The Utility of IgA-Based Serologic Markers in Diagnosing Celiac Disease in Children 24 Months of Age or Younger

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Current screening guidelines in North America for celiac disease recommend additional IgG based testing for younger children. Our multicenter retrospective study showed that the anti-tissue transglutaminase IgA antibody test should be the recommended initial test in all children, including those ≤24 months of age. *(J Pediatr 2020;224:158-61).*

The prevalence of celiac disease has increased in the last few decades. This increment is attributed to multiple factors, such as the availability of sensitive serologic tests, increased screening of high risk individuals, increased awareness of celiac disease and possibly environmental factors.[1,2] Younger children have nonspecific signs and symptoms for celiac disease. Although small bowel biopsy is considered to be the gold standard for the diagnosis of celiac disease, the usefulness of the available serologic tests is of paramount importance in evaluating these children. There are a number of commercially available serologic tests to identify individuals who may have celiac disease, which have revolutionized clinical care.[3,4] These include antibodies to gliadin, tissue transglutaminase (TTG), endomysium (EMA), and deamidated gliadin peptides (DGP).

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Based on sensitivities and specificities, current evidence-based guidelines recommend TTG-IgA as the most accurate and cost-effective initial screening test for celiac disease.[5-7] However, these recommendations apply to individuals aged ≥2 years and additional testing is recommended in children ≤24 months of age. This recommendation is based on the notion that TTG-IgA performs less well in children <2 years of age based on findings of initial studies using EMA and TTG-IgA.[8,9] Subsequently, several smaller studies have suggested a lower sensitivity for TTG-IgA among those <2 years of age when compared with older children.[10-12] Accordingly, it is recommended to include additional tests, specifically DGP-IgG, for all those <2 years of age undergoing testing for celiac disease.[6]

This age-based difference in recommendations is a source of confusion for pediatricians and general practitioners when they consider screening for celiac disease. To address this problem, we designed this study with the primary aim to determine whether TTG-IgA antibody can be used as a reliable initial test to identify children with celiac disease who are <2 years of age.

DGP	Deamidated gliadin peptide
EMA	Endomysium
TTG	Tissue transglutaminase

Methods

This multicenter study included Mayo Clinic, Nationwide Children's Hospital, and Boston Children's Hospital. Electronic medical records were reviewed retrospectively at Mayo Clinic from 1997 to 2017 and Nationwide Children's Hospital from 2000 to 2018, while Boston Children's Hospital has a prospectively maintained database of patients with villous atrophy from 2008 onward. All children ≤24 months of age with proximal small bowel mucosal findings suggestive of celiac disease on biopsy (intraepithelial lymphocytes or villous atrophy) documented in their clinical notes or pathology reports were included in the study. Abstracted data included serologic markers (serum IgA, anti-TTG IgA, anti-TTG IgG, EMA, DGP IgA, and DGP IgG), HLA genetic testing, and histologic reports. This study was approved by the local institutional review board at all 3 study sites.

A thorough chart review was done to assess if the children included in the cohort met the diagnostic criteria for celiac disease based on the North American Society of Pediatric Gastroenterology, Hepatology and Nutrition guidelines.[5] Children with suggestive signs or symptoms, elevated serologic markers with characteristic histologic findings, and subsequent resolution of symptoms and normalization of antibody levels on a gluten-free diet were considered as the

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celiac disease group. Children with small bowel mucosal findings suggestive of celiac disease who did not meet any of these criteria (ie, negative serology, no exposure to gluten, or no response to gluten-free diet) were considered as the control group and served as a comparison group. Histologic findings on small bowel biopsy were recorded as intraepithelial lymphocytosis with normal villous architecture; partial villous atrophy; and complete villous atrophy. Because various assays were used across the 3 institutions during the study time period, TTG IgA results are presented as multiples of the upper limits of normal for the assay used. Continuous variables were summarized using the median and IQRs and categorical data were summarized using counts and percentages. The Kruskal-Wallis test and χ^2 tests were used to evaluate association of TTG IgA to small bowel damage in IgA sufficient children with celiac disease. A P value of <0.05 was considered statistically significant. All analyses were performed using R statistical software (v3.4.2; The R Foundation, Vienna, Austria).

