ORIGINAL ARTICLE

Immunohistochemical analysis of intestinal biopsies in individuals with celiac disease

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Abstract

Background and Aims: The immunohistochemical application of CD3 (T lymphocytes) and CD20 (B lymphocytes) markers in duodenal biopsy can facilitate the detection of the number and distribution of intraepithelial lymphocytes along the villi, which is regarded as a key factor for accurate diagnosis of celiac disease. This study aims at finding a relationship between CD3 and CD20 immunohistochemical and histopathological alterations of celiac disease, and at investigating whether the application of those immunohistochemical stainings would improve the detection of lymphocytosis within the epithelium and add advantages to celiac disease diagnosis.
Methods: Biopsies were obtained from 100 individuals and stained with hematoxylin and eosin (H&E). They were then evaluated according to the Marsh classification. After that, staining for CD3 and CD20 was individually done and assessed.
Results: The overall mean intraepithelial lymphocyte count per 100 enterocytes for H&E was 23.1 (95% confidence interval [CI] = 19.52–26.68), and for immunohistochemistry by CD3 and CD20 was 27.84 (95% CI = 24.31–31.38). The difference was highly significant, $P = 0.001$. The expression of CD3 immunohistochemically was as follows: Less-than-half staining pattern was reported in 16% cases, and half staining pattern was seen in 26%, while most cases 58% had more than half staining pattern. This discovery was consistent with the histological classification of March III among most cases. The expression of CD20 immunohistochemically was as follows: mild crypt involvement was observed in 16% of cases, while moderate crypt involvement and intense crypt involvement were seen in 43% and 41% of cases, respectively.

Introduction

Celiac disease is an autoimmune disorder that targets the mucosal layer of the small intestine in predisposed people who possess the human leukocyte antigen (HLA DQ2 and DQ8) haplotypes following gluten consumption.[1] The chief histopathological consequences encompass shortening of villi accompanied by crypt inflammation, which is demonstrated by an increase in cell proliferation in addition to predominant lymphocytes inflammatory infiltration in the epithelium and lamina propria.[2] The presence of celiac disease antibodies (anti-tissue transglutaminase, anti-deamidated gliadin peptide, and anti-endomysial antibody) and specific human leukocyte antigen haplotypes (HLA DQ2 and DQ8) are very helpful with the clinical evaluation and diagnosis. Atypical symptoms and inconsistencies in serological and histological findings can provide a challenge in diagnosis.[3]

From a histological point of view, the alterations can be assessed according to the "modified Marsh-Oberhuber classification." Lesions can be expressed using a range of architectural, cytological, and ultrastructural characteristics that are grouped to produce a blurry range of histopathological permutations. These characteristics alone are not unique to celiac disease.[4]

Immunohistochemistry (IHC) is the key supplementary procedure for pathologists that allows visualization of the distribution and the number of certain molecules in the tissue relying on specific antigen–antibody reactions. What helps IHC to excel compared with other laboratory methods is its unique feature lying in its ability to be performed without ruining the histologic architecture.[5]

Homogenously stained aggregates of lymphoid tissue with anti-CD3 in T lineage lymphoma and anti-CD20 in B lineage lymphoma are generally regarded as confirmatory findings of malignant lymphoma infiltrates into the bone marrow, lymphocyte infiltration in tumors, and other immune diseases like Hashimoto's thyroiditis, lupus, and rheumatoid arthritis.[6]

The extra advantages of CD3 and CD20 staining in the context of diagnosing celiac disease have not been examined formerly. Hence, for the purpose of this research, we prospectively contrasted the results of H&E sections and CD3 and CD20 sections in individuals surmised to have celiac disease. Additionally, our study aims to find a relationship between CD3 and CD20 immunohistochemical expression of lymphocytes with histopathological alterations related to celiac disease.

Materials and methods

Our study was carried out at Al-Assad university hospital in Syria. The study commenced in January 2021 and concluded in April 2021.

This research was performed following the guidelines of the local medical ethical board at Damascus University, Syria.

