and approve the study protocol. The approval number and date will be logged before data collection begins.

2.5.2 Informed Consent:

All participants will be asked to provide written informed permission before being included in the research. Participants under the age of 18 will be required to provide parental/guardian agreement in addition to the child's assent. Participants will be told about the study's goals, methods, potential hazards, and benefits, as well as their freedom to withdraw at any time without penalty.

2.5.3 Confidentiality and Privacy:

To ensure anonymity, all personal identification will be replaced with coded numbers. Data and biological samples will only be used for the research reasons specified in the protocol. Records will be kept safe and available only to approved research workers.

2.5.4 Risk and Benefit Assessment:

The research has low risk associated with blood sample collection (minor discomfort or bruise at the venipuncture site). There are no direct health dangers or intrusive treatments. Participants may gain indirectly from a better knowledge of immunological systems in Type 1 diabetes, as well as future therapeutic care improvements.

2.5.5 Sample and Data Handling:

Blood samples will be taken under sterile circumstances by qualified personnel. Biological samples will be maintained in a biobank at -80°C for immunological investigation and destroyed when laboratory processes are completed. Genetic testing will not be undertaken without extra permission.

2.5.6 Voluntary Participation:

Participation is entirely optional. Refusing to participate will have no effect on medical care or the relationship between the patient and the physician.

2.6 Statistical analysis

The data was presented as mean $\pm$ SD and analyzed using one-way ANOVA. ANOVA The

post-hoc test LSD was performed to detect significant differences between means, and data was analyzed using the software package IBM SPSS Program version 20 (*SPSS Statistical Packages for the Social Sciences: Statistical Software for Windows Version 13.0*, 2001).

3. Results

3.1 antibodies and immunoglobulins

Results The current study found that there is significant increase in IA-2A and IgM in type 1 diabetes patients compared with healthy shown in table (1)

Table 1: effect of type 1 diabetes mellitus on some antibodies level and immunoglobulins (Means $\pm$ SD)

Parameters	Groups		P-value
	Patients	Healthy	
IA-2A (U/mL)	A 13.32 $\pm$ 3.51	B 4.21 $\pm$ 2.12	p < 0.001
IgM (pg/ml)	A 196.65 $\pm$ 31.10	B 98.67 $\pm$ 38.14	p < 0.001

Different letters represent a significant difference at (p $\leq$ 0. 01)

3.2 pro and anti-inflammatory cytokines

Results The current study found that there is significant increase in IFN- γ and TGF- β 1 in type 1 diabetes patients compared with healthy shown in table (2)

Table 2: effect of type 1 diabetes mellitus on some pro and anti-inflammatory cytokines (Means $\pm$ SD)

Parameters	Groups		P-value
	Patients	Healthy	
IFN- γ (pg/ml)	A 1.68 $\pm$ 0.24	B 0.41 $\pm$ 0.88	p < 0.001
TGF- β 1 (pg/ml)	A 1.49 $\pm$ 0.19	B 0.67 $\pm$ 0.15	p < 0.001

Different letters represent a significant difference at (p $\leq$ 0. 01)

3.3 correlation between study parameters in type1 diabetes mellitus patients

Results of present study showed that there is a positive significant correlation between IFN- γ and TGF- β 1, while there is a negative significant correlation between IgM and TGF- β 1 in type1 diabetes Mellitus patients shown in table (3)

Table 3: correlation coefficient between study parameters (Anti IA-2, IgM, IFN- γ and TGF- β 1) in type1 diabetes Mellitus patients

Parameters		Parameters			
		IA-2A	IgM	IFN- γ	TGF- β 1
IA-2A	r p-value	1	0.238 0.067	-0.095 0.472	-0.247 0.057
IgM	r p-value	0.238 0.067	1	-0.002 0.986	-0.383** 0.002
IFN- γ	r p-value	-0.095 0.439	-0.002 0.986	1	0.507** 0.0001
TGF- β 1	r p-value	-0.247 0.057	-0.383** 0.002	0.507** 0.0001	1

Note:** At a level of 0.01 (2-tailed), correlation is significant (pearson correlation, sig.(2-tailed))

