



Seronegative Celiac Disease in Patients with Isolated Refractory Dyspepsia and Gastroesophageal Reflux Disease

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Background/Aims: To investigate the presence of seronegative celiac disease in patients with isolated refractory dyspepsia and gastroesophageal reflux disease (GERD)-related complaints.

Methods: This was a single-center, prospective study performed at a tertiary care referral hospital. Among 968 consecutive patients, 129 seronegative patients with tissue damage consistent with Marsh IIIa classification or above were included. The patients were divided into two groups: dyspepsia (n=78) and GERD (n=51). Biopsies were taken from the duodenum regardless of endoscopic appearance, and patients with Marsh IIIa or above damage were advised to consume a gluten-free diet. The Glasgow Dyspepsia Severity (GDS) score, Reflux Symptom Index (RSI), and Biagi score were calculated at baseline and every 3 months. Control endoscopy was performed every 6 months during follow-up.

Results: The median follow-up time was 19.9 months (range, 6 to 24 months) in the dyspepsia group and 19.2 months (range, 6 to 24 months) in the GERD group. All the patients were positive for the *HLA-DQ2* and *DQ8* haplotypes. The differences between the mean GDS scores (14.3 ± 2.1 vs 1.1 ± 0.2 , respectively, $p < 0.05$), RSI scores (6.3 ± 0.8 vs 0.7 ± 0.1 , respectively, $p < 0.05$), and Biagi scores (3.1 ± 0.4 vs 0.7 ± 0.3 in the dyspepsia group and 2.5 ± 0.4 vs 0.5 ± 0.2 in GERD group) before and after implementation of the gluten-free diet were statistically significant. The decreases in the scores were consistent with improvements in the histological findings. There was no significant correlation between endoscopic appearance and histological examination results ($p = 0.487$).

Conclusions: Seronegative celiac disease may be considered in this group of patients. Even if a patient is seronegative and has normal endoscopic findings, duodenal biopsy should be considered. (*Gut Liver* 2022;16:375-383)

Key Words: Biopsy; Dyspepsia; Gastroesophageal reflux; Histopathology; Seronegative celiac disease

INTRODUCTION

Celiac disease (CD) is an immune system-related disorder, triggered by environmental factors in genetically predisposed individuals. Due to the variability in the effects of environmental factors, clinical presentations widely range from asymptomatic to severe malabsorption or extra-intestinal manifestations.¹ The diagnosis of CD is a serious challenge because of the clinical differences and a multitude of factors contributing to the pathogenesis. Current guidelines seek the presence of serum autoantibodies in addition to the signs suggestive of the disease for diagnosis

in symptomatic patients.^{2,3} Among these autoantibodies, the initial ones to be studied for screening are the anti-tissue transglutaminase immunoglobulin A (TGA IgA) and anti-endomysial antibody (EMA) IgA, which have been reported to have a high sensitivity (81% to 100% and 74% to 100%, respectively) and specificity (97% to 99% and 99% to 100%, respectively).⁴

Antibodies play an important role in diagnosis, but in a subset of patients with high clinical suspicion and tissue damage consistent with CD, antibodies are found to be negative. The term seronegative CD (CeD) is used for this patient group.⁵ The first identification of discordance be-



tween the tissue samples and serology dates back to 1999. Rostami *et al.*⁶ examined the correlation with serology use and modified the Marsh classification by describing IIIa, IIIb, and IIIc subgroups. While EMA was found to be 100% positive in patients in the IIIc group, it was as low as 29% in the IIIa group. To date, various mechanisms have been suggested for the pathogenesis of CeD. According to one hypothesis, immune complexes formed due to high affinity at the tissue level cannot enter the circulation.^{7,8} According to another hypothesis, as plasma cell maturation is insufficient in certain immune deficiency syndromes (e.g., selective IgA deficiency and common variable immune deficiency), antibody production is impossible.^{9,10} Genetic analysis (human leukocyte antigen *HLA-DQ2* and *DQ8*) or identifying immune complexes containing TGA in the tissue is beneficial to confirm the diagnosis.¹¹

The clinical presentation in CD varies so much that a stratification based on symptoms was developed. Patients in the low-risk group with dyspeptic complaints and gastroesophageal reflux disease (GERD) symptoms may be underdiagnosed.¹² The literature also backs up this hypothesis. The prevalence of CeD is reported to be around 1% in Western countries but the number increase to 1.5% in patients with dyspepsia.^{13,14} In Turkey, the prevalence of

CeD is somewhat lower, reported to be around 0.47% but in a community-based case-control study in patients with dyspepsia, the number increase to 1.44% accordingly.^{15,16}

In this study, we investigated the presence of CeD in patients with refractory dyspeptic and GERD complaints.

