ORIGINAL ARTICLE DOI: 10.20515/otd.1782586

Osmangazi Journal of Med, 2026;48(2):166-174

Osmangazi Journal of Medicine

e-ISSN: 2587-1579

Tim-3 Expression in Duodenal Mucosa: The Role in the Pathogenesis and Diagnosis of Celiac Disease As An İmmune Checkpoint Molecule

Duodenum Mukozasında Tim-3 Ekspresyonu: Çölyak Hastalığının Patogenezinde ve Tanısında İmmün Kontrol Noktası Molekülü Olarak Rolü

[1]Fatih Yılmaz, [2]Kadri Atay

[1]Mardin Training and Research Hospital, Pathology Laboratory, Mardin, Türkiye
[2]Mardin Training and Research Hospital, Adult Gastroenterology Clinic, Mardin, Türkiye

Correspondence / Sorumlu yazar:

Fatih YILMAZ

Mardin Training and Research Hospital, Pathology Laboratory, Mardin, Türkiye

e-mail: faatiihyiilmaaz@yahoo.com.tr

Received : 12.09.2025

Accepted : 01.12.2025

Abstract: Limited data exist on the expression of immune checkpoint molecules (ICMs) in celiac disease (CD). This study aims to evaluate Tim-3 expression in duodenal biopsies from CD patients and those of non-CD patients, some of whom have increased intraepithelial lymphocytes (IELs). Duodenal tissues from 214 individuals who applied to the adult gastroenterology clinic were re-evaluated. The American College of Gastroenterology Guidelines were used to confirm the diagnosis of CD. Sections were prepared from formalin-fixed paraffin-embedded duodenal tissues for immunohistochemical analyses. The area with the highest Tim-3 expression in the lamina propria was counted at 40x (HPF) magnification. Receiver operating characteristic analysis offered a cut-off value predicting CD diagnosis as ">9 per HPF" ($p<0.001$, AUC: 0.744). High Tim-3 expression was associated with higher IEL numbers and a diagnosis of CD and correlated with endoscopic and serological findings of CD ($p<0.001$) in the whole cohort. In the CD group, a correlation was found between Tim-3 positive cell numbers and mean IEL counts ($p=0.007$, Spearman's rho: 0.279). On the other hand, no difference was detected between Tim-3 expression and Marsh types ($p=0.291$), serum tTG ($p=0.482$), and EMA ($p=0.765$) titers in CD patients. In the non-CD group, Tim-3 expression was unaffected by either the increase in IEL or gastric *Helicobacter pylori* infection. In this study, we first demonstrated the expression profile of Tim-3 in the duodenal mucosa as an ICM. Confirmation of these findings with further analyses will provide a better understanding of the inflammatory cycle in the duodenal mucosa and new insights into diagnosing and understanding CD pathogenesis.

Keywords: Celiac disease, immune checkpoint molecule, intraepithelial lymphocyte, Tim-3

Özet: Çölyak hastalığında (ÇH) immün kontrol noktası moleküllerinin (İKM) ekspresyonuna ilişkin sınırlı veri bulunmaktadır. Bu çalışmada, ÇH hastalarına ve bazılarında intraepitelyal lenfosit (İEL) artışı olan ÇH olmayan hastalara ait duodenum biyopsilerinde Tim-3 ekspresyonunun değerlendirilmesi amaçlandı. Yetişkin gastroenteroloji kliniğine başvuran 214 bireyin duodenal dokuları yeniden değerlendirildi. Amerikan Gastroenteroloji Koleji Kılavuzları, ÇH tanısını doğrulamak için kullanıldı. İmmünohistokimyasal analizler için formalinle fikse edilmiş parafine gömülmüş duodenum dokularından kesitler hazırlandı. Lamina propriada en yüksek Tim-3 ekspresyonuna sahip alan 40x (BBA) büyütmede sayıldı. Receiver Operating Characteristic analizi, ÇH tanısını "BBA başına >9" ($p<0.001$, AUC: 0.744) olarak öngören bir kesme değeri sundu. Yüksek Tim-3 ekspresyonu, daha yüksek İEL ve ÇH tanısı ile ilişkiliydi ve tüm kohortta ÇH'nin endoskopik ve serolojik bulgularıyla korele idi ($p<0.001$). ÇH grubunda, Tim-3 pozitif hücre sayıları ile ortalama İEL sayıları arasında bir korelasyon bulundu ($p=0.007$, Spearman'ın rho: 0.279). Diğer taraftan, ÇH hastalarında Tim-3 ekspresyonu ile Marsh tipleri ($p=0.291$), serum tTG ($p=0.482$) ve EMA ($p=0.765$) titreleri arasında bir fark tespit edilmedi. ÇH olmayan grupta, Tim-3 ekspresyonu İEL'deki artıştan ve gastrik *Helicobacter pylori* enfeksiyonundan etkilenmemiştir. Bu çalışmada, Tim-3'ün bir İKM olarak duodenum mukozasındaki ekspresyon profilini ilk kez gösterdik. Bu bulguların daha ileri analizlerle doğrulanması, duodenum mukozasındaki inflamatuar döngünün daha iyi anlaşılmasına ve ÇH hastalarının tanısında ve patogenezinin anlaşılmasında yeni bakış açılarına olanak sağlayacaktır.