3.4 correlation between study parameters in healthy

Results of present study showed that there is a positive significant correlation between IA-2A, IgM and TGF- β 1, also there is a positive significant correlation between IgM and TGF- β 1 in healthy shown in table (4)

Table 4: correlation coefficient between study parameters (Anti IA-2, IgM, IFN- γ and TGF- β 1) in healthy

Parameters		Parameters			
		IA-2A	IgM	IFN- γ	TGF- β 1
IA-2A	r p-value	1	0.375* 0.041	-0.255 0.174	0.293** 0.006
IgM	r p-value	0.375* 0.041	1	0.065 0.733	0.668** 0.0001
IFN- γ	r p-value	-0.255 0.174	0.065 0.733	1	0.104 0.583
TGF- β 1	r p-value	0.493** 0.006	0.668** 0.0001	0.104 0.583	1

Note:** At a level of 0.01 (2-tailed), * At a level of 0.05 (2-tailed) correlation is significant (pearson correlation, sig.(2-tailed))

3.5 Distribution of type1 diabetes Mellitus patients and healthy according to age

Results of present study showed that ages (5-10), (11-15) and (16-20) year are formed percentage (45%), (41.67%) and (13.33%) respectively in type1 diabetes Mellitus compared with healthy that formed percentage (20%), (36.67%) and (43.33%) respectively shown in table (5).

Table 5: Distribution of type1 diabetes Mellitus patients and healthy according to age

Age	Study groups N(%)		x2	P-value	Total
	healthy	type1 diabetes Mellitus			
5-10 years	6(20%)	27(45%)	11.25	0.004*	33(36.67%)
11-15 years	11(36.67%)	25(41.67%)			36(40%)
16-20 years	13(43.33%)	8(13.33%)			21(23.33%)
Total	30(100%)	60(100%)			90(100%)

* Significant at a level of 0.05

3.6 Distribution of type1 diabetes Mellitus patients and healthy according to sex

Results of present study showed that percentage female and male are (70%) and (30%) respectively in type1 diabetes Mellitus compared with healthy that formed (43%) and (56.67%) respectively shown in table(6).

Table (6) Distribution of type1 diabetes Mellitus patients and healthy according to sex

sex	Study groups N(%)		x2	P-value	Total
	healthy	type1 diabetes Mellitus			
Female	13(43%)	42(70%)	5.98	0.01*	55(61.11%)
Male	17(56.67%)	18(30%)			35(38.89%)
Total	30 (100%)	60(100%)			90(100%)

* Significant at a level of 0.05

4. Discussion

The current study found that there are significant increase in IA-2A in type 1 diabetes patients compared with control, these results accompanied with (Khan *et al.*, 2021) The presence of autoantibodies, such as insulinoma-associated antigen-2 autoantibodies, in children and adolescents with type 1 diabetes mellitus. Furthermore, the presence of insulinoma-related antigen-2 autoantibodies is strongly linked with illness duration. According to one study, IA-2A is present in 89.4% of T1DM in children in the first year of diagnosis, and its prevalence is around 70% in the first three years. As a result, the occurrence of IA-2A has the highest prognostic relevance in T1DM (Borg *et al.*, 1997). The results provided suggests that IA-2A disappearance rate is quicker in individuals with an age of onset ≤ 10 years and correlates with illness duration. Therefore, to successfully differentiate between type 1 diabetes and other monogenic forms of the disease, like Wolfram syndrome and young people's maturity-onset diabetes (Yaghootkar *et al.*, 2019). As previously noted, the diagnostic sensitivity of the anti-islet autoantibody tests we examined varied with age at the onset of diabetes, with IA-2A being higher in adults and greater in children (Bravis *et al.*, 2018). Although

one investigation found that the incidence of IA-2A at and after type 1 diabetes onset was not highly associated with endogenous insulin secretion (Williams *et al.*, 2016). These findings demonstrate that the severity of the autoimmune response to IA-2 varies with age, despite the fact that both autoantigens are transmembrane proteins localized inside insulin secretory granules and their half-lives are correlated (Haneda *et al.*, 2018). The study found that GADA prevalence reduced fast in individuals under 14 years of age, but IA-2A positive declined similarly independent of age of start (Tridgell *et al.*, 2011).

