REVISTA ESPAÑOLA DE
ENFERMEDADES DIGESTIVAS
The Spanish Journal of Gastroenterology

Title:
The value of a biopsy in celiac disease follow up: assessment of the small bowel after 6 and 24 months treatment with a gluten free diet

Authors:
Amir Sadeghi, Neda Rad, Sara Ashtari, Mohammad Rostami-Nejad, Afshin Moradi, Mahrokh Haghbin, Kamran Rostami, Umberto Volta, Mohammad Reza Zali

DOI: 10.17235/reed.2019.5947/2018
Link: PubMed (Epub ahead of print)

Please cite this article as:
Sadeghi Amir , Rad Neda, Ashtari Sara, Rostami-Nejad Mohammad, Moradi Afshin, Haghbin Mahrokh, Rostami Kamran, Volta Umberto , Zali Mohammad Reza. The value of a biopsy in celiac disease follow up: assessment of the small bowel after 6 and 24 months treatment with a gluten free diet. Rev Esp Enferm Dig 2019. doi: 10.17235/reed.2019.5947/2018.

REVISTA ESPAÑOLA DE
ENFERMEDADES DIGESTIVAS
The Spanish Journal of Gastroenterology

OR 5947

The value of a biopsy in celiac disease follow up: assessment of the small bowel after 6 and 24 months treatment with a gluten free diet

Amir Sadeghi[1], Neda Rad[1], Sara Ashtari[1], Mohammad Rostami-Nejad[2], Afshin Moradi[1], Mahrokh Haghbin[2], Kamran Rostami[3], Umberto Volta[4] and Mohammad Reza Zali[1]

[1]Gastroenterology and Liver Diseases Research Center and [2]Basic and Molecular Epidemiology of Gastrointestinal Disorders Research Center. Research Institute for Gastroenterology and Liver Diseases. Shahid Beheshti University of Medical Sciences. Tehran, Iran. [3]Departments of Gastroenterology. Mid Central DHB. Palmerston Hospital. Palmerston North, New Zealand. [4] Departments of Medical and Surgical Sciences. University of Bologna. Italy

Received: 8/10/2018

Accepted: 17/9/2019

Correspondence: Mohammad Rostami-Nejad. Basic and Molecular Epidemiology of Gastrointestinal Disorders Research Center. Research institute for Gastroenterology and Liver Diseases. Shahid Beheshti University of Medical Sciences. 1985714711, Tehran, Iran
e-mail: m.rostamii@gmail.com

Author´s contribution: Mohammad Rostami-Nejad, Amir Sadeghi and Neda Rad contributed equally to this article.

ABSTRACT

Introduction: a routine small bowel biopsy (SBB) during the follow up of celiac disease (CD) is controversial. Little information is available regarding the histological changes during (gluten free diet (GFD) in the long term.

Objectives: the aim of the study was to evaluate a novel criterion to compare duodenal histology in CD patients after six months and two years of gluten withdrawal.

Methods: this was a cross-sectional study of 200 patients with confirmed Marsh I-III who were under the six months (group A, n = 100) and 24 months (group B, n = 100) of a GFD. Nineteen patients were excluded due to an inadequate adherence to the GFD and another 23 patients were excluded as they were unwilling to undergo a re-endoscopy and did not comply with the necessary criteria. Endoscopy with a duodenal biopsy, serological assays and clinical evaluation were performed and compared with baseline data in the remaining 58 patients (20 patients in group A and 38 patients in group B).

Results: a significant complete histological recovery was found in 47.4% of patients in group B compared to 30% in group A (p = 0.026). A partial histological recovery was reported in seven (35%) and eleven (28.9%) patients in groups A and B, respectively. Any changes in mucosal histology after GFD was observed in 35% of patients in group A and 23.7% in group B. Serological assessment and endoscopic appearance normalized in 78.9% *vs* 75.0% in group B and 68.4% *vs* 65.0% in group A, respectively. However, this improvement did not reach statistical significance (p > 0.05).

Conclusions: the results of this study show that histological recovery in patients with Marsh ≥ III is slow and does not correlate with symptomatic improvement. We suggest that the long-term effects of a GFD can play an important role in achieving histological improvement, especially in older patients.

Keywords: Celiac disease. Gluten-free diet. Marsh classification. Histological recovery.

INTRODUCTION

Celiac disease (CD) is permanent state of intolerance to gluten and is an immune-mediated disorder that affects approximately 1% of the Western and Middle Eastern population (1-3). The immunologic response to gluten in gluten sensitive people causes histological abnormalities of the small intestinal mucosa, including increased intraepithelial lymphocytosis, crypt hyperplasia and villous distortion (4-6). The only treatment for CD is the lifelong adherence to a gluten-free diet (GFD) that is expected to improve not only symptoms and micronutrient deficiencies but also prevent long-term complications including malignant diseases (7,8). Histological recovery of the small intestinal mucosa is assumed to occur after

starting a gluten-free diet, followed by clinical remission (9). Most patients with CD respond to GFD but symptoms and mucosal changes persist in a small percentage of patients, despite full compliance with GFD and improvement of the symptoms. Even though the histological abnormalities persist, malignant transformation is rare in patients following a GFD.

Some guidelines traditionally recommend a follow-up endoscopy and biopsies after 6-12 months on a GFD in order to document the histological improvement and confirm the clinical remission and dietary compliance (10,11). More recent guidelines advise against a routine biopsy as long as patients achieve an optimal clinical improvement (12). Meanwhile, some studies suggest that the time to a complete histological recovery is much longer than 6-12 months of a GFD (4,13). Therefore, the optimal timing for a repeat small bowel biopsy after starting a GFD is a controversial matter. It is unclear if a routine biopsy should be performed in every CD patient or only in those at high risk of complications.

