ORIGINAL ARTICLE

Enhanced Diagnostic Performance of a Combined Immunological Biomarker Panel for Celiac Disease

[1]Kawakib I. Al-zubaidy, [2]Sanaa S. Atia, [1]Fatima G. Ali*, [1]Hind A.N. Al-Ajwady
[1]Department of Biology, College of Education, Al-Qurna, University of Basrah, Basrah, Iraq
[2]Department of Biology, College of Science, University of Basrah, Basrah, Iraq

Key words:
tTG-IgA, I-FABP, and Citrulline, Celiac Disease

Corresponding Author:
Fatima Gaaid Ali
Department of Biology, College of Education, Al-Qurna, University of Basrah, Basrah, Iraq
fatimagaaid@gmail.com

ABSTRACT

Background: *Celiac disease diagnosis traditionally requires an invasive duodenal biopsy. Non-invasive diagnostic strategies utilizing serological and biochemical biomarkers offer promising alternatives by reducing the need for invasive procedures.* ***Objective:*** *The aim of this study was to compare the diagnostic performance of an integrated panel of biomarkers for the non-invasive diagnosis of celiac disease using potentially diagnostic antibodies in intestinal tissue, tissue transglutaminase IgA (tTG-IgA), in conjunction with intestinal fatty acid-binding protein (I-FABP) and plasma citrulline, against an established standard.* ***Methodology:*** *A prospective cohort study was conducted involving 120 subjects, including 65 patients with biopsy-proven celiac disease and 55 healthy controls. Standardized assays were used to measure serological and biochemical markers. Receiver operating characteristic (ROC) curve analysis was used to evaluate diagnostic performance.* ***Results:*** *The combined biomarker model demonstrated superior diagnostic value (AUC = 0.974, 95% CI: 0.948–1.000) compared to tTG-IgA alone (AUC = 0.912). The combined model showed a sensitivity and specificity of 95.4% and 96.4%, respectively. It achieved a diagnostic accuracy of 92.5% in patients with borderline tTG-IgA titers as well.* ***Conclusion:*** *The combination of tTG-IgA, I-FABP, and citrulline can improve diagnostic accuracy and may reduce the need for invasive biopsy when patients are carefully selected from high-risk groups. These findings should be validated in multicenter studies.*

INTRODUCTION

Celiac disease, or gluten-sensitive enteropathy, is a systemic, chronic autoimmune disease triggered by the intake of gluten and gluten-related proteins in genetically susceptible individuals[1]. The prevalence is estimated to be between 0.6% and 1.4% worldwide, despite the fact that a significant number of cases remain undiagnosed[2]. The clinical manifestations of the condition are broad and include classical gastrointestinal symptoms such as chronic diarrhea and malabsorption, as well as extra-intestinal manifestations including iron deficiency anemia, osteoporosis, and neurological abnormalities[3]. The systemic nature of the disorder and its potential long-term complications, including an increased risk of malignancies and other autoimmune diseases, highlight the importance of accurate and timely diagnosis[4].

The pathogenesis of celiac disease involves a complex interaction between environmental triggers (gluten), genetic predisposition associated with specific human leukocyte antigen (HLA) alleles, and innate and adaptive immune responses[5]. The majority of patients (over 95%) carry one of two HLA gene variants: HLA-DQ2 or HLA-DQ8[6]. Gluten ingestion leads to partial proteolytic degradation into peptides, including gliadin. These gliadin peptides are deamidated in the small intestine by tissue transglutaminase (tTG), particularly the T2 isoform. The resulting deamidated peptides have a high affinity for HLA-DQ2 or HLA-DQ8 molecules on the surface of antigen-presenting cells, thereby triggering the activation of gluten-specific CD4+ helper T cells[7]. The subsequent cytokine release, such as interferon-gamma (IFN-γ), leads to characteristic mucosal damage, including villous atrophy, crypt hyperplasia, and increased intraepithelial lymphocyte infiltration[8].

The diagnostic paradigm of celiac disease has historically relied on small intestinal biopsy as the sole method for confirming the characteristic enteropathy, with lesions classified according to the modified Marsh classification[9]. Although biopsy remains the gold standard, particularly in complicated cases, it is invasive and costly. The development of highly sensitive and specific serological assays has transformed the diagnostic approach to celiac disease. Serum IgA antibodies against tissue transglutaminase (tTG-IgA) form the basis of serological screening. As an initial screening test, the tTG-IgA assay demonstrates a sensitivity of 78–100% and a specificity of 90–100%, making it the preferred method in most international guidelines[10]. Additional serological markers, including

anti-endomysial IgA (EMA-IgA) and anti-deamidated gliadin peptide antibodies (DGP-IgA/IgG), may serve as complementary tests, particularly in young children or individuals with selective IgA deficiency[11].

