Quantification of Duodenal Intra-Epithelial Lymphocytes

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Abstract
Objective: To investigate the number of intra-epithelial lymphocytes in the duodenal mucosal biopsies of patients with non specific duodenitis, clinically labelled cases of celiac disease and in normal duodenal mucosa.

Material and Methods: The study included three groups. A total of 73 duodenal biopsies received in the department of histopathology, PIMS (Pakistan Institute of Medical Sciences) Islamabad, between 2008 and 2011 were placed in two groups; Group I (38 cases): Biopsy of patients with non specific duodenal symptoms, not fulfilling the clinical criteria of celiac disease and Group II (35 cases): Patients with clinical suspicion of celiac disease. Group III (20 cases) included duodenal tissue from autopsies carried out in cases of sudden death from non-GIT related causes in the department of histopathology, Armed Forces Institute of Pathology, Rawalpindi. All paraffin embedded sections were stained with Hematoxylin and Eosin and intra-epithelial lymphocytes were counted per 20 enterocytes in 5 villous tips or per 50 enterocytes in 2 villous tips (in partial villous atrophy) or per 100 enterocytes in total villous atrophy.

Results: Formal counting of the index cases and controls revealed a significant difference in IELs/100 epithelial cell count between the 3 groups. The mean villous tip IEL scores were 29.9±10.7 SD (range: 10-60) in chronic non specific duodenitis, 58.19 ± 24.7SD (range:16-130) in celiac disease and 15.7±2.79 SD (range:12-22) in autopsy cases.

Conclusion: The results of present study suggest that 40 IELs/ 100 epithelial cells is a significant finding and should be taken as a cut-off point for celiac disease in our population.

Key words: Celiac disease, Intra-epithelial lymphocytes, Non-specific duodenitis.

Introduction

Celiac disease is a chronic immune-mediated disorder induced by dietary exposure to gluten, which damages the intestinal surface and interferes with the absorption of important nutrients. This results in a lifelong adherence to gluten free diet like cereals, wheat, barley etc. It also has an association with long-term complications like enteropathy associated T-cell lymphoma. Therefore, a correct early diagnosis is a mandatory requirement in these patients. Due to the emergence of more sensitive and specific diagnostic modalities, the early diagnosis of celiac disease is on the rise. The studies carried out in Europe and North America have reported a prevalence of 0.3% to 1.2%. However significant data on prevalence is lacking in our country and although the studies carried out in our neighbourhood (India) have reported an increase in the cases of celiac disease but their true prevalence is also not known.

The diagnosis of celiac disease requires a multidisciplinary approach including, the initial serological tests with IgA tissue transglutaminase and IgA endomysial antibodies, followed by endoscopic assessment and histopathological confirmation. A raised intraepithelial lymphocyte count along with villous atrophy and crypt hyperplasia constitute the classic histopathological triad for the diagnosis of celiac disease. Amongst these, an increase in intraepithelial lymphocytes (IELs) is considered the most sensitive index of the adverse effects of gluten on the mucosa of the gastrointestinal tract, and a diagnosis of celiac disease can be favoured in the presence of intraepithelial lymphocytosis alone (Marsh stage 1). However, due to the association of intraepithelial lymphocytosis to non-gluten sensitive enteropathies such as cow’s milk protein intolerance, giardiasis, post infective malabsorption, IgA deficiency, tropical sprue, hypogammaglobulinemia, and unexplained diarrhea with failure to thrive, the sensitivity of intraepithelial lymphocytosis in celiac disease is being questioned. The cut off value for IEL is also a source of disagreement between different pathologists and different classifications are using different cut off value of IEL. The commonly used Marsh classification modified by
Oberhuber, uses an IEL count of 30 as cut-off, while a simplified classification by Corazza employs a cut-off value of 25.9,10

Taking into account that intraepithelial lymphocytosis also occurs in gluten sensitive enteropathies, we aim to investigate the intraepithelial lymphocyte count in gluten sensitive enteropathy and non-gluten sensitive enteropathy and their comparison with the controls.