The aim of the present study was to evaluate the indication and value of the small bowel biopsy (SBB) during the follow-up of CD patients on a GFD. We present clinical, serological, endoscopy and duodenal histology data in CD patients during six and 24 months of gluten withdrawal.

MATERIAL AND METHODS

Patient selection and study design

Two hundred patients with CD, who were under the six months (group A, n = 100) and 24 months (group B, n = 100) GFD, were identified in the Gastroenterology Clinic in Taleghani Hospital, Tehran (Iran), between 2015 and 2017. Demographic characteristics such as sex, age, family history and presenting signs and symptoms were recorded for each patient. At baseline, those patients with Marsh I-II and a negative IgA-tTG test were excluded. Only patients with severe histological (Marsh III) changes were included in order to achieve a better characterization, as the objective of study was to assess the value of histology and biopsies. Therefore, 100 CD patients (50 cases in each group) with Marsh III were enrolled in this cross-sectional study. GFD adherence was assessed after the diet using a validated structured questionnaire consisting of four questions about how the patients managed their GFD (14). Actual adherence to the GFD based on a questionnaire was defined as adequate with a score of 3-4 and as inadequate with a score of 0-2 (15). Nineteen patients with a score of 0-2 were

excluded from the study due to an inadequate adherence to GFD. This included ten patients in group A and nine patients in group B. Moreover, 23 patients were excluded due to the personal reason as they were unwilling to undergo a re-endoscopy after GFD. This included 20 patients in group A and three patients in group B. Therefore, endoscopy with a duodenal biopsy, serological assays and clinical evaluation were performed in the remaining 58 patients (20 patients in group A and 38 patients in group B) (Fig. 1). Duodenal histology, serological results, endoscopic features and clinical presentation in these patients after six and 24 months of a GFD were compared with the baseline data. Written informed consent was obtained from all patients and the study was approved by the Ethics Committee of the Research Institute for Gastroenterology and Liver Diseases, Shahid Beheshti University of Medical Sciences, Tehran, Iran.

Upper GI endoscopy procedure

Patients underwent an upper GI endoscopy and changes in the villous structure of the mucosa associated with CD including reduced Kerckring's folds, scalloping of the mucosa on circular folds, mucosal fissures and a mosaic pattern were recorded (16). Endoscopic appearance was reported based on normal and abnormal features. A normal endoscopic appearance was defined as a lack of alterations in the duodenal mucosal and a normal villous appearance, whereas an abnormal endoscopic appearance was defined as scalloping of the duodenal folds, a mosaic pattern of the mucosa in the second part of the duodenum and loss of the duodenal folds (17,18).

Histological evaluation

Upper gastrointestinal endoscopy and biopsies were performed with a flexible endoscope (Olympus, Japan). Six well-oriented biopsy specimens were collected from the bulb (two biopsies) and second part of the duodenum (four biopsies) from every patient. Briefly, the samples were fixed overnight in 10% formalin buffer and paraffin-embedded. Subsequently, they were stained with hematoxylin and eosin (H&E) and examined by a gastrointestinal pathologist for routine histological evaluation. The duodenal biopsies were interpreted by two expert pathologists who were not informed about the clinical status of the patients; the small

intestinal histological features were interpreted according to the Marsh classification as follows (19,20): Marsh 0: normal mucosal architecture, without significant intraepithelial lymphocytic infiltration; Marsh I: raised intraepithelial lymphocytes (IELs) with > 25 lymphocytes per 100 enterocytes; Marsh II: raised intraepithelial lymphocytes and crypt hyperplasia; and Marsh III: villous flattening (19,20).

Serological and clinical evaluation

After six or 24 months of a GFD, five milliliters of venous blood were drawn from each patient for serological evaluation. Blood samples were kept at -20 °C for the assessment of anti-tTG (IgA) and total serum IgA for those with an IgA deficiency. Determinations of tTG IgA were performed by an enzyme-linked immunosorbent assay (ELISSA, AESKULISA®, Germany) in duplicate and according to the manufacturer's instructions (the cut-off value for t-TG was > 15 U/ml). Clinical presentations were also collected using a questionnaire, before and after six or 24 months of a GFD.

Interpretation of histological results and statistical analysis

Histological outcomes during GFD were divided into three groups: normalization, remission and unchanged. Normalization or the complete histological recovery group (Marsh 0) included cases with a full recovery, without significant intraepithelial lymphocytic infiltration. The remission or partial histological recovery group included cases with an improvement or reconstitution of the villous architecture (improving towards Marsh I and II). The unchanged group was classified as the same Marsh class at diagnosis (Marsh III). Patients in the normalization and remission groups were considered as "responsive" and patients in the unchanged group were considered as "non-responsive" to GFD.

Statistical analysis was performed using the Statistical Package for Social Sciences (SPSS) 21.0 (Chicago, IL, USA) and a two tailed p-value < 0.05 was considered as statistically significant. Data are expressed as the mean, median and interquartile ranges or values as a percentage. The Kolmogorov-Simonov's test was used to assess the normal distribution of the data. Categorical variables were compared using Pearson's Chi-squared test or the Fisher's exact test, depending on the nature of the data. The Wilcoxon signed-rank test was used to compare the

histopathological results (histological and serological findings) before and after GFD in each group. In addition, the Mann-Whitney U-test was used to compare the histology recovery between two groups. Odds ratios (ORs) and 95% confidence intervals (CIs) were used to describe the association and were obtained by logistic regression analysis. Age and gender were included in the model to detect any possible factors linked to the histological and serological findings.

