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Original Article

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Influence of Age and Type 1 Diabetes Mellitus on Serological Test for Celiac Disease in Children

Anshu Maheshwari [1,2,3] **Zhaoping He** [1,2] **Melissa Nicole Weidner** [1,2,4] **Patrick Lin** [1,2,5] **Ryan Bober** [2,6] **and Fernando J. Del Rosario** [1,2]

[1]Department of Pediatrics, Division of Gastroenterology, Hepatology and Nutrition, Nemours/Alfred I. duPont Hospital for Children, Wilmington, DE, USA
[2]Department of Pediatrics, Sidney Kimmel College of Medicine, Philadelphia, PA, USA
[3]Department of Pediatrics, University of Illinois College of Medicine in Peoria and Children's Hospital of Illinois, Peoria, IL, USA
[4]Department of Pediatrics, Robert Wood Johnson University Hospital, New Brunswick, NJ, USA
[5]Department of Pediatrics, Lehigh Valley Reilly Children's Hospital, Allentown, PA, USA
[6]Department of Pediatrics, Weill Cornell Medicine, New York, NY, USA

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Correspondence to
Fernando J. Del Rosario
Division of Gastroenterology, Hepatology and Nutrition, Nemours/Alfred I. duPont Hospital for Children, 1600 Rockland Road, Wilmington, DE 19803, USA.
E-mail: Fernando.DelRosario@nemours.org

ORCID iDs
Anshu Maheshwari
https://orcid.org/0000-0001-6959-9021
Zhaoping He
https://orcid.org/0000-0002-4722-1674
Melissa Nicole Weidner
https://orcid.org/0000-0002-6966-1734
Patrick Lin
https://orcid.org/0000-0003-2663-4004
Ryan Bober
https://orcid.org/0000-0002-5693-959X
Fernando J. Del Rosario
https://orcid.org/0000-0002-5826-8013

Conflict of Interest
The authors have no financial conflicts of interest.

ABSTRACT

Purpose: Serological tests of tissue transglutaminase (TTG) and deamidated gliadin (DGP) antibodies for celiac disease diagnosis show conflicting correlation with histology in young children and in type 1 diabetes mellitus (T1DM). Tests' ability to predict histology and cutoff values based on age and T1DM was evaluated.
Methods: A retrospective study of children who had celiac serological tests between 6/1/2002 and 12/31/2014 at a pediatric hospital.
Results: TTG IgA displayed similar results in predicting histology between <4.0 and ≥4.0 years age groups with sensitivity 98% and 93%, and specificity 88% and 86%, respectively. In children <4.0 years old, sensitivity for DGP antibodies was 100% and specificity 94%; in ≥4.0 years age groups, sensitivity was 60%, 88% for DGP IgA and IgG and specificity 95%, 96%, respectively. TTG IgA had low specificity in patients with T1DM compared with non-T1DM, 42% vs. 91%. Positive TTG IgA with normal histology was associated with higher T1DM prevalence at 36% compared with negative tests at 4%. Finally, the TTG IgA cutoff value was higher in T1DM at 36 vs. 16.3 units in non-T1DM. DGP IgG cutoff showed similar values between age groups; TTG IgA and DGP IgA cutoffs were lower in <4.0 years at 8.3 and 11.9 units than ≥4.0 years at 23.4 and 19.9, respectively.
Conclusion: TTG IgA is sufficient for the <4.0 years age group and DGP antibodies had no advantage over TTG IgA in older children. The cutoff value to determine a positive TTG IgA should be higher for children with T1DM.

Keywords: Celiac disease; Serologic tests; Child; Type 1 diabetes mellitus

INTRODUCTION

Celiac disease (CD) is an autoimmune disorder elicited by gluten ingestion in genetically susceptible individuals [1,2]. The immune response is associated with the appearance of circulating CD-specific antibodies, characterized by histologic blunting of intestinal mucosa and presenting with a range of symptoms including diarrhea, abdominal pain, and weight

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loss [3,4]. The prevalence of CD is between 0.5% and 0.9%, and higher in females at 0.6% vs. males at 0.4% [5,6]. The risk of developing CD is higher in relatives of CD patients, carriers of the DQ2 or DQ8 heterodimer, and patients with autoimmune and chromosomal diseases such as Down, Williams, and Turner syndromes [4,7,8]. The average prevalence of CD among children with type 1 diabetes mellitus (T1DM) is 4.5%, ranging from 0.97 to 16.4%, which significantly exceeds that in the general population due to an overlap in the genetic susceptibility conferred by human leukocyte antigen (HLA)-DQ2 and DQ8 [9-12].