Anahtar Kelimeler: Çölyak hastalığı, İmmün kontrol noktası molekülü, İntraepitelyal lenfosit, Tim-3

Ethics Committee Approval: The study was approved by Mardin Artuklu University Clinical Research Ethics Committee (Decision no:2024/2-13, Date: 13.02.2024).

Informed Concent: Since this was a retrospective study analyzing existing histopathological data, informed consent was deemed unnecessary by the authors.

Authorship Contributions: FY: Writing – review & editing, Supervision, Methodology, Investigation, Formal analysis, Data curation, Conceptualization. KA: Supervision, Methodology, Investigation, Data curation.

Copyright Transfer Form: Copyright Transfer Form was signed by all authors.

Peer-review: Internally peer-reviewed.

Conflict of Interest: The authors declare that there is no conflict of interest.

Financial Disclosure: The authors declared that this study received no financial support.

How to cite/ Atıf için: Yılmaz F, Atay K, Tim-3 Expression in Duodenal Mucosa: The Role in the Pathogenesis and Diagnosis of Celiac Disease As An İmmune Checkpoint Molecule, Osmangazi Journal of Medicine, 2026;48(2):166-174

1. Introduction

Immune checkpoint molecules (ICMs) are critical for regulating innate and adaptive immune responses in the intestinal mucosa under homeostasis (1). Their inhibitory effect on immune cells, especially effector T lymphocytes, and their role in peripheral tolerance are vital to preventing the inflammatory response from causing tissue damage (2). Considering the destructive inflammation in celiac disease (CD), the fate of regulatory T cells (Tregs) and ICMs is essential in elucidating CD pathogenesis. The potential relationship between ICMs and CD has become more evident with the emergence of CD-like histomorphological changes in the duodenal mucosa of patients receiving ICM inhibitors (3). Furthermore, the observation of CD predisposition, especially in individuals with CTLA4 gene polymorphism, supports this opinion (4). Only a few studies investigated the roles of ICMs in CD, mainly examining the PD-L1/PD-1 axis (5, 6). However, their expression profiles regarding the pathogenesis and diagnosis of CD remain to be elucidated (7).

T cell immunoglobulin and mucin domain-containing protein 3 (Tim-3) was initially identified as a receptor expressed on interferon-γ-producing $CD4^+$ and $CD8^+$ T cells, but now, it is part of a module containing multiple co-inhibitory receptors (checkpoint receptors) expressed on many immune cells in chronic viral infections and cancers (8, 9). Many studies have reported that Tim-3 polymorphism can be associated with various autoimmune diseases, including multiple sclerosis, Graves' disease, Hashimoto's disease, autoimmune thyroid diseases, ankylosing spondylitis, idiopathic thrombocytopenic purpura, systemic lupus erythematosus, and rheumatoid arthritis (10). In addition, downregulation or blockade of Tim-3 causes exacerbation of colitis in animal models; conversely, activation causes attenuation of inflammatory activity (11, 12). This evidence highlights the importance of Tim-3 in immune regulation and autoimmunity in the gastrointestinal tract. However, in our literature review, no study examined the relationship between Tim-3 and CD in duodenal mucosa; therefore, its role in CD pathogenesis and diagnosis is unclear.

This study aims to evaluate Tim-3 expression in duodenal biopsies of CD patients and those of non-CD patients, some of which had increased intraepithelial lymphocytes (IELs), and its diagnostic potential, if any.

2. Materials and Method

2.1. Patient selection

The files of patients who applied to the adult gastroenterology clinic of Mardin Training and Research Hospital and underwent duodenal biopsy between 2021 and 2023 were reviewed. The study included patients with sufficient clinical, demographic, serological, and endoscopic findings and evaluable material in the pathology laboratory. Data were obtained from the hospital automation system, pathology, and adult gastroenterology clinic archives. Endoscopic findings (scalloping) for CD were classified as 0: absent, 1: mild, and 2: prominent. Anti-endomysium (EMA) and anti-tissue transglutaminase (tTG) IgA were used as primary serological parameters in the diagnosis and follow-up of patients. Additionally, IgG antibodies against gliadin and tTG were added to the diagnostic algorithm in patients with or suspected of IgA deficiency. The positivity was decided according to the manufacturer's thresholds.

The CD was diagnosed with supporting histological and serological findings in patients with suspicious clinical signs and symptoms (intestinal or extraintestinal) for CD. Diagnostic confirmation was performed following the recommendations of the American College of Gastroenterology Guidelines (13). Patients on a gluten-free diet due to suspected or diagnosed with CD were excluded.

2.2. Histopathological evaluation

Pathology slides were retrieved from the laboratory archive and re-evaluated with a light microscope (Olympus BX41, Japan). Duodenal mucosa was examined for changes in IELs, villi, and crypt architecture, as well as additional findings, if any. IELs were counted as the mean number per 100 enterocytes (IEL/100) using the CD3 [CONFIRM anti-CD3 (2GV6) Rabbit Monoclonal Primary Antibody] stained slides. >25 IEL per 100 enterocytes was accepted as the intraepithelial lymphocytosis. The degree of mucosal damage in CD patients was scored between 1 and 3c using the scoring system established by Marsh and modified by Oberhuber (14).