RESULTS

Demographic characteristics of patients

Fifty-eight adult CD patients (25 males, 33 females with a mean age of 39.5 ± 13.7 years) with serological positivity and flat mucosa (Marsh III) at the time of diagnosis were evaluated. The main symptoms at the time of diagnosis in all 58 CD patients in both groups were bloating (65.5%), fatigue (65.5%) and anemia (53.4%). The demographic characteristics of 58 CD patients are presented in table 1. Demographic characteristics of the study population were not significantly different between the two groups ($p > 0.05$).

Histological, serological, clinical and endoscopic findings

Table 2 shows the histological, serological, clinical and endoscopic findings in both groups, before and after six and 24 months of a GFD. Complete histological recovery (Marsh 0) after six months of GFD was found in 6/20 (30%) patients compared to 18/38 (47.4%) patients after 24 months of GFD. The difference between the two groups was statistically significant ($p = 0.026$). Partial histological recovery was found in seven (35%) and eleven (28.9%) patients in group A and B, respectively. Therefore, the histological findings showed that 13/20 (65%) and 29/38 (76.3%) patients were responsive to GFD (complete and partial histological recovery) in groups A and B, respectively. The percentage of patients without any changes in mucosal histology (remained as Marsh III) after six and 24 months of GFD was 35% and 23.7%, respectively. No statistically significant differences were found between the two groups with respect to remission and the histology remained unchanged as Marsh III ($p > 0.05$).

All 58 patients at baseline had an abnormal endoscopic appearance (villous flattening), whereas there was a recovery of the villous architecture after six and 24 months of GFD in 13 (65%) and

26 (68.4%) patients, respectively. However, the endoscopic appearance in seven (35%) and 12 (31.6%) patients remained abnormal in group A and B, respectively. In group A, seven patients with an abnormal endoscopy had scalloping features and a mosaic pattern appearance in five (71.4%) and two (28.6%) cases, respectively. On the other hand, a mosaic pattern and scalloping features were seen in seven (58.4%) and five (41.6%) patients, respectively, with an abnormal endoscopic appearance after 24 months of GFD. Serological follow up after six and 24 months of GFD showed normalization of IgA-tTG in 15 (75%) and 29 (78.9%) patients, respectively. There was a significant reduction in t-TG antibody levels in each group ($p < 0.001$).

At baseline, all patients with clinical symptoms were enrolled in the study. The clinical symptoms improved in 50% and 57.9% of patients in group A and B, respectively, during follow up after six and 24 months of GFD ($p < 0.002$). No statistically significant differences were found between the two groups (50% *vs* 57.9%, $p > 0.05$).

The most prevalent clinical symptoms at baseline in group A were fatigue (80%), weight loss (65%), bloating and diarrhea (60%). While the three most common symptoms after six months of GFD were diarrhea (35%), weight loss (20%) and bloating (15%). All symptoms were significantly improved during six months of a GFD, except for diarrhea and aphthous stomatitis ($p = 0.056$ and $p = 0.577$, respectively). At the initial presentation, bloating (68.4%), fatigue (57.9%) and anemia (55.2%) were the most common symptoms in group B. After 24 months of GFD, bloating (23.7%), diarrhea (5.2%) and weight loss (5.2%) were the three most common symptoms and all symptoms were significantly improved in this group ($p < 0.05$).

Univariate and multivariate analysis

Table 3 shows the univariate and multivariate analysis for histological and serological findings according to sex and age in the studied groups. The patients were subdivided according to age (above and below 40 years). Univariate analysis showed that the rate of histological recovery in young patients was higher than in older patients in group A ($p = 0.046$), whereas there was no statistically significant difference in the different age groups of patients according to the multivariate analysis showed ($p = 0.074$). In addition, there were no statistically significant differences between histological and serological recovery according to sex and age in group B ($p > 0.05$).

Table 4 shows the association between histological findings and endoscopic appearance, serological features and overall clinical symptoms in the studied groups. The relationship between histological and serological findings in group B was statistically significant ($p = 0.033$). There was no statistically significant difference between the two groups with regard to histological, clinical symptoms or endoscopic appearance ($p > 0.05$). In terms of the association between clinical symptoms and histology findings, 50% and 37.5% of patients with persistent histological abnormalities remained symptomatic in group A and B, respectively. Despite the higher percentage of clinical symptom improvement in patients with histological normalization in both groups, these differences were not significant between the two groups ($p > 0.05$).

DISCUSSION

The clinical, serological and histological characteristics of 58 adult CD-seropositive patients with Marsh III were assessed before and after GFD in two groups; group A and B on a GFD for six and 24 months, respectively. Complete histological recovery (Marsh 0) occurred more significantly in CD patients with 24 months of GFD than in those with six months of GFD (47.4% *vs* 30%, $p = 0.026$). Remission status or partial histological recovery (Marsh I and II) was reported in 35% and 28.9% of patients in group A and B, respectively. Moreover, 35% and 23.7% of patients still had a total villous atrophy, despite six and 24 months of GFD, respectively. GFD adherence was assessed after the diet using a validated structured questionnaire (14,15). However, it is almost impossible to be sure that all patients strictly follow the GFD. There is no consensus regarding the optimal frequency of monitoring the GFD or the best tools for assessing compliance. Available methods to assess GFD adherence markers are insufficiently sensitive and are either indirect or based on subjective estimates. The determination of gluten immunogenic peptides (GIP) in feces and urine has recently been proposed for direct verification of GFD compliance in order to reduce the bias of GFD adherence (21,22).

