International Journal of Science and Research (IJSR)
ISSN: 2319-7064
Impact Factor (2024): 7.101

Diagnostic Utility of CD3 Immunohistochemical Marker with Association to Modified Marsh-Oberhuber Classification for Malabsorption Syndrome

Dr. Vrutika Shah[1], Dr. Ujwala Maheshwari[2], Dr. Parul Jain[3], Dr. Rishabh Jain[4]

[1]Senior Resident, MGM Medical College & Hospital, Kamothe, Navi Mumbai,
Email: *drvrutikashah[at]gmail.com*

[2]Professor, MGM Medical College & Hospital, Kamothe, Navi Mumbai,
Corresponding Author Email: *maheshwarium[at]yahoo.com*

[3]Assistant Professor, MGM Medical College & Hospital, Kamothe, Navi Mumbai
Email: *paruljain309[at]gmail.com*

[4]Gastroenterologist, Pinnacle Super Specialty Clinic, Vashi, Navi Mumbai,
Email: *paruljain309[at]gmail.com*

Abstract: ***Introduction: Duodenal intraepithelial lymphocytes (IELs) are T cells found in the duodenum that play a role in the immune response. Some identifiable causes of raised IELs include coeliac disease (CD), non-steroidal anti-inflammatory drugs (NSAIDs), H. pylori, autoimmune disorders, immunodeficiency, gastrointestinal infections, and inflammatory bowel disease (IBD). Immunohistochemical (IHC) marker like CD3 is very useful for highlighting the IELs during evaluation of biopsies. Aim and Objectives: To compare IEL counts in Hematoxylin and eosin (H & E) stain and CD3 immunohistochemical (IHC) stain in duodenal biopsies of suspected malabsorption cases and compare them with clinical, immunological and biochemical parameters. The study also aims to associate it with Modified Marsh- Oberhuber Classification for Malabsorption syndrome (Celiac disease). Materials & Method: We used initial histopathological reporting for duodenal biopsies followed by immunohistochemical staining using CD3. Application of Modified Marsh- Oberhuber classification used to associate the pathological findings for celiac disease. Statistical analysis was done using Chi-square test. Observations and results: 74 duodenal biopsies were assessed, of which 26 cases showed raised IELs. Male preponderance with a median age of 33 years was noted. Most common presenting symptom was anaemia followed by chronic diarrhoea. Serological correlation was done in cases of raised IELs. Conclusion: Malabsorption syndrome is a histopathological entity that affects small bowel with microscopic and submicroscopic changes and may be associated with significant symptoms. Finding of a raised IEL count with normal villous architecture is of sufficient clinical importance so it should be highlighted in routine histopathological report of duodenal biopsies for further evaluation. Immunohistochemical demonstration of CD3 is one method of confirmation of raised IELs. Duodenal biopsy reporting requires an upgraded approach in view of the new research data of diagnosis.***

Keywords: Duodenal Biopsy, Celiac Disease, Intraepithelial lymphocytes, Malabsorption, CD3

1. Introduction

Microscopic enteritis (ME) is a multifactorial inflammatory process including a broad group of diseases. It is characterized by microscopic and submicroscopic changes in intestinal mucosa associated with malabsorption syndromes.[1, 2, 3] Raised intraepithelial lymphocytes (IELs) is considered to be one of the sensitive histopathological indicator of ME according to Bucharest consensus 2015.[1, 2, 3, 4, 5] Duodenal intraepithelial lymphocytes (IELs) are T cells found in the duodenum that play a role in the immune response. Some identifiable causes of raised IELs include coeliac disease (CD), non-steroidal anti-inflammatory drugs (NSAIDs), H. pylori, autoimmune disorders, immunodeficiency, gastrointestinal infections, and inflammatory bowel disease (IBD).[6] Finding a raised IEL count with normal villous architecture is of sufficient clinical importance so it should be highlighted in routine histopathological report of duodenal biopsies for further evaluation. Duodenal biopsies revealing mucosal damage in cases of malabsorption syndrome are classified according to Modified Marsh Oberhuber Classification.[7] Use of IHC is one of the most specific and sensitive method for demonstration of raised IEL. In evaluation of biopsies, immunohistochemical (IHC) marker like CD3 is very useful for highlighting the IELs.

