AMERICAN JOURNAL OF BIOLOGY AND NATURAL SCIENCES
https://biojournals.us/index.php/AJBNS
ISSN: 2997-7185

Article

Complete Serological Profile and Diagnostic Correctness of Celiac Disease Immunological markers in a Pediatric Population

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Citation: Kadhium, N. M. Complete Serological Profile and Diagnostic Correctness of Celiac Disease Immunological markers in a Pediatric Population. American Journal of Biology and Natural Sciences 2026, 3(5), 64-73

Received: 08th Feb 2026
Revised: 21th Mar 2026
Accepted: 18th Apr 2026
Published: 11th May 2026

Abstract: Background: Celiac disease (CD) is consider an autoimmune disease which can be initiated by gluten intake,which need an accurate serological testing and supervision. Many different antibody tests are accessible, but the ideal cut-offs and combination of tests in pediatric patients require more details explanation. Objective: To determine the diagnostic efficiency of multiple antibody used to diagnose celiac disease, determine ideal cut-off measures, and evalute the effect of gluten-free diet (GFD) compiance on immunological markers in children and teenagers. Methods: analysis of the present study we take 180 patients (ages 2-18 years) with documented demographic information, family history, and serum antibody which markers including total IgA, tTG-IgG, tTG-IgA, DGP-IgG, DGP-IgA, EMA-IgG, and EMA IgA. Statistical analyses was done by using SPSS software and the tests included logistic regression, ROC analysis correlation analysis and t-tests. **Results:** Patients who have not following GFD (n=54) revealed significantly higher serum antibody titers compared to those on GFD (n=126): tTG-IgA (58.4±28.1 vs 8.7±10.2, p<0.001), EMA IgA (62.3±18.9 vs 9.2±8.4, p<0.001). tTG-IgA showed powerful diagnostic functioning (AUC=0.94), with ideal cut-off ≥20 U/mL with 92% specificity and 88% sensitivity. Logistic regression analysis recognized that tTG-IgA (OR=1.20, 95% CI:1.13-1.27) and EMA IgA (OR=1.16, 95% CI:1.07-1.26) as significant indicators. Children (2-9 years old) had significantly higher antibody values when compared to teenagers (10-17 years old). **Conclusions:** tTG-IgA ≥20 U/mL showed perfect diagnostic efficacy for patients without treatment. Positive correlations between antibody measures suggest excessive testing could be lowered. Age-related reference ranges may inhance diagnostic correctness in pediatric patients.

Keywords: Pediatric gastroenterology, celiac disease, endomysial antibodies, pediatric gastroenterology tissue transglutaminase, gluten-free diet, diagnostic accuracy

Introduction

Celiac disease (CD) was a long lasting autoimmune created intestinal disorder approximately 1% of the global population suffering from its illness, with increasing identification in school aging populations [1]. The disease is defined by an unsaitable immune reaction to gluten which can lead to

intestinal distruction and systemic features. exact diagnosis is important for avoiding long-term complications such as growth retardation, malnutrition, increased malignancy risk and osteoporosis, [2]. Uniform terminology for CD and associated disorders have been suggested to enhance uniformity in clinical and research practice [3].

Current diagnostic procedures depend strongly on serum antibody testing, with tissue transglutaminase IgA (tTG-IgA) which considered primary screening test because its high specificity and sensitivity [3]. A big quantitative-analysis had verified tTG-IgA as the most correct single measure for pediatric CD [4]. Nevertheless, pediatric-specific difficulties in the diagnosis of CD consist of different antibody titers among age groups and the requirement for verifying measurement in vague cases [5]. on the other hand, multiple difficulties remain in the diagnosis of pediatric patients: different antibody measures across age cohorts, the requirement for confirmatory testing in undetermined cases and IgA deficiency complicating analysis[6]. furthermore, obseriving treatment response through immunological markers remains suboptimal, with ideal cut-offs for determining remission insufficiently established [7]. Indication from organized reviews assists the apply of serologic testing as a first line systematic protocol for CD in children that showing symptoms [8].

The development of celiac disease serological procedures have introduced several antibody measures, including endomysial antibodies (EMA) and deamidated gliadin peptide (DGP) antibodies , making both chances for diagnosis improvement and accurate test selection and explanation [9]. Understanding the correlations, comparative performance, and clinical utility of these measures in practical pediatric practice is important for establishing diagnostic procedures.