In cases where the gastric biopsy was also performed, the presence of gastric *Helicobacter pylori* (HP) was confirmed using HP immunohistochemistry [VENTANA® anti-

Helicobacter pylori (SP48) Rabbit Monoclonal Primary Antibody] and Giemsa histochemical staining. HP intensity was scored as 1: mild, 2: moderate, and 3: severe.

2.3. Immunohistochemical staining and analyses

4 μm-thick sections were taken from formalin-fixed paraffin-embedded duodenal biopsies for immunohistochemical staining. After deparaffinization, Tim-3 antibody [Anti-TIM3 (Hepatitis A virus cellular receptor 2) Rabbit Monoclonal antibody, Clone: RM448, Dilution: 1/150, RevMab Biosciences USA, Inc.] was applied to the slides with an automated immunohistochemistry stainer (Ventana BenchMark XT). Tonsil tissue was used as a positive control, and cytoplasmic expression was considered positive in mononuclear immune cells (Figure 1). The area of maximum expression was scanned at 4x magnification. The number of positive cells in a high-power field (40x, 0.196 mm^2) (HPF) in the hot spot area was counted.

2.4. Statistical analyses

Normality tests were used to determine the distribution pattern of numerical data. Categorical variables were summarised as frequencies and percentages, and numerical ones were mean ± SD or median (Q1-Q3). Appropriate Chi-Square tests (Pearson, Continuity correction, Fisher's exact) were used to demonstrate the relationship between categorical variables. The difference between the groups was found using the Mann-Whitney U and Kruskal-Wallis H tests, and the correlation was shown using Spearman correlation analysis. The ROC (Receiver Operating Characteristic) curve determined the most appropriate threshold value of Tim-3 expression for differential diagnosis between CD and non-CD biopsies. Medcalc statistical software (Version 22.014) was used for ROC analyses. IBM SPSS base system (SPSS, Version 25.0, USA) was used for statistical analysis. A two-tailed p-value <0.05 was considered statistically significant.

3. Results

3.1. Cohort characteristics

The study consisted of biopsy tissues from 214 individuals, 72 (33.6%) male, and 142 (66.4%) female, aged between 17 and 79 (median: 30) years. 95 (44.4%) cases were diagnosed with CD, and Marsh scores ranged between 1 to 3c. The mean IEL numbers of CD patients ranged from 29 to 125 (median: 77), while those of the non-CD group ranged from 3 to 68 (median: 22.5) (p<0.001). Thus, the mean IEL number was >25/100 in all CD patients (n=95), and it increased in 44 (37.0%) cases in the non-CD group. The median EMA and tTG titers were 95.6 and 106 in CD patients and 5.9 and 1.2 in the non-CD patients, respectively (p<0.001). Scalloping was prominent in 77 (81.1%) CD patients and mild in 10 (10.5%), while in the non-CD group, it was mild in 33 (27.7%) patients and absent in 83 (69.7%) (p<0.001). Gastric biopsy was also performed in 148 cases, and HP infection was detected in 92 (62.2%). HP was found in 34 (65.4%) of the CD patients and in 58 (60.4%) of the non-CD controls (p=0.676). HP severity ranged from 1 to 3, and frequencies were 33 (35.9%), 36 (39.1%), and 23 (25.0%), respectively. Clinicopathological features are summarized in Table 1.

Table 1. Clinicopathological characteristics.

Characteristics		**n (%)**
Age, year		
Median (IQR)		30.0 (22.0-41.0)
Gender		
	Male	72 (33.6)
	Female	142 (66.4)
IEL/100		
Median (IQR)		41.0 (21.0-71.0)
Diagnosis		
	CD	95 (44.4)
	Non-CD	119 (55.6)
Marsh types		
	1	1 (1.1)
	2	6 (6.3)
	3a	22 (23.2)
	3b	33 (34.7)
	3c	33 (34.7)
HP		
	Present	56 (26.2)

	Absent	92 (43.0)
	NA	66 (30.8)
Scalloping		
	Absent	91 (42.5)
	Mild	43 (20.1)
	Prominent	80 (37.4)
tTG (IgA)		
Median (IQR)		14.0 (1.8-112.0)
EMA (IgA)		
Median (IQR)		13.0 (5.3-114.5)
Total		214 (100.0)

CD: Celiac disease, IELs: Intraepithelial lymphocytes, HP: Helicobacter pylori, tTG: Tissue Transglutaminase, EMA: Anti-Endomysium Antibody, NA: Not assessed

3.2. Immunohistochemical findings

Tim-3 was expressed to variable degrees in biopsies from CD and non-CD patients, primarily in immune cells in the lamina propria (Figure 1). Tim-3 positive cell density was lower in tissues from non-CD (Figure 1b) patients than in CD tissues (Figure 1c-e).