Results

A total of 150 children met the study inclusion criteria (68 Boston Children's Hospital, 41 Mayo Clinic, and 41 Nationwide Children's Hospital). The median age at the time of small bowel biopsy was 18 months (range, 3-24 months) and 51% (n = 77) were male. The most common presentation was failure to thrive (**Table I**; available at www.jpeds.com). The small bowel mucosal findings were consistent with intraepithelial lymphocytosis in 3% (5/150), partial villous atrophy in 45% (68/150), and complete villous atrophy in 52% (77/150) of children. Only 18% (n = 27) had a documented family history of celiac disease. At the time of presentation, 14% (n = 20) were anemic based on reference laboratory test cut-offs for age. Genetic testing was available in 13% (n = 19/150).

Various serologic markers were used for celiac disease testing: 134 TTG-IgA; 93 EMA-IgA; 33 anti-gliadin IgA and/or IgG; and 23 DGP IgG and/or IGA. The majority of the children (n = 116) had >1 test performed. In this cohort, 127 children (85%) were confirmed to have celiac disease (celiac disease group) and 23 children (15%) did not meet the diagnostic criteria for celiac disease (control group). Of the children with celiac disease (n = 127), 115 had TTG-IgA screening done, out of which 112 (97.5%) had elevated TTG-IgA. The remaining 3 with normal serum IgA and negative TTG-IgA had positive IgG based testing (1 each with DGP-IgG, TTG-IgG, and gliadin-IgG). Of the 23 children in the control group, 19 had TTG-IgA screening done and all of them had negative TTG-IgA. In the remaining 4 children, 1 had negative anti-EMA antibodies and no serologic testing was available for 3 children (these children were <6 months of age and did not have gluten exposure before the small bowel biopsy). The **Figure** (available at www.jpeds.com) illustrates the available serologic test results for the cohort. The differences between demographic and histologic features of celiac disease vs control groups are summarized in **Table II** (available at www.jpeds.com).

Of the children with confirmed celiac disease, the total serum IgA level was available in 107 children. The median serum IgA level was 99.5 (range, 0-486). Out of these, 94.5% (101/107) were IgA sufficient and 5.5% (6/107) were IgA deficient (IgA level of <5 mg/dL). All IgA-deficient children had positive IgG-based serology (3 TTG-IgG, 2 DGP-IgG, and 1 gliadin IgG).

Histologic evaluation of patients was consistent with intraepithelial lymphocytosis in 5, partial villous atrophy in 60, and complete villous atrophy in 69 children with available TTG-IgA (n = 134). Of the children with available TTG-IgA (n = 134), the celiac disease group (n = 115) was older (median age, 19 months vs 15 months; $P = .001$) and had a higher serum TTG-IgA (median 7.4 multiples of the upper limits of normal vs 0.3 multiples of the upper limits of normal; $P < 0.001$) than those with control group (n = 19). **Table III** summarizes the laboratory test results for children with TTG IgA data by celiac disease.

Of the 23 children in the control group, 4 children were lost to follow-up, 4 children had negative genetic testing for celiac disease, 3 children had no gluten exposure, 4 children had symptom resolution despite being on a regular (gluten-containing) diet, 5 children did not respond to a gluten-free diet, and 3 children had alternate diagnosis that

Table III. Summary of laboratory tests for children with TTG IgA results available based on celiac disease status (n = 134)

Laboratory tests	No celiac disease (n = 19)	Celiac disease (n = 115)	Total (n = 134)	*P* value
TTG IgA				<.001*
Median (range)	3.0 (0.1-20.0)	100.0 (0.1-4965.0)	100.0 (0.1-4965.0)	
Multiples of the upper limits of normal of TTG IgA				<.001*
No.	19	115	134	
Median (range)	0.3 (0.0-1.0)	7.4 (0.0-248.2)	6.0 (0.0-248.2)	
EMA				<.001†
No.	14	66	80	
Negative	14 (100.0%)	13 (19.7%)	27 (33.8%)	
Positive	0 (0.0%)	53 (80.3%)	53 (66.2%)	
DGP IgA (ULN 20)				.001*
No.	6	25	31	
Median (range)	10.0 (2.8-33.0)	114.0 (1.0-2367.0)	75.0 (1.0-2367.0)	
DGP IgG (ULN 20)				.001*
No.	5	25	30	
Median (range)	15.1 (10.0-31.3)	150.0 (20.0-1936.0)	130.5 (10.0-1936.0)	
Anti-gliadin (ULN 23)				.063*
No.	3	24	27	
Median (range)	9.7 (3.0-66.3)	98.8 (1.9-235.0)	90.1 (1.9-235.0)	

ULN, upper limit of normal.
*Kruskal-Wallis rank-sum test.
†Fisher exact test for count data.