The high diagnostic accuracy of tTG-IgA, especially at levels exceeding 10-fold the upper limit of normal, has led to the development of non-biopsy-based diagnostic approaches. This strategy has been widely adopted in pediatric populations[12]. Although the use of non-biopsy diagnostic pathways in adults was previously controversial, recent systematic reviews and evolving guidelines, including the updated European Society for the Study of Coeliac Disease (ESsCD) 2025 guidelines, now support a no-biopsy approach in selected adult cases. Specifically, adults under 45 years of age with IgA anti-tTG levels exceeding the upper limit of normal (ULN), confirmed on a second sample, may be diagnosed without duodenal biopsy[13]. This reflects a paradigm shift toward greater acceptance of non-invasive diagnostic strategies in adult celiac disease.

Celiac disease screening is particularly important in high-risk populations. Active case-finding is strongly recommended in individuals at increased risk, including first-degree relatives of affected patients, individuals with autoimmune disorders such as type 1 diabetes mellitus or autoimmune thyroid disease, and those with genetic syndromes such as Down syndrome and Turner syndrome[14]. In addition, screening should be considered in patients presenting with unexplained clinical conditions that may indicate celiac disease, such as refractory iron deficiency anemia, elevated liver transaminases, or early-onset osteoporosis[15].

Based on these considerations, this study aims to conduct a comprehensive clinical evaluation of IgA anti-tissue transglutaminase as a screening tool in a well-defined cohort of high-risk adults for celiac disease. The sensitivity, specificity, positive predictive value, and negative predictive value of different tTG-IgA titers will be assessed, compared with other serological tests, and evaluated for their potential role in a non-biopsy diagnostic pathway in this patient group.

METHODOLOGY

Study Design

This study was designed as a prospective cohort study at Basrah General Hospital between January 2023 and January 2024. We prospectively recruited adult patients (age ≥ 18 years) who have clinical suspicion of celiac disease, either with gastrointestinal (chronic diarrhea, unexplained weight loss, abdominal discomfort) or extraintestinal (iron-deficiency anemia, raised liver transaminases and osteoporosis) symptoms. The risk participants were also first-degree relatives of the known patients of CD and the concomitant cases of the autoimmune diseases like type 1 diabetes mellitus or autoimmune thyroid disease.

Study Population

A potential cohort study was done with 120 subjects, including 65 with a biopsy-confirmed celiac disease and 55 normal subjects. The study could be improved in the future through the introduction of a symptomatic control group (say, those diagnosed with irritable bowel syndrome or Crohn's disease) to further delimit the discriminatory capability of the biomarkers, especially intestinal fatty acid -binding protein (I -FABP) and citrulline, the concentrations of which might be influenced by different kinds of intestinal damage. The study cohort consisted of adults stratified according to the severity of clinical manifestations, comorbidity of autoimmune diseases and biochemical defects related to malabsorption, including iron-deficiency anemia and fat-soluble vitamins deficiency.

Inclusion Criteria

Respondents were only included if they were age ≥ 18 years, had maintained a gluten-containing diet for at least six weeks before serological testing and endoscopic examination, written informed consent, and showing clinical or biochemical signs of possible celiac disease was present. The participants were all treatment-naive on the date of enrollment, and none of them had ever been diagnosed with celiac disease.

Exclusion Criteria

Patients were not included in the study when they had a documented history of celiac disease; when they had followed a gluten-free diet more than two weeks prior to enrolling in the study; and when they had a condition that was known to independently change the level of biomarkers including active gastrointestinal infections, inflammatory bowel disease (Crohn's disease or ulcerative colitis), significant renal dysfunction (estimated glomerular filtration rate <60 mL/min/1.73 m^2), or decomp Also, selective IgA deficiency was tested by evaluating the total serum IgA concentration; subjects with a level less than 0.07 g/L were not included in the main study. Pregnant women, lactating women, and those taking immunosuppressive drugs (corticosteroids, biologic) were also excluded. To ensure the specificity of I-FABP and citrulline to celiac-related mucosal disease, subjects with other plausible causes of enteropathy tropical sprue, intrinsic non-steroidal anti-inflammatory drugs, etc. than celiac disease were strictly excluded using clinical and endoscopic evaluation.

