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Article

Evaluation of Gut Permeability and Immune Responses in Celiac Disease: Correlating Intestinal Barrier Function with Systemic Immune Activation Markers

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Abstract: Background: Celiac disease is an autoimmune disorder characterized by intestinal inflammation and increased gut permeability. Here, we aimed to evaluate intestinal permeability in celiac disease patients and its correlation with systemic immune activation markers, including serum antibodies and cytokines. **Methods:** A total of 120 patients diagnosed with celiac disease were compared with 85 healthy non-celiac group (control). Demographic data, body mass index (BMI), medication use, family history and symptomatology were recorded. Intestinal permeability was assessed using the lactulose-to-mannitol (L/M) ratio, and serum levels of anti-tTG IgA, anti-DGP IgG, IL-2, TNF-α, and IFN-γ were measured. Spearman's correlation analysis was performed to explore relationships between the L/M ratio and other parameters. **Results:** The mean age of celiac disease patients was 46.55 ± 8.57 years, with no significant age difference compared to controls ($p = 0.219$). The celiac disease group exhibited a significantly higher BMI (29.96 ± 5.09) compared to controls (25.47 ± 4.51, $p < 0.0001$). Medication use was prevalent in 86.7% of celiac disease patients, contrasting with none in the control group ($p < 0.0001$). A significant family history of celiac disease was noted in 74.7% of patients ($p < 0.0001$). Intestinal permeability testing revealed a mean lactulose percentage of 0.21 ± 0.04% in celiac disease patients versus 0.08 ± 0.02% in controls ($p < 0.0001$), with a mean mannitol percentage of 13.98 ± 2.48% in celiac disease patients compared to 24.59 ± 3.39% in controls ($p < 0.0001$). Strong positive correlations were observed between the L/M ratio and anti-tTG IgA ($rs = 0.724$), anti-DGP IgG ($rs = 0.765$), IL-2 ($rs = 0.720$), TNF-α ($rs = 0.591$), and IFN-γ ($rs = 0.716$), all with p-values < 0.0001. **Conclusions:** Our findings demonstrated that increase intestinal permeability in celiac disease is significantly correlated with elevated levels of specific autoantibodies and pro-inflammatory cytokines. These findings highlight the importance of assessing gut permeability as a potential marker of disease activity and immune response in celiac disease patients.

Keywords: celiac disease, intestinal permeability, lactulose-to-mannitol (L/M) ratio, serum levels of anti-tTG IgA, anti-DGP IgG, IL-2, TNF-α, and IFN-γ.

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1. Introduction

The hallmark of celiac disease that distinguishes it from other chronic diseases, is villous atrophy coupled with small intestinal inflammation, and being an autoimmune condition, it occurs in genetically susceptible individuals and is triggered by consumption of gluten-containing foods [1, 2]. About 1% of the global population suffer with celiac disease, as the prevalence of the condition continues to grow, with epidemiologist estimating a 3% prevalence by 2030 [1, 3]. Many people go undiagnosed or are misdiagnosed despite its well-established diagnostic criteria, which include intestinal biopsies and serological markers. This has a substantial negative impact on the quality of life and places a heavy strain on health systems [3]. Clarifying the involvement of gut permeability and immune dysregulation in the pathophysiology of celiac disease has

become more important as our knowledge of the immunological and biochemical foundations of the condition has become more advanced. Studies have shown that in genetically susceptible individuals, increased intestinal permeability, commonly known "leaky gut", may occur before the onset of clinical symptoms and inflammatory response [4]. In order to preserve gut homeostasis, the intestinal epithelium acts as an essential barrier that prevents luminal antigens and pathogens from translocating. Gluten and its bioactive particles cause an abnormal immunological response in individuals with celiac disease, which activates T cells and produces antibodies against the gluten components of gliadin. Increased permeability is the outcome of this immunological response, which also damages the intestinal epithelium and releases inflammatory cytokines [5, 6].