MATERIALS AND METHODS

1. Study design

This single-center, prospective, cross-sectional study was conducted at a tertiary care referral hospital. Written informed consent was obtained from all patients. Institutional Ethics Committee approved the study protocol (approval number: 04.03.2016–51/28). The study was conducted in accordance with the principles of the Good Clinical Practice and the Declaration of Helsinki.

2. Study population

A total of 968 consecutive patients aged 18 to 75 years who were admitted to our clinic between January 2017 and May 2018 with dyspepsia and GERD-related complaints were screened. Among these, 157 patients having chronic gastrointestinal complaints, receiving proton pump inhibi-

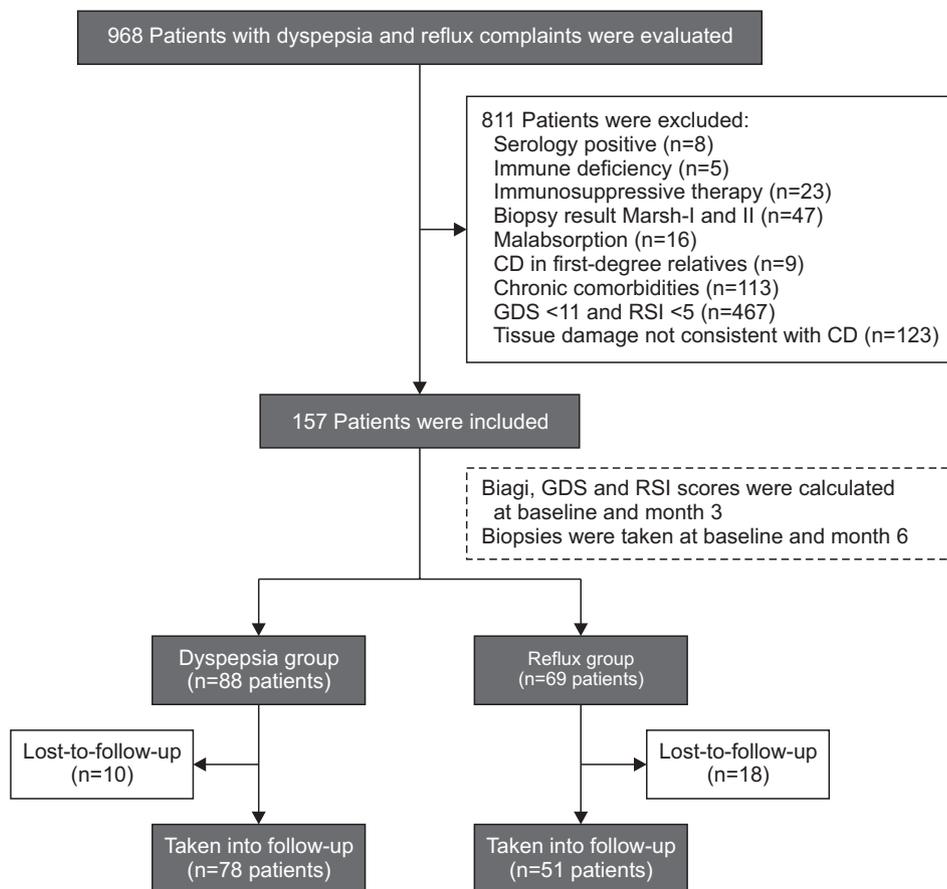


Fig. 1. Study flowchart. CD, celiac disease; GDS, Glasgow Dyspepsia Severity; RSI, Reflux Symptom Index.

tor therapy within the past ≥ 3 months, being unresponsive to treatment having a baseline Glasgow Dyspepsia Severity (GDS) score¹⁷ of more than 11 and a Reflux Symptom Index (RSI) score¹⁸ of >5 were included in this study. The patients were divided into two groups as follows: dyspepsia group (n=88) and GERD group (n=69). Due to follow-up loss, in the dyspepsia group the final patient was 78 and in the GERD group the final patient was 51. The study flow-chart is shown in Fig. 1.

3. Data collection

Baseline demographic and clinical data of all patients were recorded. The baseline GDS, RSI, and Biagi scores were calculated. In serum samples, CD antibodies (EMA and TGA), *HLA* tissue antigens, IgG, IgA, and IgM for immune deficiency screening, and albumin, calcium, complete blood count, ferritin, vitamin B₁₂, folate levels, and liver enzymes for malabsorption and liver involvement were analyzed.