Sample Size Calculation

The analysis of a priori power was done to identify the necessary sample size. Using preliminary data and available literature, we estimated that using the pooled biomarker model, the Area Under the Curve (AUC) would be 0.95 to discriminate between patients with celiac disease and the normal controls. A sample that includes 120 participants consisting of 65 with biopsy-

confirmed celiac disease and 55 healthy controls was found to be adequate to achieve a statistical power of 90%(1-β=0.90) at a significance level of 2-sided α= 0.05 and an expected attrition rate of 10 %. The power analysis was done using G Power, which is version 3.1.9.7. Though it is considered a sufficient sample size to achieve the main goal of appraising the diagnostic efficacy of the composite biomarker model, it might limit the applicability of the results and complicate the process of more specific subgroup analysis. This study therefore must be considered as an initial study, which requires external confirmation in bigger, more diversified multicenter cohort.

Novel Biomarkers and Biochemical Procedures

Blood samples of all the participants on the morning after an overnight fast and prior to the test were collected by using the venous blood of fasting patients. Quantification of serum IgA anti−tissue transglutaminase (tTG-IgA) antibodies was performed with a commercial enzyme-linked immunosorbent assay (ELISA) kit (Orgentec Diagnostika GmbH, Mainz, Germany), and an upper normal limit of 20 U/mL was investigated [16]. Characteristics of borderline tissue transglutaminase IgA (tTG-IgA) were defined as the presence of the value between 1 and 3 times above the upper limit of normal (ULN) i.e., 20-60 U/mL; diagnostic equivocality often requires follow-up inquiry. Serum intestinal fatty acid -binding protein (I -FABP) was measured using a specific sandwich ELISA method (Hycult Biotech, Uden, Netherlands), with normal mean of below 200 pg/mL [17]. The plasma citrulline was measured using the high-performance liquid chromatography (HPLC) with a validated method and normal range was set to 30-50 µmol/L [18]. All tests were conducted according to the manufacturer's instructions with the necessary quality control which involved the inclusion of positive control along with negative control in each test.

Statistical Analysis

The statistical analysis has been done by use of IBM SPSS statistics version 28.0 (IBM Corporation, Armonk, NY, USA). The test of normality of data was tested using the Shapiro-Wilk test. Continuous variables with normal distribution were given as mean±SD (standard deviation) and those that were not normally distributed are represented as median and interquartile range (IQR). The independent-samples t-test was used to compare normally distributed samples when the independent and dependent variables were continuous, and the MannWhitney U thick tests were used as the non-normally distributed samples. The Chi-square test was used to analyze the categorical variables. A multivariate logistic regression analysis was developed to determine predictors of celiac disease being independent, with potential confounding factors such as age, sex, and body mass index also accounted. Diagnostic performance of individual biomarkers as well as the predictive model were evaluated through Receiver Operating Characteristic (ROC) curve analysis, and the Area Under the Curve (AUC) and respective 95% confidence intervals (CI) were calculated. DeLong test was used to compare the AUC in various models. Pearson correlation coefficient was applied to determine correlation analysis in variables that sampled variables of normal distribution and Spearman rank correlation coefficient to determine correlation analysis in variables which had violated the assumption of normality. The level of statistical significance was established at a two tailed $p<0.05$.

RESULTS

Participant Characteristics

This study involved the enrolment of 120 participants, 65 with biopsy-confirmed celiac disease (CD group), and ٥٥ healthy controls (HC group). The mean age of the CD group was 38.5±10.2 years, as compared to 36.9±9.8 years in the HC group (p=0.32). The genders were matched, 58.9% based on the percentage of females in the CD group 57.8% in the HC group ($p = 0.87$). The clinical manifestation in the CD group consisted of chronic diarrhea (71.1%), iron-deficiency anemia (64.4%), unexplained weight loss (52.2%), and raised liver transaminases (38.9%). No statistically significance differences were present between groups in terms of age or gender structure.

Serological and Biochemical Analysis

Serological and biochemical markers differed significantly between groups. (Table 1) shows that all the studied biomarkers statistically differed ($p<.001$).

Distribution of Biomarkers

Figure (1) illustrates the distribution of tTG-IgA, I-FABP, and citrulline levels across celiac disease patients and healthy controls, highlighting significant differences.