One hundred patients with clinical features pointing to celiac disease were included. Every patient has undergone esophagogastroduodenoscopy (OGD) at the gastrointestinal centers.

Biopsies from the duodenum were procured from patients and preserved in 10% formalin solution. Following this, the biopsies underwent the routine protocol of tissue grossing, processing, paraffin-embedding, and sectioning. The slides were stained with hematoxylin and eosin (H&E) and two slides of each case were stained for immunohistochemical markers CD3 and CD20. For immunohistochemistry, blocks were stained with mouse monoclonal antibodies kit (Bio-SB Company, USA) using the Dako Agilent Autostainer Link 48 slide stainer (Agilent Technologies, USA). The slides were examined for the histopathological features of the biopsy and immunohistochemical characteristics of IELs.

The degree of histopathological alterations including chronic inflammation, activity, and atrophy was evaluated based on the modified Marsh-Oberhuber classification and correct biopsy orientation was considered for all samples included in the study.[4]

The results of anti-tissue transglutaminase antibodies (tTGA) besides the clinical information of the patients were obtained from their medical records.

Results

The demographic characteristics of celiac disease patients registered in the current study are outlined in Table 1. The mean age was 19.12 ± 13.71 years and there was almost even distribution of cases according to age intervals. The group comprised 51 (51%) males and 49 (49%) females.

The presenting symptoms of the patients are summarized in Table 2.

Table 1 Demographic characteristics of celiac disease patients included in the study

Characteristic	Result
Number of cases	100
Age (years)	
Mean ± SD	19.12 ± 13.71
Range	5–65
<15, n (%)	52 (52%)
15–25, n (%)	28 (28%)
26–40, n (%)	12 (12%)
41–55, n (%)	2 (2%)
56–65, n (%)	6 (6%)
Gender	
Male, n (%)	51 (51%)
Female, n (%)	49 (49%)

Table 2 Clinical features of patients

Clinical findings	Number of patients (%)
Anemia	35 (35%)
Bloating	16 (16%)
Abdominal pain	12 (12%)
Diarrhea	22 (22%)
Weight loss	16 (16%)
Short stature	6 (6%)
Anorexia	11 (11%)
Constipation	10 (10%)
Dyspepsia	4 (4%)
Reflux	9 (9%)
History of gastritis	10 (10%)
Vitamin B12 deficiency	3 (3%)

Results according to the levels of anti-tissue transglutaminase, villous atrophy severity, and number of patients are shown in Table 3.

The overall mean IEL count per 100 enterocytes for H&E was 23.1 (95% confidence interval [CI] = 19.52–26.68), and 27.84 for IHC by CD3 and CD20 (95% CI = 24.31–31.38). The difference was highly significant ($P = 0.001$).

CD3 immunohistochemical expression is shown in Table 4 and CD3 staining in Figure 1. Less-than-half staining pattern was observed in 16 (16%) cases, half staining pattern was approximately reported in 26 (26%) cases, while the majority of cases (58%) exhibited more-than-half staining pattern. The last finding coincided with the histological grading of Marsh III classification in most cases.

CD20 immunohistochemical expression is shown in Table 5 and CD20 staining is shown in Figure 2.

Regarding the crypts, mild involvement was observed in 16% of sections, while moderate and severe involvement was observed in 43% and 41% of sections, respectively.

The variance between routine H&E sections and CD3 & CD20 staining was revealed in eight (8%) cases. In six (6%) patients, celiac disease diagnosis (Marsh II and III) was forsaken after analyzing CD3 and CD20 staining (diagnosis of three patients changed from Marsh I to II, two patients changed from II to III A, and one patient changed from III A to III B).

As for the other two (2%) patients, in whom isolated IELs (Marsh I) was detected scoring a degree of Marsh I on CD3 and CD20 sections, this finding was missed with routine sections (the diagnosis changed from Marsh 0 to Marsh I).