4. Excluded clinical conditions

- Seropositivity
- History of consuming gluten-free diet (GFD)
- Diagnosis of immunodeficiency with IgG, IgA, and IgM levels below normal
- Having immunosuppressive therapy (e.g., azathioprine, 5-fluorouracil, ipilimumab)^{19,20}
- Chronic comorbidities (e.g., hypertension, coronary artery disease, autoimmune disorders) to rule out possible drug-associated enteropathy (such as olmesartan, thiazide diuretics, non-steroidal anti-inflammatory drugs) and to rule out conditions, which occur more frequently in CD patients than in the general population^{2,19,20}
- Biopsy results of Marsh I and Marsh II to rule out other causes of lymphocytic duodenitis and crypt hyperplasia without villous atrophy in the duodenum (e.g., food intolerance, allergy enteropathy, inflammatory bowel disease, small intestine bacterial overgrowth, *Helicobacter pylori*)²¹
- Signs of malabsorption (i.e., low levels of ferritin, vitamin B₁₂, folic acid, and calcium) to find the isolated dyspeptic and GERD patients
- GDS score of <11 , RSI score of <5
- Lost to follow-up

5. Endoscopic procedures

Seronegative patients with Marsh IIIa or above based on histological examination consumed GFD. An expert dietitian instructed the patients about GFD and dietary compliance was monitored with Biagi score every 3 months after

(a score ≤ 1 was considered diet compliant while a score ≥ 2 was considered diet non-compliant). Follow-up visits were scheduled every 3 months. The GDS and RSI scores were calculated at baseline and every 3 months during follow-up. After 6 months of diet, control endoscopy was performed, and four biopsy samples were taken from the duodenum and two from the duodenal bulb. In those having a GDS score of >11 , RSI score of >5 , and Biagi score ≥ 2 during follow-up, control endoscopy was performed and biopsy samples were taken every 6 months until the reversal of tissue damage was seen. Patients with a GDS score of <2 , RSI score of ≤ 1 , and Biagi score of ≤ 1 were considered dietary compliant, and control endoscopy was performed to take biopsy samples whether the tissue damage was reversed.

6. Histological examination

A gastrointestinal pathologist who was blinded to the patients' clinical and laboratory data examined the tissue samples. In the presence of a pathological sign during the examination, another blinded pathologist re-evaluated the samples. Tissue samples were investigated for intraepithelial gamma/delta lymphocytes using immunohistochemistry. After the conditions causing villous atrophy (e.g., tropical sprue, autoimmune enteropathy, Whipple disease, collagenous sprue, Crohn's disease, eosinophilic enteritis, intestinal lymphoma, intestinal tuberculosis, infectious enteritis [e.g., giardiasis], and graft-versus-host disease)²² were excluded with histological examination, intra-observation variability between the pathologists was calculated for the patients classified as IIIa, IIIb, and IIIc according to the Marsh classification. The same pathologists assessed follow-up biopsies using the same procedure.

7. Serological measurements

Eu-tTG[®] IgA and Eu-tTG[®] IgG commercial enzyme-linked immunosorbent assay kits (Eurospital, Trieste, Italy) were applied for IgA (<9 AU/mL negative, 9–16 AU/mL borderline, >16 AU/mL positive) and IgG (<20 AU/mL negative, ≥ 20 AU/mL positive) tissue TGA measurements, while QUANTA Life[®] h-TG IgA (Inova Diagnostics, Inc., San Diego, CA, USA) commercial enzyme-linked immunosorbent assay kits were applied for IgA EMA measurements (<20 AU/mL negative, ≥ 20 AU/mL positive), according to the manufacturer's instructions.

8. HLA-DQ2 and DQ8 measurements

To determine the *HLA* haplotypes, Genequality CD-Type v2.0[®] (AB Analitica, Padova, Italy) commercial kit was applied according to the manufacturer's instructions. The multiplex polymerase chain reaction and reverse line-

blotting technique were used for genotyping. Typing was performed using specific probes through the amplification of the second exons of genes encoding *DQA1*, *DQB1*, and *DRB1*, and the biotinylated primer.

9. Statistical analysis

Statistical analysis was performed using the SPSS for Windows version 21.0 software (IBM Corp., Armonk, NY, USA). Descriptive data were expressed in mean±standard deviation, median (min-max) or number and frequency, where applicable. Normality assumption was checked using the Shapiro-Wilk test. The chi-square test was used to analyze categorical variables for the univariate analysis, while the Mann-Whitney U test was used to analyze non-categorical variables for independent samples. The correlation coefficient between the pathologists was calculated using the Spearman correlation. A p-value of <0.05 was considered statistically significant at 95% confidence interval.