Aim: The aim of the study is to compare IEL counts in Hematoxylin and Eosin (H & E) stain and CD3 immunohistochemical (IHC) stain in duodenal biopsies showing raised IELs and compare them with clinical, immunological and biochemical parameters. It also associates the findings with Modified Marsh- Oberhuber Classification for Malabsorption syndrome.

2. Material and Methods

2.1 Study details

A prospective study conducted in Department of Pathology, Mahatma Gandhi Mission's

Medical College and Hospital, Navi Mumbai over a period of one year between 2023 and 2024.

2.2 Study design

We received 74 duodenal biopsies in a year with an inclusion criteria of clinical presentation of nutritional deficiencies, chronic diarrhoea, dyspepsia, fever, vomiting and loss of appetite. Duodenal biopsies from polyps, neoplastic conditions, superficial inadequate biopsies, and unoriented tissues were excluded.

2.3 Method

Duodenal biopsies were processed using routine histopathological process. Multiple filter paper mounted duodenal specimens fixed in 10 % buffered formalin were processed and embedded in paraffin blocks. Uniform 4-5µm thick sections were cut and stained with haematoxylin and eosin stain. Immunohistochemical staining for CD3 was done for all the cases. The antibodies and chemicals were procured from Dako, Denmark (Rabbit Anti- CD3 Monoclonal Antibody- Clone EP41).

Other supportive serological tests were correlated whenever possible. IELs were counted from five consecutive villous tips and bases of villi (total 100 enterocytes with enterocytes at each villous tip or base). Tip: 20/100 enterocytes. Base: 18/100 enterocytes.[8]

Duodenal biopsy reporting format included:

1) Number and Site of the biopsy specimens.
   - Are the specimen Normal/ Abnormal?
   - What is the villous height and architecture: Normal, broad or blunted?
2) Normal Villous to crypt (V:C) ratio: Ranges from 3:1 to 5:1
3) Presence of crypt hyperplasia
4) Surface enterocytes: Normal, flattened or damaged.
5) Brush borders: preserved or lost.
6) IEL count in form of singly scattered, nests (5 clustered lymphocytes) and plaques (5 adjacent enterocytes overrun by lymphocytes)
7) Gastric metaplasia in chronic duodenitis.
8) Presence of micro-organisms: Giardia, cryptosporidia, microsporidia, Mycobacterium, etc (Appropriate special stains to be done).
9) Other features of reactive atypia, lymphoid aggregation, muscle disarray etc was noted.

Modified Marsh Oberhuber Classification was correlated. Duodenal biopsies with CD3 stains were categorised as Infiltrative, Hyperplastic and Atrophic stages recognized in Classification. (Figure 1. a & b)

Serological studies with tissue transglutaminase, anti- gliadin antibodies and other biochemical markers were compared wherever possible.

	Type 0	Type 1	Type 2	Type 3a	Type 3b	Type 3c
No of IEL	<40	>40	>40	>40	>40	>40
Crypt	Normal	Normal	hypertrophy	hypertrophy	hypertrophy	hypertrophy
Villous	Normal	Normal	Normal	mild atrophy	Obvious atrophy	Absent

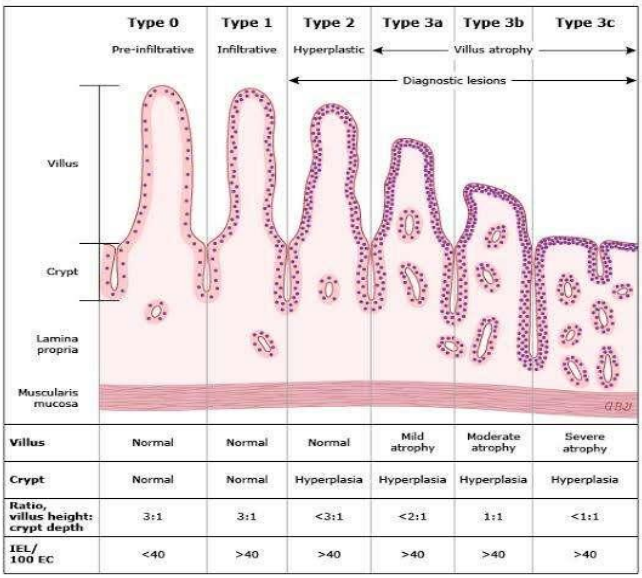

Figure 1: (a & b) Modified Marsh Oberhuber Classification. Courtesy: https://www.uptodate.com/contents/diagnosis-of-celiac-disease-in-children/print