Research Gap

In spite of extensive publicationon celiac serology, several deficiencies remain:

Age-specific cut-off levels for antibody measures in children

When compared between diagnostic functioning of all available examinations in the same group. Influence of exact gluten-free diet on the variety of antibody types ideal test combinations for clinical diagnosis and follow up. In addition to the association

patterns between different antibody measures.

The present study covers these gaps by complete analyzing a group of 180 pediatric patients with complete antibody panel.

Study Objectives

First - Line Objectives:

To identify the diagnostic correctness of tTG-IgG, tTG-IgA, DGP-IgG, DGP-IgA, EMA IgG, and EMA-IgA for celiac disease

To take ideal cut-off levels for each exam.

To evaluate the influence of gluten-free diet and how it can effect the antibody levels.

Second-Line Objectives:

To assess antibody levels differences between age group. To test the association between different antibody measures.

To create a indicative model for celiac disease using population related and serological parameters.

To evaluate the effect of family history on disease manifestation.

Materials and Methods

Prospective cohort study

Celiac disease follow up study analyzed collected clinical data from patients diagnosed for celiac disease between January 2023 and December 2025. The present study was performed according to the Declaration of Helsinki and ethical approval was received from the Institutional Review Board (IRB#: CD-2023-045).

Study Population

Inclusion Criteria:

Patient are aged range from 2-18 years suspected of celiac disease (have the same symptoms family history and done screening tests) and clinically diagnosed celiac disease by pediatric doctor . Complete serum antibody tests were taken. All patient either have GFD or regular diet.

Exclusion Criteria:

Other intestinal tract illnesses hypersensitivity reaction to gluten and known IgA deficiency (total IgA <20 mg/dL). in addition to other autoimmune disorders affecting antibody interpretation

latest gluten intake less than 6 weeks.

Sample Collection

5ml of vein-puncture were collected and separated at the speed of 3000X centrifuge for serum separation serum samples were kept for serological analysis.

Demographic Variables:

Age (years),gender or sex (female/male), clinical symptom of celiac disease and family history of celiac disease (yes/no)

Serological Variables: serum antibodies measured in the prenent study are the followings:

Total IgA (mg/dL), tTG-IgA (U/mL), tTG-IgG (U/mL), DGP-IgA (U/mL), DGP-IgG (U/mL), EMA IgA (titer/U/mL)and EMA-IgG (titer/U/mL)

Clinical Variable:

Following Gluten-free diet (yes/no)

Operational Definitions:

Propable Celiac Disease: Patients that are not following GFD with tTG-IgA ≥20 U/mL and/or EMA IgA ≥15 U/mL.

GFD compliance : Patient/parent notify of gluten avoidance strictly for more than 3 months. Family History: First-degree relative (brother or sister) with celiac disease confirmed by biopsy.

Laboratory Methods

The present study serological procedures were done in a private labortories in AL-Diwaniyh provice/ Iraq. The tests included are the following:

tTG IgA and tTG IgG: ELISA CHORUS)

DGP IgA and DGP IgG: ELISA (CHORUS)

EMA IgA and EMA IgG: ELISA , CHORUS

Total IgA: Nephelometry IgA assy kit are designed for nephelometric/ turbidimetric analyzers (SPAPLUS/BN II/ reagent antisera kit)

Statistical Analysis

The present study results analyses were conducted using IBM SPSS Statistics 28.0. Continuous data was evaluted for normality through Shapiro-Wilk test. summary statistics covered by means with standard deviations for normally distributed data and medians with interquartile ranges for non-normally distributions.

Specific Analyses:

Comparisons between groups: Mann-Whitney U tests for non-normal distributions,Independent t-tests for normally distributed variables.

Diagnostic performance: Receiver Operating Characteristic (ROC) curve analysis with calculation of area under the curve (AUC), specificity, sensitivity,negative predictive value (NPV) and positive predictive value (PPV).

Analysis for Correlation: Spearman correlation for non-normal distributions and Pearson correlation for normally distributed variables.

Predictive analysis: backword elimination was used in Binary logistic regression.

Age Grouping Analysis: post-hoc Tukey tests with One-way ANOVA .

Factor Analysis: main component analysis done by using Varimax rotation

Statistical significance evaluation was taken out at $p<0.05$ (two-tailed). For more than one comparisons,in the present study we used Bonferroni correction. The sample size was an sufficient to carry out age-stratified examination as suggested by current diagnostic research [10].

Sample Size Justification:

According to predictive AUC = 0.90 for tTG-IgA, with 80% power and alpha=0.05, a minimum of 50 control and 50 cases was necessary. The present study sample of 180 yields adequate force for all scheduled analyses.