RESULTS

The study population consisted of 78 patients in the dyspepsia group and 51 patients in the GERD group. The median follow-up was 19.9 months (range, 6 to 24 months) in the dyspepsia group and 19.2 months (range, 6 to 24 months) in the GERD group. Table 1 shows the demographic and clinical characteristics of the patients. Patients in the dyspepsia group were younger and male dominant. Laboratory values of malabsorption were within normal limits and the difference was not statistically significant between the groups. Before the diagnosis of CeD was made, the patients had endoscopic examinations with a mean 4.1±0.4 times in the dyspepsia group and 3.2±0.7 times in the GERD group and the difference was statistically significant (p=0.041). Before the diagnosis of CeD was made, the patients were admitted to the hospital due to their complaints (e.g., severe epigastric pain, nausea-vomiting, and heartburn) with mean 3.3±0.4 times in the dyspepsia group and 2.1±0.3 times in the GERD group and the difference was statistically significant (p=0.044).

Table 1. Demographic and Clinical Characteristics of the Patients

Characteristics	Dyspepsia group (n=78)	GERD group (n=51)	p-value
Sex			0.031
Male	42 (53)	24 (47)	
Female	36 (47)	27 (53)	
Age, yr	37 (18–71)	43 (18–75)	0.027
Follow-up, mo	19.9 (6–24)	19.2 (6–24)	0.346
Hemoglobin, mg/dL	14.9±2.9	14.7±2.5	0.411
Albumin, g/dL	3.8±0.4	3.9±0.3	0.665
Ferritin, mg/dL	77.5±23.7	61.4±28.5	0.45
Vitamin B ₁₂ , ng/mL	287.9±47.7	279.1±53.8	0.378
Folate, mg/dL	7.11±0.74	8.23±1.16	0.489
Calcium, mg/dL	9.3±0.2	9.5±0.3	0.771
No. of endoscopies performed before CeD diagnosis	4.1±0.4	3.2±0.7	0.041
No. of hospital admissions before CeD diagnosis	3.3±0.4	2.1±0.3	0.044
Endoscopic appearance			
Normal	64 (82)	43 (84)	0.556
Flattening	10 (12)	6 (12)	0.887
Scalloping	4 (6)	2 (4)	0.723
Histology			
Marsh-IIIa (n=92)	55 (70)	37 (72)	0.618
Marsh-IIIb (n=26)	16 (21)	10 (20)	0.603
Marsh-IIIc (n=11)	7 (9)	4 (8)	0.577
GDS			<0.001
Before diet	14.3±2.1		
After diet	1.1±0.2		
RSI			<0.001
Before diet		6.3±0.8	
After diet		0.7±0.1	
Biagi score			<0.001
Before diet	3.1±0.4	2.5±0.3	
After diet	0.7±0.3	0.5±0.2	

Data are presented as number (%), median (range), or mean±SD.

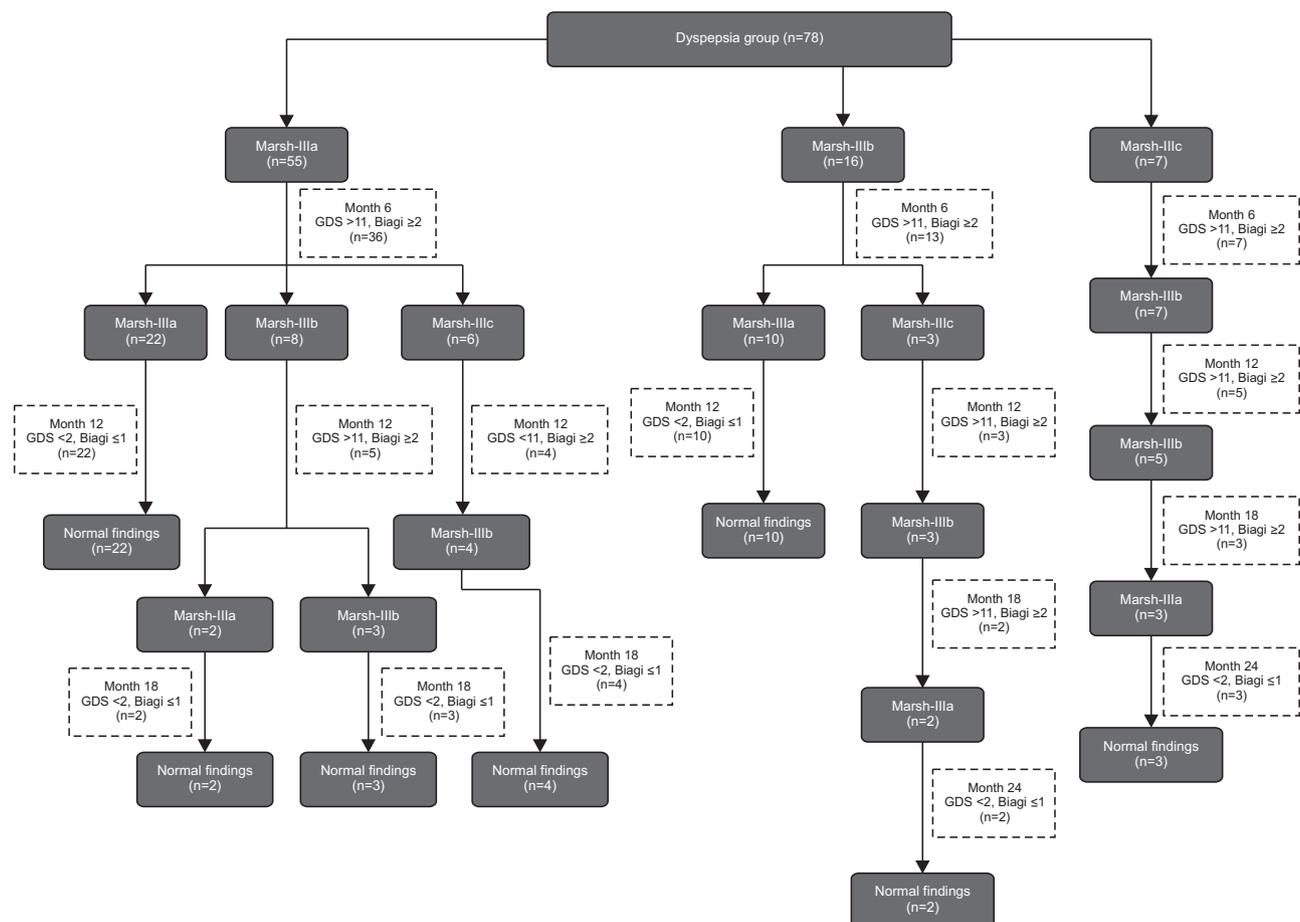
GERD, gastroesophageal reflux disease; CeD, seronegative celiac disease; GDS, Glasgow Dyspepsia Severity; RSI, Reflux Symptom Index.