2.4 Statistical analysis

All the categorical variables were expressed as frequency and percentage whereas the quantitative variables with symmetrical distribution were expressed as mean ± SD. To compare any two categorical variables, either Chi - square test or Fisher's exact test was used. Statistical significance was defined as $p < 0.05$. All the analyses were carried out using standard formulas in Microsoft Excel.

3. Observations and results

A Total 74 cases of duodenal biopsies were studied. Out of which 26 cases had increased IEL histologically. Out of 16 cases, only 2 cases were diagnosed as Celiac disease (CD) histologically and serologically. Rest were non celiac disease (NCD) of different aetiologies. Males (54,72.9%) are affected more than females (20, 27.02%) with a ratio of 2.7:1.

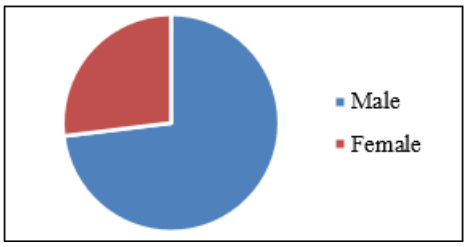

Figure 2: Gender distribution in cases of raised IELs.

The maximum cases affected in our study group was in the age range of 4-54 years (mean 33years). The mean age in CD cases in our study was 32.5 years (Figure 3).

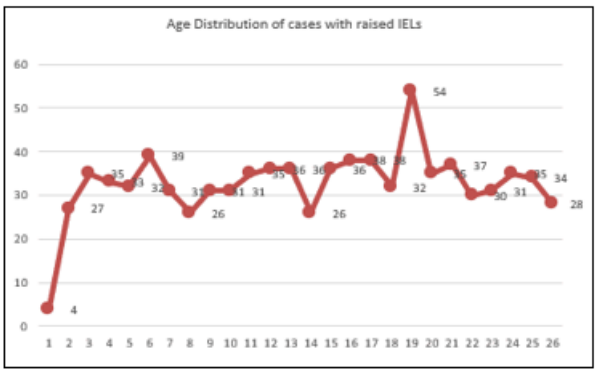

Figure 3: Age Distribution in cases of raised IELs.

There was no significant correlation between any of the symptoms in patients of IELs and non-IELs disease. Pallor was present in 10 (38.46%) cases of raised IELs (Figure 4).

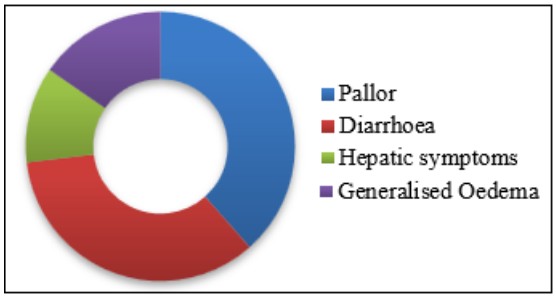

Figure 4: Clinical Presentation of patients showing raised IELs

Serology (raised anti-Ttg,IgA,IgG) was raised in 2 (100%) cases of raised IELs. Majority of the raised IEL cases had normal appearing mucosa in endoscopy. IEL at villous tip in H & E and CD3 was raised in both the Celiac Disease cases. (Figure 5).