Result and Discussions

Results

Population and Clinical Characteristics

The present study involved 180 participents with a mean age of 10.2±4.3 years (range: 2-18 years). The sample was equally distributed by gender category (53.3% female) and family celiac disease history (48.9% positive). 70% of patients were undergoing a gluten-free diet at the time of serological testing (Table 1).

Table 1. Characteristics of Study Population (N=180)

Characteristic	Category	n (%)	Mean ± SD
Age (years)			10.2 ± 4.3
Age Groups	Children (2-9)	78 (43.3%)	6.23 ± 2.38
	Adolescents (10-17)	92 (51.1%)	13.13 ± 2.26
	Adults (18+)	10 (5.6%)	18 ± 0.0
Gender category	Female	96 (53.3%)	-
	Male	84 (46.7%)	-
Family History	Yes	88 (48.9%)	-
	No	92 (51.1%)	-
Gluten-Free Diet	Yes	126 (70.0%)	-
	No	54 (30.0%)	-

Antibody Measurements

Table 2 shows the illustrative statistics for all antibody measures. All antibody distributions were positive-skewed, with means considerably higher than medians, showing most participents had low results with a subgroup appear to have very high measured values.

Table 2. Antibody measures in the study cohort.

Test	Mean ± SD	Median (IQR)	Range	Skewness	Kurtosis
Total IgA	112.4 ± 43.2	111.5 (78-145)	45-254	0.85	0.62
tTG-IgA	23.5 ± 29.8	6.6 (1.8-35)	0.1-101	1.42	0.95
tTG-IgG	30.2 ± 37.1	12.0 (3.4-45)	0.9-121	1.25	0.43
DGP-IgA	26.1 ± 25.4	8.5 (3.9-38)	0.6-88	1.12	0.21

Test	Mean ± SD	Median (IQR)	Range	Skewness	Kurtosis
DGP-IgG	29.4 ± 36.8	6.8 (2.8-40)	0.6-99	1.08	-0.15
EMA IgA	24.3 ± 26.5	4.7 (2.8-34)	1.8-87	1.34	0.82
EMA IgG	28.6 ± 33.2	6.6 (3.6-38)	3.2-99	1.21	0.31

Influence of Gluten-Free Diet

Patients not undergoing to a gluten-free diet had significant higher measures of all tested antibodies when compared to those that undergo a GFD ($p<0.001$ for all participents). The degree of difference was greatest for EMA IgG and EMA IgA (Table 3). The higher decrease in EMA antibodies when compared to DGP antibodies over a GFD had been likewise reported in time-based pediatric groups [11].

Table 3. Antibody measures by Diet Status

Measurment	No GFD (n=54)	On GFD (n=126)	Mean Difference (95% CI)	p-value	Effect Size (Cohen's d)
tTG-IgA	58.4 ± 28.1	8.7 ± 10.2	49.7 (42.3-57.1)	<0.001	2.35
tTG-IgG	71.2 ± 30.5	12.8 ± 15.3	58.4 (50.2-66.6)	<0.001	2.54
DGP-IgA	54.3 ± 22.8	14.2 ± 11.5	40.1 (33.8-46.4)	<0.001	2.28
DGP-IgG	66.8 ± 28.4	13.4 ± 14.2	53.4 (45.9-60.9)	<0.001	2.51
EMA IgA	62.3 ± 18.9	9.2 ± 8.4	53.1 (48.2-58.0)	<0.001	3.42
EMA IgG	77.4 ± 22.1	11.3 ± 10.6	66.1 (60.4-71.8)	<0.001	3.68

Diagnostic Accuracy

ROC curve analysis showed an excellent diagnostic effectivness for all tested antibodies in differentiating such as celiac disease patients (those that havnt undergo GFD showed high antibody measures) from others. EMA IgA exhibited the highest AUC (0.96) then followed by tTG-IgA (0.94) (Table 4).

Table 4. Diagnostic Accuracy of Antibody Tests

Test	AUC (95% CI)	Optimal Cut-off	Sensitivity	Specificity	PPV	NPV
tTG-IgA	0.94 (0.91-0.97)	≥20 U/mL	88%	92%	91%	89%
EMA IgA	0.96 (0.94-0.98)	≥15 U/mL	92%	94%	93%	93%
tTG-IgG	0.92 (0.88-0.95)	≥25 U/mL	85%	90%	88%	87%

Test	AUC (95% CI)	Optimal Cut-off	Sensitivity	Specificity	PPV	NPV
DGP-IgA	0.89 (0.85-0.93)	≥30 U/mL	82%	88%	85%	85%
DGP-IgG	0.91 (0.87-0.94)	≥35 U/mL	80%	92%	90%	83%
EMA IgG	0.95 (0.92-0.97)	≥20 U/mL	90%	93%	92%	91%

Correlation Analysis

All celiac-specific antibodies exhibited strong positive correlations with each other (r=0.84-0.89, p<0.001). Total IgA demonstrate moderate correlations with all antibody tests (r=0.38-0.42, p<0.001). Age exhibited weak negative correlations with EMA IgA and tTG-IgA (Table 5).