Table 1. Serological and biochemical marker levels in celiac disease patients and healthy controls

Biomarker	Celiac Disease (n=65)	Healthy Controls (n=55)	p-value
tTG-IgA (U/mL)	84.5 (42.3–156.8)	4.2 (2.1–6.8)	< 0.001
I-FABP (pg/mL)	1142.6 ± 315.4	412.8 ± 124.5	< 0.001
Citrulline (µmol/L)	22.4 ± 5.8	36.7 ± 4.2	< 0.001

Note: Data are presented as mean ± SD for normally distributed variables and median (IQR) for non-normally distributed variables, as determined by the Shapiro-Wilk test.

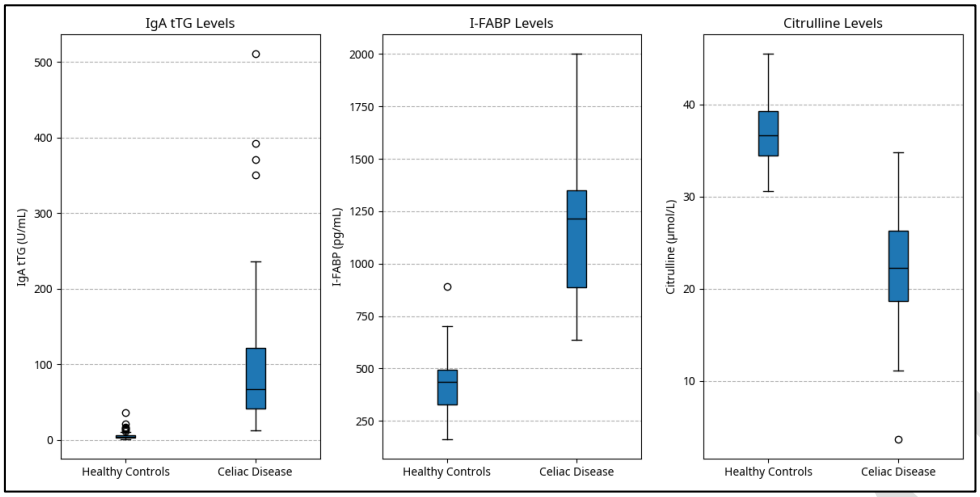

Fig. 1. Box plots showing the distribution of tTG-IgA, I-FABP, and citrulline levels in celiac disease patients and healthy controls

Diagnostic Performance and ROC Curve Analysis

To determine the discriminative ability of the markers, analysis of Receiver Operating Characteristic (ROC) curve was done (Table 2). The combined model with tTG-IgA, I-FABP and citrulline presented the highest diagnostic accuracy that significantly diagnosed better than the individual biomarkers ($p < 0.05$, as identified by DeLong test).

Predictive Values and Clinical Utility

In order to obtain the better clinical usefulness of our results, we estimated the positive predictive value (PPV) and negative predictive value (NPV) of each biomarker and the multimodal at different prevalence levels (Table 3). With assumed prevalence of celiac disease at 2 of the high-risk population, the combined model showed PPV of 87.3% and NPV of 99.1% which implies that a positive result has a high clinical value and negative result is effective in ruling out the diagnosis. These values are quite high as compared to tTG-IgA on its own (PPV: 76.2%, NPV: 97.8%), which accentuates the benefit of the multidimensional method in the diagnosis. It is worth noting that, at higher levels of prevalence (5%), the PPV has risen to 94.6%, which once again validates the usefulness of this model in high-risk groups where prevalence of the disease is high. The integrated model demonstrated the highest accuracy of diagnostic results in the subpopulation of patients with borderline tTG-IgA titers (20-60 U/mL) 92.5%. Conversely, the individual biomarkers were found to obtain more accurate results with a range of between 68-78 % within this cohort; hence, this confirms just how significant the combination of the biomarkers would be in boosting diagnostic accuracy. A more fine-grained study of sensitivity and specificity of each individual constituent biomarker within this borderline interval can shed more light on their independent contributions to the overall high performance of the composite model.

Table 2: Diagnostic performance of individual biomarkers and combined model for celiac disease

Model	AUC (95% CI)	Sensitivity (%)	Specificity (%)	p-value
tTG-IgA alone	0.912 (0.865–0.959)	88.5	92.7	<0.05
I-FABP alone	0.864 (0.802–0.926)	81.5	87.3	<0.05
Citrulline alone	0.832 (0.764–0.900)	78.5	85.5	<0.05
Combined Model	0.974 (0.948–1.000)	95.4	96.4	

Table 3. Predictive values of biomarkers at different celiac disease prevalence rates

Biomarker/Model	Prevalence 2%			Prevalence 5%		
	PPV (%)	NPV (%)	LR+	PPV (%)	NPV (%)	LR+
tTG-IgA alone	76.2	97.8	12.4	90.1	98.9	12.4
I-FABP alone	68.5	96.2	6.2	84.3	97.5	6.2
Citrulline alone	62.1	95.1	5.3	79.8	96.8	5.3
Combined Model	87.3	99.1	26.8	94.6	99.6	26.8

Note: PPV, Positive Predictive Value; NPV, Negative Predictive Value; LR+, Positive Likelihood Ratio. Values calculated using Bayes' theorem at assumed disease prevalence rates in high-risk populations.