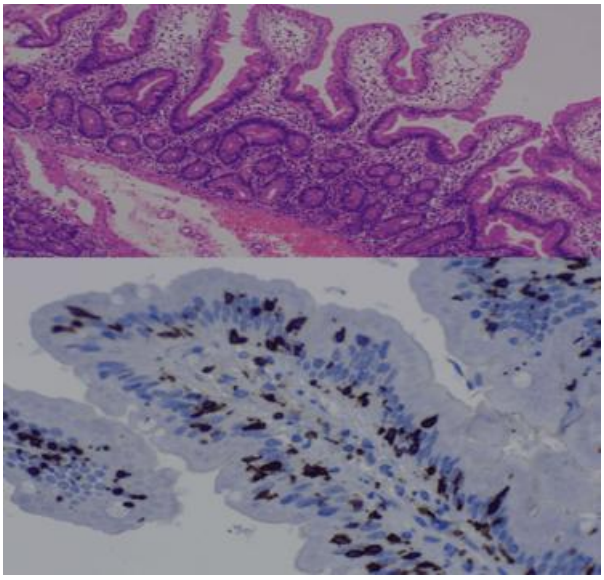

Figure 5 (a): 10x magnification of H & E slide shows raised IELs and mild crypt hyperplasia. Confirmed with IHC CD3 stain. Case was categorized as Hyperplastic category according to Modified Marsh Oberhuber Classitfication.

At base of villi in H & E it was raised in 5 cases and in CD3 it was raised in 7 cases thus increasing the diagnostic accuracy of Celiac disease.[11] The sensitivity and specificity of detecting IEL by CD3 IHC is much more than H&E stain. The specificity of IEL at tip in Celiac Disease is 97.3% and 100% in both H&E and CD3 IHC respectively. According to Modifed Marsh Oberhuber Classification, of the 26 cases of raised IELs, 16 cases were histologically noted to be in Infiltrative stage, 9 cases belonged to Hyperplastic stage and 1 case categorized as Atrophic stage.

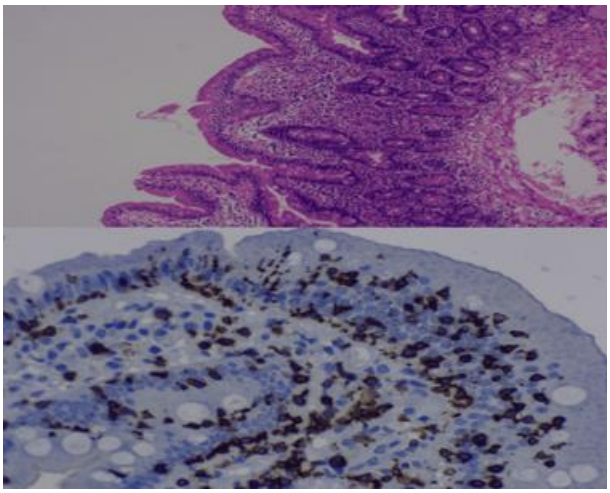

Figure 5 (a) 10x magnification of H & E slide shows raised IELs, crypt hyperplasia and villous atrophy. Confirmed with IHC CD3 stain. Case was categorized as Atrophic category according to Modified Marsh Oberhuber Classification.

4. Discussion

Increase in villous intraepithelial lymphocytes in duodenum when present, whether carries significance or not is a matter of debate since last two decades. According to a few authors it is nonspecific contradictory to few other studies.[9] However, numerous studies concur on the diagnostic significance of CD3 marker in diagnosis of malabsorption syndrome,

specifically Celiac Disease. A careful algorithmic approach to reporting duodenal biopsies can help in screening various etiologies of raised IELs. Furthermore, work-up of suspected cases of Malabsorption syndrome can help diagnose Celiac disease.

Comparison of our study with other study is described in Table 1.

