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Assessment of the serum level of anti-tissue transglutaminase antibodies and the HLA-DQ2 gene in Iraqi children with type 1 diabetes mellitus

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Abstract

Type 1 diabetes mellitus (T1DM) and celiac disease (CD) are two of the most prevalent autoimmune disorders affecting children and adolescents, with a notable tendency to co-occur in the same individual. This co-occurrence is thought to be attributed to the shared genetic predisposition underlying both conditions. This study aimed to evaluate the serum levels of anti-tissue transglutaminase antibodies and the impact of the human leukocyte antigen-DQ2 (*HLA-DQ2*) gene on their levels in diabetic pediatric populations in Wasit Province, Iraq. A total of 100 patients with type T1DM and 60 healthy individuals, both sexes, under the age of 16 years, were recruited. Blood samples (5 mL) were collected from each participant to determine the level of tTG-IgA and tTG-IgG and to detect *HLA-DQ2* genes using direct ELISA and conventional PCR, respectively. Additionally, fasting blood glucose (FBG), random blood glucose (RBG), and glycated haemoglobin (HbA1C) levels were also measured. The study found a significant difference in the mean levels of tTG-IgA and tTG-IgG between patients and controls, with patients having a higher mean value of tTG-IgA than controls (16.87±72.40 versus 2.47±1.78). Similarly, tTG-IgG levels were also higher in patients (14.52±51.23 versus 2.05±2.29), although no significant correlation was found between *HLA-DQ2* gene present and sample type, nor between the means of selected autoantibodies between positive and negative *HLA-DQ2* gene analysis among patient groups. The study reveals a significant difference in anti-tTG autoantibodies among T1DM patients compared to healthy controls, increasing their risk of developing CD. Additionally, a high percentage of T1DM children carried the *HLA-DQ2* gene, suggesting genetic screening isn't mandatory.

Keywords: Celiac disease; *HLA DQ2*; transglutaminase antibodies; type 1 diabetes mellitus.

تقييم مستوى الأجسام المضادة لإنزيم الترانسغلوتاميناز النسيجي وجين HLA–DQ2 في مصل الأطفال العراقيين المصابين بداء السكري من النوع الأول

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الخلاصه

داء السكري النوع الاول ومرض حساسية الحنطة هما من أكثر اضطرابات المناعة الذاتية انتشارا التي تؤثر على الأطفال والمراهقين، مع ميل ملحوظ إلى الحدوث المشترك للمرضين في نفس الفرد. يعتقد أن هذا الحدوث المشترك يعزى إلى الاستعداد الوراثي المشترك الكامن وراء كلتا الحالتين. تركز هذه الدراسة على تقييم مستويات المصل من الأجسام المضادة لإنزيم الترانسغلوتاميناز النسيجي وتأثير جين (HLA-*DQ2*) على هذه المستويات في مجموعات الأطفال المصابين بداء السكري من النوع الأول في محافظة واسط، العراق. تم أخذ 100 مريض مصاب بداء السكري من النوع الأول و60 شخصًا سليمًا، من كلا الجنسين وتحت سن 16 عامًا. تم جمع خمس مليلترات من الدم من كل مشارك لتحديد مستوى الأجسام المضادة لإنزيم الترانسغلوتاميناز النسيجي نوع A وG وللكشف عن جينات *HLA-DQ2* باستخدام اختبار مقايسة الامتصاص المناعي المرتبط بالأنزيمات المباشر واختبار التفاعل التسلسلي للبوليمراز التقليدي، على التوالي. بالإضافة الى ذلك تم أيضًا قياس مستويات السكر في الدم اثناء الصيام، والسكر في الدم العشوائي والسكر التراكمي (الهيموغلوبين السكري). وجدت الدراسة فرقًا ملحوظًا في المتوسط المعياري لمستويات الأجسام المضادة لإنزيم الترانسغلوتاميناز النسيجي نوع A وG بين المرضى والأصحاء، حيث كان لدى المرضى قيمة متوسط معياري أعلى من الأجسام المضادة لإنزيم الترانسغلوتاميناز النسيجي نوع A مقارنة بالأصحاء (72.40 ± 16.87مقابل 1.78±2.47). وبالمثل كانت مستويات الأجسام المضادة لإنزيم الترانسغلوتاميناز النسيجي نوع G في المرضى هي الأعلى ايضا (51.23 ±14.52 مقابل 2.29±2.05)، بالرغم من ذلك لم يتم العثور على ارتباط ملحوظ بين وجود جين *HLA-DQ2* ونوع العينة، ولا بين متوسطات الأجسام المضادة المحددة بين التحليل الإيجابي والسلبي لجين *HLA-DQ2* بين مجموعات المرضى. تكشف الدراسة عن فرق كبير في الأجسام المضادة لإنزيم الترانسغلوتاميناز النسيجي بين مرضى داء السكري من النوع الأول مقارنة بالأصحاء، مما يزيد من خطر إصابتهم بمرض حساسية الحنطة. بالإضافة إلى ذلك، يحمل نسبة عالية من الأطفال المصابين بداء السكري من النوع الأول جين *HLA-DQ2*، مما يشير إلى أن الفحص الجيني ليس إلزاميًا.

