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A Cross-Sectional Analytical Study of Duodenal Biopsy in Malabsorption Syndrome

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ABSTRACT

Duodenal biopsies are a cornerstone in the diagnostic evaluation of malabsorption syndromes, providing invaluable histopathological insights that cannot be achieved through clinical assessment and laboratory tests alone. In present study, we aimed to study role of duodenal biopsy in malabsorption syndrome at a tertiary hospital. Present study was single-center, prospective and retrospective, observational study, conducted all endoscopic duodenal biopsy samples from patients presenting with malabsorption syndrome, underwent histopathology and Immunohistochemistry (IHC) evaluation. Among 101 cases, middle-aged group (18-50 years) constitutes the largest segment at 63.3%. The male-to- female ratio was 1.46:1, with a male predominance. The largest number of cases by H&E staining were in grade 0 (87%) followed by Grade 3a (6.9%) and grade 2, 3b and 3c. However, after IHC with CD3 and re- enumeration, the number of cases of grade 0 drastically fell to 23.7% followed by 21.7% grade 3a, grade 1, grade2, grade3b and grade 3c in decreasing order. Final distribution of M-O grades in the series after IHC was 23.7% (24/101) in grade 0, 21.7% in grade 3a followed by 20.7% (21/101) grade 1, 16.8% grade 2 (17/101), 10.8% (11/101) in 3b and 5.9(6/101) in grade 3c. By employing a detailed evaluation using the Marsh-Oberhuber classification, alongside advanced immunohistochemistry (IHC) techniques, we were able to describe various histological grades associated with malabsorption syndromes, particularly celiac disease.

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Key Words

Marsh-Oberhuber classification, duodenal biopsy, malabsorption syndromes, histopathological analysis, celiac disease

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INTRODUCTION

Malabsorption syndromes encompass a broad spectrum of disorders characterized by impaired nutrient absorption in the gastrointestinal tract, leading to significant clinical manifestations such as chronic diarrhea, weight loss and nutrient deficiencies. In clinical practice, malabsorption syndrome is significant due to its diverse etiology and the broad spectrum of clinical manifestations. Common causes include celiac disease, tropical sprue, Crohn's disease, chronic pancreatitis and infections such as Whipple's disease and giardiasis[1].

Diagnosing malabsorption syndrome requires a comprehensive clinical evaluation, including a detailed patient history, physical examination, laboratory tests and imaging studies. One of the most critical diagnostic tools is the histopathological examination of duodenal biopsies, which can reveal characteristic mucosal changes associated with specific malabsorptive conditions[2].

Duodenal biopsies are a cornerstone in the diagnostic evaluation of malabsorption syndromes, providing invaluable histopathological insights that cannot be achieved through clinical assessment and laboratory tests alone[3,4]. These biopsies involve the endoscopic collection of tissue samples from the duodenum, the first part of the small intestine, which is often the primary site of pathology in malabsorptive disorders[3,4]. It is hypothesized that IHC could play a significant role in the enumeration of T- lymphocytes in the duodenal mucosa in the evaluation of Malabsorption syndromes and in the enumeration of NE cell population[3,4]. In present study, we aimed to study role of duodenal biopsy in malabsorption syndrome at a tertiary hospital.

MATERIAL AND METHODS

Present study was single-center, prospective and retrospective, observational study, conducted in Department of Pathology, at Bharati Vidyapeeth (Deemed to be University) Medical College, Hospital and Research Centre, Pune, India. Study duration was of 18 months (Sept, 2022 to Mar, 2024). The study was initiated after obtaining approval from the institutional ethics committee.

Inclusion criteria: All endoscopic duodenal biopsy samples from patients presenting with malabsorption syndrome, which were received by the Department of Pathology from the Department of Gastroenterology.

Exclusion criteria:

- Inadequate and non-representative samples
- Patients under long-term use of antibiotics
- Patients with a history of major gastrointestinal surgery
- Patients using nonsteroidal anti-inflammatory drugs (NSAIDs)

Histopathology: The endoscopic biopsy samples were processed for histopathological examination using standard techniques. Biopsies were fixed in neutral buffered formalin for 2-6 hrs. Following fixation, the samples were processed using an Automated Tissue Processor and paraffin blocks were prepared. Sections of 2-4 microns thickness were cut and mounted on glass slides, then stained with Hematoxylin and Eosin (H&E) using standard protocols. These slides were assessed for features indicative of malabsorption, including mucosal changes in villi, lamina propria and inflammatory changes. The biopsies were scored using the Marsh-Oberhuber criteria.