Using clinical presentation and specific endoscopic findings also play a role in identifying duodenal pathologies. Hallmark endoscopic finding is patchy villous atrophy with scalloping/ notching of mucosal folds.[11]

Table 1: Comparison of study with other research articles

Findings	*Gupta et al* [3]	*S. Brown*[10]	*Balasubraman et al* [8]	*Mahadev et al* [12]	*Yadav et al* [7]
Raised IELs/ Serologically positive CD	105/164, 11/105	150	16/101	124/626	61/94
Mean age	40-59 Years (44.5)	1-84 years, 14.7% above 60 years	36	21.8	21.8
Gender	Too less to opine	Female	Males	-	Males
Symptom	No significant correlatiion	-	Anaemia	Anaemia	-
Stand- out feature of article	emphasizes that the subtle histopathological changes in duodenal biopsies in correlation with robust clinical, biochemical, serological and endoscopic findings may be significantly useful in identifying many subclinical CD patients.	CD can present can be in late age also	Similar to Gupta et al	They found raised transaminase level in celiac patients. This could be due to mild dyfunction of liver and a histological picture of nonspecific reactive hepatitis (known as celiac hepatitis) in CD cases	The high prevalence of CD may be primarily due to particular diet in Northern India in addition to increased awareness amongst people, better and sensitive screening techniques and recognizing the early changes in histopathology of biopsy specimen.

In our study, a male preponderance was noted in deference to other studies. A mean age of 32.5 years was noted, possibly due to the chronicity and time associated development of the etiology of raised IELs. Duodenal pathologies have a tendency to present with diarrhoea, nutritional anaemia, abdominal pain, bloating etc theorizing to the function of small intestines. Activation of innate immune response and adaptive immune response by intra- epithelial lymphocytes expressing Natural Killer (NK) receptors MHC Class I and HLA on epithelial cells, eventually lead to destruction of the epithelium. Interferon and cytokine activation contributes to the destruction too. This reduces absorption of vitamins and minerals resulting in characteristic clinical presentation.

The initiation of malabsorption syndrome occurs with increased immune response, reflected by increased intra-epithelial T lymphocytes. T lymphocytes normally occur in the duodenal mucosa in the range of less than 25/ 100 enterocytes. This increase specifically occurs at the tip of the villi. Our study also had similar findings on histology which were more specifically confirmed using IHC CD3 stain. Architectural abnormalities according to severity of the etiology were noted in order of crypt hyperplasia followed by villous shortening associating to the classification for celiac disease.

Study by Mokhtar included 60 patients. Their age ranged from 2 to 70 years with a mean of 19.5 years (±15.7 SD). The most common age group was below 10 years old (41.6%). Male and female are equally affected. The most common clinical presentation was chronic diarrhoea (55.0%), followed by iron deficiency anemia (41.7%). The degree of villous atrophy ranged from complete atrophy (45.0%), marked atrophy (38.3%) to mild atrophy (16.6%). Marsh grade IIIC was the most common grade. The younger age-groups had a higher prevalence of iron deficiency anaemia and higher Marsh grade.[11]

One of the limitations in our study was that cases were lost on follow- up at the stage of serological work- up. Thus, the diagnosis of Malabsorption syndrome, particularly Celiac disease relied only on histological factors. Sergio et al states that serological marker (TTG) may give false positive and false negative results in suspected Celiac disease cases.[13] False negative TTG levels may be seen in IgA deficiency disease. False- positive results may occur in the setting of patients suffering from inflammatory bowel disease (IBD), primary biliary cirrhosis, cardiovascular disease, autoimmune enteropathy and other immune-mediated disorders.[13, 15, 16] Clinical correlation is of significance. In the absence of supportive findings, a strong clinical suspicion requires evaluation by detection of HLA, HLA- DQ2 or DQ8 with gluten withdrawal. Neutrophilic crypt abscesses are usually not seen in celiac disease, but are frequently seen in its mimickers, such as patients with infection, peptic duodenitis or autoimmune enteropathy.[13]

A recent study by Camarero et al found use of flow cytometry (IEL lymphogram) as useful test for monitoring the natural progression of the disease and predicting the transition from potential celiac to overt Celiac Disease. [14]

5. Conclusion

Malabsorption syndrome is a histopathological entity that affects small bowel with microscopic and submicroscopic changes and may be associated with significant symptoms. Findings of a raised IEL count with normal villous architecture is of sufficient clinical importance so it should be highlighted in routine histopathological report of duodenal

biopsies for further evaluation. Use of IHC is the most specific and sensitive method for demonstration of raised IEL. Raised IELs should be investigated and correlated with a detailed clinical history, complete hematological, biochemical investigations. Duodenal biopsy reporting requires an upgraded approach in view of the new research data of diagnosis.