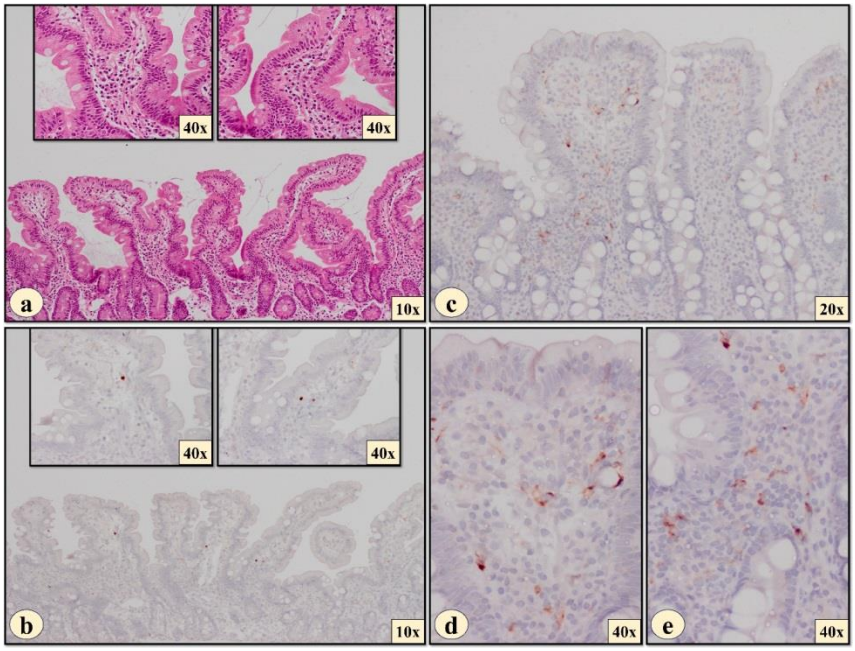

Figure 1. Low Tim-3 expression in a non-CD sample. H&E (**a**) and Tim-3 (**b**) stained sections at 10x and 40x magnifications. High Tim-3 expression in a CD mucosa at 20x (**c**) and 40x (**d-e**) magnifications.

The median positive cell count per HPF was 13.0 (9.0-17.0) in CD patients and 7.0 (4.0-11.0) in the non-CD group ($p<0.001$) (Figure 2a). While no difference was found in Tim-3 positive cell counts between histological Marsh types in CD patients ($p=0.291$) (Figure 2b), a correlation was detected with IEL numbers ($p=0.007$, Spearman's Rho: 0.279) (Figure 2c). On the other hand, no association was found between Tim-3 positivity with serum tTG ($p=0.482$) and EMA ($p=0.765$) titers in CD patients. In the non-CD group, Tim-3 expression was not affected by either the increase in IEL ($p=0.161$) (Figure 2d), the presence ($p=0.298$) (Figure 2e), or the severity ($p=0.299$) (Figure 2f) of gastric HP infection.

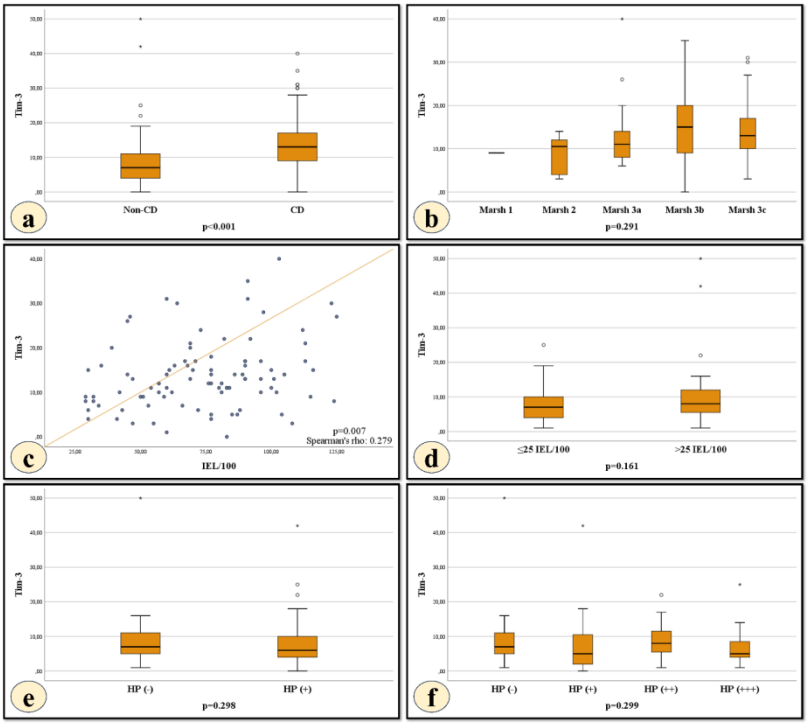

Figure 2. Comparison of Tim-3 expression between in CD and non-CD samples (**a**), Tim-3 expression in biopsies of CD patients according to Marsh types (**b**), and correlation of Tim-3 with IEL/100 (**c**). Comparison of Tim-3 expressions in non-CD biopsies according to 25 IEL/100 (**d**), presence (**e**), and severity (**f**) of gastric HP infection.

ROC analysis offered a cut-off predicting CD diagnosis as ">9" (p<0.001, Area under the ROC curve (AUC): 0.744) (Table 2, Figure 3).

Table 2. ROC curve results of Tim-3 expression predicting celiac disease.