The difference of Biagi scores before and after diet in both groups were statistically significant (3.1 ± 0.4 vs 0.7 ± 0.3 in dyspepsia group, $p < 0.001$ and 2.5 ± 0.3 vs 0.5 ± 0.2 in GERD group, $p < 0.001$). The difference between the mean GDS scores calculated before and after diet in the dyspepsia group (14.3 ± 2.1 vs 1.1 ± 0.2 , respectively, $p < 0.001$) and the mean RSI scores calculated before and after the diet in the GERD group (6.3 ± 0.8 vs 0.7 ± 0.1 , respectively, $p < 0.001$) was statistically significant. GDS scores according to Marsh damage were as follows: 13.4 ± 1.2 for Marsh IIIa, 14.1 ± 1.3 for Marsh IIIb, and 15.6 ± 0.9 for Marsh IIIc. RSI scores according to Marsh damage were as follows: 5.9 ± 0.5 for Marsh IIIa, 6.5 ± 0.8 for Marsh IIIb, and 7.1 ± 0.3 for Marsh IIIc. Although there was a numerical increase in the severity of symptoms as the damage increased according to the Marsh score in both groups, the difference was not statistically significant ($p = 0.554$ and $p = 0.633$, respectively).

During the endoscopic examination, 82% ($n = 64$) of the patients in the dyspepsia group had a normal appearance of duodenum, 12% ($n = 10$) had flattening of the mucosal folds, and 6% ($n = 4$) had scalloping. A total of 84% ($n = 43$)

of the patients in the GERD group had a normal appearance of the duodenum, 12% ($n = 6$) had flattening of the mucosal folds, and 4% ($n = 2$) had scalloping. In the histological examination, 70% ($n = 55$) of the patients in the dyspepsia group had Marsh-IIIa, 21% ($n = 16$) had Marsh-IIIb, and 9% ($n = 7$) had Marsh-IIIc damage. Additionally, 72% ($n = 37$) of the patients in the GERD group had Marsh-IIIa, 20% ($n = 10$) had Marsh-IIIb, and 8% ($n = 4$) had Marsh-IIIc damage. The correlation coefficient between the pathologists was calculated to be 0.96, 0.87, 0.95, and 0.95, 0.88, 0.97, respectively. There was no statistically significant correlation between the endoscopic appearance and histological examination ($p = 0.487$).