Although definitive diagnosis relies on intestinal biopsy, serological testing to measure CD-specific antibodies has been widely used to screen patients with suspected gluten sensitive enteropathy, as well as for monitoring dietary compliance [13,14]. The most commonly ordered antibody tissue transglutaminase (TTG) shows good specificity and sensitivity for histological findings in children [4,13,14], while other studies have demonstrated variable results in young children [15,16]. Recent studies suggest that deamidated gliadin peptide (DGP) antibodies had an increased diagnostic accuracy in young children [17,18], though others illustrated that it increased neither the sensitivity nor the specificity in predicting histology results compared with TTG antibodies [19-21].

One of the well-recognized concerns of the serological tests is the positive serology found in individuals who had normal mucosa [13,14], though in occasional cases, this might be the result of patchy distribution of disease with inadvertent biopsies of normal mucosa, or 'latent CD' if patients eventually developed villous atrophy later [22,23]. This is particularly notable in T1DM patients with a high positive rate of TTG IgA without mucosal villous atrophy [24-27]. The TTG IgA antibody is routinely performed to screen children with T1DM diagnosis for CD whether symptomatic or asymptomatic [12,28]. In the presence of positive results, upper endoscopy with small bowel biopsy is performed to confirm diagnosis of CD. However, studies have revealed that some of these initial positive serologies with normal mucosa became negative or remained at low levels in repeat tests [25,26,29] and CD never developed [27,30]. Liu et al. [31] suggested that the threshold of TTG IgA for screening purposes should be set higher than for clinical diagnosis to avoid unnecessary biopsy. Positive serology has been proposed to be a distinct and TIDM-specific milder phenotype of CD or a temporary positive state in children with diabetes [24,26,32,33]. However, no studies have been conducted to verify this.

In this retrospective study using electronic medical record data collected over a 10-year period, we conducted our study with the following aims. 1) Analyze test accuracy of TTG and DGP antibodies in predicting histological findings by age group and T1DM diagnosis. 2) Examine underlying factors that are associated with positive serological tests with normal histological results. 3) Evaluate cut-off values of serologic tests that distinguish a positive from a negative result based on age groups and T1DM diagnosis in a non-CD cohort using serology tests from recent years.

MATERIALS AND METHODS

Study subjects and groups

Study subjects were from a tertiary care pediatric hospital located in the USA. This retrospective study was approved by the Institutional Review Board (IRB numbers 704031 and 341359) and the informed consent was waived.

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Inclusion

Males and females <20 years of age who had one or all of the celiac serological markers (TTG, DGP, and gliadin antibodies) ordered between June 1, 2002 and December 31, 2014 at Nemours/Alfred I. duPont Hospital for Children were included in the study. The serology tests were ordered for suspected CD based on the presence of chronic abdominal pain and diarrhea. The serology tests were also ordered in those individuals with medical conditions with a high risk of association with CD such as T1DM, short stature, and family history of CD with or without symptoms.

Exclusion

Patients with only follow-up data and missing initial evaluations were excluded from the study. In patients who had multiple serological tests or biopsy, the repeated results were excluded for analysis, and only initial work-up results were used. The rationale for these exclusions was to specifically investigate if serological markers are effective at the initial screen and diagnosis.

Study cohorts

Based on history and examination findings of clinical parameters/features as described in inclusion/exclusion above, 2,224 patients met the criteria and were included in the study. Two sub-cohorts were formed from the 2,224 study subjects.

1. The histological cohort (n=602)

In serology positive cases and in cases that were serology negative but with strong suspicion of CD, endoscopy with biopsies from the second part of the duodenum as our standard procedure was performed several days up to several months after serology tests while on a gluten-containing diet. Genetic analysis of HLA was conducted in a portion of the cases when serology did not correlate with histology results and/or strong clinical suspicion. In this cohort, serology tests were performed in various labs and with different assay kits between 2002 and 2014.