		95% Confidence Interval
Associated criterion	>9	
Significance level P (Area=0.5)	<0.0001	
Area under the ROC curve (AUC)	0.744	0.680 – 0.801
Sensitivity	70.5	60.3 – 79.4
Specificity	68.9	59.8 – 77.1
PPV	64.4	
NPV	74.5	

NPV: Negative predictive value, PPV: Positive predictive value

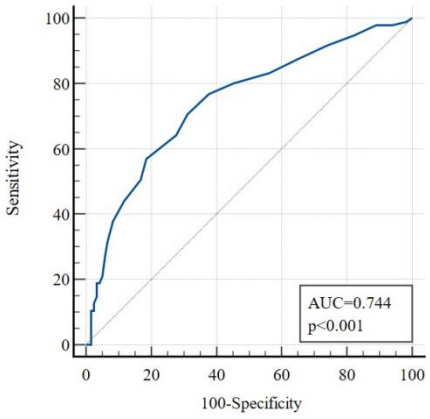

Figure 3. ROC curve of Tim-3 expression predicting the diagnosis of CD.

Sixty-seven (70.5%) of CD patients showed high Tim-3 expression, while 82 (68.9%) of non-CD controls showed low. Therefore, high Tim-3 expression was associated with CD diagnosis and higher IEL count and correlated with endoscopic and serological findings of CD (p<0.001) in the whole cohort (**Table 3**).

Table 3. Tim-3 expression and clinicopathological characteristics.

			Tim-3 expression		
Characteristics		**n (%)**	**≤9/HPF**	**>9/HPF**	**p**
n (%)		214 (100.0)	110 (51.4)	104 (48.6)	
Age					
Median (IQR)			31 (22.0-41.0)	30 (22.0-40.0)	0.495
Gender	Male	72 (33.6)	37 (51.4)	35 (48.6)	0.998
	Female	142 (66.4)	73 (51.4)	69 (48.6)	
IEL/100					
Median (IQR)			30.0 (18.0-53.0)	68.5 (44.3-90.0)	**<0.001**
Diagnosis	CD	95 (44.4)	28 (29.5)	67 (70.5)	**<0.001**
	Non-CD	119 (55.6)	82 (68.9)	37 (31.1)	
Marsh types	1	1 (1.1)	1 (100.0)	0 (0.0)	0.420
	2	6 (6.3)	3 (50.0)	3 (50.0)	
	3a	22 (23.2)	6 (27.3)	16 (72.7)	
	3b	33 (34.7)	10 (29.4)	24 (70.6)	
	3c	33 (34.7)	8 (24.2)	25 (75.8)	
HP	Present	92 (43.0)	51 (55.4)	41 (44.6)	0.269
	Absent	56 (26.2)	37 (66.1)	19 (33.9)	
	NA	66 (30.8)			
Scalloping	Absent	91 (42.5)	62 (68.1)	29 (31.9)	**<0.001**
	Mild	43 (20.1)	24 (55.8)	19 (44.2)	
	Prominent	80 (37.4)	24 (30.0)	56 (70.0)	
tTG (IgA)					
Median (IQR)			2.9 (0.8-104.5)	72.7 (12.2-120.0)	**<0.001**
EMA (IgA)					
Median (IQR)			8.7 (4.7-34.3)	54.9 (9.0-127.0)	**<0.001**

CD: Celiac disease, IEL: Intraepithelial lymphocytes, HP: *Helicobacter pylori*, HPF: High Power Field, tTG: Tissue Transglutaminase, EMA: Anti-Endomysium Antibody, NA: Not assessed, Significant values ($p < 0.05$) are marked in bold.

In the CD group, a correlation was found between Tim-3 positive cell numbers and mean IEL counts (p=0.007, Spearman's rho: 0.279).

4. Discussion

Considering that ICMs are the main actors of immune regulation in the gastrointestinal tract, it is surprising that our knowledge about their roles in CD pathogenesis is extremely limited (5). On the other hand, it is reasonable to assume that aberrant ICM expressions and/or signaling are involved in chronic inflammatory gut diseases, such as Crohn's disease, ulcerative colitis, CD, and chronic infections, such as HP (1). In addition, the widespread use of agents that block ICM pathways and autoimmune exacerbations occurring in various systems in some patients emphasize the importance of ICMs in immune regulation and their diagnostic and possible therapeutic potential in autoimmune diseases (8). The association of CTLA-4 polymorphism with CD susceptibility and a CD-like morphology encountered in the small intestine in patients using anti-ICMs supports this opinion (3, 4). ICMs and $FOXP3^+$ Tregs work together to maintain peripheral tolerance (15). The immunosuppressive effect of Tregs is mediated by cell-cell contact via various ICMs in addition to the release of inhibitory cytokines (16). ICMs support Treg functions and differentiation (17), while Tregs can express multiple types of ICMs, including Tim-3 (7, 8, 18, 19). However, these mechanisms are inadequate in suppressing the inflammatory response triggered by gluten in CD, which results in destruction and dysfunction in the intestinal mucosa. Several studies reported that despite the increase in Tregs in the duodenal tissues of CD patients, their inhibitory functions are impaired (20-23). IL-15 is a critical factor that increases proinflammatory and cytotoxic properties and inhibits anti-inflammatory mechanisms in CD (24). It also renders effector $CD4^+$ and $CD8^+$ T lymphocytes resistant to the inhibitory effects of Tregs (22, 25), and this can

explain why their inhibitory functions remain insufficient despite the increased number of Tregs in CD patients. On the other hand, the functional status and expression levels of ICMs remain poorly understood in CD.

