



Clinicopathological and Microbiological Profile of Patients with Chronic Diarrhoea and Malabsorption with Special Emphasis on Duodenal Biopsies: A Study from Tertiarycare Hospital in South India

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Abstract:

Background: Chronic diarrhoea and malabsorption continue to be a major health problem worldwide. Due to conflicting reports regarding the clinicohistological spectrum of malabsorption, this study was conducted to study the spectrum of malabsorption.

Objective: To study the morphologic spectrum of duodenal biopsies and stool microbiological profile in patients with chronic diarrhoea and malabsorption.

Materials and Methods: The study included 106 patients aged above 18 years, presenting with chronic diarrhoea or malabsorption. Duodenal mucosal biopsy obtained was evaluated for villous atrophy, crypt architecture and Intraepithelial lymphocyte count (IEL) by H&E and immunohistochemistry for CD3. Stool samples collected were subjected to wet mount and culture examination. Serological testing for antibodies to coeliac sprue were also performed.

Results: This descriptive study included 57 males and 49 females. Duodenal biopsy spectrum consisted of chronic duodenitis (48), duodenitis associated with autoimmune conditions (20), histomorphology of coeliac sprue (5), duodenitis with villous atrophy (14), only increased IEL (11) and miscellaneous (8). CD3 stained sections (n=90) offer a slightly better advantage over H & E-stained sections in detecting IEL. Stool microscopy and culture were positive in 6 cases. Coeliac sprue antibodies were positive in 1 case with Marsh type 1 lesion.

Conclusion: Duodenal biopsy is a useful parameter in the study of malabsorption which must be adjuncted by microbiological and serological tests. Coeliac sprue is a rare entity in our population as per this study.

Key words: Chronic diarrhoea, Malabsorption, Duodenal biopsy, Serological testing

Introduction:

Chronic diarrhoea and malabsorption continue to be a major health problem worldwide.(1) Malabsorption is an important cause of mortality and morbidity in the tropics.(2) The current trends suggest that coeliac disease and inflammatory bowel disease are also emerging as potential causes of malabsorption.(3) Previously it was thought to be more common in the western population with an incidence of about 1%.(4)

One of the investigations in the evaluation of malabsorption and chronic diarrhoea is the study of small intestinal biopsy (5). Duodenal biopsy may show villous and crypt abnormalities. Increased intraepithelial lymphocytes (IEL) may be increased in varying cases such as peptic duodenitis, inflammatory bowel disease etc. and may be a nonspecific finding.(6) Serological testing for coeliac disease antibodies such as anti-gliadin, anti endomysial and anti-tissue transglutaminase help in distinguishing between the close mimics of coeliac sprue despite overlapping serology between coeliac sprue and autoimmune enteropathy.(7)

We intended to study the morphologic spectrum of duodenal biopsies adjuncted by Immunohistochemistry (IHC) for IEL with CD3 antibody and to assess the microbiological profile of stool samples in patients with chronic diarrhoea and malabsorption. We also wished to study the prevalence of coeliac sprue by serological testing for antibodies seen in coeliac disease.

Materials and Methods

A descriptive study was conducted in the Department of Pathology, Microbiology and Medical Gastroenterology, JIPMER from June 2016 to May 2018. After approval by the institutional ethics committee, patients aged more than 18 years presenting with chronic diarrhoea and/ or malabsorption were recruited

for the study. After obtaining informed consent from the participants, demographic details and clinical characteristics like features of nutritional deficiency were noted. Hemogram, biochemical and serological parameters were also collected. Criteria applied for malabsorption included chronic diarrhoea lasting for ≥ 4 weeks, anaemia defined by haemoglobin $< 10\text{g/dl}$, steatorrhea, oedema/ hypoalbuminemia/ hypoproteinaemia, clinical features of nutritional deficiencies and autoimmune diseases.(8)

Duodenal biopsy was obtained endoscopically in the Department of Medical Gastroenterology. It was received in 10% neutral buffered formalin in a glass vial. The tissues were subjected to standard processing protocols and embedded in paraffin wax, sectioned at $4\mu\text{m}$ thickness and stained with Haematoxylin and Eosin (H&E) stain. Histopathological parameters like villous architecture and atrophy, crypt architecture, lamina propria infiltrate and IEL count per 100 enterocytes were observed. Villous atrophy was classified as mild, moderate/ marked and total /severe corresponding to Villous to Crypt (V:C) ratio of 2:1, 1:1 and 0:1 respectively (9) and Modified Marsh Oberhuber classification was applied (10). Marsh 1 lesions show increased IEL, Marsh 2 lesions show crypt hyperplasia and Marsh 3 lesions show villous atrophy in addition to Marsh 1 and 2 lesions. Crypt architecture was analysed as either normal or hyperplastic wherein hyperplastic crypts were elongated with increased number of mitoses. (11) Lamina propria infiltrate was graded subjectively into three grades as mild, moderate and severe (12).