Introduction

Type 1 diabetes mellitus is an autoimmune disease that leads to insulin deficiency due to beta cell destruction of the pancreas, accounting for 5–10% of diabetes cases globally [1]. Patients with T1DM are at a heightened risk for developing other autoimmune disorders, with celiac disease being the most common autoimmune disorder diagnosed in T1DM patients after autoimmune thyroiditis [2]. Celiac disease is a chronic inflammatory condition of the small intestine triggered by dietary gluten and related prolamin exposure in genetically predisposed individuals, affecting 0.5–1% of the general population. It is characterized by enteropathy in the small intestine, systemic symptoms related to malabsorption and/or immune activation, CD biomarkers such as specific antibodies against tissue transglutaminase, and genetic markers such as *HLA-DQ2* and *HLA-DQ8* [3]. The relationship between CD and T1DM has been documented in pediatric populations. The etiology of both conditions is complex; they may arise from exposure of genetically predisposed individuals to unknown environmental substances, which may lead to stimulation of an abnormal inflammatory reaction in the small intestinal mucosa and islet cells of the pancreas, causing CD and T1DM, respectively [4]. However, the actual reasons for the association between these conditions were not fully understood. One possible explanation is that both conditions share the same genetic background represented by HLA and non-HLA genes [5], with *HLADQ2* being the predominant allele seen in individuals with both T1DM and CD [6]. HLA molecules are dimers of class II major histocompatibility complex molecules located on the surface of antigen-presenting cells (APCs); they present gluten-derived peptides to CD T

cells, triggering strong inflammatory responses. *HLA-DQ2* is found in 95% of patients with CD [7], and it is also observed in one-third of T1DM patients [6]. The prevalence of CD in patients with T1DM varies between 1.1% and 16% [8]. The prevalence of CD in diabetic populations ranges from 1.5% to 4.6% and 2% to 4.1% in children and adults, respectively, according to the European studies [9]. However, two studies in north India estimate that the prevalence of CD in individuals with T1DM is between 11% and 17% [4]. CD prevalence in diabetes patients in Iran, Jordan, Saudi Arabia, and Turkey is 5%, 16.6%, 15.9%, and 4.4%, respectively [9–12], with a range of 10-11.2% in Iraqi T1DM patients. Early identification and treatment can help reduce the risk of hypoglycemia and other complications [13].
Although a definitive diagnosis of CD requires an intestinal biopsy, various serologic biomarkers are commonly used for screening and monitoring dietary compliance in suspected cases [14]. TTG is a commonly used antibody for screening CD, providing good specificity (up to 96%) and sensitivity (98%). It also has a 90% positive predictive value, particularly in type 1 diabetic children [15]. TTG plays a crucial role in wound repair as well as in the development of celiac disease, as it targets anti-endomysium antibodies and enhances binding affinity between gliadins and HLA molecules on APCs [16]. This study aimed to assess the serum levels of anti-tTG antibodies in children and adolescents with T1DM and assess the impact of the *HLA-DQ2* gene on these levels in Wasit Province, Iraq.

Subjects, material and methods

The cross-sectional study was conducted from September 1, 2023, to January 1, 2024, in Wasit Province, Iraq. It involved a selection of 160 children and adolescents, aged under 16 years; both sexes were included, males and females. This population was divided into two groups, with 100 subjects who were already diagnosed with Type-1 diabetes mellitus and the remaining 60 as apparently healthy controls who were free from CD signs and symptoms and had no history of any chronic disease. The inclusion criteria specified that only individuals clinically diagnosed with type-1 diabetes mellitus for a period of four years or less within the designated age range. Any patient not meeting these criteria was excluded from the study. Prior to the inclusion of the potential subjects in the study, written consent was obtained from the participants or their parents (guardians) for participation in the study.