Immunohistochemistry (IHC): Immunohistochemistry was performed on all cases. Antibodies for CD3 and synaptophysin were used with the peroxidase-anti-peroxidase technique to perform IHC. Quantitative assessments of T-lymphocytes and neuroendocrine cells were carried out. The histopathological and IHC results were correlated with clinical and endoscopic features to compare endoscopic and histopathological diagnoses. The biopsies were rescored using the Marsh- oberhuber criteria and differences, if any, were noted. The NE cell population was correlated with the diagnosis and Marsh stage.

Data were collected using test request forms, endoscopy and serology reports for prospective cases. For retrospective cases, histopathology records, old paraffin blocks and old test request forms were utilized. Histopathology registers were also referenced for retrospective data collection.

The collected data were coded and entered into Microsoft Excel and analyzed using SPSS version 25.0 software. Results were presented in tabular and graphic formats. For qualitative data, various rates, ratios and percentages were calculated. The Chi-square test was used to find associations between two or more attributes for qualitative variables. A $p<0.05$ was considered significant.

RESULTS

The study comprised 101 cases of Malabsorption syndrome in whom duodenal biopsies were carried out as per inclusion and exclusion criteria. IHC was carried out with CD3 and synaptophysin antibodies. The middle-aged group (18-50 years) constitutes the largest segment at 63.3%, followed by the elderly (>50 years) at 25.7%. Pediatric cases account for 10.8%. The male-to- female ratio is 1.46:1, with a male predominance in both pediatric and elderly age groups (Table 1).

The majority of biopsies, 52.47% (53 specimens), were taken from the second part of the duodenum (D2 site), followed by the unspecified site (D site) (37.6%), first part of the duodenum (D1 site) (9.9%) (Table 2).

Notable conditions include gastric issues (e.g., erosions, gastritis, ulcers, outlet obstruction) in 34 cases and celiac disease in others. Clinical diagnoses of malabsorption syndrome, anemia under evaluation and melena were noted in 29 cases (Table 3).

The grading of all 101 cases was carried out by the Marsh-Oberhuber (M-O) criteria and classified after H&E stain and correlated with IELs. The largest number of cases by H&E staining were in grade 0 (87%) followed by Grade 3a (6.9%) and grade 2, 3b and 3c. However, after IHC with CD3 and re- enumeration, the number of cases of grade 0 drastically fell to 23.7% followed by 21.7% grade 3a, grade 1, grade2, grade3b and grade 3c in decreasing order (Table 4).

Final distribution of M-O grades in the series after IHC was 23.7% (24/101) in grade 0, 21.7% in grade 3a followed by 20.7% (21/101) grade 1, 16.8% grade 2 (17/101), 10.8% (11/101) in 3b and 5.9(6/101) in grade 3c (Table 5).

Abdominal pain was the most common presenting symptom in 59 cases followed by vomiting and weight loss in 21 and 20 cases respectively. 9 cases each showed diarrhea or features of anemia. In a few cases multiple symptoms were seen. Abdominal pain, vomiting and diarrhea was seen more often in cases of lower grades (0-3a). Weight loss was seen in large number of cases of malabsorption due to coeliac/sprue (Table 6).

In grade 0 patients the most common finding was duodenal ulcer/erosion. In grade 1 similarly the most common findings were either duodenal lesions or a normal or a normal picture. Cases in grade 2 were more often normal (8/13) but grade 3a showed duodenal lesions as the most common finding. Nodularity on endoscopy was seen in only 10 cases (9.9%) and atrophy in only 7 cases (6.9%). 36 patients showed a normal endoscopy with grades ranging from grade 1 to grade 3. In 15 cases endoscopic information was not available as patients were lost to follow up after biopsy (Table 7).