IHC for CD3 (Dako, polyclonal) was done to analyse the IEL count and complemented with the counts on H&E-stained sections. They were interpreted as normal (0-25), borderline (26-29) or increased (≥ 30) for the entire

villous per 100 enterocytes (6,13,14) and normal (0-5) or increased (≥ 6) at the villous tip per 20 enterocytes (15). Stool samples were collected and were subjected to wet mount microscopy and culture examination. At the same time blood samples were collected in a serum tube. They were centrifuged and serum was separated and preserved in 3 aliquots of 0.5ml each at -80°C . Serum ELISA was done to detect the presence of IgA anti gliadin, IgA anti endomysial (EMA) and IgA anti tissue transglutaminase (tTG) antibodies (BIOCODON).

A total of 106 patients were included in the study after excluding patients with biopsy showing neoplastic lesions. Statistical analysis

was done using IBM -SPSS software version 19.0. Level of agreement of intraepithelial lymphocyte count by haematoxylin and eosin-stained sections were done by kappa statistics. All the statistical analysis was carried out at 5% level of significance with p value < 0.05 as statistically significant.

Results

This descriptive study included 106 consecutive patients presenting with diarrhoea or malabsorption during the study period. The morphologic spectrum of duodenal biopsies in patients with chronic diarrhoea and malabsorption was grouped as follows: (Table 1).

Table 1: Distribution of Morphologic spectrum of Duodenal Biopsies in patients with Chronic Diarrhoea and Mal absorption

S.NO.	MORPHOLOGICAL DIAGNOSIS	NUMBER OF CASES (%)
1.	Duodenitis associated with autoimmune conditions	20 (18.9)
	i) Chronic Active Duodenitis pattern	15
	ii) Coeliac disease like pattern	04
	iii) Graft versus host disease like pattern	01
	iv) Mixed pattern	-
2.	Villous atrophy with increased IEL (Marsh 3) (Histomorphology suggestive of coeliac disease)	05 (4.7)
3.	Chronic duodenitis (No villous atrophy)	48 (45.3)
4.	Duodenitis with villous atrophy	14 (13.2)
	i) Mild villous atrophy	07
	ii) Moderate villous atrophy	05
	iii) Total villous atrophy	02
5.	Only increased IEL (Marsh 1)	11 (10.4)
6.	Miscellaneous causes	08 (7.5)
	i) Eosinophilic duodenitis	04
	ii) Duodenitis associated with inflammatory bowel disease	02
	iii) Brunner gland hyperplasia	01
	iv) Nematode	01

The study included 57 males (53.8%) and 49 females (46.2%). In duodenitis associated with autoimmune conditions there was a female preponderance constituting around 70%. The age of the study population ranged between 19-

90 years with mean age of 40.49 ± 15.30 . Majority of the males and females were in the age range of 18 – 30 years constituting 33.3% and 32.7% respectively. The age distribution in

different morphologic groups did not have marked variation.

The most common symptom was diarrhoea accounting for 86 patients (81.1%). Anaemia was the second most common manifestation and was observed in 69 (65.1%) cases. Nutritional deficiency was seen in 39 (36.8%) patients. The mean haemoglobin was 10.3 ± 2.87 expressed in g/dl. MCV was found to be raised in two (2.8%) patients, however there were no megaloblastic epithelial changes in the duodenal biopsies studied. 24 patients (22.6%) had peripheral blood eosinophilia with 20 (18.9%) of which only 4 patients had eosinophilic duodenitis. Serum protein and serum albumin were available in 97 patients of which 45 (46.4%) had hypoproteinaemia. Hypoalbuminemia was observed in 35 (36.1%)

patients. All subjects were negative for HIV. Endoscopically, organisms (hookworms) were seen in two patients. Scalloped folds were observed in 16 (15.1%) of which two patients showed villous atrophy suggestive of coeliac disease. 7 out of 24 patients with histologic evidence of atrophy showed scalloped mucosal folds on endoscopy.