Sample collection and processing

Blood samples were collected from each participant through venipuncture. The samples were then divided into two categories for analysis: 2.5 ml in an EDTA anticoagulant tube for DNA extraction by using the FavorPrep Genomic DNA Mini Kit, Germany. The *HLA-DQ2* gene was detected using a conventional PCR technique with sequence-specific primers (F: 5'- GG ACA GAG GTG CGC CGT CTT -3'; R: 5'- GC TTT CCT CCG CTC GAT CAG G-3') for all samples according to the manufacturer's instructions, the PCR conditions are shown in Table 1. The PCR products were then subjected to 1.5 g agarose gel electrophoresis at 72 volts for 80 minutes and visualized under ultraviolet light, and 2.5 ml was placed in a gel tube for serum separation. The blood was allowed to coagulate naturally at room temperature for 30 minutes, followed by centrifugation for 10 minutes at 1500 Xg (G-force) for serum separation. The separated serum was collected and divided into several sterile Eppendorf tubes, which were stored at -20 °C until use. Subsequently, each participant underwent two serologic tests: anti-tTG IgA and anti-tTG IgG antibody tests by the direct enzyme-linked immunosorbent assay (ELISA) technique using commercially available kits of the Aeskulisa company (AESKULISA, Germany) with a cut-off value of >18 U/ml for each test. The results were analyzed according to the manufacturer's instructions regarding principles, procedures, and result interpretation

Ethical approval

This study was performed in accordance with the ethical rules for medical research involving human participants of the Declaration of Helsinki (1964). The study protocol was approved by the institutional guidelines and the Ethics Committee of the Medical College at Wasit University in July 2023, and the Wasit Health Department (1233) on 25/7/2023. Written consent or a substitute was collected from each participant or their parents/guardians prior to their inclusion in the study.

Statistical analysis

Data were statistically analyzed using the Statistical Package for Social Sciences program (SPSS; Version 26). The data were tested to assess if the continuous variables were normally distributed or not. Data were expressed as mean± standard deviation values or median (interquartile range) for continuous numerical and as percentages and frequencies for categorical variables. A p-value of < 0.05 was considered statistically significant for all tests performed.

Table 1: PCR conditions, including the PCR program utilized in the thermo-cycler

Gene	Initial Denaturation	Denaturation	Annealing	Extension	Final Extension	Hold	Cycle
HLA-DQ2	94 ˚C/5 min	94 ˚C/30 sec	54˚C/30sec	72˚C/30sec	72˚C/5min	4˚C	35

Results

Demographic characteristics

The results of this study are based on the analysis of data collected from 160 samples, comprising 100 patients with diabetes and 60 healthy controls. Table 2 presents sociodemographic features in both study groups. It was observed that males had the highest percentages in both groups; they represented 52% and 55% of both patients and controls, respectively. While females represented 48% of patients and 45% of controls. The largest percentage in both groups was found in the range of 11 to 15 years, while those under 5 years old represented the lowest percentages, and the age groups from 6 to 10 years old only represented 33% of patients and 38.3% of controls. Figure 1 illustrates the duration of disease among diabetic children, showing that 50% had been diagnosed with diabetes for 1-2 years, followed by 29% who had the disease for less than one year, and 21% of patients who were diagnosed for 3-4 years.

Table 2: Socio-demographic features of the study groups (patients=100, controls=60).

Variables		Sample type No. (%)		Total No. (%)	*P*-value
		Patients (n=100)	Controls (n=60)		
Sex	Male	52 (52%)	33 (55%)	85 (53.1%)	0.460
	Female	48 (48%)	27 (45%)	75 (46.9%)	
Age groups (years)	1-5	17 (17%)	13 (21.7%)	30 (18.8%)	0.713
	6-10	33 (33%)	23 (38.3%)	56 (35%)	
	11-15	50 (50%)	24 (40%)	74 (46.3%)	