2. The normal non-CD cohort (n=1,854)

Among the 2,224 patients in the study cohort, patients who had serological tests done between 2013 and 2014 in the same lab using the same Elisa kits (Inova Diagnostics, San Diego, CA, USA) and who did not have CD diagnosis were included in the normal non-CD cohort. In this cohort, most serology tests (n=1,280) were ordered for screening purposes while some were ordered due to the presence of CD symptoms (n=574). Patients were determined not to have CD based on negative serological tests, lack of CD clinical parameters/features, negative medical history, and endoscopically normal mucosa if available. Biopsy was only done in a portion of the non-CD cohort. Only normal biopsy cases (n=233) were included in the non-CD cohort.

Study comparison groups

The above two cohorts were also futher divided into two study groups. 1) Age groups: <4 and ≥4 years of age. The breakdown of 4 years of age was mainly based on the first quartile of age in the entire cohort. This was also within the range of age breakdown between 2 and 7 years in several previous studies [15-19]. 2) T1DM and non-T1DM groups: Patients were classified into the T1DM group if the diagnosis was reported in the patients' records by their endocrinologists based on standard criteria at the time of the initial serological tests. Those who had no records indicating T1DM were included in the non-T1DM group.

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Study definitions

1. Biopsy results classifications

Patients' pathology reports were evaluated by attending pathologists. Abnormal histology was categorized by the Marsh classification. Stage 0: Small-intestinal biopsy specimens appeared normal. Stage 1: There was an increase in the number of intraepithelial lymphocytes to more than 30 per 100 enterocytes. Stage 2 (crypt hyperplasia): In addition to the increased intraepithelial lymphocytes, there was an increase in crypt depth without a reduction in villous height. Stage 3 (villous atrophy): ranging from partial to subtotal, to total atrophy. Marsh stage 3 (n=257) and stage 2 (n=25) were described as having abnormal histology results (total n=282) and stages 0 and 1 as having normal histological status.

2. Positive serology tests

In the histology cohort, serological tests were performed by various labs and test results above the reference values of the individual labs were considered positive. In the non-CD cohort, serology tests were performed between 2013 and 2014 by the gastrointestinal clinical lab at Nemours/Alfred I. duPont Hospital for Children using the same Elisa kits, and positive tests were above the reference value of 20 units.

3. Criteria for CD diagnosis

Patients with at least one or more positive serological CD antibodies test and confirmed with Marsh classification stages 3 or 2 in duodenal biopsies were classified as CD by their gastroenterologists. A few patients who had negative serological tests but were positive for HLA-DQ2 or HLA-DQ8 and had abnormal histology (Marsh class 2 or 3) were also defined as having CD according to their gastroenterologists.

4. False positive tests

Patients who had positive serological tests and normal mucosa (Marsh stage 0 and 1) were described as “false positive tests.” This did not rule out or rule in latent CD, which had to be retrospectively determined if villous atrophy developed later on repeat biopsy [22,34] or in cases of patchy distribution of abnormal mucosa.

Clinical Data Collection

Clinical data were collected from the electronic medical record. Demographic data included age, sex, and family history of CD. Laboratory test results included celiac genetic testing and celiac serology results. Histology reports from upper gastrointestinal endoscopies with biopsies were reviewed by pathologists. CD symptoms including diarrhea, abdominal pain, and other diagnoses including T1DM, short stature, failure to thrive, constipation, Down syndrome, and autoimmune thyroiditis were collected and recorded for the study.

Statistical analysis

Sensitivity, specificity, and test accuracy for the serological markers (TTG IgA and DGP antibodies) in predicting histological results were calculated for children in the histology cohort. Continuous variables were expressed as mean±standard deviation, and categorical variables as a percentage of the number of patients. Calculation of celiac test means was performed only in the normal non-CD cohort. Variables with abnormal distribution were converted to Log10 to obtain a *p*-value for significance, but raw means were shown in the results. Data were analyzed with descriptive statistics to compare variables between groups. The comparison was assessed by Student's *t*-test for continuous variables and by chi-square contingency analysis for categorical variables. A *p*-value less than 0.05 was used to indicate

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statistical significance. The cutoff value to distinguish a negative from a positive value was established using the reference interval-the threshold values within which the test results of the majority (95%) of healthy individuals (non-CD) would fall [35]. The one-sided reference interval was set at 2.5 percentile with highest results since only high values are of clinical concern. Statistical analysis was conducted using IBM SPSS Statistics for Windows, Version 25.0 (IBM Co., Armonk, NY, USA).