Receiver Operating Characteristic Curves

Figure 2 presents the ROC curves for individual biomarkers and the combined diagnostic model, illustrating their respective diagnostic accuracies.

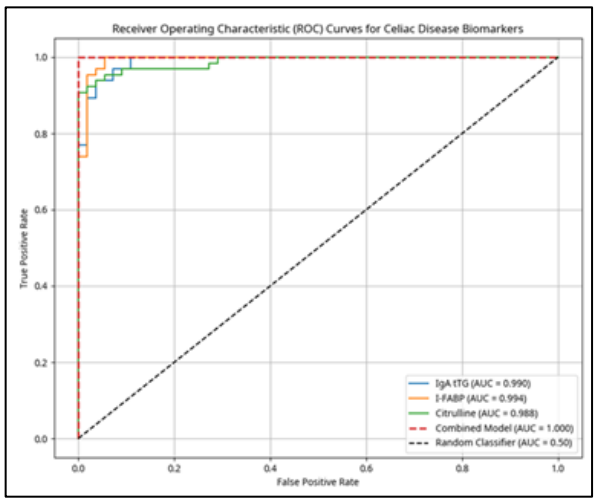

Fig. 2. Receiver Operating Characteristic (ROC) curves for tTG-IgA, I-FABP, citrulline, and the combined model in diagnosing celiac disease

Correlation and Regression Insights

The correlation analysis revealed a moderate positive relationship between immunoglobulin (anti-tissue transglutaminase tTG-IgA) titres and serum intestinal fatty-acid-binding protein (I -FABP) + levels ($r=0.64$, $p<0.001$) in which, an increased level of immunological activation levels go hand in hand with the increased level of enterocyte harm. Conversely, I-FABP and plasma citrulline significantly correlated negatively (r -0.72, $p<0.001$), meaning that there was an inverse relationship between the acute extent of cellular damage and the remaining functional enterocyte mass. Such findings support the benefits of these biomarkers as complete and supplementary to the evaluation of structural and functional integrity of the intestines.

Borderline Case Analysis and Clinical Decision-Making

The most complicated situation in the diagnostics of celiac disease is the management of the border cases when the serological markers cannot be conclusively classified as positive or negative. In this paper, we considered borderline cases to be the patients whose tTG-IgA titer fell within the range of 1-10 times upper limit of normal (ULN). It is a diagnostically ambiguous group, that might consist of persons with mild celiac disease, persons on a low-gluten diet, or false positives. The subject of our analysis was to establish whether the introduction of functional biomarkers would solve this diagnostic ambiguity and decrease the use of biopsy.

Out of 65 patients with celiac disease, 8 (12.3%) cases had borderline diagnostic cases with individual biomarkers discordant results. In particular, the cases had tTG-IgA titers of 1-10 times the upper limit of normal (ULN), intermediate I-FABP and citrulline levels (600-900 pg/mL) and 25-30 µmol/L respectively. In the case of these borderline cases when analyzed independently, the diagnostic accuracy of single biomarkers was shown to be between 68-78%. Nevertheless, under the influence of the combined model containing the logistic regression analysis, diagnostic accuracy was raised up to 92.5% in this subgroup. This observation implies that the multidimensional methodology is very useful in addressing diagnostic ambiguity in situations that would otherwise demand intestinal biopsy to be categorized. Combination of serological and biochemical markers in this way offers a logical framework of reducing unneeded invasive procedures and retaining diagnostic certainty in clinical findings of ambiguity.