The relationship between intestinal permeability, immunological activity, and celiac disease symptoms has been the subject on an expanding corpus of research. For instance, a study conducted by Kaczmarczyk et al. (2021) showed that children who subsequently acquired celiac disease had increased intestinal permeability, indicating that changes in the permeability may occur prior to the development of the disease [7]. Furthermore, research has shown a correlation between disease activity and mucosal damage and levels of certain serological markers, such as anti-tissue transglutaminase (anti-tTG) [8-10]. Patients with active celiac disease have also been found to have higher levels of inflammatory cytokines such as interleukin-6 (IL-6), (IL-18) and tumour necrosis factor-alpha (TNF-α), which may indicate the degree of mucosal damage and contribute to the pathophysiology of the disease [10-12]. The dynamic interaction between immune response and intestinal permeability in celiac disease has been demonstrated in previous studies. Increased permeability makes it possible for macromolecules like gluten peptides to cross the epithelial barrier and cause an exaggerated immune response. Increased permeability may also be caused by changes in the composition of the gut microbiota, as highlighted in several studies. This is because dysbiosis is linked to immunological dysregulation and increased inflammation [13, 14]. However, more research is necessary to determine how the composition of the microbiota and its metabolites may affect the function of the gut barrier and in turn, immunological responses in celiac disease.

Furthermore, there is a immunological is complicated link between immunological indicators and celiac disease clinical outcomes. Although the existence of anti-tTG antibodies is an essential diagnostic tool, new research suggests that in order to properly assess disease activity, a thorough evaluation of both serological and inflammatory indictors is required. For example, research as shown that active celiac disease is associated with elevated levels of IL-15, a cytokine that stimulates intraepithelial lymphocytes (IELs), which are correlated with villous atrophy [15]. Measuring intestinal permeability combined with immune response indicators is important since it may improve knowledge on celiac disease diagnosis and pathogenesis. The integration of gut permeability measurement with comprehensive immune profiling in celiac disease patients relative to healthy controls is critically underrepresented in literature. Closing these gaps is essential to improving patient outcomes and creating tailored treatment strategies.

Here, we recruited a cohort of celiac disease patients and matched them with healthy controls in order to examine the connection between intestinal permeability and systemic immune activation markers in those with the disease. Our research will enable us to clarify the immunological and biochemical markers if celiac disease and offer important new information on how these markers relate to clinical symptoms. This could open up opportunities for innovative treatment strategies that target the underlying barrier dysfunction in addition to the autoimmune components of celiac disease.

2. Materials and Methods

Study subjects

A total of 120 patients diagnosed with celiac disease from histological examination and serological findings, attending the Karbala Center for Digestive System and Liver Diseases and Surgery, were recruited in the study from August to November 2024. For the control group, 85 age-matched healthy individuals clinically confirmed to be without gastrointestinal disorders were recruited. Information regarding duration of disease, symptoms and medication use was obtained from the patients' medical records while socio-demographic data such as age, marital status, smoking status, and family history was obtained from the patients and the control group through questionnaire-based interview. Exclusion criteria included patients < 18 years of age, pregnancy or breast-feeding women, renal failure, liver cirrhosis, nephrotic syndrome, congestive heart failure, diabetes and thyroid conditions that affected intestinal motility, water and solute flow, and absorption in order not to interfere with intestinal permeability tests.

Ethical approval was sought and obtained from the Research and Ethics Committee of the

College and the Hospital Research Committees. Written informed consent was obtained from the patients and well as the health participants prior to the commencement of the research.

Assessment of intestinal permeability

To conduct the intestinal permeability test, both patients and control group were fasted for 10 hours, after which they completely eliminated residual urine. They then ingested an isosmolar solution containing 6.0 g of lactulose and 3.0 g of mannitol, and urine collected over 6 hours. After collection, a 2.5 mL of the urine was preserved with 0.6 mg thimerosal and stored in liquid nitrogen. The analysis of urinary lactulose and mannitol was performed using high-performance liquid chromatography (HPLC) (Mitsubishi Chemical Corporation, Tokyo, Japan). The urine samples were filtered, treated with ion-exchange resin, and injected into the HPLC system, which included specific columns for separating the substances and a refractive index detector for result interpretation. For standardization of the test and adequate interpretation of the data reported as g/L, an equation was generated using the areas under the curves calculated by the workstation and a line was drawn for the determination of the two substances.

The results were presented in percentage of urinary excretion of both probes and the final result was presented as the ratio of urinary excretion of the two probes, as this ratio is a better indicator of intestinal permeability, unaffected by premucosal and postmucosal factors.

Measurement of inflammatory cytokines and serological markers for celiac disease.