RESULTS

Characteristics of study cohorts

A total of 2,224 subjects met the inclusion criteria as described in the methods and were included in the study, from which two cohorts were formed (**Fig. 1**). The histology cohort (n=602) had both serology and biopsy performed, in which 282 displayed abnormal (Marsh 2/3) mucosa and 320 normal (Marsh 0/1) mucosa (**Table 1**). The normal non-CD cohort (n=1,854) had serology tests performed with the same Elisa kit between 2013 and 2014 and CD was ruled out by negative serology tests and other clinical information or normal biopsy when available as described in methods. Patients were evaluated for abdominal pain or diarrhea and were screened for various reasons (**Table 1**). Among several diagnoses and conditions commonly seen in CD, T1DM was the largest group (n=82 in the histology cohort; n=439 in non-CD).

Characteristics of abnormal and normal biopsy in the histology cohort

No significant difference was observed in age and sex between the two groups (**Table 2**). In the abnormal histology group, 277 (98.2%) had one or more positive serological tests and were diagnosed as having confirmed CD, and five (1.8%), who had negative serological tests but positive genetic tests and responded to a gluten-free diet, were diagnosed with CD by their gastroenterologists. In the normal histology group, 80 patients (25.0%) had at least one positive serological test and none had confirmed CD (**Table 2**). Family history and positive genetic tests were significantly higher in the abnormal histology group compared with the normal group (p<0.001; **Table 2**). The abnormal histology group had a higher prevalence of T1DM at 19.9% compared with 8.1% in the normal group (p<0.001). Correlation was not observed in other underlying conditions, such as short stature or failure to thrive (data not shown).

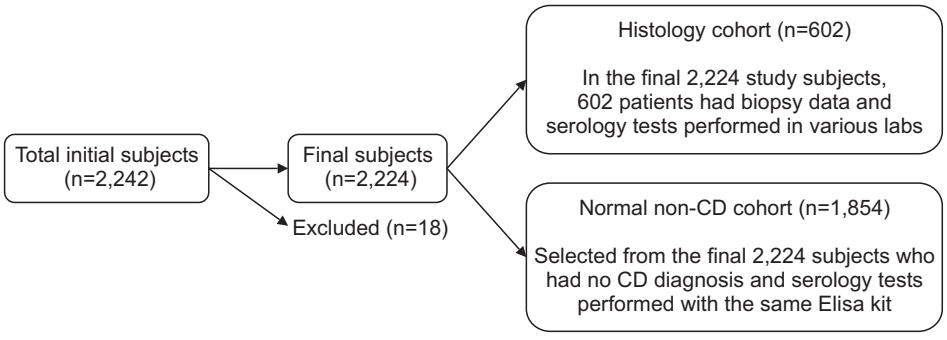

Fig. 1. Study cohorts. 1) Histology cohort: Among 2,224 patients, 602 patients who had histology data formed the histology cohort and serology was performed in different labs. 2) Normal non-CD cohort: Among 2,224 patients, 1,854 patients had serological tests performed recently in the same lab using the same Elisa kits, and the majority (n=1,621) had no biopsy and a small portion (n=233) had biopsy with normal results. The absence of CD was determined by the patients' physicians based on serological tests, clinical profiles, and medical history in 1,621 patients who did not have biopsy and together with normal biopsy results in 233 patients.
CD: celiac disease.