Histological recovery in CD after starting a GFD takes time and the optimal timing of the follow-up biopsy remains unclear. According to the US and UK guidelines, follow-up by small bowel biopsy after 4-6 months on GFD is considered as the gold standard (23-25). However, other studies have shown that the small bowel mucosa does not generally normalize within 4-6 months (4,9,13,26,27). Grefte et al. reported that the recovery of the intestinal mucosa in 22

CD patients continued from nine to 19 months and was still incomplete after 2-4 years (28). Similar to our findings, Wahab et al. found (20) that only 65% of 158 patients achieved histological improvement (Marsh 0-II) within two years (4). Furthermore, Tursi et al. found that 32/42 of CD patients (72%) had a normal small bowel histology after two years on a GFD (29). The findings by Wahab et al. and Tursi et al. are in accordance with our findings and show that repeating the duodenal biopsy six months after diagnosis is too early to achieve a complete histological normalization. The most frequently reported symptoms such as fatigue, diarrhea, weight loss, bloating and anemia at the time of diagnosis were similar to those reported in previous studies (30-33). These results confirmed that the increased duration of adhering to GFD is significantly correlated with the improvement the symptoms.

According to previous studies, age at diagnosis is a factor that can affect histological healing in CD patients (4,34,35). Tursi et al. reported that young patients (15-30 years old) showed an improved histology within 12 months ($p < 0.034$) compared to older patients (> 30 years old). However, this difference was not statistically significant (26). In our study, patients under 40 years at diagnosis in group A showed a more significant improvement in histology recovery than patients older 40 years (50% *vs* 0%, $p = 0.046$), according to the univariate analysis. However, no statistically significant differences were found in different age groups of patients in the multivariate analysis ($p = 0.074$). In addition, there were no statistically significant differences between histological and serological recovery according to sex and age in group B ($p > 0.05$). Therefore, we concluded that the degree of histological recovery was independent of age, although a more complete mucosal recovery (Marsh 0) occurred in CD adult patients over 40 years of age within 24 months of a GFD than in those with six months of a GFD. This is probably due to the long-term effects of a GFD. We suggest that the long-term of effects of a GFD can play an important role in achieving a histological improvement, especially in older patients. It is likely that younger patients have a greater ability to adapt to the gluten-free diet and that the diet was not optimal in older patients. Therefore, the optimal timing to reach a serological and histological recovery in older CD patients (patients aged over 40 years) is higher than in younger patients. Nevertheless, larger studies are needed to further clarify this relationship.

The response to gluten withdrawal in patients is variable. Notably, clinical, histological and serological responses often do not occur in parallel and equally. Several studies have shown that serum CD associated antibodies, such as anti-transglutaminase and/or anti-endomysium (t-TG/EMA) positivity after the start of a GFD, are more frequently associated with intestinal damage (13,36-38). Moreover, antibody negativity is not necessarily related to histological recovery and may in fact be associated with false-negative results (9,39-43). In our study, a poor correlation was found between histological recovery and other outcomes such as serological, endoscopic features and clinical symptoms. The presence or absence of clinical symptoms in patients with CD is not related to their pathological outcome.

Antibody seroconversion and the normality of the endoscopic appearance after six and 24 months of a GFD was not an indicator of a complete resolution of the histological damage. This is due to the fact that antibody negativity was found in 57.1% and 44.4% of Marsh III patients with no histological changes in group A and B, respectively. In addition, antibodies were still positive in 11.1% of patients in group B with a complete recovery of histological damage.

Furthermore, a normal endoscopic appearance was found in 42.9% and 44.4% of patients with a no-change status (Marsh III) in group A and B, respectively. These findings suggest the possibility of antibody positivity in patients with a complete mucosal recovery as previously reported (44-46). The determination of antibodies and endoscopic appearance as alternative markers for histological improvement and GFD should be used with caution. Therefore, a negative CD serology and normal endoscopic features in patients with Marsh III are not reliable predictors of a histological responsive outcome. These observations suggest that duodenal histology may be a necessary tool to assess the efficacy of GFD and also support the need for further information on the extent and the factors that influence mucosal healing during GFD. A limitation of this study is that the association of a complete histological recovery with mild or more severe forms of histological damage could not be determined, as patients with Marsh I and Marsh II were excluded from the study.

In conclusion, this study has shown that serological and endoscopic recovery is faster than histological improvement in adults with CD who undergo a GFD. Histological recovery in CD after starting a GFD takes more time. In fact, our results show that repeating the duodenal biopsy six months after diagnosis and starting a GFD is not sufficient to achieve a complete

histological recovery. Therefore, repeating the duodenal biopsy 24 months after diagnosis and starting the GFD in CD patients is considered as important to monitor histological recovery, although reversal to normality of the small intestinal histology requires a prolonged and strict adherence to GFD, for even more than two years.

ACKNOWLEDGMENTS

We would like to thank the Gastroenterology and Liver Diseases Research Center of the Research Institute for Gastroenterology and Liver Diseases, Shahid Beheshti University of Medical Sciences (Tehran, Iran), for their support of the study.

AUTHOR CONTRIBUTIONS

AS, NR, MRN and MRZ planned the study. AS, NR and AM performed the histological evaluation and preparation of the samples. MH and SA prepared the database and data extraction and analysis. KR, UV and MRN supervised the work and prepared the manuscript. All authors edited and revised the manuscript and approved the final version of manuscript.