Follow-up of the patients with GDS > 11 , Biagi score ≥ 2 and abnormal findings on histological examination in the dyspepsia group are presented in Fig. 2. The decrease in the GDS and Biagi scores was found to be consistent with the improvement in the histological findings. Of 36 dietary non-compliant patients classified as Marsh-IIIa at baseline, 22 were still found to be Marsh-IIIa, eight progressed to Marsh-IIIb, and six progressed to Marsh-IIIc at month 6. Of 13 dietary non-compliant patients classified as Marsh-IIIb at baseline, 10 were still found to be Marsh-IIIa, three progressed to Marsh-IIIc at month 6. Of 7 dietary non-compliant patients classified as Marsh-IIIc at baseline, 5 were still found to be Marsh-IIIb, and two progressed to Marsh-IIIa at month 6. At month 12, 22 patients with Marsh-IIIa at baseline were still Marsh-IIIa, 5 progressed to Marsh-IIIb, and 4 progressed to Marsh-IIIc. At month 12, 10 patients with Marsh-IIIb at baseline were still Marsh-IIIa, 3 progressed to Marsh-IIIc, and 7 progressed to Marsh-IIIb. At month 12, 7 patients with Marsh-IIIc at baseline were still Marsh-IIIb, 5 progressed to Marsh-IIIa, and 2 progressed to Marsh-IIIb. At month 18, 2 patients with Marsh-IIIa at baseline were still Marsh-IIIa, 3 progressed to Marsh-IIIb, and 4 progressed to Marsh-IIIc. At month 18, 3 patients with Marsh-IIIb at baseline were still Marsh-IIIb, 3 progressed to Marsh-IIIa, and 4 progressed to Marsh-IIIc. At month 18, 3 patients with Marsh-IIIc at baseline were still Marsh-IIIb, 3 progressed to Marsh-IIIa, and 2 progressed to Marsh-IIIb. At month 24, 2 patients with Marsh-IIIa at baseline were still Marsh-IIIa, 3 progressed to Marsh-IIIb, and 4 progressed to Marsh-IIIc. At month 24, 3 patients with Marsh-IIIb at baseline were still Marsh-IIIb, 3 progressed to Marsh-IIIa, and 2 progressed to Marsh-IIIb. At month 24, 2 patients with Marsh-IIIc at baseline were still Marsh-IIIb, 3 progressed to Marsh-IIIa, and 2 progressed to Marsh-IIIb.



The tissue-level damage continued and even progressed, as the noncompliance to diet persisted; however, histological improvement was achieved, when dietary compliance was established. Similar findings were also present in the patients classified as Marsh-IIIb and Marsh-IIIC in the initial assessment.

Follow-up of the patients with RSI >5, Biagi score ≥2 and abnormal findings on histological examination in the GERD group are presented in Fig. 3. Similar to the correlation between the GDS score and the histological examination in the dyspepsia group, the RSI scores were consistent with histological findings. Seven patients classified as Marsh-IIIa at baseline who were not dietary compliant at month 6 with an RSI score of >5 and Biagi score ≥2, showed progression to Marsh-IIIb. Symptoms and histological examination findings gradually improved, as evidenced by decreased RSI and Biagi scores with dietary compliance. Similarly, seven of the patients who were initially classified as Marsh-IIIb and not compliant to dietary regimen progressed to Marsh-IIIC at month 6. Subsequently, the patients returned to normal gradually as evidenced by decreased RSI and Biagi scores with dietary compliance.

All the patients were detected to be positive for *HLA-DQ2* and *DQ8* haplotypes; *DR5* 37% (n=47), *DR3-DQ2* 31% (n=39), *DR4-DQ8* 15% (n=19), *DR7-DQ2* 15% (n=18), *DR8-DQ7* 1% (n=3), and *DR8-DQ8* 1% (n=3). Detailed *HLA* alleles of the patients are shown in Table 2.

DISCUSSION

First, it should be noted that the issues of CeD must be approached with great care. It overlaps significantly with the issue of non-celiac gluten sensitivity, seronegative food

Table 2. Distribution of *HLA* Alleles and Haplotypes

DQB1	DQA1	DRB1	Haplotype	No. (%) (n=129)
03	05	11	<i>DR5</i>	47 (37)
02	05	03	<i>DR3-DQ2</i>	39 (31)
0302	03	04	<i>DR4-DQ8</i>	19 (15)
02	0201	07	<i>DR7-DQ2</i>	18 (15)
0301	06	08	<i>DR8-DQ7</i>	3 (1)
0302	03	04	<i>DR8-DQ8</i>	3 (1)