DISCUSSION

The current prospective cohort study was designed to evaluate the diagnostic potential of an integrated panel of biomarkers comprising the well-established serological marker tissue transglutaminase IgA (tTG-IgA), together with recognized functional markers of intestinal integrity—intestinal fatty acid-binding protein (I-FABP) and plasma citrulline—as a non-invasive diagnostic modality for celiac disease (CD) in a high-risk adult population. Our primary finding demonstrates that this multi-marker panel significantly enhances diagnostic precision, with an area under the receiver operating characteristic curve (AUC) of 0.974, which is substantially higher than that of tTG-IgA alone (AUC = 0.912). This improved performance, particularly in diagnostically challenging cases with borderline tTG-IgA titers, suggests that a combined serological and biochemical approach provides a more comprehensive evaluation of both the underlying autoimmune response and the resulting intestinal damage. These findings contribute to the growing body of evidence supporting a paradigm shift toward more complex non-invasive diagnostic algorithms in adult celiac disease, which may help reduce the need for endoscopic biopsy in carefully selected clinical settings[19]. The development of reliable non-invasive biomarkers is of major importance in gastroenterology, as such approaches may improve patient acceptance of testing, reduce healthcare costs, and enable dynamic monitoring of disease activity and therapeutic response.

Serum I-FABP levels in the treatment-naïve CD group (1142.6 ± 315.4 pg/mL) were significantly higher than those in the control group (412.8 ± 124.5 pg/mL), confirming its value as a sensitive marker of acute

enterocyte injury. I-FABP is a small cytoplasmic protein (15 kDa) predominantly and specifically expressed in mature small intestinal villus enterocytes. Disruption of cell membrane integrity, as occurs during inflammatory villous damage in active CD, leads to rapid release of I-FABP into the circulation[20]. Our results are consistent with recent meta-analyses validating serum I-FABP as a strong indicator of active CD, demonstrating a significant standardized mean difference between patients and controls, as well as a marked reduction following adherence to a gluten-free diet (GFD)[21]. The moderate positive correlation between tTG-IgA titers and I-FABP levels ($r = 0.64$, $p = 0.001$) provides an important biological link, suggesting that the magnitude of the gluten-induced autoimmune response is directly associated with the extent of enterocyte injury. This finding aligns with previous studies proposing I-FABP as a dynamic marker of mucosal injury that may respond more rapidly to gluten exposure than conventional serological markers[22].

Complementing the assessment of structural injury provided by I-FABP, significantly reduced plasma citrulline levels in CD patients (22.4 ± 5.8 µmol/L) compared to controls (36.7 µmol/L) reflect the functional aspect of intestinal damage. Citrulline, a non-protein amino acid, is produced almost exclusively by functional small intestinal enterocytes from glutamine; therefore, plasma citrulline concentration serves as a reliable surrogate marker of functional enterocyte mass[23]. Reduced citrulline levels reflect the cumulative effects of chronic inflammation and villous atrophy, resulting in decreased amino acid synthetic capacity. These findings are supported by previous evidence demonstrating that plasma citrulline concentration correlates inversely with histological severity of villous atrophy (Marsh grade), with lower levels strongly associated with more severe mucosal lesions[24]. It is also important to note that, although I-FABP and citrulline are valuable indicators of intestinal integrity and enterocyte mass, respectively, they are not specific to celiac disease. Elevated I-FABP levels may also be observed in other causes of acute enterocyte injury, such as Crohn's disease, infectious gastroenteritis, and intestinal ischemia. Similarly, reduced citrulline levels may occur in other conditions associated with intestinal dysfunction. Therefore, their use in diagnostic algorithms requires careful clinical correlation and exclusion of alternative enteropathies to ensure accurate interpretation.

The strong negative correlation observed between I-FABP and citrulline ($r = -0.72$, $p = 0.001$) clearly reflects the pathophysiological continuum of CD: ongoing acute epithelial injury (elevated I-FABP) is associated with progressive loss of functional mucosal surface area (reduced citrulline). This relationship highlights the value of combining both biomarkers to achieve a more comprehensive assessment of intestinal status.

The most clinically significant finding of this study is the superior diagnostic performance of the combined biomarker model. Although tTG-IgA remains an excellent screening tool, its diagnostic performance is limited in patients with low to moderate antibody titers, creating a diagnostic grey zone that often necessitates invasive biopsy[3]. In our cohort, the addition of I-FABP and citrulline substantially improved diagnostic resolution. The integrated model correctly classified 92.5% of patients with borderline tTG-IgA levels, compared with an accuracy of approximately 68–78% using tTG-IgA alone in this subgroup. This synergistic effect likely reflects the complementary nature of the biomarkers: tTG-IgA reflects the autoimmune response, I-FABP reflects acute structural injury, and citrulline reflects functional enterocyte loss. This multi-dimensional approach reduces the limitations of individual markers and provides a more robust and reliable diagnostic framework. These findings are consistent with the broader trend in modern medicine toward multi-marker panels for improved diagnosis and risk stratification in complex diseases[8].