Table 5. Correlation analysis between Study Variables

Variable	1	2	3	4	5	6	7	8
1. Age	1.00	0.08	-0.15*	-0.12	-0.10	-0.09	-0.14*	-0.11
2. Total IgA	0.08	1.00	0.42**	0.38**	0.36**	0.35**	0.39**	0.41**
3. tTG-IgA	-0.15*	0.42**	1.00	0.87**	0.82**	0.81**	0.89**	0.86**
4. tTG-IgG	-0.12	0.38**	0.87**	1.00	0.79**	0.83**	0.84**	0.88**
5. DGP-IgA	-0.10	0.36**	0.82**	0.79**	1.00	0.86**	0.78**	0.80**
6. DGP-IgG	-0.09	0.35**	0.81**	0.83**	0.86**	1.00	0.77**	0.84**
7. EMA IgA	-0.14*	0.39**	0.89**	0.84**	0.78**	0.77**	1.00	0.85**
8. EMA IgG	-0.11	0.41**	0.86**	0.88**	0.80**	0.84**	0.85**	1.00

*p<0.05, **p<0.001

Predictive analysis

Binary outcome regression was used for tTG-IgA and EMA IgA as a strong significant determinants of suspected celiac disease after managing the demographic variables. The model predicts disease risk with 92.2% classification correctness (Table 6).

Table 6. Logistic Regression Model for determining suspected Celiac Disease cases.

Variable	B	SE	Wald	p-value	Odds Ratio	95% CI
Age	-0.12	0.06	4.00	0.045	0.89	0.79-0.99
tTG-IgA	0.18	0.03	36.00	<0.001	1.20	1.13-1.27
EMA IgA	0.15	0.04	14.06	<0.001	1.16	1.07-1.26

Variable	B	SE	Wald	p-value	Odds Ratio	95% CI
Constant	-5.23	1.12	21.80	<0.001	0.01	-

Model Statistics:
Nagelkerke $R^2 = 0.78$
Model $\chi^2 = 142.36$, df = 3, $p < 0.001$
Hosmer-Lemeshow: $\chi^2 = 6.24$, $p = 0.621$
Overall Classification Accuracy: 92.2%

Age-Related Differences

Children (2-9 years) had significantly higher tested antibody measures when compared to adolescents (10-17 years) for tTG-IgG, tTG-IgA and EMA IgA ($p<0.05$ for all participants) (Table 7).

Table 7. Age Group Comparisons

Test	Children (2-9)	Adolescents (10-17)	Adults (18+)	F	p-value	Post-hoc
tTG-IgA	31.2 ± 32.4	18.5 ± 26.8	14.2 ± 21.3	4.23	0.016	C > A*
tTG-IgG	38.7 ± 39.2	24.8 ± 34.6	18.9 ± 28.4	3.87	0.022	C > A*
EMA IgA	31.8 ± 28.9	19.4 ± 23.8	15.6 ± 19.2	4.56	0.012	C > A*

*C = Children, A = Adolescents; $p<0.05$

Factor Analysis

Main component analysis showed one dominant factor (eigenvalue = 4.23) describing 70.5% of the dispersion, with high loadings from all tested antibodies (>0.75), implying they measure a prevalent underlying cause (celiac autoimmunity).

Diagnostic Classification

Based on integrated measures, 48 patients (26.7%) were classified as established likely celiac disease, 32 (17.8%) as suscpected celiac, and 100 (55.6%) as imprbable celiac (Table 8).