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Table 1. Characteristics of study cohorts

Variable	Histology cohort* (n=602)	Normal non-CD† (n=1,854)
Age (yr)	9.3±5.0	10.0±5.1
Age	5 mo to 20 yr	2 mo to 20 yr
Sex (male/female)	239/363	880/974
Indications for		
Diarrhea and/or abdominal pain	364 (60.5)	574 (31.0)
Screen for various reasons	238 (39.5)	1,280 (69.0)
Other diagnosis and conditions		
Diabetes mellitus type 1	82 (13.6)	439 (23.7)
Failure to thrive	68 (11.3)	104 (5.6)
Short stature	48 (8.0)	101 (5.4)
Constipation	37 (6.1)	361 (19.5)

Values are presented as mean±standard deviation, number only, or number (%).
CD: celiac disease.
*Patients underwent endoscopy with biopsy. †Patients without CD diagnosis based on the note from patients' physicians or normal histology finding (Marsh stage 0–1) if performed.

Table 2. Characteristics of histologically abnormal and normal subjects

Variable	Abnormal* (n=282)	95% CI	Normal† (n=320)	95% CI	*p*-value
Age (yr)	8.8±4.5	8.2–9.3	9.8±5.4	9.2–10.4	0.432
Sex (male/female)	35/65 (100/182)	0.7–1.0	43/57 (139/181)	1.0–1.4	0.056
Lab positive‡	277 (98.2)	10.3–30.3	25.0 (80)	0.1–0.2	<0.001
Genetic positive§	81 (98.8)	1.6–71.4	48 (73.8)	0.3–0.5	<0.001
Celiac disease¶	282 (100.0)	NA	NA	NA	NA
Family history	75 (26.6)	1.7–2.3	19 (5.9)	0.2–0.5	<0.001
T1DM	56 (19.9)	1.3–1.9	26 (8.1)	0.4–0.8	<0.001

Values are presented as mean±standard deviation, number only, or number (%).
CI: confidence interval, T1DM: type 1 diabetes mellitus, NA: not available.
*Marsh stage 2–3. †Marsh stage 0–1. ‡One of the serological tests positive, all determined by tissue transglutaminase and deamidated gliadin antibodies except eight by gliadin antibody. §One or both human leukocyte antigen (HLA) DQ2 and DQ8 (HLA-DQ locus) genes positive and only performed in a portion of patients. ¶Marsh stage 2–3 and elevated serological and/or positive genetic results.

Serology tests in predicting mucosa histology by age and type 1 diabetes mellitus

In the histology cohort, 585 patients had TTG IgA tests performed, and a portion had DGP IgA and IgG (**Table 3**). The sensitivity in predicting histological results for TTG IgA was 94% and specificity, 86%. Similar performance characteristics were observed in DGP IgG, but with better specificity at 96%. The overall performance of DGP IgA was not as promising due to very low sensitivity at 65%.

Table 3. Correlating serology with biopsy results in histology cohort in age and T1DM groups

Groups (n)	Sensitivity % (95% CI)	Specificity % (95% CI)	Accuracy % (95% CI)
TTG IgA (583)	94 (90–96)	86 (82–90)	90 (87–92)
DGP IgA (208)	65 (50–77)	95 (90–98)	88 (83–92)
DGP IgG (209)	90 (77–96)	96 (91–98)	94 (90–97)
TTG IgA			
<4 yr (106)	98 (87–100)	88 (77–94)	92 (84–96)
≥4 yr (477)	93 (89–96)	86 (81–90)	90 (87–92)
DGP IgA			
<4 yr (40)	100 (48–100)	94 (81–99)	95 (83–99)
≥4 yr (168)	60 (44–75)	95 (90–98)	86 (80–91)
DGP IgG			
<4 yr (40)	100 (48–100)	94 (81–99)	95 (83–99)
≥4 yr (169)	88 (74–96)	96 (91–99)	94 (89–97)
TTG IgA			
Non T1DM (504)	93 (88–96)	91 (86–94)	91 (87–94)
T1DM (79)	100 (93–100)	42 (23–63)	81 (71–89)

T1DM: type 1 diabetes mellitus, CI: confidence interval, TTG: tissue transglutaminase, DGP: deamidated gliadin peptide.