References

[1] K Rostami, D Aldulaimi, G Holmes, MW Johnson, M Robert, A Srivastava, Microscopic enteritis: Bucharest consensus, World J Gastroenterol, 201521925932604.
[2] E Ierardi G Losurdo A Iannone D Piscitelli A Amoruso M Barone Lymphocytic duodenitis or microscopic enteritis and gluten-related conditions: what needs to be explored? Ann Gastroenterol, 201730438092
[3] Gupta R, Raman S, Mallick BB, Senapati U. Diagnostic evaluation of microscopic enteritis in duodenal biopsies of suspected malabsorption cases with clinico-immunohistological correlation. Indian J Pathol Oncol 2022;9(1):3-9.
[4] R Patterson E Shmidt AS Oxentenko FT Enders TC Smyrk Normal Villous Architecture With Increased Intraepithelial Lymphocytes. A Duodenal Manifestation of Crohn DiseaseAm J Clin Pathol2015143344550
[5] STG Hammer, JK Greenson, The clinical significance of duodenal lymphocytosis with normal villus architecture, Arch Pathol Lab Med2013137912169
[6] Aziz I, Evans KE, Hopper AD, *et al*, OC-080 A systematic approach identifying the causes of duodenal intraepithelial lymphocytosis, *Gut* 2010;59:A33.
[7] Yadav P, Das P, Mirdha BR, Gupta SD, Bhatnagar S, Pandey RM, et al. Current spectrum of malabsorption syndrome in adults in India. Indian J Gastroenterol. 2011;30(1):22–8. doi:10.1007/s12664-011-0081-0.
[8] Balasubramanian P, Badhe BA, Ganesh RN, Panicker LC, Mohan P. Morphologic Spectrum of Duodenal Biopsies in Malabsorption: A Study from Southern India. J Clin Diagn Res. 2017;11(7):17–21.
[9] Eugenia Shmidt, Thomas C. Smyrk, Christopher L. Boswell, Felicity T. Enders, Amy S. Oxentenko, Increasing duodenal intraepithelial lymphocytosis found at upper endoscopy: time trends and associations, Gastrointestinal Endoscopy, Volume 80, Issue 1, 2014, Pages 105-111.
[10] I S Brown J Smith C Rosty Gastrointestinal Pathology in Celiac DiseaseA Case Series of 150 Consecutive Newly Diagnosed PatientsAm J Clin Pathol20121381429
[11] Mokhtar MA, Mekki SO, Mudawi HM, Sulaiman SH, Tahir MA, Tigani MA, Omer IA, Yousif BM, Fragalla IA, Mohammed Z, Dafaalla M. Histopathological features of coeliac disease in a sample of Sudanese patients. Malays J Pathol. 2016 Dec;38(3):267-272. PMID: 28028297.
[12] S Mahadeva J I Wyatt P D Howdle Is a raised intraepithelial lymphocyte count with normal duodenal villous architecture clinically relevant?J Clin Pathol2002556424810.1136/jcp.55.6.424
[13] Sergi C, Shen F, Bouma G. Intraepithelial lymphocytes, scores, mimickers and challenges in diagnosing gluten-sensitive enteropathy (celiac disease). *World J Gastroenterol* 2017; 23(4): 573-589 [PMID: 28216964 DOI: 10.3748/wjg.v23.i4.573]
[14] Camarero C, De Andrés A, García-Hoz C, Roldán B, Muriel A, León F, Roy G. Assessment of Duodenal Intraepithelial Lymphocyte Composition (Lymphogram) for Accurate and Prompt Diagnosis of Celiac Disease in Pediatric Patients. Clin Transl Gastroenterol. 2021 Nov 10;12(11):e00426. doi: 10.14309/ctg.0000000000000426. PMID: 34757327; PMCID: PMC8585297.
[15] Murch S. Recent Advances in Celiac Disease. Indian J Pediatr. 2016; 83: 1428-1435.
[16] Pai RK. A practical approach to small bowel biopsy interpretation: celiac disease and its mimics. Semin Diagn Pathol. 2014; 31: 124-136.