Blood samples were obtained by venipuncture following disinfection of the antecubital fossa with 70% ethanol from all the study participants. Five millilitres of blood were drawn and dispensed into a gel tube and was allowed to rest for 15 to 30 minutes at room temperature prior to serum preparation. Serum was prepared by centrifugation at 10,000 g for 15 minutes and the supernatants were dispensed into Eppendorf tubes and stored at -20 °C until use. Serum levels of IL-2, IL-10, and IFN-γ were measured using commercial ELISA kits: the human IL-2 ELISA kit (CytImmune Sciences Inc., Rockville, MD), the human IL-10 ELISA kit (Cell Sciences Inc., Canton, MA), and the Human Interferon gamma ELISA Kit (IFN-γ) from Sigma-Aldrich (Massachusetts, United States), adhering to the manufacturers' protocols. To quantitatively detect the presence of circulating IgA autoantibodies to tissue trans glutaminase (tTG) antigen as well as IgG antibodies to deamidated gliadin antigen in the serum samples, the indirect enzyme linked immune reaction using the Anti-tTG Ab ELISA Kit (Bio-Rad Laboratories Inc., California, United States) and DGP IgA/IgG ELISA Kit (Eagle Bioscience, New Hampshire, United States) was used as per manufacturer's protocols.

Statistical analysis

For descriptive statistics such as sex, smoking status, symptoms, and use of medication Chi-squared test was used after Shapiro-Wilk test for normality. Student's t-test or Mann-Whitney U test was used for continuous variables (i.e., age, intestinal permeability, serum cytokines and antibodies levels) to compare means between the celiac disease group and the control group. Pearson correlation analysis was used to assess the relationship between gut permeability (from lactulose/mannitol ratio) and levels of inflammatory cytokines as well as serological markers. Statistical significance was set at $p < 0.05$, utilizing IBM SPSS Statistics v.25.0 (IBM Corporation, Armonk, New York).

3. Results

Table 1 shows the general characteristics of the study participants. The mean age to the 120 celiac disease patients recruited in this study was 46.55±8.57 years, while the control group had a mean age of 47.39±5.43 years. The difference in age between the two groups was not statistically significant ($p = 0.219$). Regarding sex distribution, the celiac disease patients included 49 males (40.8%) and 71 females (59.2%). The control group constituted 33 (38.8%) and 52 females (61.2%), showing no significant difference between the groups ($p = 0.772$). The BMI was significantly higher among the celiac disease patients, with a mean BMI of 29.96±5.09 kg/m2, compared to 25.47±4.51 kg/m2 in the control group ($p < 0.0001$). Medication use differed significantly between groups; 104 participants (86.7%) among the celiac disease patients reported current medication usage, while none were reported in the control group. Conversely, the control group included 85 participants (100%) who did not use medication compared to 16 (13.3%) celiac disease patients. Family history of celiac disease was present in 89 patients (74.7%), compared to 23 (27.1%) in the control group, a difference that was statistically significant ($p < 0.0001$). The symptomatology varied among celiac disease patients with the following percentages reporting specific symptoms: diarrhea (35.8%), abdominal pain (48.3%), iron-deficiency anemia (10.8%), fatigue (61.7%), weight loss (35.8%), abdominal distention (25.0%), flatulence (22.5%), abnormal liver function (7.5%), neurologic dysfunction (6.7%), vomiting (11.7%), constipation (11.7%), nausea (5.0%), and osteopenia or osteoporosis (4.2%).

Table 1. General characteristics of the study participants

	Celiac Disease *(n = 120)*	**Control** *(n = 85)*	**p-value**
Age, *years (Mean±SD)*	46.55±8.57	47.39±5.43	0.219
Sex, n *(%)*			
Male	49 *(40.8)*	33 *(38.8)*	0.772
Female	71 *(59.2)*	52 *(61.2)*	
BMI, *Kg/m²*	29.96±5.09	25.47±4.51	< 0.0001
Use of medication, n *(%)*			
Yes	104 *(86.7)*	0 *(0.0)*	
No	16 *(13.3)*	85 *(100.0)*	
Family history, n *(%)*			
Yes	89 *(74.7)*	23 (27.1)	< 0.0001
No	31 *(25.3)*	62 *(72.9)*	
Symptoms, *n (%)*			
Diarrhea	43 *(35.8)*		
Abdominal pain	58 *(48.3)*		
Iron-deficiency anaemia	13 *(10.8)*		
Fatigue	74 *(61.7)*		

Weight loss	43 *(35.8)*
Abdominal distention	30 *(25.0)*
Flatulence	27 *(22.5)*
Abnormal liver function	9 *(7.5)*
Neurologic dysfunction	8 *(6.7)*
Vomiting	14 *(11.7)*
Constipation	14 *(11.7)*
Nausea	6 *(5.0)*
Osteopenia or osteoporosis	5 *(4.2)*

BMI; body mass index, SD; standard deviation.