FUNDING

The present article is financially supported by the Research Department of the School of Medicine of the Shahid Beheshti University of Medical Sciences (Grant No. 9729).

REFERENCES

1. Fasano A, Catassi C. Clinical practice. Celiac disease. N Engl J Med 2012;367(25):2419-26. DOI: 10.1056/NEJMcp1113994

2. Asadzadeh H, Sadeghi A, Rostami-Nejad M. Celiac disease. Arvand J Health Med Sci 2016;1(2):57-60. DOI: 10.22631/ajhms.2016.43214

3. Rostami Nejad M, Karkhane M, Marzban A, et al. Gluten related disorders. Gastroenterol Hepatol Bed Bench 2012;5(Suppl 1):S1-7.

4. Wahab PJ, Meijer JW, Mulder CJ. Histologic follow-up of people with celiac disease on a gluten-free diet: slow and incomplete recovery. Am J Clin Pathol 2002;118(3):459-63. DOI: 10.1309/EVXT-851X-WHLC-RLX9

5. Marsh MN. Gluten, major histocompatibility complex, and the small intestine. A molecular and immunobiologic approach to the spectrum of gluten sensitivity ("celiac sprue"). Gastroenterology 1992;102(1):330-54.

6. Sadeghi A, Ehsani-Ardakani MJ, Jamshidfar N, et al. Refractory celiac disease. Diagnosis, treatment and clinical manifestations. Govaresh 2017;22:7-16.

7. Rostami K, Aldulaimi D, Rostami-Nejad M. Gluten free diet is a cure not a poison! Gastroenterol Hepatol Bed Bench 2015;8(2):93-4.

8. Francavilla R, Cristofori F, Stella M, et al. Treatment of celiac disease: from gluten-free diet to novel therapies. Minerva Pediatr 2014;66(5):501-16.

9. Bardella MT, Velio P, Cesana BM, et al. Coeliac disease: a histological follow-up study. Histopathology 2007;50(4):465-71. DOI: 10.1111/j.1365-2559.2007.02621.x

10. Revised criteria for diagnosis of coeliac disease. Report of Working Group of European Society of Paediatric Gastroenterology and Nutrition. Arch Dis Child 1990;65(8):909-11. DOI: 10.1136/adc.65.8.909

11. Rubio-Tapia A, Hill ID, Kelly CP, et al. ACG clinical guidelines: diagnosis and management of celiac disease. Am J Gastroenterol 2013;108(5):656-76;quiz 77. DOI: 10.1038/ajg.2013.79

12. United European Gastroenterology. When is a coeliac a coeliac? Report of a working group of the United European Gastroenterology Week in Amsterdam, 2001. Eur J Gastroenterol Hepatol 2001;13(9):1123-8.

13. Ciacci C, Cirillo M, Cavallaro R, et al. Long-term follow-up of celiac adults on gluten-free diet: prevalence and correlates of intestinal damage. Digestion 2002;66(3):178-85. DOI: 10.1159/000066757

14. Biagi F, Andrealli A, Bianchi PI, et al. A gluten-free diet score to evaluate dietary compliance in patients with coeliac disease. Br J Nutr 2009;102(6):882-7. DOI: 10.1017/S0007114509301579

15. Biagi F, Bianchi PI, Marchese A, et al. A score that verifies adherence to a gluten-free diet: a cross-sectional, multicentre validation in real clinical life. Br J Nutr 2012;108(10):1884-8. DOI: 10.1017/S0007114511007367

16. Murray JA, Rubio-Tapia A, Van Dyke CT, et al. Mucosal atrophy in celiac disease: extent of involvement, correlation with clinical presentation, and response to treatment. Clin

Gastroenterol Hepatol 2008;6(2):186-93;quiz 25. DOI: 10.1016/j.cgh.2007.10.012

17. Lee SK, Green PH. Endoscopy in celiac disease. Curr Opin Gastroenterol 2005;21(5):589-94. DOI: 10.1097/01.mog.0000174218.00333.19

18. Balaban DV, Popp A, Vasilescu F, et al. Diagnostic yield of endoscopic markers for celiac disease. J Med Life 2015;8(4):452-7.

19. Rostami K, Marsh MN, Johnson MW, et al. ROC-king onwards: intraepithelial lymphocyte counts, distribution & role in coeliac disease mucosal interpretation. Gut 2017;66(12):2080-6. DOI: 10.1136/gutjnl-2017-314297

20. Marsh MN, Johnson MW, Rostami K. Mucosal histopathology in celiac disease: a rebuttal of Oberhuber's sub-division of Marsh III. Gastroenterol Hepatol Bed Bench 2015;8(2):99-109

21. Moreno ML, Rodriguez-Herrera A, Sousa C, et al. Biomarkers to monitor gluten-free diet compliance in celiac patients. Nutrients 2017;9(1). DOI: 10.3390/nu9010046

22. Rodrigues M, Yonamine GH, Fernandes Satiro CA. Rate and determinants of non-adherence to a gluten-free diet and nutritional status assessment in children and adolescents with celiac disease in a tertiary Brazilian referral center: a cross-sectional and retrospective study. BMC Gastroenterol 2018;18(1):15. DOI: 10.1186/s12876-018-0740-z

23. Gastroenterology BSo. Guidelines for the Management of Patients with Coeliac Disease. 2002. Accessed in Nov 2009. Available from: http://wwwbsgorguk