The duodenal biopsies show broadened villi in 19 (17.9%) and flattened in five (4.7%) patients. Mild atrophy was observed in 12 (11.3%) patients (Figure 1A), moderate/marked atrophy in 7 (6.6%) patients (Figure 1B) and severe/total atrophy in 5 (4.7%) patients (Figure 1C).

Crypt hyperplasia was seen in 14 (13.2%) of patients (Figure 1D) and cryptitis was observed in three (2.8%) patients.

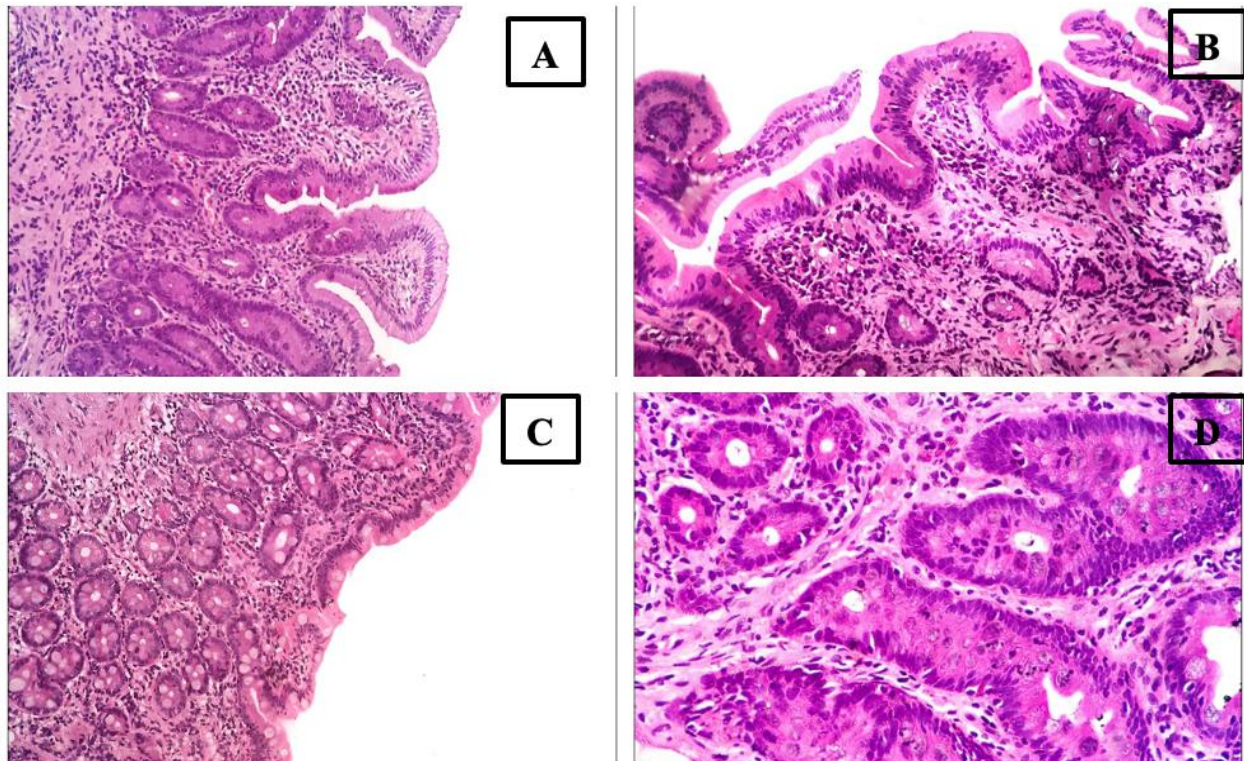


Figure 1: (A) Photomicrograph showing mild villous atrophy with V:C ratio 2:1 (H&E 200x), (B) Photomicrograph showing moderate villous atrophy with V:C ratio 1:1 (H&E 200x), (C) Photomicrograph showing total villous atrophy with virtually no villi (H&E, 200x), (D) Photomicrograph showing crypt hyperplasia with crypt elongation and crypt mitosis (H&E 400x)