Table 2 presents the results of the intestinal permeability test between the celiac disease patients relative to the control group. For lactulose percentage, the celiac disease patients had a mean percentage of 0.21±0.04%, which as significantly higher than that of the control group 0.08±0.02% ($p < 0.0001$). The mean percentage of mannitol in celiac disease patients was 13.98±2.48% which as significantly lower than the recorded for the control group ($p < 0.0001$). The lactulose-to-mannitol ratio in patients with celiac disease was 0.015±0.004 which was significantly higher than the 0.003±0.001 measure in the control group ($p < 0.0001$).

Table 2. Intestinal permeability test between the celiac disease patients relative to the control group

	Celiac Disease *(Mean±SD)*	**Control** *(Mean±SD)*	**p-value**
Lactulose *(%)*	0.21±0.04	0.08±0.02	< 0.0001
Mannitol *(%)*	13.98±2.48	24.59±3.39	< 0.0001
Lactulose/Mannitol	0.015±0.004	0.003±0.001	< 0.0001

SD; standard deviation.

Table 3 summarizes the results of the serum antibody and cytokine measurements in the celiac disease patients compared to the control group. The patients exhibited a mean anti-tTG IgA level of 193.59±36.25 U/mL, which is significantly higher than the control group with mean level of 36.91±8.11 U/mL ($p < 0.0001$). The mean anti-DGP IgG level in celiac disease patients was 88.11±17.18 U/mL, while the control group had a much lower mean of 15.99±3.75 U/mL

Table 3. The serum antibody and cytokine measurements in the celiac disease patients compared to the control group

	Celiac Disease *(Mean±SD)*	**Control** *(Mean±SD)*	**p-value**
Anti-tTG IgA, *(U/mL)*	193.59±36.25	36.91±8.11	< 0.0001
Anti-DGP IgG, *(U/mL)*	88.11±17.18	15.99±3.75	< 0.0001
IL-2, *(pg/mL)*	12.60±3.22	4.69±1.25	< 0.0001
TNF-α, *(pg/mL)*	9.03±2.63	4.81±1.29	< 0.0001
IFN-γ, *(pg/mL)*	6.87±2.16	2.59±0.94	< 0.0001

Anti-tTG IgA: anti tissue transglutaminase Immunoglobulin A, Anti-DGP IgG: anti-deamidated gliadin peptide Immunoglobulin G, IL-2: interleukin 2, TNF-α: tumour necrosis factor – alpha, IFN-γ: interferon – gamma, SD: standard deviation.

($p < 0.0001$). This data is graphically presented in Figure 1

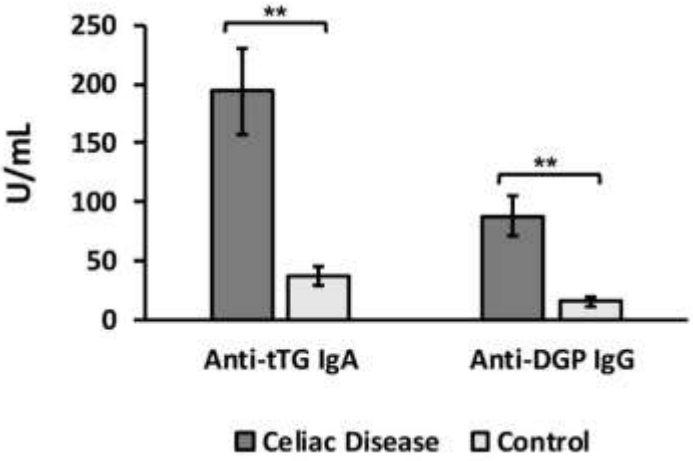

Figure 1. Serum levels of anti-tTG IgA and anti-DGP IgG in patients with celiac disease compared to healthy controls

Bars represent the mean concentrations; error bars represent ± standard deviation for each group. The celiac disease group demonstrates significantly higher levels of both anti-tTG IgA and anti-DGP IgG compared to the control group, indicated by $p < 0.0001$. Statistical significance is marked by double asterisks (**).