Table IV. Serologic markers and diagnosis of patients without celiac disease (n = 23)

No.	TTG-IgA (ULN)	TTG-IgG (ULN)	EMA	DGP-IgA (ULN)	DGP-IgG (ULN)	AGA-IgA (UNL)	AGA-IgG (UNL)	Nonceliac disease (alternate diagnoses or reason for exclusion from celiac disease group)
1	<1.0 (20)	2.3 (20)	Negative	2.8 (20)	13.8 (20)	–	–	Negative genetic testing
2	<1.0 (20)	5.1 (20)	Negative	–	–	**66.3 (23)**	15.6 (23)	Negative genetic testing
3	<1.2 (20)		Negative	–	–	–	–	Negative genetic testing
4	<20 (20)	**100 (5)**	Negative	–	–	–	–	Negative genetic testing
5	<1.2 (4)	2.3 (6)	–	10 (20)	**31.3 (20)**	–	–	No gluten exposure, (CMPA)
6	–	–	–	–	–	–	–	No gluten exposure, (CMPA)
7	3.5 (4)	16 (6)	Negative	–	–	–	–	No gluten exposure (EoE)
8	5.2 (20)	–	Negative	–	–	–	–	Symptoms resolved on regular diet
9	<1.2 (4)	–	–	–	–	–	–	Symptoms resolved on regular diet
10		–	–	–	–	–	–	Symptoms resolved on regular diet. Repeat small bowel biopsy normal on regular diet
11	<1.2 (4)	–	Negative	–	–	–	–	Symptoms resolved on regular diet. Repeat small bowel biopsy normal on regular diet
12	0.1 (4)	0.6 (6)	Negative	–	–	–	–	No response to GFD, (GERD)
13	<1.2 (4)	<1.2 (6)	Negative	10 (20)	10 (20)	–	–	No response to GFD, (GERD)
14	3.0 (4)	–	Negative	–	–	–	–	No response to GFD
15	<20 (20)	–	Negative	–	–	–	–	No response to GFD
16	<1.2 (4)	**9.1 (6)**	Negative	10 (20)	**20.8 (20)**	–	–	No response to GFD, No change on biopsy on GFD (trisomy 21)
17	<1.2 (4)	1.2 (6)	–	13.2 (20)	15.1 (20)	–	–	Infectious diarrhea
18	–	–	–	–	–	–	–	Congenital diarrhea
19	9.6 (20)	–	–	–	–	–	–	VEO-IBD
20	<20 (20)	–	Negative	**33 (20)**	–	–	–	Lost to follow-up
21	–	–	Negative	–	–	–	–	Lost to follow-up
22	3.0 (20)	3.0 (20)	–	–	–	3.0	–	Lost to follow-up
23	3.0 (20)	–	Negative	–	–	9.7 (23)	–	Lost to follow-up

AGA, gliadin; *CMPA*, cow milk protein allergy; *EoE*, eosinophilic esophagitis; *GERD*, gastroesophageal reflux disease; *GFD*, gluten-free diet; *ULN*, upper limit of normal; *VEO-IBD*, very early onset inflammatory bowel disease.

explains their small bowel biopsy findings. The available serologic markers and reasons for nonceliac diagnosis for children in the control group are summarized in Table IV.

Discussion

TTG-IgA is considered to be the most sensitive serologic test available for celiac disease.[13,14] Despite high sensitivity of TTG-IgA in adults and older children, there has historically been hesitation about the application of this test in younger children <2 years of age.[5,15,16] The current pediatric celiac disease guidelines from North America still recommend that TTG-IgA should be combined with other IgG-based markers, such as DGP-IgG to improve diagnostic accuracy in these younger children.[5,6,17,18] This multicenter study demonstrates that TTG-IgA is highly useful for celiac disease screening in younger children with suspected celiac disease. Our study showed that about 98% of children had elevated TTG-IgA with corresponding histologic findings. The TTG-IgA titers were significantly higher in celiac disease group as compared with the control group ($P < 0.001$). These findings are consistent with recently published European guidelines, which endorse that TTG-IgA should be used as the initial screening test regardless of age.[19] Thus, TTG-IgA alone is an efficient initial screening test for celiac disease in IgA-sufficient children <24 months of age. IgG-based serologic tests were useful to identify celiac disease in IgA-deficient children.