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The predictive value of the tests was analyzed in age and T1DM groups (**Table 3**). There was no dramatic performance change of TTG IgA between children <4.0 and ≥4.0 years of age. The DGP IgA displayed better sensitivity and accuracy in <4.0 years age group at 100% and 95%, respectively compared with the ≥4.0 years group at 60% and 86%, respectively, while the specificity remained similar. There was no significant difference for DGP IgG, except slightly better sensitivity in <4.0 years at 100% compared with 88% in ≥4.0 years of age. The specificity of TTG IgA in predicting histology was significantly lower in T1DM at 42% compared with non-T1DM at 91% or the entire histology cohort at 86% and the sensitivity was similar between T1DM and non-T1DM. In an alternative calculation, T1DM children with positive TTG IgA (n=68) exhibited a high percentage of normal biopsies at 22.1% (15/68) compared with 11.7% (27/230) normal biopsies found in TTG IgA positive tests of non-T1DM (p=0.0456; data not shown). Due to very small sample size, DGP antibodies were not analyzed for the T1DM group.

Correlating tissue transglutaminase IgA with demographic, type 1 diabetes mellitus, and Marsh stage 1 in normal histology

Of the 320 normal histology cases from the histology cohort, 311 patients had TTG tested with 269 negative and 42 positive results, hence a false positive rate of 13.5% (42/311; data not shown). No significant age difference existed between TTG IgA positive and negative groups (**Table 4**). The positive group had slightly more females (71%) than males (29%), whereas there was a similar sex distribution in the negative group (p=0.046). Positive TTG IgA had higher prevalence of T1DM at 36% (n=15) compared with the negative group at 4% (p<0.001). In an alternative calculation, TTG IgA positive with normal biopsy (false positive rate) was 57.7% (15/26) in T1DM patients, which was higher than 9.5% (27/285) in non-T1DM (p<0.001; data not shown). In addition, positive TTG IgA was also associated with a higher percentage of Marsh stage 1 classification at 45% compared with the TTG IgA negative group at 18% (p<0.001). T1DM was not associated with Marsh stage 1 status or sex (p=1.00 or p=0.220, respectively; data not shown).

Serological test cutoff value in different age groups and type 1 diabetes mellitus diagnosis

The non-CD cohort (n=1,854) was used to generate the cutoff value (2.5% reference interval) for TTG IgA and DGP antibodies (**Table 5A**). The TTG IgA cutoff in the entire cohort was 21.8 units, which was very close to the lab reference value of 20 units according to Inova's Elisa kit. In the <4.0 years of age group, TTG IgA cutoff was 8.3 units, 2.8-fold lower than the 23.4 units in ≥4.0 years. The cutoff value for the T1DM diagnosis group was 36.6 units, much higher than non-T1DM at 16.3 units. The DGP IgA cutoff was 11.0 units in the <4.0 years of age group, lower than the ≥4.0 years group at 19.9 units or the entire cohort at 18.6 units. There was no significant age-associated difference in DGP IgG at 18.9 units for <4.0 and 19.1 for ≥4.0 years age groups. The DGP antibody tests were not analyzed for T1DM due to small sample size. Mean values of TTG IgA and DGP IgA were slightly lower in the <4.0 years age group than in the ≥4.0 years group, while DGP IgG was slightly higher in the <4.0 years age

Table 4. Comparing demographic, T1DM, and Marsh stage 1 between TTG IgA positive and negative in normal histology patients*

Variable	Positive (n=42)	95% CI	Negative (n=269)	95% CI	p-value
Age (yr)	8.8±4.7	7.3–10.2	10.0±5.2	9.3–10.7	0.167
Sex (male)	12 (2.6)	0.29–1.01	121 (45.0)	1.00–1.19	0.046
T1DM	15 (35.7)	3.70–9.91	11 (4.1)	0.30–0.73	<0.001
Marsh stage 1	19 (45.2)	1.75–5.18	48 (17.8)	0.68–0.93	<0.001

Values are presented as mean±standard deviation or number (%).
T1DM: type 1 diabetes mellitus, TTG: tissue transglutaminase, CI: confidence interval.
*Total n=311 TTG IgA tests were performed with normal biopsy.