Celiac patients showed a mean IL-2 level of 12.60±3.22 pg/mL, compared to 4.69±1.25 pg/mL in the control group, while mean TNF-α levels for celiac disease patients was 9.03±2.63 pg/mL, as the control group reported a lower mean of 4.81±1.29 pg/mL. Similarly, the celiac disease patients had a mean IFN-γ level of 6.87±2.16 pg/mL, with the control group recording a mean value of 2.59±0.94 pg/mL. The difference between the two study groups with respect to the three cytokines reflects statistical significance with $p < 0.0001$; an indication of enhanced proinflammatory activity in celiac disease relative to the control group. This data is graphically presented in Figure 2.

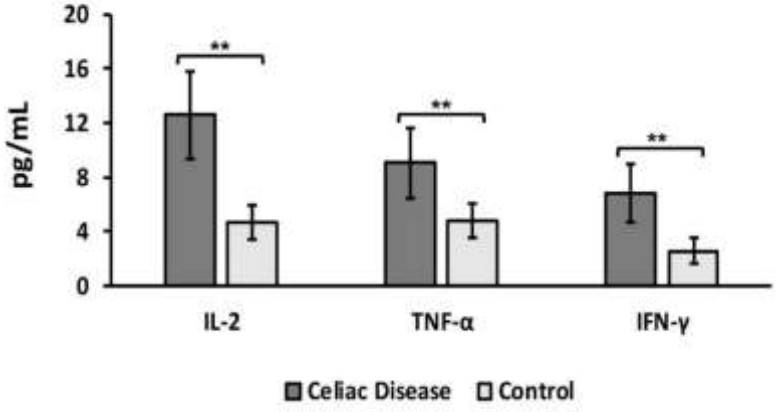

Figure 2. Serum cytokine levels of IL-2, TNF-α, and IFN-γ in patients with celiac disease compared to healthy controls

Bar graphs represent the mean concentrations; error bars represent ± standard deviation for each group. The celiac disease group shows significantly higher levels of IL-2, TNF-α, and IFN-γ compared to the control group, indicated by ($p < 0.0001$). Statistical significance is marked by double asterisks (**).

Spearman's correlation analysis showing the association between age, BMI, serum antibody levels, cytokine levels, and intestinal permeability (measured by the L/M ratio). For each parameter, the Spearman correlation coefficient (rs) and matching two-tailed p-value (p) are presented in Table 4.

Table 4. Spearman's correlation analysis showing the association between age, BMI, serum antibody levels, cytokine levels, and intestinal permeability (L/M ratio)

Parameter	rs	p (2-tailed)
Age	0.091	0.216
BMI	0.397	< 0.0001
Anti-tTG IgA	0.724	< 0.0001
Anti-DGP IgG	0.765	< 0.0001
IL-2	0.72	< 0.0001
TNF-α	0.591	< 0.0001
IFN-ɣ	0.716	< 0.0001

BMI: body mass index, Anti-tTG IgA: anti tissue transglutaminase Immunoglobulin A, Anti-DGP IgG: anti-deamidated gliadin peptide Immunoglobulin G, IL-2: interleukin 2, TNF-α: tumour necrosis factor – alpha, IFN-γ: interferon – gamma.

There was a weak, non-significant positive connection between age and the L/M ratio, as indicated by a correlation coefficient of 0.091 ($p = 0.216$). A p-value < 0.0001 and a correlation coefficient of 0.397 indicated a moderately positive relationship between intestinal permeability and BMI. The correlation coefficients for anti-tTG IgA and anti-DGP IgG were 0.724 and 0.765, respectively ($p < 0.0001$), suggesting a strong positive relationship in which higher levels of anti-tTG IgA and anti-DGP IgG is associated with increased intestinal permeability. TNF-α demonstrated a moderate association with the L/M ratio ($rs = 0.591$), whereas IL-2 and IFN-γ showed a high positive association with intestinal permeability, with correlation coefficients of 0.716 and 0.720, respectively ($p < 0.0001$).