Mild lamina propria infiltrate was seen in 30 (28.3%), moderate infiltrate in 48 (45.3%) and dense infiltrate in 28 (26.4%) patients. Brunner gland hyperplasia was observed in one patient who presented with chronic diarrhoea (Figure 2A) and endoscopy showed mucosal nodularity. Incidentally, stool wet mount examination of this patient showed cysts of *Entamoeba*. Also, one of the duodenal biopsies revealed cross sections of adult forms of nematode in the crypts and lamina propria (Figure 2B). Rheumatoid arthritis and seronegative arthritis were the most common autoimmune disorders encountered in this study. 2 cases of rheumatoid arthritis showed villous atrophy with a coeliac disease like pattern. Remaining cases showed chronic inflammation with a duodenitis like pattern. There were 5 cases in the study showing features of villous atrophy with increased IEL (Histomorphology suggestive of coeliac

disease). Of these, 3 cases showed moderate villous atrophy, and a single case each of mild and total villous atrophy. The mean IEL for 100 enterocytes was found to be 14.53 ± 8.49 . Increased IEL was observed in 12 (11.3%) patients with borderline increase in 4 patients (3.8%) and 8 (7.5%) patients had markedly increased IEL ($>30/100$ enterocytes) (Figure 2C). IHC for CD3 was performed in 90 patients (Figure 2D). IEL counts were increased in 15 (16.7%) and increased in borderline range in 2 (2.2%) and normal in 73 (81.1%) cases. The level of agreement between IEL on H & E-stained sections and CD3 stained section was done using Kappa statistics. Kappa value was 0.644 in our study indicating a moderate agreement. This indicates that CD3 stained sections offer a slightly better advantage over H & E-stained sections in detecting IEL.