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Table 5A. Test cutoff values and mean in age and T1DM groups in the normal non-CD cohort

Variable	Entire cohort	<4 years	≥4 years	DM1	Non-DM1	Lab value*
TTG IgA	1,818 (21.8)	288 (8.3)	1,530 (23.4)	438 (36.6)	1,380 (16.3)	20
DGP IgG	929 (19.1)	222 (18.9)	707 (19.1)	NA	NA	20
DGP IgA	929 (18.6)	222 (11.9)	707 (19.9)	NA	NA	20

Values are presented as number (%).
T1DM: type 1 diabetes mellitus, CD: celiac disease, TTG: tissue transglutaminase, DGP: deamidated gliadin peptide, NA: not available.
*Elisa kit reference value.

Table 5B. Mean±SD in age and TIDM groups in the normal non-CD cohort

Variable	Entire cohort	<4 years	≥4 years	DM1	Non-DM1	*p*-value†
TTG IgA	5.8±8.3	4.2±5.8	6.1±8.7	8.0±14.5	5.0±4.7	<0.001
DGP IgG	5.3±4.8	5.9±5.7	5.1±4.5	NA	NA	<0.001
DGP IgA	5.5±6.0	4.5±4.4	5.8±6.3	NA	NA	<0.001

Values are presented mean±SD.
SD: standard deviation, T1DM: type 1 diabetes mellitus, CD: celiac disease, TTG: tissue transglutaminase, DGP: deamidated gliadin peptide, NA: not available.
†$p<0.001$ when comparing between age groups and between T1DM and non-T1DM.

than in the ≥4.0 years group; nevertheless, the differences were significant. Mean TTG IgA was 8.0±14.5 in T1DM, significantly higher than 5.0±4.7 units in non-T1DM (**Table 5B**).

DISCUSSION

Although TTG IgA is the obligatory test for correlation with histology and for screening at risk populations for CD in the presence of IgA sufficiency, there is a debate whether TTG IgA is a reliable test in younger children, given concerns about age-related changes in immunogenicity [15,16]. We showed that mean TTG IgA in the <4.0 years of age group was slightly but significantly lower than the ≥4.0 years of age group when analyzed in a normal non-CD cohort, suggesting there was an age-related difference in TTG IgA levels. However, our data showed that TTG IgA had similar sensitivity and specificity in predicting histology findings for both <4.0 and ≥4.0 years age groups, suggesting that TTG IgA was adequate for younger children in predicting histology as previously reported [18-21], and fluctuating TTG IgA levels seen in younger children appear to have no significant impact on the histologic correlation. DGP antibodies (IgA and IgG) had better specificity for the younger age group compared with TTG IgA, but sensitivity was similar. In the older group, DGP IgG displayed parallel performance as TTG IgA, but DGP IgA had low sensitivity at 60%. Overall, our data supported the consensus that DGP antibodies had some advantage over TTG IgA in younger children [19-21], but there was no benefit in older children. In fact, DGP IgA in older children had low sensitivity. We believe that the age-related discrepancy among different investigations could be due to assay sensitivity, sample size, or how an age group was defined. The cutoff values of TTG IgA and DGP IgA were lower in the <4 years of age group compared with the ≥4.0 years of age group in our study. However, we could not validate if age-based cutoff values could further improve the test sensitivity and specificity in predicting histology results because only a small portion of <4.0 years of age children had tests done in the same lab and also had biopsy data. A large sample size study is needed to determine an age-related cutoff value that can maximize the benefits of each serological test.

It is well recognized that patients with T1DM have a higher prevalence of CD compared with the general population in part due to the strong inherent autoimmune predisposition with specific HLA alleles associated with both diseases [9-12]. Hagopian et al. [32] proposed that pre-existing T1DM autoimmunity might elicit CD autoimmunity via the upregulation of TTG

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expression in the stressed and inflamed islets, which in turn might trigger autoimmunity to TTG due to the close proximity and shared draining lymph nodes between the duodenum and pancreas. The interplay between T1DM autoimmunity and other environmental and genetic factors can lead to the development of CD with the release of TTG IgA into circulation and abnormal mucosal morphology.