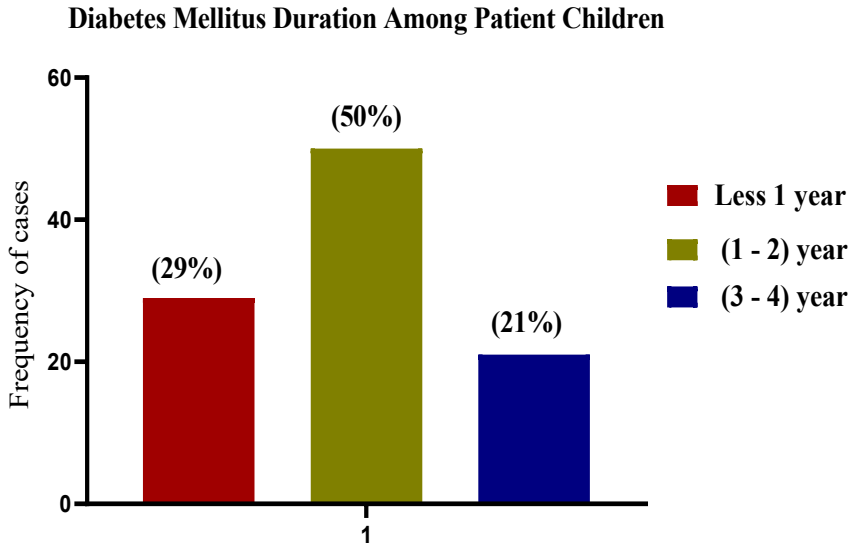

Figure 1: Frequency distribution of diabetes mellitus duration among patient children (n=100).

Biochemical test

Table 3 shows a statistically significant difference in biochemical tests related to DM between the patient and control groups, with a *P*-value less than 0.001 for all the selected tests. Diabetic children exhibited higher levels of FBG, RBG, and HbA1C.

Table 3: Mean differences of the biochemical test among 160 children sample (patients=100, controls=60).

biochemical tests	Patients Mean ±SD	Controls Mean ±SD	*P*-value
FBG (mg/dl)	165.16±30.51	87.34±8.35	<0.001
RBG (mg/dl)	473.20±130.32	103.24±9.44	<0.001
HbA1C (%)	10.09±2.15	3.88±0.97	<0.001

SD= standard deviation, FBG= Fasting Blood Glucose, RBG= Random Blood Glucose, HbA1C= glycated haemoglobin.

Anti-tissue transglutaminase antibody (tTG)

There is a significant difference in the mean levels of tTG-IgA and tTG-IgG between patients and controls (*P* value < 0.05), as shown in Table 4. Patients had a significantly higher mean value of tTG-IgA compared to controls (16.87±72.40 versus 2.47±1.78). Similarly, a higher mean value was also observed in tTG-IgG (14.52±51.23 versus 2.05±2.29).

Table 4: Difference between patients and controls in autoantibodies levels in the study sample (patients=100, controls=60).

Autoantibodies	Patients		Controls		*P*-value
	Mean (SD)	Median (IQR)	Mean (SD)	Median (IQR)	
tTG-IgA (U/ml)	16.87(72.40)	1.07(1.17)	2.47(1.78)	2.36(1.90)	<0.001
tTG-IgG (U/ml)	14.52(51.23)	1.44(3.61)	2.05(2.29)	1.31(1.93)	0.04

SD = Standard deviation, IOQ= Interquartile range, tTG-IgA= anti-tissue transglutaminase immunoglobulin A, tTG-IgG= anti-tissue transglutaminase immunoglobulin G.

HLA-DQ2 gene

Table 5 found no significant association between the presence of the HLA-DQ2 gene and the type of sample (*P*-value = 0.209). The highest percentage of patients (65%) was found to have positive genes; the same was observed in controls, where 55% of them demonstrated positive genes.

Table 5: Association between disease status (patients and controls) and gene analysis (patients=100, controls=60).

HLA-DQ2 gene	Sample No. (%)		Total No. (%)	*P*-value
	Patient	Control		
Positive	65(65%)	33(55%)	98(61.3%)	0.209
Negative	35(35%)	27(45%)	62(38.8%)	

HLA-DQ2= human leukocyte antigen-DQ2.