The prevalence of selective IgA deficiency is reported to be higher in patients with celiac disease as compared with the general population.[5,13,20] However, the true incidence of IgA deficiency in younger children is not well-known, because low IgA levels might be transient.[21] In our cohort, only 5.5% children were IgA deficient (serum IgA of <5 mg/dL), which is comparable with other patients with celiac disease. We found that that TTG IgA is a useful screening test in IgA-sufficient children.

Our study showed that 15% of the included children did not meet the celiac disease diagnostic criteria despite having suggestive histologic findings for celiac disease. All of these children had negative TTG-IgA with only few children having other markers positive (Table IV). Multiple conditions like peptic ulcer disease, food hypersensitivity, inflammatory bowel disease, infections, and bacterial overgrowth can produce histologic mimics of celiac disease.[13,22] Similar underlying etiologies were seen in our study in children in the control group. Thus, the children with a clinical presentation and mucosal findings suggestive of celiac disease who do not meet the diagnostic criteria for celiac disease should be evaluated for other causes of villous atrophy.

The main limitation of our study is its retrospective design, resulting in incomplete data for some children and incomplete evaluation of children in the control group with villous atrophy, because some of them were lost to follow-up. The main strength of our study is that it was multicenter study in a relatively large cohort of younger children with celiac disease.

In conclusion, TTG-IgA is a useful marker of celiac disease and should be the initial screening test for celiac disease across the lifespan, including children ≤24 months of age. Total serum IgA should also be obtained so that those who are IgA deficient can have IgG-based testing performed. ■

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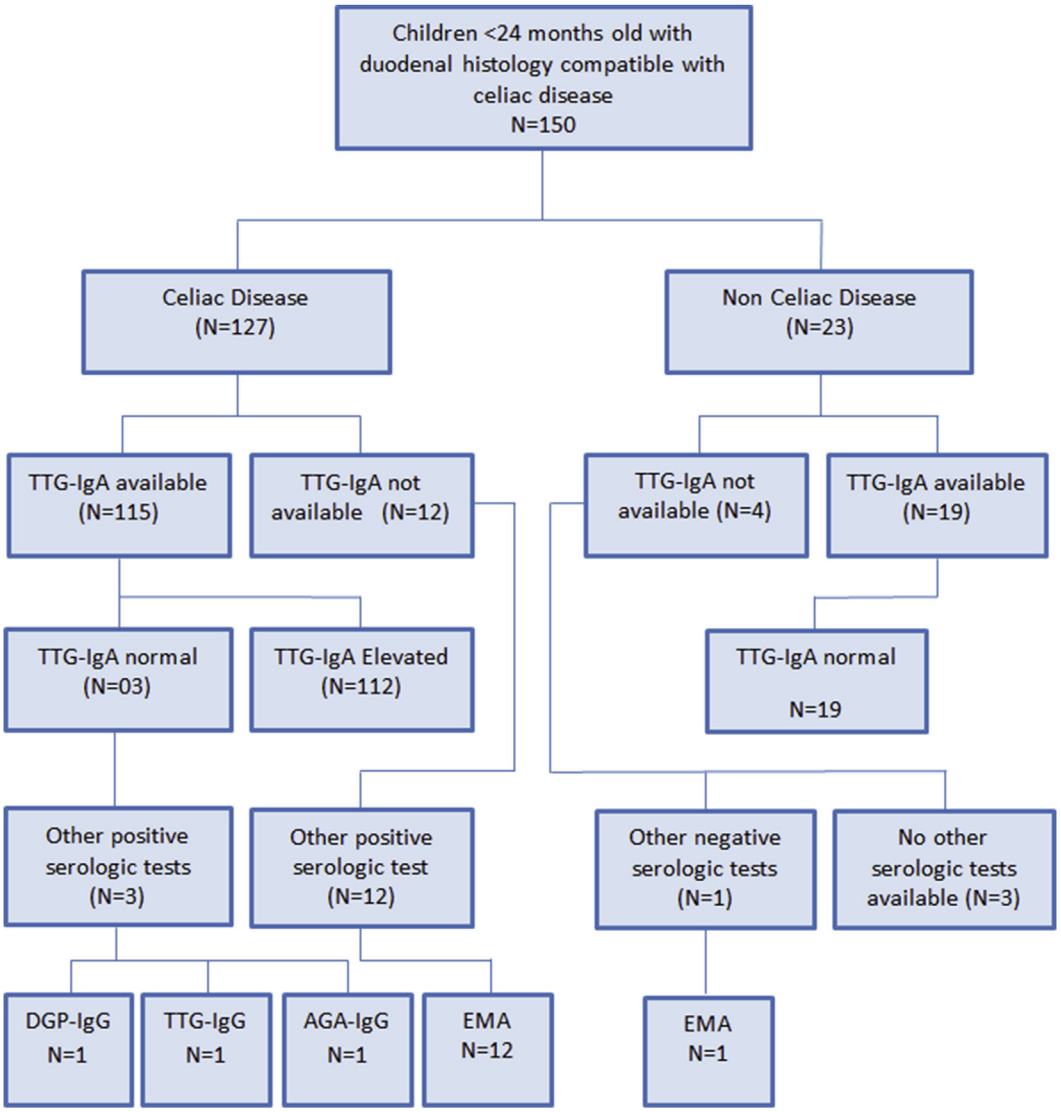