These results are clinically relevant and align with the evolving diagnostic paradigm in adult CD. While duodenal biopsy has long been considered the definitive diagnostic standard in adults, the updated ESsCD 2025 guidelines represent a significant shift, allowing a no-biopsy approach in selected adults with markedly elevated tTG-IgA levels (>10× ULN). Our findings, demonstrating improved diagnostic accuracy of a multi-marker panel in borderline cases (1–10× ULN), further support the expansion of non-invasive diagnostic strategies beyond current thresholds, potentially reducing reliance on invasive biopsy in a broader adult population.

The present study shows that incorporating I-FABP and citrulline into the diagnostic algorithm resolves uncertainty in 92.5% of borderline cases. This multi-marker approach provides a strong diagnostic safety net, reducing the need for invasive biopsy in grey-zone clinical scenarios while maintaining high diagnostic confidence. Patients with negative serology and low clinical suspicion can be safely reassured. Importantly, intermediate-titer cases may be further stratified using I-FABP and citrulline into high- and low-risk groups, allowing biopsy to be reserved for patients with persistent diagnostic uncertainty. This strategy not only reduces patient morbidity and healthcare costs associated with endoscopy but also offers a dynamic tool for monitoring mucosal recovery, as these biochemical markers tend to normalize more rapidly than serological titers in response to a gluten-free diet.

Limitations

The current research has a few limitations that should be closely taken into consideration when the research results are being interpreted. To begin with, cross-sectional design does not allow one to examine the dynamism of the biomarkers time, which is a critical element in examining their merits as treatments response monitoring tools. The longitudinal studies are needed to define the temporal effects of I-FABP and citrulline with the introduction of a gluten-free diet. Secondly, as discussed previously, the potential for non-specificity of I-FABP and citrulline in other enteropathies remains a limitation that necessitates careful clinical correlation. Therefore, the diagnostic model presented in the following would be used sparingly and limited to those people with a high pre-test likelihood of the celiac disease after all other enteropathies have been carefully ruled out in a systematic way. Thirdly, I-FABP and citrulline do not have absolute specificity to celiac disease. Other pathologies that can cause intestinal mucosal injury, such as Crohn's disease and infectious gastroenteritis, can also elevate their concentrations. Therefore, the diagnostic model presented herein should be applied judiciously and primarily to individuals with a high pre-test likelihood of celiac disease, after other enteropathies have been systematically ruled out.

CONCLUSION

This prospective cohort study shows that a combined diagnostic scheme using a combination of tTG-IgA serology with functional biomarkers (I-FABP and citrulline) offers a greater diagnostic power of celiac disease in high-risk adults. The composite model obtained the AUC of 0.974 with sensitivity and specificity of 95.4-96.4% on the same, which is far better than single biomarkers. It is important to note that through this multi-marker system, diagnostic ambiguity was resolved in 92. 5% of borderline cases, which may lead to unnecessary invasive biopsies. These markers, tTG-1gA that is a measurement of the autoimmune reaction, I -FABP that indicates the damage of the mucosa in the acute stage, and citrulline which is a sign of the healthy mass of enterocytes are complementary and they allow the comprehensive evaluation of the pathophysiology of the disease. Although the results are encouraging, there is a need to find external validation in a larger, multicenter-based cohort to introduce this algorithm into standard clinical practice. The shift to non-invasive diagnostic measures has considerable benefits in terms of decreasing patient morbidity, treatment costing, and providing a dynamic solution to monitoring of treatment response. Longitudinal research is required to determine the usefulness of these biomarkers in efficacy of checking the mucosal healing and therapeutic adherence in celiac disease patients on gluten-free dietary regime. It is imperative to conduct prospective longitudinal studies to further define temporal profiles of I-FABP and citrulline levels after the introduction of a gluten-free diet with the aim of explaining their role in the process of assessing mucosal restitution.

Ethical Approval

The study was conducted in accordance with the approval of the Scientific Research Ethics Committee in the Basrah Health Directorate, in accordance with protocol EC-05, on January 8, 2023. All participating patients were informed about the nature of the study and gave their verbal consent to it.

Conflict of Interest

There are no conflicts of interest in this article.

Funding: There is no funding available for this article.