The present study discovered that there are significant increase in IgM in type 1 diabetes patients compared with healthy, these results accompanied with the study conducted in India by (Gorus *et al.*, 1998) This validated the higher concentration of immunoglobulins in diabetes patients compared to the control group. The study conducted in Saudi Arabia by (Anil, 2006) indicated that diabetic individuals with gingivitis had higher concentrations of immunoglobulins IgA, IgM, and IgG than equivalent patients without diabetes. The study (Al-Greti, 2008) , conducted in Iraq, demonstrated higher levels of immunoglobulins in chronic conditions such as diabetes and bacterial infections. Furthermore, (Pishdad & Faghiri, 1995) shown that elevated levels of IgG and IgM in the sera of patients with type 1 diabetes are important indicators of the presence of multiple variables that contribute to the pathological problems associated with type 1 diabetes. Increased immunoglobulin levels are associated with the presence of the following markers: chronic inflammatory illnesses, liver diseases, haematological disorders, infections, and malignancies (Dispenzieri *et al.*, 2001). Individual studies indicate that a percentage of Type 1 diabetes individuals have low IgG and IgM levels (Bruno *et al.*, 1976). Two investigations have reported unusually low blood IgG and IgM levels among diabetics needing insulin (YULDASHEV, 1980). Patients with low IgG and IgM levels frequently fail to exhibit humoral responses to particular antigens (Petty *et al.*, 1979).

The present study showed that there are significant increase in TGF- β in type 1 diabetes patients compared with healthy, these results accompanied with (Zorena *et al.*, 2013) persons with type 1 diabetes have much higher TGF- β concentrations than healthy persons. TGF- β levels were shown to be significantly higher in patients with type 1 diabetes, supporting previous research findings. Hyperglycemia in diabetes leads to increased expression of TGF- β in numerous cells, including macrophages (Tesch, 2007). It has been demonstrated that a considerable increase in glucose levels in the body activates the kinase protein in the cytoplasm. This leads to the synthesis of TGF- β , which binds to Smad proteins and receptors, enters the nucleus, and forms a complex that regulates gene expression (Gomes *et al.*, 2014). The Smad protein transfers extracellular signals from the TGF- β pathway to the nucleus (Lan, 2012). TGF- β has a regulatory function in type 1 diabetes patients by inhibiting the development of immunological pathogenesis of self-antigens while maintaining immune response (Li *et al.*, 2006). TGF- β 1 is generated by several cells, including regulatory T cells and dendritic cells (Wan & Flavell, 2006). Regulatory T cells (Tregs) prevent type 1 diabetes by secreting TGF- β (You *et al.*, 2006). In studies including newborns whose parents had T1D, repeated measurements of aTGF- β 1 in sera were obtained (AN, 1981), siblings of probands with type 1 diabetes and newborns from the general population who are genetically at risk (Buzzetti *et al.*, 2004). In Type 1 Diabetes, elevated TGF- β 1 levels can exacerbate inflammation and initiate an autoimmune attack on pancreatic β -cells (Tavakkoly Bazzaz & al., 2014). The role of TGF- β 1 in type 1 diabetes is complex. Its immunosuppressive properties help maintain tolerance and protect against autoimmune responses against pancreatic β cells (Ma *et al.*, 2016). TGF- β 1 has been investigated as a medicinal medication for reducing autoimmune against β cells (Phillips *et al.*, 2021). The study