Figure. Flow diagram showing available serologic test results for the cohort. *AGA*, gliadin.

Table I. Primary presenting symptoms of children at the time of evaluation for celiac disease

Presentations	Number (n = 150)	Percentage
Failure to thrive	84	56
Diarrhea	26	17.3
Vomiting	10	6.6
Abdominal pain	9	6.0
Constipation	6	4.0
GERD	4	2.6
Anemia	1	0.7
Positive family history	1	0.7
Short stature	1	0.7
Diabetes mellitus type 1	1	0.7
Missing	7	4.7

GERD, gastroesophageal reflux disease.

Table II. Comparison between celiac disease and control groups (demographics, histologic features)

Variables	No celiac disease (n = 23)	Celiac disease (n = 127)	Total (n = 150)	*P* value
Age at diagnosis (mo)	14.0 (3.0 to 24.0)	19.0 (4.8 to 24.0)	18.6 (3.0 to 24.0)	**<.001***
Female	12 (52.2)	61 (48.0)	73 (48.7)	.389†
Weight (Z score) at diagnosis				.905*
No.	23	121	144	
N-Missing	0	6	6	
Median (range)	−0.7 (−5.9 to 4.1)	−0.7 (−3.6 to 16.6)	−0.7 (−5.9 to 16.6)	
Height at diagnosis				**.011***
No.	16	99	115	
N-Missing	7	28	35	
Median (range)	71.3 (59.0-88.3)	79.0 (69.7-126.0)	78.9 (59.0-126.0)	
Family history of celiac disease				.078†
Family history present	1 (4.3)	26 (20.5)	27 (18.0)	
Family history not documented	22 (95.7)	101 (79.5)	123 (82.0)	
HLADQ2/DQ8 testing				**<.001**‡
Positive	9 (39.1)	6 (4.7)	15 (10.0)	
Negative	4 (17.4)	0 (0.0)	4 (2.7)	
Unknown	10 (43.5)	121 (95.3)	131 (87.3)	
Anemia	5/21 (24)	15/121 (12)	20/142 (14)	.165‡
Biopsy findings				**<.001**‡
Intraepithelial lymphocytosis	1 (4.3)	4 (3.1)	5 (3.3)	
Partial villous atrophy	21 (91.3)	47 (37.0)	68 (45.3)	
Complete villous atrophy	1 (5.3)	76 (58.0)	77 (51.3)	

Values are number (%) or median range unless otherwise indicated. Significant *P* values (<.05) are in bold text.
*Kruskal-Wallis rank-sum test.
†Fisher exact test for count data.
‡Pearson χ^2 test.