Ponce de León and colleagues reported that PD-L1 showed high expression in superficial intestinal epithelial cells and lamina propria in biopsies from CD patients immunohistochemically (5). At the same time, PD-1 was not detected in these tissues. On the other hand, they reported that sPD-1 and sPD-L1 titers were significantly higher in the serum of patients with CD compared to healthy controls. Although it is unknown what factors contribute to deregulated PD-1 expression, they suggested that overexpression of sPD-1 may act as an "antibody" to block the PD-1/PD-Ls pathway and lead to abnormal T cell proliferation in patients with CD. However, although their study's most striking finding was the loss of PD-1 expression in CD patients, they did not mention information about the expression status in control biopsies. This makes it challenging to understand the role of the PD-1/PD-L1 pathway in CD pathogenesis and to reveal its diagnostic potential. In our study, Tim-3 expression was observed in the lamina propria of both control and CD tissues. In addition, Tim-3 expression was significantly higher in samples from CD patients than in those from the control group. In this context, although Tim-3 expression showed characteristics similar to those of PD-L1 in our cohort, the study's limitation was that we could not evaluate Tim-3 ligands, such as Galectin 9, Phosphatidylserine, HMGB, and CEACAM1.

We also investigated the association of Tim-3 expression with inflammatory conditions from non-CD causes, such as HP. Historically, gastric HP colonization has been considered one of the most common non-CD causes of increased duodenal IEL (26). However, recent studies have suggested that this effect does not yield a statistically significant difference and should not prevent research into other etiological causes of increased duodenal IEL (27). Studies report that the prevalence of HP in CD patients ranges from 12.5% to 89%, whereas it ranges from 17.3% to 97% in non-CD controls (28-31). Additionally, some studies have reported lower HP prevalence in CD patients than in controls and have even suggested that HP positivity may be protective against CD (32). In our recent study, we reported, for the first time, that a dual effect may be observed depending on the severity of gastric HP colonization, which may explain the inconsistent results in the literature (33). In this context, we compared the relationship between Tim-3 expression and HP with the severity, in addition to the presence of HP in this cohort. In this study, the prevalence of gastric HP in CD patients was 65.4%, while in non-CD controls it was 60.4% (p=0.676). In CD patients, there was a weak correlation between Tim-3 positivity and duodenal IEL counts (p=0.007, Spearman's Rho: 0.279), whereas no differences were observed with Marsh types (p=0.291), serum tTG (p=0.482), or EMA (p=0.765) titers. In non-CD controls, the presence or severity of gastric HP colonization did not affect Tim-3 expression. Additionally, the increase in IEL does not affect Tim-3 expression in these cases. Although increased PD-L1 expression in immune cells in the gastric mucosa has been reported in cases of HP-induced gastritis (34), the effect of gastric HP status on ICMs in the duodenal mucosa is unknown. In this respect, our study demonstrates for the first time the potential effect of the presence and severity of gastric HP on ICM expressions in the duodenal mucosa.

It is observed that suppression or induction of Tim-3 produces results similar to those of other ICM molecules and the Treg marker FOXP3 in the gut. Loss of function of Tregs associated with mutations in the FOXP3 causes IPEX syndrome, characterized by immune dysregulation, polyendocrinopathy, enteropathy, and X-linked inheritance (35, 36). Conversely, many inflammatory or autoimmune diseases, including CD, are associated with loss of Treg function even in the absence of gene alterations (19, 37, 38). The frequency of $FOXP3^+$ Tregs is reported to increase significantly in the inflamed mucosa of active or inactive Crohn's disease and ulcerative colitis (39, 40). Yu et al. reported that $FOXP3^+CD4^+$ T cells are increased in the lamina propria of inflamed and non-inflamed regions of the ulcerative colitis colon compared to the normal colonic samples, but their suppressive activity may be abrogated in vivo (41). Similar to these observations, Tim-3 was significantly increased in macrophages from patients with inflammatory bowel disease (42), and blocking the Tim-3 signaling pathway exacerbated colitis and the inflammatory response (11, 12). Conversely, activating it attenuates the progression of colitis in animal models (11), so this pathway can be promising as a new treatment option for CD patients (7). Similarly, in addition to higher Tim-3 expression in samples from CD patients, we also found a positive correlation between Tim-3 expression and IEL in these tissues. However, no difference in Tim-3 expression was detected between Marsh types. Like Tim-3, we recently reported a higher density of $FOXP3^+$ cells in tissues from CD patients than non-CD controls, which correlated with IEL in CD

patients (43). Moreover, similar to Tim-3, FOXP3 expression was unaffected by the IEL increase or gastric HP status in the non-CD samples. However, distinct from it, $FOXP3^+$ positive cell density correlated with Marsh types.