Crypt hyperplasia was seen in 47.5% (48/101) of the cases studied with maximum distribution in grade 3a (19/22), grade 3b (9/11) and grade 3c (4/6). In 16 of 48 cases crypt hyperplasia was noted in lower M-O grades of 0-2. 38.6% (39/101) cases showed varying degree of villous atrophy. 18/19 cases showed mild

Table 1: Distribution of cases by age and sex

	Sex		
Age category	Male	Female	Total
<18	9	2	11
18-50	33	32	65
>50	18	7	25
TOTAL	60	41	101

Chi-square value: 5.991, p-value: 0.0511 and Not significant

Table 2: Distribution of cases as per site

Specimen biopsy	Frequency	Percentage
D2	53	52.47
D1	10	9.90
D	38	37.60

Chi-square value: 29.863, p<0.001 and Highly significant

Table 3: Distribution of cases as per Provisional diagnosis

Provisional diagnosis	Frequency
Anemia under evaluation	17
Erosive gastritis	11
Duodenal ulcer	10
Gastritis	10
Celiac disease	10
Gastric ulcer	9
Duodenitis	7
Duodenal fissures	7
Malena	6
Malabsorption syndrome	6
Gastric outlet obstruction	4
Crohn's disease	1
K/C/O Downs syndrome	1
Graft vs host disease	1
K/C/O Carcinoma cervix	1

Chi-square value: 45.31, p<0.001 and Highly significant

Table 4: Histological grades as per Marsh-Oberhuber classes by H&E and IHC

	0		1		2		3a		3b		3c		Total	
IELs	H&E	IHC	H&E	IHC	H&E	IHC	H&E	IHC	H&E	IHC	H&E	IHC	H&E	IHC
<30	86	24	-	-	-	-	-	-	-	-	-	-	86	25
30-40	2	-	-	17	-	11	7	12	1	8	1	4	11	52
>40	-	-	-	4	2	5	-	10	1	3	1	2	4	24
Total	88	24	-	21	2	17	7	22	2	11	2	6	101	101

Chi-square value: 0.54, p>0.05 and Not significant

Table 5: Change in m-o grade after IHC (CD3)

IELs	No change	Grade 0-1	Grade 0-2	Grade 0-3a	Grade 0-3b	Grade 0-3c	Total
<30	24	0	1	0	0	0	25
30-<40	02	17	11	11	8	3	52
40 & more	11	4	3	4	1	1	24
Total	37	21	15	15	9	4	101

Chi-square value: 64.31, p<0.001 and Highly significant

Table 6: Distribution of cases as per symptoms and signs

Symptoms	G0	G1	G2	G3a	G3b	G3c	Total
Abdominal pain	13	13	13	11	6	3	59
Vomiting	7	2	3	5	4	0	21
Weight loss	5	1	2	8	1	3	20
Anemia	0	4	3	0	1	1	09
Diarrhea	1	3	1	3	1	-	09

Chi-square value: 32.54, p>0.05 and Not significant

Table 7: Relationship of endoscopic findings with M-O grades

Endoscopic finding	Marsh grade 0	Marsh grade 1	Marsh grade 2	Marsh grade 3a	Marsh grade 3b	Marsh grade 3c	Total
Duodenal ulcer/erosion	9	8	3	10	1	2	33
Nodularity	3	3	0	2	2	0	10
Atrophy	1	1	2	1	0	2	7
Normal	5	9	8	7	6	1	36
Not available	6	0	4	2	2	1	15
Total	24	21	17	22	11	6	101

Chi-square value: 25.69, p>0.05 and Not significant

Table 8: Correlation of M-O grade with histopathological findings

HPE finding	Grade 0 (N = 24)	Grade 1 (N = 21)	Grade 2 (N = 17)	Grade 3a (N = 22)	Grade 3b (N = 11)	Grade 3c (N = 6)	Total (N = 101)
Crypt hyperplasia	4	3	9	19	9	4	48
Villous atrophy							
Absent	23	21	17	1	0	0	62
Mild	1	0	0	18	0	0	19
Moderate	0	0	0	3	11	0	14
Complete	0	0	0	0	0	6	6