4. Discussion

Gut permeability plays a crucial role in the pathophysiology of celiac disease by allowing gluten and other substances to enter the bloodstream through an impaired intestinal barrier function, thus facilitating the immune response to gluten and contributing to the symptoms and progression of the disease in genetically predisposed individuals [6]. Understanding the interrelationship between gut permeability and immune profiling of celiac disease is central to improving our knowledge on the pathophysiology, diagnosis and management of the disease. Here, we show the connection between intestinal barrier function and systemic levels of immune activation markers.

First, we investigated various demographic and clinical characteristics of the celiac disease patients in comparison with non-celiac control group. Our findings revealed that the mean age of the participants in both groups was similar, with no statistically significant difference. This observation is consistent with the findings of many previous studies, suggesting that celiac disease can affect individuals across a broad age range [16, 17]. The sex distribution in our study showed a higher prevalence of females among celiac disease patients compared to males; an indication that celiac disease affects both sexes, albeit with a notable female preponderance [18]. In contrast with previous studies, which often associates celiac disease with underweight or malnourished patients due to malabsorption [19], we observed that the BMI of the celiac disease was significantly higher than that of the control group. However, recent studies have indicated that some celiac disease patients may present with a higher BMI, possibly due to dietary changes or the

consumption of gluten-free products that are often higher in calories and fats [20]. The family history of celiac disease was significantly higher in the celiac disease group compared to the control group, which is consistent with the genetic predisposition associated with celiac disease. A positive family history is a well-established risk factor for developing celiac disease, and our findings reinforce the importance of family screening in at-risk populations [21]. Symptomatology among celiac disease patients varied, with fatigue and abdominal pain being the most commonly reported symptoms. As highlighted in previous studies, fatigue is a prevalent yet often overlooked symptom in celiac disease patients [22]. The range of gastrointestinal and extraintestinal symptoms reported in our study agrees with the diverse clinical presentation of celiac disease, which can complicate diagnosis and management.

The results of the intestinal permeability test in this study provide significant insights into the differences in permeability between patients with celiac disease and health controls. Our finding indicate that celiac disease patients have markedly higher mean percentage of lactulose compared to controls. This finding is consistent with earlier studies that found celiac disease is characterized by increased intestinal permeability. For example, Fasano (2020) showed that individuals with celiac disease have reduced intestinal permeability, frequently as a result of inflammation and mucosal damage brought on by gluten consumption [4]. Moreover, the mean percentage of mannitol was significantly lower in celiac compared to controls. Mannitol is typically used as a marker for the absorptive capacity of the intestinal mucosa, hence, the impaired absorption of mannitol in celiac disease patients is indicative of a compromised intestinal mucosa [23], further reflecting the physiological disturbances characteristic of the disease. These findings corroborate earlier studies that have reported similar alterations in mannitol absorption in individuals with celiac disease [24, 25].

The L/M ration is critical for interpreting the results of intestinal permeability tests, as it provides insights into the relative integrity of the intestinal mucosa. In our study, celiac disease patients exhibited a significantly higher L/M ratio compared to controls. Elevated L/M ratios in celiac disease patients indicate a higher degree of permeability or compromised integrity of the intestinal barrier, as reported in previous studies [26, 27]. This suggests that celiac disease patients not only have increased absorption of larger molecules like lactulose but also retain lower levels of smaller molecules like mannitol. The substantial differences in intestinal permeability parameters observed in our study have clinically relevant implications. Increased intestinal permeability has been linked not just to celiac disease but also to various other autoimmune conditions. Research has shown that heightened permeability may contribute to the pathogenesis of other disorders such as Type 1 diabetes mellitus and inflammatory bowel disease [28]. Therefore, understanding the mechanisms underlying altered intestinal permeability in celiac disease can inform potential therapeutic interventions and monitoring strategies.

Regarding the measurements of serum antibodies and cytokine levels between the patients and the control group, our findings indicate markedly elevated levels of anti-tTG IgA and anti-DGP IgG in the celiac disease group, corroborating the role of these antibodies as key biomarkers for diagnosis. Our previous studies and others have consistently shown that anti-tTG IgA is a highly sensitive and specific marker for celiac disease, making it the cornerstone of serological diagnosis [5, 10]. Our results are in agreement with those of Galli et al. (2024), in which they reported similar findings in a cohort of adults with celiac disease, further supporting the use of anti-tTG IgA levels in clinical practice [29]. It is particularly noteworthy to state that because anti-DGP IgG antibodies can be detected even in cases where anti-tTG IgA is negative, its measurement is of crucial diagnostic value, especially in patients with IgA deficiency [30, 31]. Therefore, testing for both anti-tTG IgA and anti-DGP IgG antibodies provides a more comprehensive assessment of celiac disease.