ICMs, including Tim-3, can be expressed by different cell types, and our inability to show which cell type plays a more significant role in this population can be considered a limitation of this study. On the other hand, despite having different In this study, we demonstrated the expression profile of Tim-3 in duodenal mucosa for the first time. We believe that the significant increase in Tim-3 expression in biopsy samples from CD patients compared to non-CD patients, and the fact that its expression is not affected by inflammatory conditions in non-CD cases, may be informative subtypes and being expressed in various cell types, they serve similar purposes in the gut. One of the main suppressive mechanisms of Tregs is the inhibitory effects of ICMs through cell-cell contact via co-inhibitory receptors (44). Consequently, elucidating which regulatory mechanism plays a specific role in CD will help us understand its pathogenesis and offer hope for treatment.

5. Conclusion

about the role of ICMs in the pathogenesis and diagnosis of CD. Confirmation of these findings with further analyses and elucidation of the pathogenetic pathways will provide a better understanding of the inflammatory cycle in the duodenal mucosa and new insights into diagnosing and understanding CD pathogenesis.

REFERENCES

1. Chulkina M, Beswick EJ, Pinchuk IV. Role of PD-L1 in Gut Mucosa Tolerance and Chronic Inflammation. International journal of molecular sciences. 2020;21(23).
2. Keir ME, Butte MJ, Freeman GJ, et al. PD-1 and its ligands in tolerance and immunity. Annual review of immunology. 2008;26:677-704.
3. Lerner A, Benzvi C. Checkpoint Inhibitors and Induction of Celiac Disease-like Condition. Biomedicines. 2022;10(3).
4. Song GG, Kim JH, Kim YH, et al. Association between CTLA-4 polymorphisms and susceptibility to Celiac disease: a meta-analysis. Human immunology. 2013;74(9):1214-8.
5. Ponce de León C, Angel López-Casado M, Lorite P, et al. Dysregulation of the PD-1/PD-L1 pathway contributes to the pathogenesis of celiac disease. Cellular & molecular immunology. 2019;16(9):777-9.
6. Ponce de León C, Lorite P, López-Casado MÁ, et al. Significance of PD1 Alternative Splicing in Celiac Disease as a Novel Source for Diagnostic and Therapeutic Target. 2021;12.
7. Isabel T, Miguel Ángel López C, Teresa P, et al. Immune Checkpoints as a Novel Source for Diagnostic and Therapeutic Target in Celiac Disease. In: Jianyuan C, editor. Celiac Disease. Rijeka: IntechOpen; 2021. p. Ch. 3.
8. Wolf Y, Anderson AC, Kuchroo VK. TIM3 comes of age as an inhibitory receptor. Nature reviews Immunology. 2020;20(3):173-85.
9. Zhao L, Cheng S, Fan L, et al. TIM-3: An update on immunotherapy. International immunopharmacology. 2021;99:107933.
10. Liu R, Wang X, Chen X, et al. TIM-3 rs1036199 polymorphism increases susceptibility to autoimmune diseases: evidence based on 4200 subjects. Bioscience reports. 2018;38(6).
11. Shi F, Guo X, Jiang X, et al. Dysregulated Tim-3 expression and its correlation with imbalanced CD4 helper T cell function in ulcerative colitis. Clinical Immunology. 2012;145(3):230-40.
12. Li X, Chen G, Li Y, et al. Involvement of T cell Ig Mucin-3 (Tim-3) in the negative regulation of inflammatory bowel disease. Clinical immunology (Orlando, Fla). 2010;134(2):169-77.
13. Rubio-Tapia A, Hill ID, Semrad C, et al. American College of Gastroenterology Guidelines Update: Diagnosis and Management of Celiac Disease. The American journal of gastroenterology. 2023;118(1):59-76.
14. Oberhuber G, Granditsch G, Vogelsang H. The histopathology of coeliac disease: time for a standardized report scheme for pathologists. European journal of gastroenterology & hepatology. 1999;11(10):1185-94.
15. Zhang B, Chikuma S, Hori S, et al. Nonoverlapping roles of PD-1 and FoxP3 in maintaining immune tolerance in a novel autoimmune pancreatitis mouse model. Proceedings of the National Academy of Sciences of the United States of America. 2016;113(30):8490-5.
16. Gianchecchi E, Fierabracci A. Inhibitory Receptors and Pathways of Lymphocytes: The Role of PD-1 in Treg Development and Their Involvement in Autoimmunity Onset and Cancer Progression. Frontiers in immunology. 2018;9:2374.
17. Francisco LM, Salinas VH, Brown KE, et al. PD-L1 regulates the development, maintenance, and function of induced regulatory T cells. The Journal of experimental medicine. 2009;206(13):3015-29.
18. Anderson AC, Joller N, Kuchroo VK. Lag-3, Tim-3, and TIGIT: Co-inhibitory Receptors with Specialized Functions in Immune Regulation. Immunity. 2016;44(5):989-1004.
19. Colamatteo A, Carbone F, Bruzzaniti S, et al. Molecular Mechanisms Controlling Foxp3 Expression in Health and Autoimmunity: From Epigenetic to Post-translational Regulation. 2020;10.