For the patients, the concluding diagnosis depending on CD3 and CD20 staining was in agreement with serological results except for one patient who had negative serology, and who was suspected to have grade Marsh I on H&E sections. His diagnosis was taken back after further studying the CD3 and CD20 sections.

Discussion

Even after the latest renewal of the ESPGHAN guidelines for the diagnosis of celiac disease, stating that the diagnosis can be done without a biopsy in individuals showing symptoms and having high tTGA levels, positive anti-endomysial antibodies (EMA)

Table 3 Results of anti-tissue transglutaminase according to Marsh grading

Marsh degree	Anti-tissue transglutaminase (mean ± SD)
Grade 1	71 ± 35
Grade 2	98 ± 40
Grade 3A	158 ± 47
Grade 3B	224 ± 82
Grade 3C	498 ± 221

Table 4 CD3 immunohistochemical expression

| CD3 expression	Frequency (%)
<Half	16 (16%)
Approximately half	26 (26%)
More than half	58 (58%)
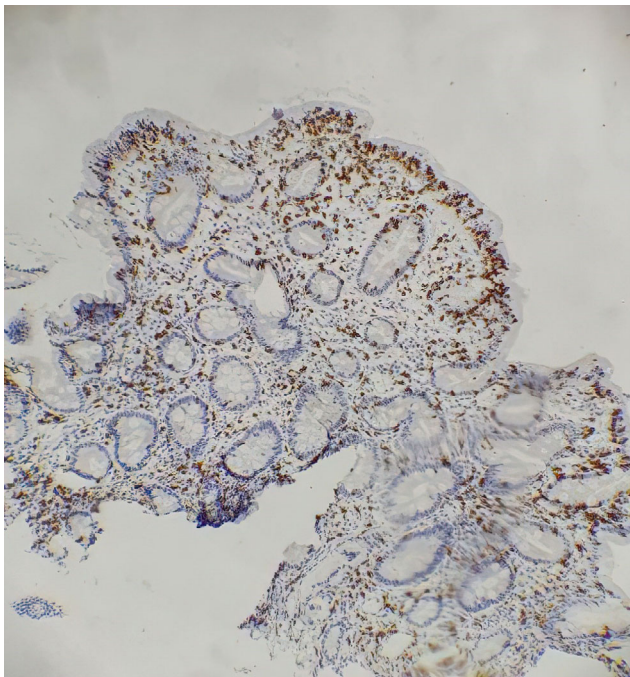
Figure 1 Photomicrograph showing CD3 immunohistochemical staining (×400) highlights markedly increased intraepithelial lymphocytes. Total effacement of villi crypt hyperplasia, consistent with celiac disease Marsh 3C.

and disease-specific human leukocyte antigens duodenal biopsies are indeed still imperative for making the final diagnosis in most patients. In this regard, the evaluation of lymphocytosis within the epithelium remains necessary and important.[7]

In our study, regular histological staining methods facilitated the histological diagnosis of celiac disease, apart from certain cases in the early stages of the disease in which histological alterations and degree of inflammation were difficult to detect and clarify. However, discerning such mild early stage cases were allowed using CD3 and CD20 immunohistochemistry.

Table 5 CD20 immunohistochemical expression

CD 20 expression	Number (%)
Mild crypt involvement	16 (16%)
Moderate crypt involvement	43 (43%)
Intense crypt involvement	41 (41%)
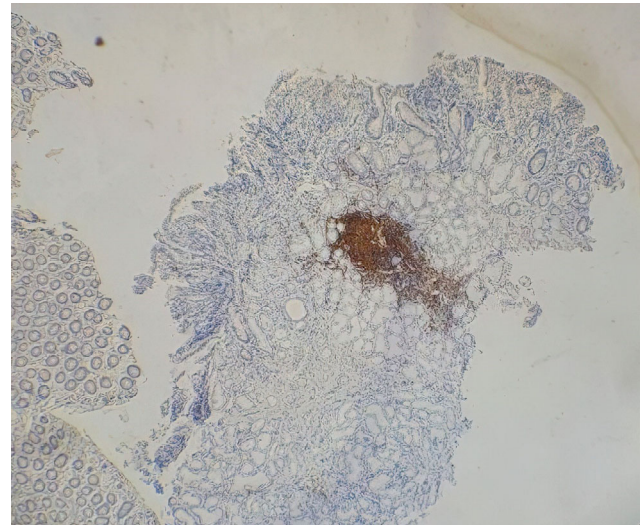
Figure 2 Photomicrograph showing CD 20 positive lymphocyte aggregate in lamina propria. Total effacement of villi crypt hyperplasia, CD20 is negative in the epithelial lining of villi, consistent with celiac disease Marsh 3C.