Cytokine profiles of celiac disease patients can also demonstrate useful information on the role of systemic inflammation in the pathophysiology of the disease. In our study, the mean level of IL-2 was significantly higher in the celiac disease relative to the control. This elevation in IL-2 suggests a heightened immune response, consistent with the inflammatory milieu observed in celiac disease. Previous studies have indicated that IL-2 is involved in immune activation and may play a role in the pathogenesis of celiac disease by promoting the proliferation of tissue-resident T cells and exacerbating the inflammatory response [32]. Similarly, we noted increased levels of TNF-α in celiac disease patients compared to the control group indicating enhanced pro-inflammatory activity. TNF-α is a key mediator in the inflammatory process associated with celiac disease, contributing to tissue damage and the perpetuation of the inflammatory response [33]. The upregulation of TNF-α further suggests that celiac disease patients may experience higher levels of intestinal inflammation, which can impact their overall health and symptomatology [34]. Finally, the levels of IFN-γ were also significantly elevated in the celiac disease patients, providing evidence of a TH-1 polarized immune response in celiac disease. Moreover, increased production of IFN-γ is well-documented as part of the immune response to gluten in genetically predisposed individuals and is associated with intestinal damage seen in the condition [34, 35].

The Spearman's correlation analysis in this study reveals significant relationship between intestinal permeability, indicated by the L/M ratio, suggesting that higher BMI is linked to intestinal permeability in celiac disease patients. Previous studies have reported the connection between obesity and altered gut permeability [36, 37]. The rise in BMI may relate to the consumption of gluten-free products, which, although marketed as healthier, can lead to higher caloric intake and changes in gut function [38]. Strong positive correlation was also identified between anti-tTG IgA, anti-DGP IgG and intestinal permeability, underscoring the role of these antibodies as indicators of mucosal damage and immune activation in celiac disease [39, 40]. Elevated levels of these antibodies are associated with ongoing inflammation and permeability issues, suggesting their potential as predictors of gut permeability in clinical contexts [41]. Additionally, significant correlations between proinflammatory cytokine IL-2, TNF-α, and IFN-γ and the L/M ratio further emphasize the inflammatory nature of celiac disease. The relationship between IL-2 and intestinal permeability is indicative of the its role in the pathogenesis of the disease as an altered intestinal integrity is allows gluten into the blood stream and leading to an IL-2 mediated activation of T-cell [33, 42]. Additionally, the correlation of TNF-α with the L/M ratio supports the theory that increased TNF-α levels contribute to mucosal damage and increased permeability, as documented in other autoimmune diseases [5]. IFN-γ is known to promote the destruction of the intestinal epithelium, leading to barrier dysfunction, a critical feature of celiac pathophysiology [43]. Collectively, these findings suggest that as immune response intensifies – with greater antibody levels and cytokine production – intestinal permeability also increase, contributing to the cyclical nature of inflammation and mucosal damage in celiac disease patients.

5. Conclusion

This study highlights noteworthy differences in BMI, medication use, family history, and symptoms between celiac disease patients and healthy participants, emphasizing the need for comprehensive clinical evaluation and management strategies. It underscores the importance of intestinal barrier function by noting differences in lactulose, mannitol and the L/M ration, advocating for more research on the interplay between intestinal permeability, diet and autoimmune processes. Additionally, elevated levels of anti-tTG IgA, anti-DGP IgG, IL-2, TNF-α, and IFN-γ in patients reflect an altered immune response, supporting the clinical relevance of serological biomarkers in diagnosing and managing celiac disease. Spearman's correlation analysis revealed strong associations between intestinal permeability and various clinical factors, reinforcing the complexity of the

disease's mechanisms. Future studies could continue to investigate the longitudinal implications of these relationships, their roles in pathophysiology of celiac disease, and their potential in guiding therapeutic strategies and dietary interventions.

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