Table 8. Final Diagnostic Classification

Category	n	%	Criteria	Mean tTG-IgA
established Celiac	48	26.7%	tTG-IgA ≥20 + No GFD	67.8 ± 24.5
suspected Celiac	32	17.8%	tTG-IgA 10-19.9 OR EMA IgA ≥15	14.3 ± 4.2
Imprbable Celiac (on GFD)	100	55.6%	All antibodies <10 + On GFD	3.2 ± 2.8

Discussion

Main Findings

This complete analysis of the serology of celiac disease in a pediatric population under 18 years gives many important findings:

The present study verify the important diagnostic efficiency of tTG-IgA (AUC=0.94), assisting its effect as the first line screening test for clinically suspected celiac disease. The ideal cut-off of ≥20 U/mL yields a suitable balance of specificity (92%) and sensitivity (88%), in agreement with new pediatric guidelines [12]. The remarkable reduction in all antibody values in patients that undergo gluten-free diet (5-7 fold diminutions) highlights the usefulness of these indicators for clinical observations for treatment response. EMA antibodies presented the largest effect sizes, suggesting particular utility in clinical observation of nutritional compliance. There are strong correlations between all antibody measures (r=0.77-0.89) which indicate significant redundancy in present testing for clinical practices. This finding has significant implications for cost control in celiac disease laboratory diagnosis. The differences associated with age in antibody measurements propose that age-specific reference ranges may enhance diagnostic correctness, especially in younger children who showed higher levels. Highly positive tTG-IgA measures had also been related to an additional severe histopathology (Marsh 3) in cild- related CD [13].

Clinical Implications

Based on the present study findings, the following clinical protocol was suggested:

Primary Screening: tTG-IgA as initial test with cut-off ≥20 U/mL

Confirmatory measuring: EMA IgA for borderline or suspected cases (tTG-IgA 10-20 U/mL)

Patients that have IgA-Deficiency : tTG-IgG as first line test with cut-off ≥25 U/mL

Treatment follow up: EMA IgA or tTG-IgA every 6-12 months.

Age related Considerations: take into account lower thresholds for teenagers.

The present study results suggest that routine screening of both DGP antibodies and both isotypes of tTG and EMA antibodies may not yield additional diagnostic measure exceeding tTG-IgA and EMA IgA in the majority of cases. This match with the meta-analysis results yielding similar performance between tTG and DGP tests, even though have some differences in particular populations [14].

Comparison with Previous Studies

The current study finding line up with major research in the field:

The elevated diagnostic precision of tTG-IgA (AUC=0.94) aligns with the reports from the European Society for Pediatric Gastroenterology [15]. The powerful effect of GFD on antibody measures corroborates observation studies by Leonard et al. [16].

Differences in age group support outcome from the TEDDY study group [17] and other potential longitudinal studies [25]. However, the ideal cut-off of ≥20 U/mL is higher than some pediatric research's recommending ≥10 U/mL [18]. This difference may indicate differences in assay methodology or study population features. More over, extended diagnostic delays still common in pediatric CD and the serological profiles might be affected at presentation [19].

Mechanisms and Biological rational

The powerful correlations between different antibody measures likely demonstrate shared immunological mechanisms in celiac disease pathogenesis. All examinations measure antibodies that directed against neo-antigens developed by transglutaminase-mediated deamidation of gluten peptides [20]. The differential reaction to gluten-free diet (especially effect on EMA antibodies) may link to examination characteristics: EMA measures antibodies that directed against tissue transglutaminase in its native structure, which might be considered more specific to clinically active disease [21][22].

The present study found differences between age groups could result from multiple factors such as: higher gluten ingestion per body weight in participant of younger children, growth related changes in immune response, or differences in illness duration and strength [23]. The disease course of CD autoimmunity indicate that seroconversion frequently happened in early years of childhood, which might explain higher antibody measures in younger children [24][25].

Strengths and Limitations

Strengths:

The present study have complete serological diagnositc panel for all participant. Pediatric sample size was relatively big

Real-world clinical serological examination from multiple-center, several analytical methods were used and obvious operational description.

Limitations:

Retrospective design to monitor disease developmental stages.

Lack of biopsy confirmation in most cases

Subjective assessment of GFD adherence

Limited adult group for comparison .

Recommandations

Forward looking Validation: assess accuracy of the patients confirmed by biopsy.

Continues Studies: monitor antibody alteration with nutritional interventions

Age-group Analysis: Establishment of age-specific standard ranges

Financial efficiency : Evalute economic effect of streamlined measuring.

Hereditary Correlates: check HLA and non-HLA genetic outcome on antibody measures.

Conclusion

The present study provides complete confirmation supporting tTG-IgA ≥20 U/mL as the best cut-off for celiac disease diagnostic screening in pediatric cohort . The powerful correlations between antibody examinations suggest chances for making diagnostic algorithms much easier, while the remarkable response to gluten-free diet reinforces serological observing of following treatment . Age-related factors should be included into clinical explanation, especially the higher antibody measures seen in younger age children. These results have immediate consequences for medical practice and laboratory testing procedures.

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