Since the serological tests became available for screening, studies have shown that patients with T1DM also have positive tests in the absence of CD diagnosis [24-27]. In a pediatric study, TTG IgA was found to be transient or to fluctuate in patients with T1DM and became normal when repeated later and most had an infiltrative lesion or entirely normal mucosa upon histological confirmation [26]. In a small sample, Chan et al. [27] noted that among 16 T1DM children with positive TTG IgA, 25.0% (4/16) had normal biopsies and CD did not develop in follow up. They also showed that among their 66 non-T1DM with normal biopsy, only 6.1% (4/66) were TTG IgA positive (false positive). A more recent study also revealed that among 24 T1DM children with positive TTG IgA, 41.7% (10/24) had normal biopsies [30]. We also observed a similar rate of normal biopsies among T1DM children with positive TTG IgA and a similar false positive rate in the non-T1DM population. In addition, our data showed that T1DM with normal biopsies had a higher positive rate of TTG IgA in comparison to non-T1DM.

The appearance of TTG IgA in the circulation can precede the intestinal morphology changes, thus positive serology with normal mucosa could be latent CD if abnormal mucosa was detected later [22,23]. There is no obvious explanation for the normal mucosa with positive TTG IgA or subsequent normalization of TTG IgA in T1DM patients without development of CD. In a study of T1DM patients with normal intestinal mucosa, Maglio et al. [33] showed that mucosal TTG IgA deposits were detected in both serum positive and negative TTG IgA patients, but were significantly higher than in the non-disease controls. Further analysis showed that only TTG IgA from a gene family that was typically produced in patients with CD was detected in the positive serum TTG IgA, not in the serology negative cases [33]. The study hints that there is an inflammatory state in the structurally normal intestine of patients with T1DM. It is possible that positive TTG IgA is a manifestation of T1DM autoimmunity, and fluctuation of TTG IgA over a period of time might reflect the degree of inflammation associated with the islets as suggested previously [24,26,32,33]. In the non-CD cohort, we also revealed that mean TTG IgA was significantly higher in T1DM than non-T1DM, further supporting a T1DM-specific serology status in the absence of CD.

We propose that the poor specificity of TTG IgA and false positives observed in T1DM may in part be due to an established threshold that does not take into account the potential effects of inflammation associated with T1DM on the levels of TTG IgA. One recent study also indicated the need for thresholds of TTG IgA levels to decide if biopsy is necessary among T1DM [30]. An early study proposed that an immediate intestinal biopsy should only be warranted if anti-TTG antibody values were up to at least three times the upper normal limit [29]. Liu et al. [31] suggested that to reduce the frequency of unnecessary biopsies for CD, the threshold of TTG IgA for screening purposes should be set higher than for clinical diagnosis. We were able to show that T1DM had a higher cutoff value at 36.6 units compared with non-T1DM at 16.3 units. Our results and previous studies imply that it is fruitful to analyze TTG IgA thresholds in T1DM. Unfortunately, we could not test if a higher cutoff value could improve test specificity because we had a very small number of patients with T1DM who had both histology data and serology test done in the same lab with same assay kits. A larger sample size is needed to further examine thresholds in T1DM population.

Collectively, our results were in agreement with previous findings on the advantages of DGP antibodies in younger children and we recommend an age-based strategy to determine which serological antibody testing should be ordered. Furthermore, we found that TTG IgA in children with T1DM had high false positive rate and low specificity in predicting histology findings, which may necessitate higher cutoff values. We strongly advocate that each lab should establish its own cutoff value based on the populations that they serve therefore strengthening the accuracy of the test.

Study limitations

Our study is limited by its retrospective nature. Patient data collected through our electronic medical records were not specifically recorded for the purpose of this study and sometimes were not complete. Therefore, several variables may have affected our results. 1) The duration between serological tests and endoscopic confirmation was not consistent among patients, which could influence the correlation between histologic findings and serological tests, even though the patients were all compared during their evaluation periods and were not on a gluten-free diet. 2) The decision to undergo biopsy evaluation was dependent on the gastroenterologist's discretion, though often performed in serology positive cases or in serology negative cases with a strong suspicion. Therefore, there was selection bias. However, our results largely agreed with previous studies as we discussed above. 3) The majority of our normal non-CD population was not evaluated with biopsies to rule out CD because most of these patients were screened for various reasons. CD diagnosis was ruled out based on negative serological test results and the lack of clinical features. Only a small portion of these patients had biopsies, confirming that they did not have CD. Since we had a large sample size, we believed these variations had minimal impact on the results.

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