24. ACG clinical guidelines: diagnosis and management of celiac disease. Am J Gastroenterol 2017;108(5):1977-80.

25. Ludvigsson JF, Bai JC, Biagi F, et al. Diagnosis and management of adult coeliac disease: guidelines from the British Society of Gastroenterology. Gut 2014;63(8):1210-28. DOI: 10.1136/gutjnl-2013-306578

26. Tursi A, Brandimarte G, Giorgetti GM, et al. Endoscopic and histological findings in the duodenum of adults with celiac disease before and after changing to a gluten-free diet: a 2-year prospective study. Endoscopy 2006;38(7):702-7. DOI: 10.1055/s-2006-925178

27. Hutchinson JM, West NP, Robins GG, et al. Long-term histological follow-up of people with coeliac disease in a UK teaching hospital. QJM 2010;103(7):511-7. DOI: 10.1093/qjmed/hcq076

28. Grefte JM, Bouman JG, Grond J, et al. Slow and incomplete histological and functional recovery in adult gluten sensitive enteropathy. J Clin Pathol 1988;41(8):886-91. DOI: 10.1136/jcp.41.8.886

29. Tursi A, Brandimarte G, Giorgetti GM, et al. Endoscopic features of celiac disease in adults and their correlation with age, histological damage, and clinical form of the disease. Endoscopy 2002;34(10):787-92. DOI: 10.1055/s-2002-34255

30. Pulido O, Zarkadas M, Dubois S, et al. Clinical features and symptom recovery on a gluten-free diet in Canadian adults with celiac disease. Can J Gastroenterol 2013;27(8):449-53. DOI: 10.1155/2013/741740

31. Mandile R, Discepolo V, Scapaticci S, et al. The effect of gluten free diet on clinical symptoms and the intestinal mucosa of patients with potential celiac disease. J Pediatr Gastroenterol Nutr 2018;66(4):654-6. DOI: 10.1097/MPG.0000000000001745

32. Cranney A, Zarkadas M, Graham ID, et al. The Canadian Celiac Health Survey. Dig Dis Sci 2007;52(4):1087-95. DOI: 10.1007/s10620-006-9258-2

33. Green PHR, Stavropoulos SN, Panagi SG, et al. Characteristics of adult celiac disease in the USA: results of a national survey. Am J Gastroenterol 2001;96(1):126-31. DOI: 10.1111/j.1572-0241.2001.03462.x

34. Lanzini A, Lanzarotto F, Villanacci V, et al. Complete recovery of intestinal mucosa occurs very rarely in adult coeliac patients despite adherence to gluten-free diet. Aliment Pharmacol Ther 2009;29(12):1299-308. DOI: 10.1111/j.1365-2036.2009.03992.x

35. Lebwohl B, Murray JA, Rubio-Tapia A, et al. Predictors of persistent villous atrophy in coeliac disease: a population-based study. Aliment Pharmacol Ther 2014;39(5):488-95. DOI: 10.1111/apt.12621

36. Lebwohl B, Sanders DS, Green PHR. Coeliac disease. Lancet 2018;391(10115):70-81.

37. Galli G, Esposito G, Lahner E, et al. Histological recovery and gluten-free diet adherence: a prospective 1-year follow-up study of adult patients with coeliac disease. Aliment Pharmacol Ther 2014;40(6):639-47. DOI: 10.1111/apt.12893

38. Kaukinen K, Sulkanen S, Maki M, et al. IgA-class transglutaminase antibodies in evaluating the efficacy of gluten-free diet in coeliac disease. Eur J Gastroenterol Hepatol 2002;14(3):311-5. DOI: 10.1097/00042737-200203000-00017

39. Nikzamir M, Rostami Nejad M, Sadeghi A, et al. The correlation between endoscopy manifestations and pathology outcome for diagnosis of celiac disease. Int J Celiac Dis 2017;5(3):101-3.

40. Rashid M, Lee J. Serologic testing in celiac disease: practical guide for clinicians. Can Fam Physician 2016;62(1):38-43.

41. Leffler DA, Schuppan D. Update on serologic testing in celiac disease. Am J Gastroenterol 2010;105(12):2520-4. DOI: 10.1038/ajg.2010.276

42. Sharkey LM, Corbett G, Currie E, et al. Optimizing delivery of care in coeliac disease - Comparison of the benefits of repeat biopsy and serological follow-up. Aliment Pharmacol Ther 2013;38(10):1278-91. DOI: 10.1111/apt.12510

43. Dickey W, Hughes DF, McMillan SA. Disappearance of endomysial antibodies in treated celiac disease does not indicate histological recovery. Am J Gastroenterol 2000;95(3):712-4. DOI: 10.1111/j.1572-0241.2000.01838.x

44. Lahdeaho ML, Maki M, Laurila K, et al. Small-bowel mucosal changes and antibody responses after low- and moderate-dose gluten challenge in celiac disease. BMC Gastroenterol 2011;11:129. DOI: 10.1186/1471-230X-11-129

45. Leonard MM, Weir DC, DeGroote M, et al. Value of IgA tTG in predicting mucosal recovery in children with celiac disease on a gluten-free diet. J Pediatr Gastroenterol Nutr 2017;64(2):286-91. DOI: 10.1097/MPG.0000000000001460

46. Haines ML, Anderson RP, Gibson PR. Systematic review: the evidence base for long-term management of coeliac disease. Aliment Pharmacol Ther 2008;28(9):1042-66. DOI: 10.1111/j.1365-2036.2008.03820.x

REVISTA ESPAÑOLA DE
ENFERMEDADES DIGESTIVAS
The Spanish Journal of Gastroenterology