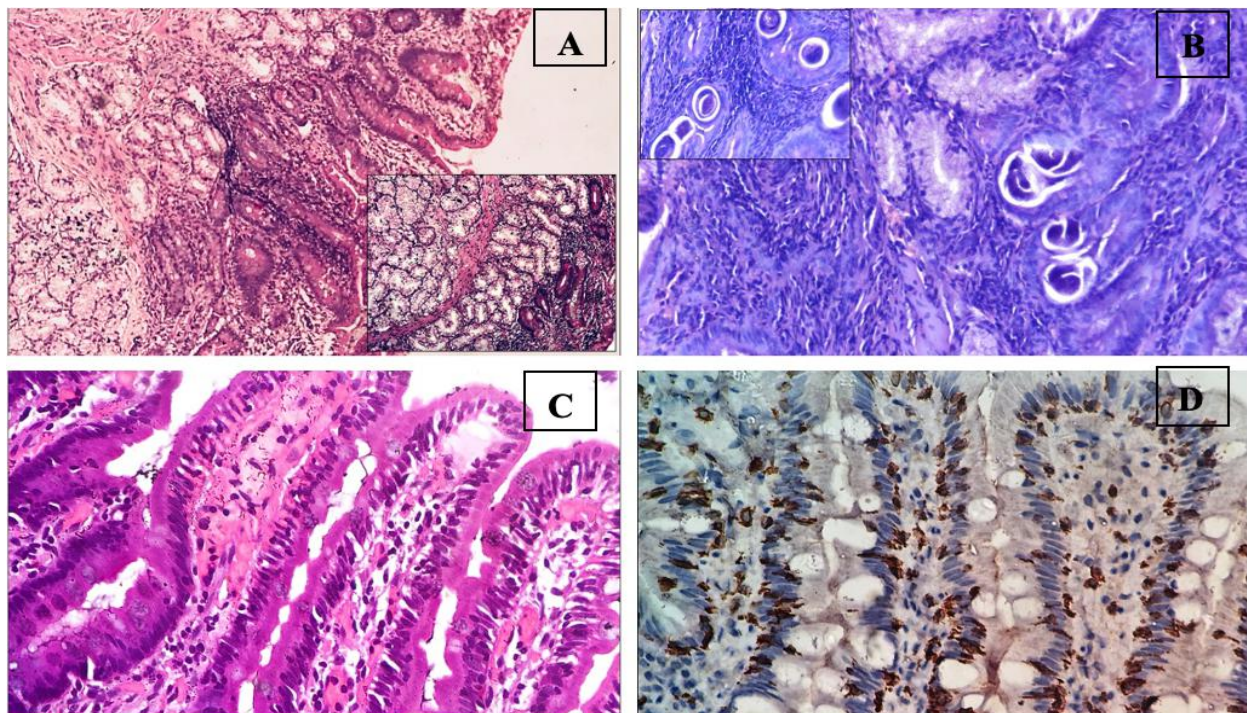


Figure 2: (A) Duodenal biopsy showing brunner glands prolapsing into lamina propria. (H&E, 100x). Inset: shows proliferation of brunner glands (H&E, 400x), (B) Duodenal biopsy with nematode (*Strongyloides*) infestation in the crypt (H&E 200x) Inset : Adult worms of nematode (H&E, 400x), (C) Photomicrograph with villi showing increased intraepithelial lymphocytes (H&E, 400x), (D) Villi showing intraepithelial lymphocytes highlighted by CD3 (IHC, 400x)

Stool microscopic examination was done for all 106 patients and majority of them were negative. 4 patients had evidence of steatorrhea. Wet mount examination revealed organisms in 6 cases, comprising of 3 cases of *Entamoeba*, 2 cases of hook worm and a single case of *Strongyloides*. Stool culture was positive in 6 cases comprising of 5 cases of *Salmonella* and 1 case of *Aeromonas*. Serological examination was done for IgA antigliadin antibody on 89 cases, IgA anti tTG on 90 cases and IgA anti EMA on 87 cases. IgA anti gliadin antibody was positive in one case with a median of 2.11 (0.6088), IgA anti tTG antibody did not show positivity in any case with a median of 10.17 (4.5577) and IgA anti EMA antibody was positive in one case with a median of 20.3643 (15.1433). IgA anti gliadin antibody and IgA anti EMA antibody were positive in the same case. IgA anti tTG antibody showed high normal value in the same case. Histomorphology along with IHC revealed Marsh type 1 lesion.

Discussion

Chronic diarrhoea is defined as a change in stool consistency or frequency for more than or equal to four weeks (16,17). Malabsorption occurs due to failure of nutrient absorption because of mucosal disease or structural disorder (18). Tropical sprue, coeliac sprue, infections and immunodeficiencies are the common causes of malabsorption. The criteria for malabsorption used in this study was similar to a previous study conducted by Balasubramanian P *et al* (8).

The age range in the present study was 19-90 years with a mean of 40.49 ± 15.30 years however majority of patients were in the age range of 18-30 years. Other similar studies from India showed a mean range of 37.8 ± 13.2 years and 36.5 ± 12.6 years (1,3). In a study from CMC Vellore, the age range was 31.9 ± 16 years (2). The gender distribution in the present study was similar to a Lucknow based study which showed a male dominance with

61% (3). Another study from Mumbai showed a male dominance with 49% (1).

Chronic diarrhoea and anaemia were the most common manifestations accounting for 81.1% and 65.1% respectively. In two other studies on duodenal biopsy in malabsorption, 84% and 71 % patients presented with chronic diarrhoea (8,19). Nutritional deficiency has been noted in 36.8% of patients in contrast to 12.8% of patients in a previous study (8). The mean haemoglobin observed was 10.3 ± 2.87 g/dl. Although, no specific histological features were identified in anaemic patients, this study is justified in subjecting patients with iron deficiency anaemia to routine endoscopic biopsy as this screening helps in identifying an additional 6.63% of patients with malabsorption (20). The mean differences in haemoglobin was statistically significant between atrophy and normal villous architecture group by chi square test with a p value of 0.015. In this study hypoproteinaemia was observed in 42.5% of patients and low serum albumin levels in 33% of cases. In two other studies hypoalbuminemia was seen in 40.3% and 12% of cases (2,21). The most common endoscopic findings in the present study were either normal mucosa or scalloped duodenal mucosal folds. A study on endoscopic features in coeliac disease documented that mosaic pattern and scalloped folds have maximum sensitivity and specificity for suspecting coeliac disease. (22) However, in this study, only 7 patients with scalloped folds on endoscopy showed villous atrophy. There was no significant association between the endoscopic findings and villous atrophy with a p value of .146.