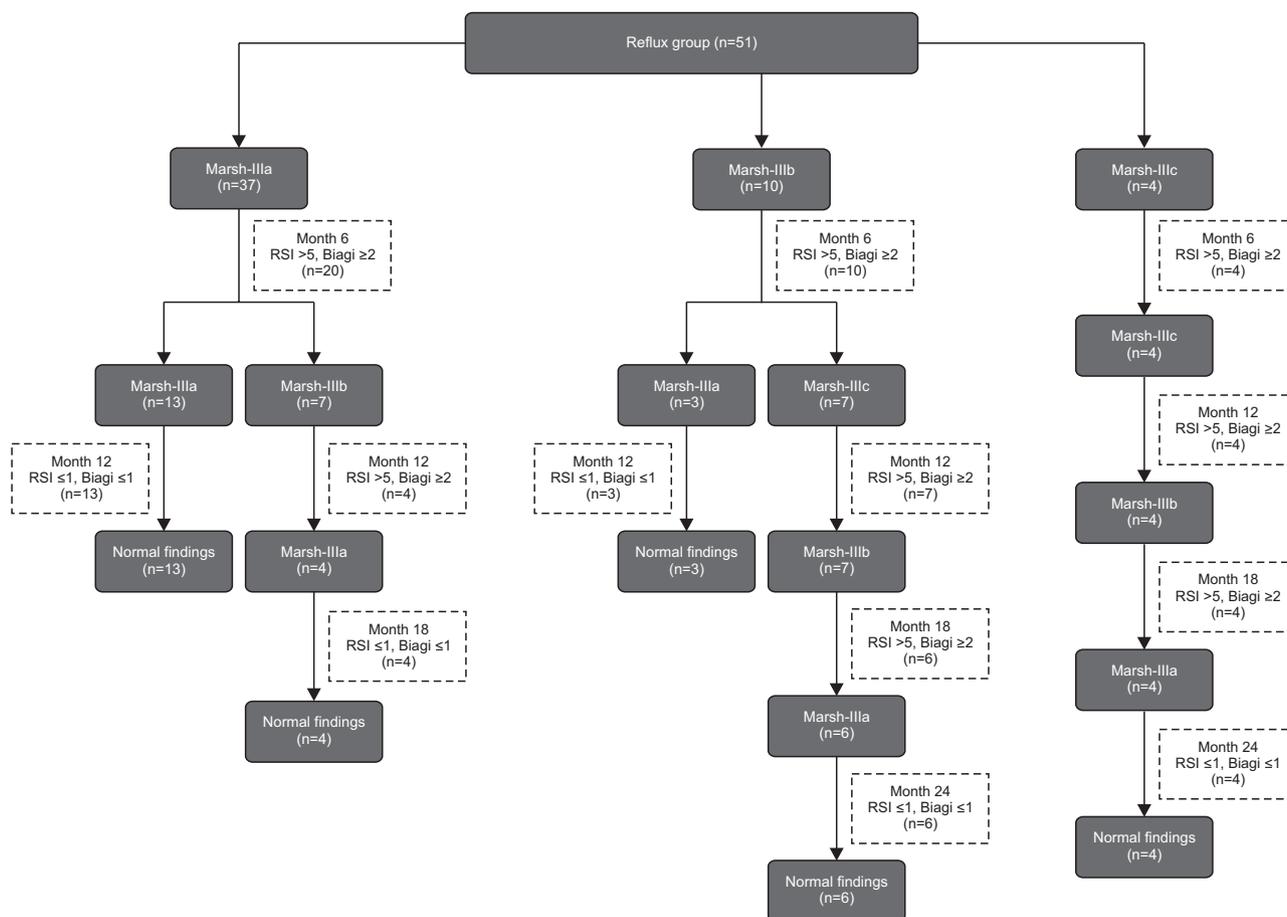


Fig. 3. Histological findings of the patients in the gastroesophageal reflux disease group during follow-up. RSI, Reflux Symptom Index.

allergy and foods containing fermentable, oligosaccharides, disaccharides, monosaccharides, and polyols (FODMAPs) intolerance.^{23,24} Unproven conclusions can quickly lead to unnecessary dietary restrictions in patients with diverse clinical presentations, which can affect their quality of life and can also lead to misdiagnosis of other underlying pathologies.

In this study, we investigated the presence of CeD in patients with isolated refractory dyspeptic and GERD complaints. This group of patients had isolated dyspepsia or isolated GERD-related symptoms without malabsorption, were seronegative, had tissue damage consistent with CD and benefited from a GFD. In this study, we did not classify these patients as non-celiac gluten-sensitive or food allergic, as all of them had increased intraepithelial lymphocyte count, villous atrophy, and crypt hyperplasia consistent with the Marsh classification. We did not categorize these patients as seronegative villous atrophy either, as all conditions known to cause villous atrophy were ruled out in the tissue samples, and both symptomatic and histological improvement were achieved with GFD.²⁵ Low FODMAP diet has a beneficial effect on symptoms in patients with refractory dyspepsia and GERD and GFD may consist of possible low FODMAP regimens²⁶ but all of our patients had tissue damage.