Table 1. Demographic characteristics of 58 celiac patients

Demographic characteristics		*Group A 6 months of GFD 20 (%)*	*Group B 24 months of GFD 38 (%)*	*Total 58 (100%)*	*p-value*
Sex	Male	9 (36)	16 (64)	25 (100)	0.832*
	Female	11 (33.3)	22 (66.7)	33 (100)	
Age	Mean ± SD	40.4 ± 11.8	39.1 ± 14.7	39.5 ± 13.7	0.720†
	range	19-57	13-67	13-67	
Age group	≤ 40	12 (36.4)	21 (63.6)	33 (100)	0.729*
	> 40	8 (32)	17 (68)	25 (100)	
Family history of CD	Yes	1 (12.5)	7 (87.5)	8 (100)	0.241‡
	No	19 (38)	31 (62)	50 (100)	

Data are expressed as the number of total (%), mean ± standard division and range. *Pearson's Chi-squared test. †Student's t-test. ‡Fisher's exact test.

REVISTA ESPAÑOLA DE
ENFERMEDADES DIGESTIVAS
The Spanish Journal of Gastroenterology

Table 2. Histological, serological, clinical and endoscopic findings in the studied groups

Histopathological results		*Group A 20 (%)*		*p-value*	*Group B 38 (%)*		*p-value*
		Initial presentation	*6 months of GFD*		*Initial presentation*	*24 months of GFD*	
Histological findings	Marsh III	20 (100)	7 (35)	< 0.001*	38 (100)	9 (23.7)	< 0.001*
	Marsh II	0	0		0	1 (2.6)	
	Marsh I	0	7 (35)		0	10 (26.3)	
	Normal histology	0	6 (30)[#]		0	18 (47.4)[†]	
IgA-tTG test	Positive	20 (100)	5 (25)	< 0.001*	38 (100)	9 (21.1)	< 0.001*
	Negative	0	15 (75)		0	29 (78.9)	
Endoscopic appearance	Normal	0	13 (65)	< 0.001*	0	26 (68.4)	< 0.001*
	Abnormal	20 (100)	7 (35)		38 (100)	12 (31.6)	
Clinical symptoms	Anemia	10 (50)	1 (5)	0.004	21 (55.2)	1 (2.6)	< 0.001
	Osteoporosis	7 (35)	0	0.005	10 (26.3)	0	< 0.001
	Diarrhea	12 (60)	7 (35)	0.056	18 (47.4)	2 (5.2)	< 0.001
	Weight loss	13 (65)	4 (20)	0.001	16 (42.1)	2 (5.2)	< 0.001
	Fatigue	16 (80)	0	< 0.001	22 (57.9)	1 (2.6)	< 0.001
	Aphthous stomatitis	2 (10)	1 (5)	0.577	7 (18.4)	1 (2.6)	0.012
	Bloating	12 (60)	3 (15)	0.001	26 (68.4)	9 (23.7)	< 0.001

p-value < 0.05 were considered as statistically significant. *Wilcoxon signed-rank test was used to compare the histological and serological findings before and after GFD in each group. [†]The comparison of the histology recovery between the two groups was assessed by the Mann-Whitney U-test and the p-value was significant (30% *vs* 47.4%, p = 0.026).

REVISTA ESPAÑOLA DE
ENFERMEDADES DIGESTIVAS
The Spanish Journal of Gastroenterology

Table 3. Univariate and multivariate analysis of the histological and serological findings according to sex and age in the studied groups

Variables		*Univariate analysis*		*Multivariate analysis*	
		OR for histological findings (95% CI)	*p-value*	*OR for histological findings (95% CI)*	*p-value*
Group A (6 months of GFD)					
Age	≤ 40	1 ref.	-	1 ref.	-
	> 40	8.333 (1.034-62.142)	0.046*	7.169 (0.828-62.044)	0.074
Gender	Female	1 ref.	-	1 ref.	-
	Male	0.343 (0.048-2.552)	0.287	0.583 (0.063-5.387)	0.634
Group B (24 months of GFD)					
Age	≤ 40	1 ref.	-	1 ref.	-
	> 40	1.771 (0.392-8.002)	0.458	2.045 (0.425-9.831)	0.372
Gender	Female	1 ref.	-	1 ref.	-
	Male	0.306 (0.054-1.730)	0.180	0.280 (0.048-1.634)	0.157
		OR for serological findings (95% CI)	*p-value*	*OR for serological findings (95% CI)*	*p-value*
Group A (6 months of GFD)					
Age	≤ 40	1 ref.	-	1 ref.	-
	> 40	0.091 (0.008-1.077)	0.057	0.115 (0.009-1.459)	0.095
Gender	Female	1 ref.	-	1 ref.	-
	Male	4.571 (0.409-51.138)	0.217	2.706 (0.192-38.198)	0.461
Group B (24 months of GFD)					
Age	≤ 40	1 ref.	-	1 ref.	-
	> 40	0.565 (0.125-2.552)	0.458	0.568 (0.125-2.584)	0.465
Gender	Female	1 ref.	-	1 ref.	-

	Male	0.882 (0.195-3.987)	0.871	0.928 (0.202-4.264)	0.923

**p*-value < 0.05 was considered as statistically significant. Odds ratios (ORs) and 95% confidence intervals (CIs) were used for logistic regression.