Immunohistochemistry can provide a highly sensitive technique for the characterization of Marsh I cases of celiac disease. The occurrence of intraepithelial lymphocytosis by itself is not specific to celiac disease and can be seen in other forms of intestinal inflammation such as Giardiasis.[7] Therefore, the existence of IELs should be seriously considered only when there are highly suggestive clinical features and positive serological results for celiac disease. In our study, cases with mild early stages had positive serological findings and highly suggestive clinical features. Thus, it was easy to combine histological, immunohistochemical, and serological investigation and clinical features to clearly diagnose celiac disease.[8]

Increased counts of IELs are encountered in many pathological conditions like drug reactions, infections, and a variety of autoimmune disorders, but the considered methods used to assess IELs vary.[9] Some authors recommend taking counts along the entire length of the villous per 100 enterocytes, and according to current guidelines, counts more than 25 per 100 enterocytes are considered abnormal.[10,11] Other studies counted IELs only in villous tips.[12,13] Moreover, some authors favor the application of immunohistochemical stains for T and B lymphocytes even among histologically normal duodenal biopsies despite the lack of supporting evidence.[14,15]

In our study, the overall mean IEL count per 100 enterocytes for H&E was 23.1 (95% CI = 19.52–26.68), and for IHC by CD3

and CD20 27.84 (95% CI = 24.31–31.38). The difference was highly significant ($P = 0.001$) and the counts were lower than the counts in the study conducted by Cooper *et al.* in the United Kingdom,[16] which were 35.2 (95% CI = 30.0–40.8) by H&E and 49.7 (95% CI = 44.1–55.3) by CD3 IHC.

The present study showed a highly significant difference between the overall counts of IELs using H&E and CD3 IHC, with higher counts detected by CD3 and CD20 IHC, which might favor the combination of both techniques for better detection of IEL counts. This goes in agreement with what Balasubramanian *et al.*[17] and Nasseri-Moghaddam *et al.*[18] had suggested on how the utilization of CD3 and CD20 IHC would aid in the detection of IELs and make it much easier as some IELs have irregular nuclear outlines that might mimic polymorphonuclear cells, and others may resemble epithelial cells.

Moreover, a gluten challenge can cause mucosal changes that permit celiac disease diagnosis in numerous patients with Marsh I celiac disease.[19] Indeed, several research studies have demonstrated that affected individuals scoring Marsh I can profit, at least in the short term, from a gluten-free diet.[20] Immunohistochemical studies of CD3, CD8, CD4, and CD56 lymphocytes have been studied in celiac disease patients in comparison with normal healthy mucosa, and the results indicated that CD3 was the best diagnostic marker of celiac disease cases.[21,22]

Through this study, we have demonstrated how CD20 B lymphocytes are primarily linked with cryptic location and lymphoid follicle formation, a finding previously presented by other authors.[23] Indeed, the use of CD20 immunohistochemistry in our current study has shown a significant role for B lymphocytes in the pathogenesis of this autoimmune disease.

Finally, immunohistochemical staining for CD3 and CD20 plays an ancillary part in the perception of celiac disease histological consequences. To take a comprehensive note of the whole range of lesions connected with celiac disease, CD3 and CD20 staining is befitting to be done in all cases where dissimilarities exist among serological and histological examinations on routine sections.

Acknowledgments

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