In this study, before the patients were diagnosed with CeD, they were admitted to the hospital due to their complaints with at least an average of three times in the dyspepsia group and at least an average of twice in the GERD group. We also found that patients were examined by endoscopy at least an average of four times in the dyspepsia group and at least an average of three times in the GERD group. Endoscopic appearance of the duodenum was not correlated with histological examination and findings consistent with CD were present in the samples taken from normal-appearing mucosa. In their study, Giangreco *et al.*²⁷ showed that, in patients with prolonged dyspeptic complaints, the incidence of CD diagnosis was 2-fold higher than the incidence of the general population, as evidenced by histological analysis. The authors recommended that tissue samples should be examined in this group of patients. Considering these findings, it is appropriate to take biopsy samples from the duodenum in patients who have prolonged dyspepsia or GERD-related symptoms, even if the endoscopic appearance is normal. Taking biopsies can avoid increasing treatment costs, overdiagnosis of functional dyspepsia, and inability to diagnose CeD.

Although CD is mostly ruled out in seronegative patients in routine clinical practice, the role of serology in diagnosis is still controversial. EMA and TGA are not specific for CD, do not always develop secondarily to gluten,

and may also be seen in other autoimmune diseases, such as type 1 diabetes. Furthermore, these antibodies can be detected to be positive in the blood in cases with no villous atrophy detected in the tissue samples.²⁸ Tissue antibodies may be positive, while serum antibodies are negative, and even non-celiac patients may have positive tissue samples.²⁹ Taken together, we can speculate that using EMA and TGA for CD diagnosis and screening may not be adequate, and negative results do not fully rule out CD diagnosis and tissue samples should be examined. Nevertheless, further well-designed, large-scale studies are needed to confirm this subject.

In clinical practice, it is recommended to investigate the *HLA* genes in the differential diagnosis of seronegative cases, and CD diagnosis is ruled out in *HLA*-negative cases, despite the fact that the distribution of *HLA* is highly dependent of the ethnic origin.^{2,30} Similar to the literature data,^{31,32} in this study, we detected *HLA*-related genes in all patients. Combining these results with tissue findings adding the fact that the reversal of both symptoms and tissue damage, this can be considered the proof of impaired immunity in these patients although the fact that we did not check for other markers of autoimmunity.

In the current study, histological progression was observed in dietary non-compliant patients with a gradual regression and normalization upon dietary compliance during follow-up. As indicated by the scores, the symptoms of these patients regressed and improved. Considering these findings, we hypothesize that this gradual increase and improvement are related to a phenomenon, which we call “overflow effect” that refers to the occurrence of progressive tissue damage and symptoms after prolonged exposure to gluten and exceeding the personal threshold value, and the return of these changes to normal with a GFD. Further comprehensive studies are needed to evaluate this hypothesis.

This study has certain limitations. First, this is a single-center, cross-sectional study without a placebo arm. Second, contrary to guideline recommendations, deamidated gliadin antibodies, which are recommended to be studied before tissue sampling in seronegative cases and TGA deposits in the tissue samples, were not examined in this study. However, the symptoms improved and reversal of tissue damage was achieved after GFD, and deamidated gliadin antibodies are also positive in up to 10% in healthy individuals.³³ We were unable to perform the hydrogen breath test for small intestine bacterial overgrowth. As the patients’ symptoms improved with diet although the lack of rifaximin treatment and as changes consistent with the Marsh classification, small intestine bacterial overgrowth diagnosis was indirectly ruled out in our patient popula-

tion. Another limitation is the relatively short follow-up period. The median follow-up was 19.9 months (range, 6 to 24 months) in the dyspepsia group and 19.2 months (range, 6 to 24 months) in the GERD group. Although we found symptomatic and histological improvement in all patients at the end of 24 months in both groups, histologically complete recovery may take longer.³⁴ Finally, we could not get the data on past endoscopy. It would be interesting to see whether duodenal biopsies were taken and correlated with our results.

Based on these findings, the following conclusions can be reached: (1) CD may be considered in patients who have isolated refractory dyspeptic and GERD-related complaints; (2) taking duodenal biopsies in these patients should be considered, even if the patient is seronegative and has a normal endoscopic appearance; and (3) the “overflow effect” may be present in seronegative patients. Further large-scale, prospective, randomized-controlled studies are warranted to draw a firm conclusion on this subject.

CONFLICTS OF INTEREST

No potential conflict of interest relevant to this article was reported.

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AUTHOR CONTRIBUTIONS

Conceptualization: S.T., Y.G. Methodology: S.T., Y.G. Formal analysis: S.T., B.B. Data collection: S.T., B.B. Data interpretation: S.T., B.B. Writing: S.T. Obtained funding: S.T., Y.G. Original draft: S.T. Supervision: Y.G. All authors read and approved the final manuscript.

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