REVISTA ESPAÑOLA DE
ENFERMEDADES DIGESTIVAS
The Spanish Journal of Gastroenterology

Table 4. Association between the histological findings and endoscopic appearance, serological features and overall clinical symptoms in the studied groups

Findings presentation		*Histological results*			*Total*	*p-value*
		Normalization	*Remission*	*No change*		
Group A (6 months of GFD)						
Endoscopic appearance	Normal	5 (38.5)	5 (38.5)	3 (23.1)	13 (100)	0.283
	Abnormal	1 (14.3)	2 (28.6)	4 (57.1)	7 (100)	
Serologic test (IgA-tTG)	Negative	6 (40)	5 (33.3)	4 (26.7)	15 (100)	0.198
	Positive	0	2 (40)	3 (60)	5 (100)	
Clinical symptoms	No	4 (40)	4 (40)	2 (20)	10 (100)	0.351
	Yes	2 (20)	3 (30)	5 (50)	10 (100)	
Group B (24 months of GFD)						
Endoscopic appearance	Normal	15 (57.7)	7 (26.9)	4 (15.4)	26 (100)	0.113
	Abnormal	3 (25)	4 (33.3)	5 (41.7)	12 (100)	
Serologic test (IgA-tTG)	Negative	16 (55.2)	9 (31)	4 (13.8)	29 (100)	0.033*
	Positive	2 (22.2)	2 (22.2)	5 (55.6)	9 (100)	
Clinical symptoms	No	11 (50)	8 (36.4)	3 (13.6)	22 (100)	0.195
	Yes	7 (43.8)	3 (18.8)	6 (37.5)	16 (100)	

Data are expressed as the number of total (%). *p-value < 0.05 was considered as statistically significant.

REVISTA ESPAÑOLA DE
ENFERMEDADES DIGESTIVAS
The Spanish Journal of Gastroenterology

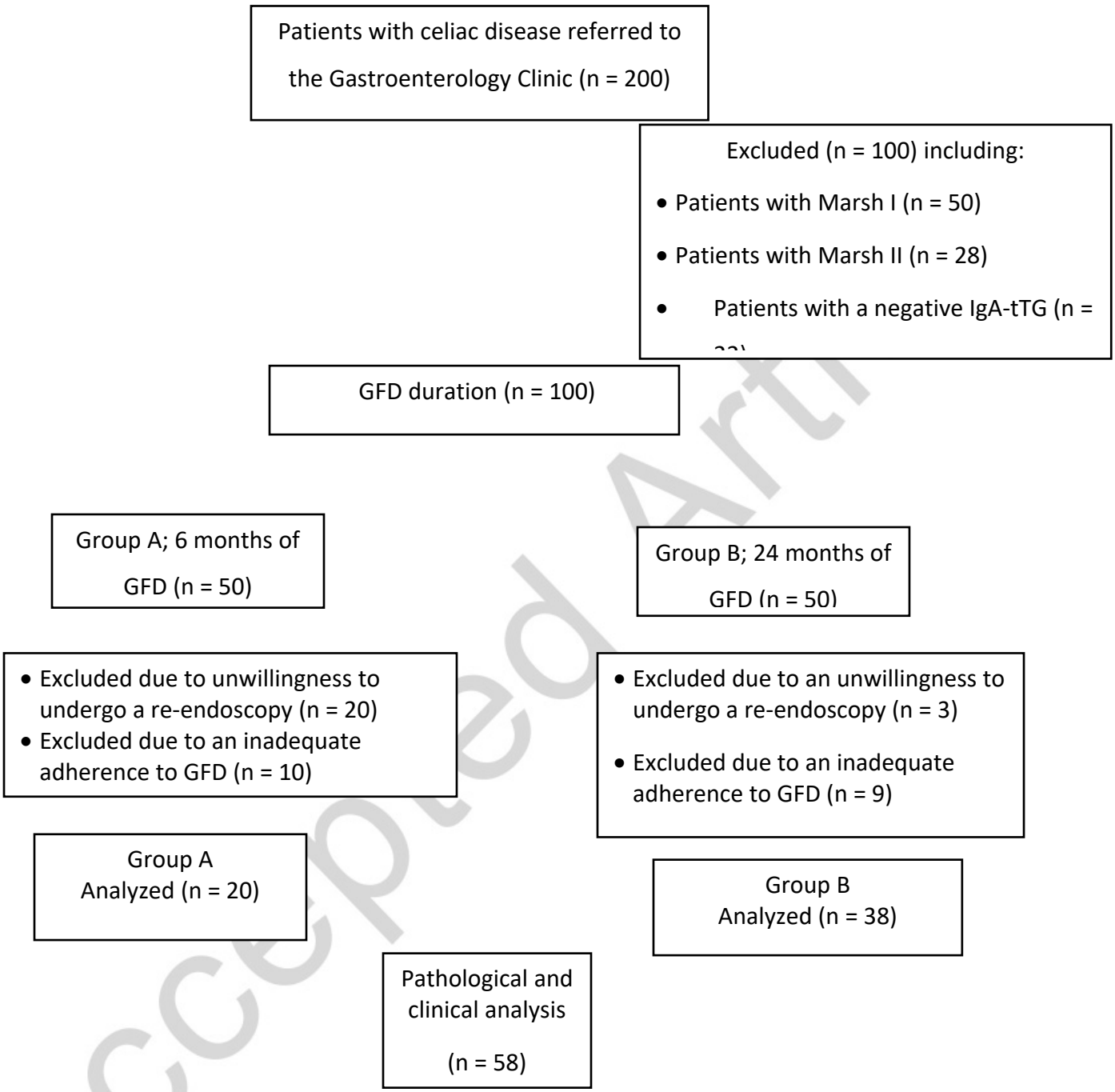

Fig. 1. Flow chart of participants in the study.