20. Zanzi D, Stefanile R, Santagata S, et al. IL-15 interferes with suppressive activity of intestinal regulatory T cells expanded in Celiac disease. The American journal of gastroenterology. 2011;106(7):1308-17.
21. Granzotto M, dal Bo S, Quaglia S, et al. Regulatory T-cell function is impaired in celiac disease. Digestive diseases and sciences. 2009;54(7):1513-9.
22. Ben Ahmed M, Belhadj Hmida N, Moes N, et al. IL-15 renders conventional lymphocytes resistant to suppressive functions of regulatory T cells through activation of the phosphatidylinositol 3-kinase pathway. Journal of immunology (Baltimore, Md : 1950). 2009;182(11):6763-70.
23. Benahmed M, Meresse B, Arnulf B, et al. Inhibition of TGF-beta signaling by IL-15: a new role for IL-15 in the loss of immune homeostasis in celiac disease. Gastroenterology. 2007;132(3):994-1008.
24. Levescot A, Malamut G, Cerf-Bensussan N. Immunopathogenesis and environmental triggers in coeliac disease. Gut. 2022;71(11):2337-49.
25. Abadie V, Jabri B. IL-15: a central regulator of celiac disease immunopathology. Immunological reviews. 2014;260(1):221-34.
26. Chang F, Mahadeva U, Deere H. Pathological and clinical significance of increased intraepithelial lymphocytes (IELs) in small bowel mucosa. 2005;113(6):385-99.
27. Guz-Mark A, Zevit N, Morgenstern S, et al. Duodenal intraepithelial lymphocytosis is common in children without coeliac disease, and is not meaningfully influenced by Helicobacter pylori infection. 2014;39(11):1314-20.
28. Agin M, Batun I, Ozdemir S, et al. Prevalence of Helicobacter pylori in Turkish children with celiac disease and its effect on clinical, histopathological, and laboratory parameters. 2019;15(6):1475-81.
29. Lasa J, Zubiaurre I, Dima G, et al. HELICOBACTER PYLORI PREVALENCE IN PATIENTS WITH CELIAC DISEASE: results from a cross-sectional study. Arquivos de gastroenterologia. 2015;52(2):139-42.
30. Diamanti A, Maino C, Niveloni S, et al. Characterization of gastric mucosal lesions in patients with celiac disease: a prospective controlled study. The American journal of gastroenterology. 1999;94(5):1313-9.
31. Simondi D, Ribaldone DG, Bonagura GA, et al. Helicobacter pylori in celiac disease and in duodenal intraepithelial lymphocytosis: Active protagonist or innocent bystander? Clinics and research in hepatology and gastroenterology. 2015;39(6):740-5.
32. Amlashi FI, Norouzi Z, Sohrabi A, et al. A systematic review and meta-analysis for association of Helicobacter pylori colonization and celiac disease. PloS one. 2021;16(3):e0241156.
33. Yilmaz F, Atay K, Çirkin G, et al. The impact of gastric Helicobacter pylori infection on duodenal mucosa: New evidence on the alteration of intraepithelial lymphocytes. American Journal of Clinical Pathology. 2025;164(3):443-54.
34. Go D-M, Lee SH, Lee S-H, et al. Programmed Death Ligand 1-Expressing Classical Dendritic Cells Mitigate Helicobacter-Induced Gastritis. Cellular and Molecular Gastroenterology and Hepatology. 2021;12(2):715-39.
35. Bennett CL, Christie J, Ramsdell F, et al. The immune dysregulation, polyendocrinopathy, enteropathy, X-linked syndrome (IPEX) is caused by mutations of FOXP3. Nature genetics. 2001;27(1):20-1.
36. van der Vliet HJ, Nieuwenhuis EE. IPEX as a result of mutations in FOXP3. Clinical & developmental immunology. 2007;2007:89017.
37. Mercer F, Unutmaz D. The biology of FoxP3: a key player in immune suppression during infections, autoimmune diseases and cancer. Advances in experimental medicine and biology. 2009;665:47-59.
38. Granzotto M, dal Bo S, Quaglia S, et al. Regulatory T-Cell Function Is Impaired in Celiac Disease. Digestive diseases and sciences. 2009;54(7):1513-9.
39. WANG Y, LIU XP, ZHAO ZB, et al. Expression of CD4+ forkhead box P3 (FOXP3)+ regulatory T cells in inflammatory bowel disease. 2011;12(4):286-94.
40. Ban H, Andoh A, Shioya M, et al. Increased number of FoxP3+CD4+ regulatory T cells in inflammatory bowel disease. Molecular medicine reports. 2008;1(5):647-50.
41. Yu QT, Saruta M, Avanesyan A, et al. Expression and functional characterization of FOXP3+CD4+ regulatory T cells in ulcerative colitis. Inflammatory Bowel Diseases. 2007;13(2):191-9.
42. Wang F, Zhou F, Peng J, et al. Macrophage Tim-3 maintains intestinal homeostasis in DSS-induced colitis by suppressing neutrophil necroptosis. Redox Biology. 2024;70:103072.
43. Yilmaz F, Atay K. FOXP3 expression in duodenal mucosa: Unique role in pathogenesis and differential diagnosis of celiac disease. Annals of Diagnostic Pathology. 2024:152393.
44. Camarca A, Rotondi Aufiero V, Mazzarella G. Role of Regulatory T Cells and Their Potential Therapeutic Applications in Celiac Disease. International journal of molecular sciences. 2023;24(19).