In the present study, there was mild villous atrophy in 11.3% of patients, moderate villous atrophy in 6.6% and severe atrophy in only 4.7%. A Mumbai based study, demonstrated more moderate villous atrophy (24%) than mild villous atrophy (18%) and similar percentage of severe villous atrophy.(21) A large duration study from CMC Vellore

showed villous architectural abnormality in 16.1% of cases whereas the present study showed 22.6% of cases with villous atrophy. (2) Anaemia and crypt hyperplasia was compared between the cases with villous atrophy and cases with normal villous architecture and were found to be statistically significant in this study. Lamina propria inflammatory infiltrate was predominantly moderate in about 45.3% of cases. A study from Lucknow noticed that there was increased mononuclear inflammatory infiltrate in 96% of patients compared to 71.7% in our study. (3) Those cases with Marsh type 3 lesions had moderate to severe lamina propria inflammation. Majority of the cases associated with autoimmune disorder had mild to moderate inflammation which was comparable to another similar study from South India. (8) The mean number of eosinophils in lamina propria was 14.92 ± 11.64 . Few studies have even taken ≥ 20 eosinophils/hpf with peripheral eosinophilia as diagnostic of eosinophilic duodenitis.(23) In the present study, eosinophilic duodenitis was diagnosed when there were >50 eosinophils/hpf in the presence of peripheral eosinophilia and absence of secondary causes of eosinophilia in symptomatic patients. In our study all patients (four cases) diagnosed with eosinophilic duodenitis had mild to moderate degree of peripheral blood eosinophilia with no secondary causes of eosinophilia.

The mean IEL count per 100 enterocytes was 14.53 ± 8.49 . The mean IEL count in the group with histology of coeliac sprue was 29.80 ± 7.76 . One study showed that the mean IEL was 20.93 in Coeliac disease group and another study revealed a mean of 28.2 ± 7.7 . (8,24) In the present study, increased IEL by H & E stain was seen in only 11.3% with borderline increase in 3.8% and definite increase in 7.5% of cases in contrast to another Indian study showing 38% cases with increased IEL and borderline increase in 10%.(21) On CD3 stained sections, 15 (16.7%) cases showed

definite increase and 2 (2.2%) showed borderline increase in IEL. CD3 gave an additional advantage in identifying 17 cases compared to H & E staining where only 12 cases showed increased IEL. The mean IEL by CD3 immunostaining was 21.64 ± 9.71 whereas in a study on CD3 staining the mean IEL count was 25.4 ± 4.1 . The study documented a high correlation between the IEL counts per 100 enterocytes with respect to H & E and CD3 staining.(25) However in our study there was only moderate agreement with a p value of 0.644 between H & E and CD3 by kappa statistics which indicated that there is a slight advantage of using CD3 staining to detect IEL.

The incidence of coeliac disease in Indian population is just about 1%.(26) In our study, we encountered 5 cases with histology of coeliac disease (Marsh type 3 lesion). However, serology for three antibodies in coeliac disease were negative. The possible reasons for negative serology may be that they were partially treated or have refractory coeliac disease or seronegative coeliac sprue.(27) One case was positive for IgA anti gliadin, and IgA anti EMA antibodies and high normal IgA tTG. Duodenal biopsy revealed borderline increase in IEL but there was no evidence of crypt hyperplasia or villous atrophy. This patient could indicate an early form of potential coeliac disease with Marsh type 1 lesion. This patient symptomatically improved after strict gluten free diet. Thus, the incidence of coeliac disease in our population is relatively low even when adjuncted by serological tests.

Stool microscopy and culture yielded positive results in 6 cases each and they were independent of endoscopic and histological findings. One of the reasons attributed for the low yield of positive stool microbiological profile could be due to assessment made on a single stool sample. It has been suggested that a minimum of three to six stools samples are necessary for parasite identification in stool.(28) Also more advanced tests of stool

examination like stool antigen detection by ELISA and other molecular tests could be employed in future studies to achieve better comparative results.

Limitations

The criteria employed for suspecting cases of malabsorption should have been more stringent with the use of faecal fat estimation and urine D-xylose estimation. This study focused only on the morphological and serological analysis. The study employed IgA antibodies for serological testing and there is a small possibility of IgA deficient individuals.

Conclusion

Duodenal biopsy in the study of malabsorption is a useful parameter, as 54.7% of duodenal biopsies showed abnormal findings in the form of villous atrophy, increased IEL and duodenitis. The use of serological tests to identify the prevalence of coeliac disease indicated that coeliac disease is still rare in our population despite few recent studies documenting its increasing prevalence. We concluded that a constellation of clinical/endoscopic/serologic/microbiologic/histopathologic findings is mandatory before we arrive at a final diagnosis.

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