**Material and Methods**

Seventy three consecutive duodenal biopsies received in the department of histopathology, PIMS from 2008 to 2011 were retrospectively reviewed and placed in two groups;

- Group I comprised of duodenal biopsy specimens of 38 patients with non specific duodenal symptoms, not fulfilling the clinical criteria of celiac disease (due to negative serology or no association with gluten diet)
- Group II included duodenal biopsy specimens of 34 patients with clinical suspicion of celiac disease based on positive serology or improvement in symptoms after gluten withdrawal.
- Controls included duodenal tissue from 20 autopsies carried out from 2009 to 2011, in cases of sudden death from non-GIT related causes in the department of histopathology, Armed Forces Institute of Pathology, Rawalpindi.

Exclusion criteria:

Since it was a retrospective study, those cases in which the serology or clinical details were not available were excluded from the study or if the biopsy was scanty for the evaluation of IELs.

All paraffin embedded sections were stained with Hematoxylin and Eosin and intra-epithelial lymphocytes were formally counted per 20 enterocytes in 5 villous tips or per 50 enterocytes in 2 villous tips (in partial villous atrophy) or per 100 enterocytes in total villous atrophy. The formal counting was done by the trainee resident and confirmed by the consultant histopathologist.

A statistical analysis was done on SPSS version 16.0. Categorical variables were compared using the $\chi^2$ test. A P value of less than .05 was used to determine statistical significance.

**Results**

The mean age of patients of celiac disease was 40.9 years ±19.6SD (Range: 15–92), while patients of chronic non-specific duodenitis had a mean age of 26.40 years ±11.9SD (Range: 6-50). The control group with a normal duodenal biopsy had a mean age of 60.1 years ±14.8 SD (Range: 28–88) as shown in table 1.

The mean IELs/100 epithelial cells were 29.9±10.7SD in chronic non-specific duodenitis (Range: 10-60), while in celiac disease it was 58.19 ±24.7SD (Range: 16-130). Compared to these values, IEL count was significantly low in controls with a mean of 15.7 ±2.79 SD (Range: 12-22) as shown in table 2.

**Discussion**

We conducted this study to quantitate the IEL count in gluten sensitive enteropathy and chronic non-specific duodenitis and their comparison with the normal in a small portion of our population. We have found that celiac disease shows a higher mean IEL count as compared to the mean IEL count observed in chronic duodenitis and normal duodenal biopsies.

<table>
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<th>Table 1: Age distribution of the patients (n=73)</th>
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<tr>
<td>Celiac Disease (n= 35)</td>
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<td>Mean age</td>
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<td>Minimum age</td>
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In 35 cases having a strong suspicion of celiac disease, 82.5% (29 cases) showed an IEL count above 40. There were 6 cases (17.2%) which showed a low IEL count below 40, most of which showed total villous atrophy.

Twenty nine (76%) out of 38 cases having a history of malabsorption, but with anti transglutaminase antibodies in normal range, showed an IEL count between 25 and 40. There were 4 cases (10.5%) having a count below 25, and 5 cases (13.1%) having a count above 40. The villous atrophy was variable in these cases. The upper limit in normal duodenal biopsy was 22, which is similar to reported in other studies.11,12
Amongst the classic triad of histologic features of celiac disease, intraepithelial lymphocytosis is the most important parameter of diagnostic value. However, recent studies have reported its low specificity due to its association with other non-celiac disease related conditions as well.13, 14

Hasan et al reported increased intraepithelial lymphocytes in association with both ulcer-associated and nonspecific duodenitis.15

Memeo et al found raised intraepithelial lymphocyte counts in patients with H. pylori-associated lymphocytic gastritis.16

Corazza and Villanacci suggest that IEL count above 25 per 100 enterocyte should be taken as abnormal for celiac disease, however in the Marsh classification and Oberhuber modification, the cut-off value for diagnosing celiac disease is 40 IEL/100 enterocytes in the duodenum. Our study also indicates that marginally raised IEL are commonly seen in chronic non specific duodenitis cases. Lowering the cut off value of IEL will significantly over diagnose the celiac disease cases.17,18

The pattern of intraepithelial lymphocyte in a non pathological biopsy is a decrescendo pattern, which is reversed in celiac disease as well as in chronic duodenitis. This reversed pattern of intraepithelial lymphocytosis was observed in our study, but the count variability was quite different in either disease with a significant p value.19,20

**Conclusion**

We conclude that a raised IEL count is also seen in patients with chronic non specific duodenitis and a count of 40 IEL/ 100 enterocytes should be taken as the lower limit for celiac disease in our population.

**Conflict of Interest**

This study has no conflict of interest to declare by any author.

**References**