Chi-square value: 111.25, p<0.001 and Highly significant

Table 9: Study of synaptophysin in Neuroendocrine and correlation with M-O grade

NE cell population	Grade 0	Grade 1	Grade 2	Grade 3a	Grade 3b	Grade 3c	Total
Increased	2	3	3	9 (1 NET)	5	2	24
Not increased	21	16	14	13	6	4	74
Decreased	1	2	0	0	0	0	3

Chi-square value: 18.36, p>0.05 and Not significant

Table 10: Correlation of M-O grade with serology

Serology Finding	0	1	2	3a	3b	3c	Total
Negative	5	2	3	2	0	0	12
Positive	0	0	1	0	0	0	2

Fisher's Exact test: 0.276, p>0.05 and Not significant

atrophy in grade 3a. 11/14 cases showed moderate atrophy in Grade 3b and all 6 cases of 3c showed complete atrophy (Table 8).

23.7% (24/101) cases showed increase in NEC population distributed predominantly amongst grade 3a to 3c (16/24 cases). 8 of 24 cases showed an increase in lower grades of 0-2. Mild increase in the population was noted in grade 0 and grade 1 (Table 9).

Estimation of serological test for TTG (IgA) was possible in only 14 patients. TTG positivity was seen in 16.6% (2/14) only. The two positive cases were in grade 2 and grade 3c each. The case graded 3c was a suspected case of coeliac disease and grade 2, a case clinically suffered from gastritis (Table 10).

DISCUSSION

In our study, the age and sex distribution of patients undergoing duodenal biopsies for malabsorption syndrome was analyzed. The majority of patients fell within the 18-50 age category, accounting for 65 cases (33 males and 32 females). This is consistent with findings from previous studies, which also report a higher incidence of malabsorption disorders in adults compared to pediatric and elderly populations. In a study conducted by Ramakrishna *et al*.[5] the peak age for the diagnosis of celiac disease, a common cause of malabsorption, was reported to be between 30 and 50 years.

Our data also indicates a male predominance in malabsorption syndrome across all age categories, with a total of 60 males compared to 41 females. Similar gender distribution trends have been observed in another research. Tarar *et al*.[6[noted a slightly higher prevalence of celiac disease among males in certain geographic regions, although the overall global prevalence shows a slight female preponderance. This discrepancy might be attributed to differences in healthcare-seeking behavior and diagnostic practices between genders.

In our study, the distribution of cases as per the biopsy site was analyzed to determine the prevalence of malabsorption syndromes based on duodenal biopsy location. The data revealed that the majority of biopsies were taken from the second part of the duodenum (D2), accounting for 52.47% of cases. This finding aligns with the established clinical practice of targeting the D2 region for biopsy in suspected malabsorption disorders, as it provides a representative sample of the mucosal changes associated with conditions such as celiac disease. Research by Maglio *et al*.[7] supports this approach, emphasizing the diagnostic accuracy of D2 biopsies in identifying villous atrophy and other characteristic histopathological changes.

Biopsies from the first part of the duodenum (D1) were less common in our study, comprising only 9.9% of cases. This lower frequency is consistent with the notion that D1 biopsies are often less diagnostic due to the potential for sampling errors and less pronounced mucosal changes in this region. A study by Elli *et al*.[8] demonstrated that while D1 biopsies can be useful, they should not be solely relied upon and multiple biopsies including from the D2 region are recommended for a thorough evaluation.

The provisional diagnosis of cases undergoing duodenal biopsies in our study reflects a diverse range of clinical indications, highlighting the multifaceted nature of malabsorption syndrome and related gastrointestinal disorders. Among the 101 cases, the most frequent provisional diagnosis was anemia under evaluation, accounting for 17 cases. This finding is consistent with the established association between malabsorption syndromes and various forms of anemia, particularly iron deficiency anemia, which often prompts further investigation via duodenal biopsy. A study by Hershko *et al*.[9] emphasizes the importance of considering malabsorption in the differential diagnosis of unexplained anemia, especially when initial workups are inconclusive.

The histological grading of duodenal biopsies in our study, as assessed by both Hematoxylin and Eosin (H&E) staining and Immunohistochemistry (IHC), provides a comprehensive view of the mucosal changes associated with malabsorption syndromes. Our findings, categorized according to the Marsh-Oberhuber classification, highlight significant discrepancies between H&E and IHC results, which underscore the enhanced diagnostic sensitivity of IHC in detecting subtle histopathological changes.