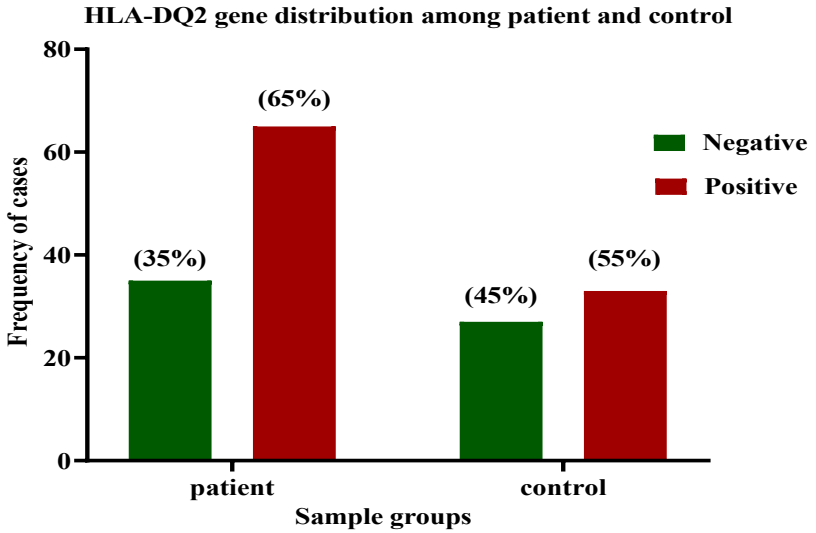

Figure 2: Frequency distribution of *HLA-DQ2* gene among patient and control children (patients=100, controls=60).

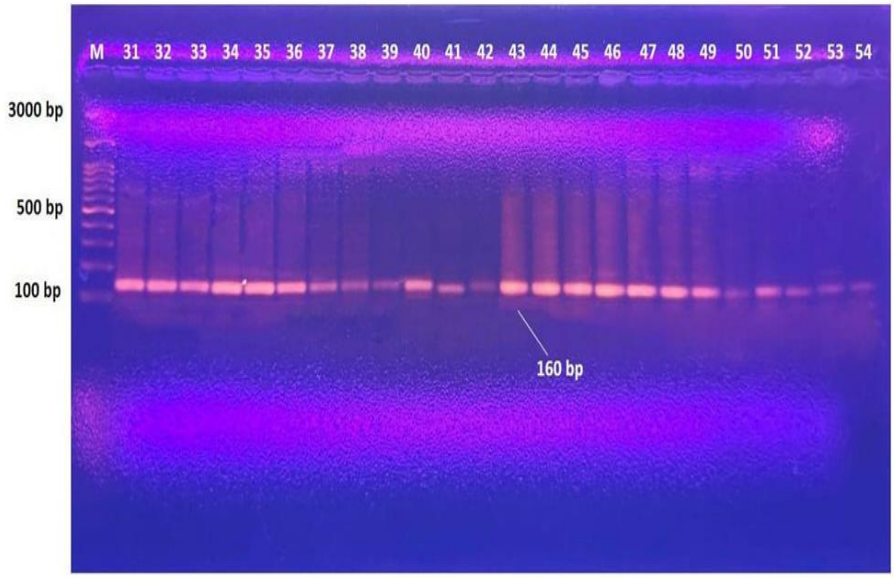

Figure 3: Analysis of *HLA-DQ2* gene (160 pb) amplification by 1.5% agarose gel electrophoresis, 31-54 represented samples, M (DNA molecular size marker (3000 bp ladder).

The correlation between *HLA-DQ2* gene and anti-tissue transglutaminase antibody

There is no significant difference in the means of the selected autoantibodies between positive and negative *HLA-DQ2* genes among the patient groups. Additionally, no correlation was found between the presence of the gene and the two selected autoantibodies, as seen in Table 6.

Table 6: Correlation between *HLA-DQ2* gene and autoantibodies in the patient groups (n=100).

	Autoantibodies	*HLA-DQ2* gene				*P*-value	R
		Positive		Negative			
		Mean (SD)	Median (IQR)	Mean (SD)	Median (IQR)		
Patients	tTG-IgA U/ml	24.67(88.94)	1.06(0.99)	2.38(6.37)	1.08(1.45)	0.820	0.023
	tTG-IgG U/ml	21.15(62.69)	1.29(4.11)	2.21(2.08)	1.51(1.60)	0.928	0.009

SD = Standard deviation, IOQ= Interquartile range, R=Correlation Coefficient, *HLA-DQ2*= human leukocyte antigen-DQ2, tTG-IgA= anti-tissue transglutaminase immunoglobulin A, tTG-IgG= anti-tissue transglutaminase immunoglobulin G.