found that type 1 diabetes patients had higher mean TGF- β 1 levels than healthy individuals. Previous study (Chang *et al.*, 2016) suggests that TGF- β 1 has a significant role in the inflammatory response associated with autoimmune diseases, including type 1 diabetes. The involvement of TGF- β 1 in autoimmune illnesses is complex. It may contribute to the development of long-term diabetes complications, such as diabetic nephropathy, and helps regulate inflammatory responses (Frangogiannis, 2020). Previous studies have demonstrated that glycemic control, medication type (such as insulin), genetics, and environmental factors can all affect TGF- β 1, leading to heterogeneous results among trials (Yadav & al., 2015). Research by (Xu & al., 2016) focused on TGF- β 1 expression during the initial stages of fracture healing in people with type 1 diabetes. Diabetes may reduce TGF- β 1 signalling, which can impede normal recovery. Diabetic patients had significantly lower TGF- β 1 response to inflammation compared to controls at all time points after surgery. Research has focused on the involvement of TGF- β 1 in diabetic nephropathy, a frequent complication of diabetes. The study primarily looked at the expression of TGF- β 1 during the early phases of type 1 diabetes fracture repair. Diabetes may inhibit normal healing by reducing TGF- β 1 signaling. Diabetic patients had significantly lower TGF- β 1 response to inflammation compared to controls at all time points after surgery. Research has explored the involvement of TGF- β 1 in diabetic nephropathy, a frequent complication of diabetes (Cristovam *et al.*, 2012). The role of TGF- β 1 in male patients with type 1 diabetes may be clarified by controlled research evaluating TGF- β 1 expression in individuals with lengthy bone fractures (depending on age and gender) (Kaiser *et al.*, 2012).

The present study noted that there is significant increase in IFN- γ in type 1 diabetes patients compared with healthy, these results accompanied with (Kikodze *et al.*, 2013) Type 1 diabetes has significantly higher levels of IFN- γ compared to the healthy group. Numerous investigations confirm that damaged beta cells are linked to elevated levels of pro-inflammatory cytokines such TNF- α , TNF- β , IL-1, IL-2, IL-12, IFN- γ , and INF- α (Hussain *et al.*, 1996). Research indicates that beta cells in the pancreas rely heavily on cellular kinetics like IFN- γ , TNF- β , and IL-1. These cellular dynamics have cytostatic effects on beta cells in the pancreas, limiting insulin synthesis and secretion. However, removing these cellular dynamics will abolish the activities that impact beta cells. Furthermore, these cellular dynamics can have a cytotoxic impact on beta cells in the pancreatic islets, culminating in their death in infants with type 1 diabetes (Foulis *et al.*, 1991). In type 1 diabetes, cellular dynamics, including interferon-gamma, trigger and accelerate beta cell death, which can occur directly or indirectly. The direct mechanism of destruction by Th1 cytokines, including interferon-gamma, has a primary effect on macrophages, resulting in increased infiltration of these cells at pancreatic islet cell sites and accelerating beta cell destruction via the release of nitric oxide and oxygen radicals (Karlson *et al.*, 2000). Alternatively, increasing the activation of cytotoxic T cell filtration CD8 specific to the MHC class I molecule expressed on the surfaces of pancreatic islet cells results in the rapid death of beta cells in humans (Seewaldt *et al.*, 2000). IFN- γ promotes the demise of beta cells by direct or indirect mechanisms. Th1 mediators, such as IFN- γ , directly activate macrophages to infiltrate pancreatic islet cells. This will speed up the killing of beta cells by mediators produced in the new route, such as free oxygen radicals and nitric oxide, or it induces T lymphocytes to identify MHC-I carrying islets and be identified only by CD8 cells. Increased expression of IFN- γ and TNF causes beta cell tissue damage in humans and mice (Amrani *et al.*, 2000).

5. Conclusion

Present results showed that among patients in Kerbala Province, Type 1 Diabetes Mellitus is

substantially correlated with IA-2A, IFN- γ , TGF- β 1, and IgM. When compared to healthy persons, these values were significantly higher in those with both newly diagnosed and established T1DM, confirming their function as trustworthy immunological indicators of β -cell autoimmunity. The increased frequency of these markers in patients emphasizes the need of using IA-2A testing in early diagnosis, risk assessment, and clinical monitoring of T1DM in this population and further shows the continuous autoimmune destruction of pancreatic β -cells. Additionally, IFN- γ and TGF- β 1 have a positive significant correlation, but IgM and TGF- β 1 have a negative significant correlation. These findings support the autoimmune character of type 1 diabetes and could help develop better regional approaches to early identification and preventative measures.

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