The majority of cases (86 out of 101) showed intraepithelial lymphocyte (IEL) counts of less than 30 when assessed with H&E staining, with only 24 cases showing similar counts with IHC. This discrepancy suggests that IHC, which utilizes specific antibodies to highlight IELs, may offer a more accurate assessment of lymphocyte infiltration. This is supported by studies like that of Volta *et al*.[10] which emphasize the improved sensitivity of IHC in identifying early mucosal changes in celiac disease, often missed by H&E staining alone.

The distribution of cases across the various Marsh-Oberhuber classes further illustrates the utility of IHC. Notably, Marsh 3 lesions (subdivided into 3a, 3b and 3c) were more frequently identified using IHC. Marsh 3b and 3c lesions were detected in 11 and 6 cases, respectively using IHC, compared to only 2 and 2 cases, respectively with H&E staining. This improved detection rate is crucial for accurate staging of celiac disease, as highlighted by a study from Tarar *et al*.[6[which recommends the use of IHC to complement traditional histological methods for a more accurate classification of villous atrophy and crypt hyperplasia. Overall, 64 cases experienced a grade change after IHC application, highlighting the substantial diagnostic impact of this technique. These findings suggest that incorporating IHC, particularly with CD3 staining, into routine diagnostic protocols for malabsorption syndromes can significantly enhance the accuracy of histopathological grading. This enhanced diagnostic precision can lead to more appropriate clinical management and better patient outcomes, as supported by the work of Tarar ZI et al.,[6] who advocated for the combined use of traditional and immunohistochemical methods in the diagnosis of gastrointestinal disorders.

The correlation between NE cell population changes and histological grades supports the hypothesis that neuroendocrine dysregulation may be more pronounced in severe malabsorption conditions. The increased NE cell population in higher Marsh grades indicates a potential compensatory response to extensive mucosal damage and inflammation, as suggested by various studies including those by Gnodi *et al*.[11] which highlighted the role of neuroendocrine cells in gut immunity and mucosal repair mechanisms.

The overall correlation between histological grades and serological findings in our study highlights the complementary roles of these diagnostic modalities. While serological tests are crucial for initial screening and identifying potential cases of celiac disease, they may miss cases with lower antibody levels or those in the early stages of mucosal damage. This is supported by findings from Al-Hussaini *et al*.[12] who suggested that a combination of serological testing and duodenal biopsy provides a more comprehensive diagnostic approach, ensuring that cases with mild or patchy mucosal damage are not overlooked.

The data underscores the need for a combined diagnostic strategy involving both histology and serology to accurately diagnose and manage malabsorption syndromes. In clinical practice, reliance solely on serological tests could lead to underdiagnosis or delayed diagnosis, particularly in patients with negative serology but significant histopathological changes. This reinforces recommendations from studies such as those by Chaudrey *et al*.[13] which advocate for routine biopsies in patients with clinical suspicion of celiac disease, irrespective of serological results.

Future research should continue to explore the complex interplay between clinical symptoms, histopathological changes and serological markers in malabsorption syndromes. Longitudinal studies could provide further insights into the progression of these conditions and the impact of early intervention on patient outcomes. Additionally, the development of more sensitive and specific diagnostic tools, including advanced imaging techniques and novel biomarkers, could further enhance the accuracy and efficiency of diagnosing malabsorption syndromes.

Limitations of study were small sample size, limitations of endoscopy in detecting all cases of mucosal abnormalities, lack of follow-up data and study was conducted within a specific institutional

context, which might influence the diagnostic and management practices. Differences in protocols and healthcare infrastructure across institutions could affect the applicability of the study findings in different settings.

CONCLUSION

By employing a detailed evaluation using the Marsh-Oberhuber classification, alongside advanced immunohistochemistry (IHC) techniques, we have been able to delineate the various histological grades associated with malabsorption syndromes, particularly celiac disease. The integration of serological testing with histopathological analysis further augmented the diagnostic accuracy, offering a more nuanced understanding of the disease's progression and its impact on patient outcomes.

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