Discussion

Routine screening for CD is recommended for asymptomatic children with T1DM due to their increased risk [17]. Screening these high-risk groups can be beneficial, as it may enhance diabetic management, support growth control, and prevent extra-intestinal symptoms of CD, as well as reduce the effects of CD on the quality of life in patients with diabetes [4]. The current study demonstrated significant differences in mean levels of tTG autoantibodies between T1DM patients and healthy controls, particularly in tTG IgA (P value < 0.001), including high risk for CD development [18]. This is significant because anti-tTG IgA tests have the highest sensitivity and specificity for identifying patients with CD [19–21]. Other studies have suggested that high levels of IgA-tTG and IgG-tTG antibodies were related to the grade of mucosal villous atrophy with high accuracy and reduced the need for biopsy in suspected CD cases [22–23]. Our findings align with those of Maheshwari *et al.,* who also revealed that mean tTG IgA was significantly higher in T1DM than non-T1DM, and the anti-tTG antibody test has very high sensitivity and specificity for the diagnosis of CD in T1D children [14]. However, IgA deficiency can lead to false negative results for anti-tTG IgA. In such cases, anti-tTG IgG has high sensitivity and specificity for CD diagnosis, even in patients with IgA deficiency [24], consistent with other studies that reported similar findings [22]. Different results were reported by Alzahrani, who reported that there was a higher proportion of CD seropositivity among non-diabetic participants compared to those diagnosed with T1D (11% versus 4.6%) with no statistically significant difference (*p*-value =.082) [25], which is in disagreement with the presented study. Furthermore, certain studies showed that negative results of serological tests do not rule out the possibility of CD in patients with T1DM [26].

However, the *HLA-DQ2* gene is a significant factor in the development of CD in children with T1DM [27]. However, our results showed no significant association between T1DM patients and healthy controls regarding the *HLA-DQ2* gene. Therefore, it cannot serve as a primary diagnostic tool for CD, as it provides no further information on children with T1D [28]. Furthermore, the International Society for Pediatric and Adolescent Diabetes (ISPAD) doesn't recommend HLA typing as a first-line screening test for CD, though it rarely allows the exclusion of CD in patients with T1D [29–30], which is consistent with our findings. Conversely, some studies have indicated that the absence of *HLA DQ2* has a high negative

predictive value, excluding CD development with almost 100% probability [31]. Similarly, other authors report the same results [32–33].

In the present study, most T1DM patients (65%) carried the *HLA-DQ2* gene. Similar to our findings, Lewandowska *et al.* found that 63.10% of T1DM children carry alleles that code for DQ2 molecules [34]. This observation corresponds with the findings of another study, which showed that the *HLA-DQ2* haplotype was found in 50% of T1DM children [35], which aligns with previous studies that demonstrated the same results [24], [36]. This co-occurrence may be attributed to the fact that both conditions share a common genetic susceptibility, which increases the likelihood of an individual developing both diseases [32]. In the control group, our results showed that 55 percent of them carry *HLA-DQ2*, and they are considered a risk group for developing celiac disease in the future [27]. This finding aligns with other studies that predict less than 5% of these controls will develop CD [37].

The mechanisms by which the DQ2 alleles affect CD pathogenesis remain partially unclear [11]. In T1DM patients, our study found no significant correlation between the presence or absence of *HLA-DQ2* genes and mean levels of tTG-IgA and tTG-IgG autoantibodies, which is in line with other authors, Lee *et al.*, who indicated that *HLA-DQ2* was not the exclusive genotype associated with antibody production. This lack of correlation may be attributed to the involvement of other non-HLA genetic factors and environmental factors that contribute to the CD phenotype [38]. Similar results were previously described by other studies, confirming that there is no association between the presence of the *HLA-DQ2* gene and anti-tTG antibody levels [39]. As compared with our study, previous findings reported a close relationship between *HLA-DQ2* gene and anti-tTG antibodies, suggesting that patients with *HLA-DQ2* gene have a higher antibody titer at diagnosis [31], [40]. This discrepancy between our findings and other studies could be due to differences in the frequencies of HLA haplotypes in the analyzed populations as well as in test conditions or might be due to combinations of population-related genetic and environmental factors [41].

Conclusion

The study indicates a significant difference in anti-tTG autoantibodies between patients with T1DM and health control, suggesting that these patients are at high risk for developing CD. A substantial percentage of children with T1DM carry the *HLA-DQ2* gene, which makes them more likely to develop CD, suggesting that genetic screening is not mandatory for diabetic children.

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Availability of data and material

The datasets used and analyzed in the current study are available from the corresponding author on reasonable request.

Competing interests

The authors declare that they have no competing interests.

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