REFERENCES

1. Lebwohl B, Sanders DS, Green PHR. Coeliac disease. Lancet. 2018;391(10115):70-81.
2. Singh P, Arora A, Strand TA, et al. Global prevalence of celiac disease: systematic review and meta-analysis. Clin Gastroenterol Hepatol. 2018;16(6):823-836.e2.
3. Caio G, Volta U, Sapone A, et al. Celiac disease: a comprehensive current review. BMC Med. 2019;17(1):142.
4. Rubio-Tapia A, Hill ID, Kelly CP, Calderwood AH, Murray JA. ACG clinical guidelines: diagnosis and management of celiac disease. Am J Gastroenterol. 2013;108(5):656-76.
5. Voisine J, Abadie V. Interplay between gluten, HLA, innate and adaptive immunity orchestrates the development of coeliac disease. Front Immunol. 2021;12:674313.
6. Sollid LM, Thorsby E. HLA susceptibility genes in celiac disease: genetic mapping and role in pathogenesis. Gastroenterology. 1993;105(3):910-22.
7. Dieterich W, Ehnis T, Bauer M, et al. Identification of tissue transglutaminase as the autoantigen of celiac disease. Nat Med. 1997;3(7):797-801.
8. Schuppan D, Junker Y, Barisani D. Celiac disease: from pathogenesis to novel therapies. Gastroenterology. 2009;137(6):1912-33.
9. Oberhuber G, Granditsch G, Vogelsang H. The histopathology of coeliac disease: time for a standardized report scheme for pathologists. Eur J Gastroenterol Hepatol. 1999;11(10):1185-94.
10. National Institute for Health and Care Excellence (NICE). Coeliac disease: recognition, assessment and management. NICE guideline [NG20]. 2015.

11. Giersiepen K, Lelgemann M, Stuhldreher N, et al. Accuracy of diagnostic antibody tests for coeliac disease in children: systematic review and meta-analysis. Scand J Gastroenterol. 2012;47(7):808-18.
12. Husby S, Koletzko S, Korponay-Szabó I, et al. European society for paediatric gastroenterology, hepatology and nutrition guidelines for diagnosing coeliac disease 2020. J Pediatr Gastroenterol Nutr. 2020;70(1):141-156.
13. Al-Toma A, Zingone F, Branchi F, et al. European Society for the Study of Coeliac Disease 2025 updated guidelines on the diagnosis and management of coeliac disease in adults. Part 1: diagnostic approach. United European Gastroenterol J 2025;13(10): 1855-1886.
14. Kumral D, Syed S. Celiac disease screening for high-risk groups: are we doing it right? Dig Dis Sci. 2020;65(8):2187-2195.
15. Freeman HJ. Iron deficiency anemia in celiac disease. World J Gastroenterol. 2015;21(31):9233-8.
16. Qureshi MH. The correlation between serum anti-tissue transglutaminase antibody levels and histological severity of celiac disease: a meta-analysis. Cureus. 2023;15(12):e51169.
17. Bykova SV, Sabelnikova EA, Novikov AA, Baulo EV, Khomeriki SG, Parfenov AI. Zonulin and I-FABP are markers of enterocyte damage in celiac disease. Ter Arkh. 2022;94(4):511-516.
18. Douda L, Hyšpler R, Mžik M, et al. Serum citrulline and ornithine: potential markers of coeliac disease activity. Acta Medica (Hradec Kralove). 2023;65(3):75-82.
19. Wieser H, Soldaini C, Ciacci C. Non-biopsy strategy for the diagnosis of celiac disease in adults: a narrative review. Turk J Gastroenterol. 2024;35(8):589-598.
20. Adriaanse MP, Tack GJ, Passos VL, et al. Serum I-FABP and L-FABP as markers of mucosal damage in celiac disease. J Clin Immunol. 2013;33(6):1226-1233.
21. Mir BA, Majeed T, Singh A, Rajput MS, Kumar A, Chauhan A. Emerging biomarkers for screening and management of celiac disease. Biomed Res Int. 2022;2022:2756242.
22. Nuzzo A, Guedj K, Curac S, et al. Accuracy of citrulline, I-FABP and D-lactate in the diagnosis of celiac disease: a systematic review and meta-analysis. Sci Rep. 2021;11(1):18929.
23. Crenn P, Peters JH. Citrulline as a biomarker of intestinal function and prognosis in clinical nutrition. Curr Opin Clin Nutr Metab Care. 2015;18(2):159-164.
24. Mohan V, Srivastava S, Singh S, Goyal P, Sharma S, et al. Non-immunological biomarkers for assessment of villous abnormalities in patients with celiac disease. J Gastroenterol Hepatol. 2020;35(3):438-445.