SHORT REPORT

Quantitation of intraepithelial lymphocytes in human duodenum: what is normal?

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Background: An increase in intraepithelial lymphocytes (IELs) is mandatory for the histological diagnosis of coeliac disease (CD). Currently, duodenal biopsies are used almost exclusively to establish the diagnosis, yet published work continues to cite an upper limit of 40 lymphocytes/100 epithelial cells, a figure derived from jejunal biopsies over 30 years ago.
Aim: To establish the normal range for IEL counts in distal duodenal biopsies.
Materials/Methods: Twenty subjects (seven men, 13 women; median age, 34 years; range, 20–65) with a normal sugar permeability test and concurrent distal duodenal biopsies were identified. The number of IELs and epithelial cell nuclei in an uninterrupted length of surface (villous) epithelium (> 500 cells) was counted. An image analysis system was used to assess villous architecture by calculating the villous height to crypt depth ratio.
Results: The range of IEL counts in 20 subjects was 1.8–26/100 villous epithelial cells, with a mean value of 11 and SD of 6.8. The mean villous to crypt ratio was 1.82 (SD, 0.38; range, 1.22–2.46). There was no correlation between IEL counts and villous to crypt ratio (Spearman rank correlation, –0.066; p = 0.80).
Conclusions: These results suggest that 25 IELs/100 epithelial cells (mean +2 SD) should be taken as the upper limit of the normal range for duodenal mucosa.

In most gastroenterology units, duodenal biopsies obtained at endoscopy have almost entirely replaced capsule biopsies of jejunal mucosa for the diagnosis of coeliac disease (CD). An increase in intraepithelial lymphocytes (IELs) is the most sensitive index of the adverse effects of gluten on the mucosa of the gastrointestinal tract,[1] and is therefore a mandatory feature for the histological diagnosis of CD. Raised IELs are the first abnormality seen after gluten challenge, and high IELs alone may be a form of gluten sensitivity.[2] Certainly, an increasingly recognised clinical scenario is the patient with normal villi and raised IELs. However, the normal range for IELs in duodenal mucosa has not been established. Published work continues to cite an upper limit of 40 lymphocytes/100 epithelial cells, a figure derived from jejunal biopsies over 30 years ago.[3 4]

Therefore, the aim of this study was to establish the normal range for IEL counts in distal duodenal biopsies.

> "Raised intraepithelial lymphocytes (IELs) are the first abnormality seen after gluten challenge, and high IELs alone may be a form of gluten sensitivity"

MATERIALS AND METHODS

Twenty subjects (seven men, 13 women; median age, 34 years; range, 20–65) with no clinical evidence of malabsorption, a normal sugar permeability (cellobiose-mannitol) test,[5] and concurrent distal duodenal biopsies were identified from the gastrointestinal research and pathology records. These patients had completely normal small bowel function because the sugar permeability test is very sensitive in picking up even minor degrees of enteropathy. All biopsies were fixed in 10% buffered formalin, embedded in paraffin wax, and 4 μm sections were cut at three levels and stained with haematoxylin and eosin. These sections were examined by light microscopy (×400 magnification) and the number of IELs and epithelial cell nuclei in a randomly chosen, uninterrupted length of surface (villous) epithelium (> 500 cells) was counted (by MH). Counts based on 500 epithelial cells gave a mean for the number of IELs/100 cells within 10% of the mean for 600–1000 cells in "cumulative mean" counts on five cases. Counts in excess of 500 cells were therefore considered to be an adequate sample. A previous morphometric study of the duodenal mucosa in CD also used IEL counts based on 500 epithelial cells.[6] We assessed villous architecture on the same sections by calculating the mean villous height to crypt depth ratio using a Leica Qwin image analysis system (by AC).

RESULTS

The range of IEL counts in the 20 subjects was 1.8–26/100 villous epithelial cells, with a mean value of 11 and SD of 6.8. The mean villous to crypt ratio was 1.82 (SD, 0.38; range, 1.22–2.46). This lies within the previously determined normal range for duodenal mucosa.[7] There was no correlation between IEL counts and villous to crypt ratio (Spearman rank correlation, –0.066; p = 0.80).

DISCUSSION

The recognition of an increased density of IELs is important in the diagnosis of CD and even in the presence of normal villous architecture may reflect lesser degrees of gluten intolerance. On the basis of these results, we suggest that 25 IELs/100 epithelial cells is taken as the upper limit of the normal range (mean +2 SD) for duodenal mucosa when counting profile densities in standard histological sections. Although it has been established that these counts overestimate the absolute IEL to enterocyte ratio by a factor of two,[8] methods that provide absolute values are impracticable for routine assessment. Indeed, we would not advocate that detailed quantification (as performed here) is carried out to corroborate a subjective impression of raised IELs. Having established that 25 IELs/100 epithelial cells is the upper limit of normal, it would suffice in routine practice to estimate whether a density ratio of 1 : 4 lymphocytes to epithelial cell nuclei is exceeded at several points in the surface epithelium. Interestingly, an identical value of 25% has been suggested as the threshold that must be exceeded for the diagnosis of lymphocytic gastritis.[9]

Abbreviations: CD, coeliac disease; IEL, intraepithelial lymphocyte

Take home messages

- An increase in intraepithelial lymphocytes (IELs) is mandatory for the histological diagnosis of coeliac disease
- Although duodenal biopsies are currently used for diagnosis, 40 lymphocytes/100 epithelial cells is used as the upper limit of the normal range, a figure derived from jejunal biopsies over 30 years ago
- Our results suggest that 25 IELs/100 epithelial cells should be taken as the upper limit of the normal range (mean +2 SD) for duodenal mucosa when counting profile densities in standard histological sections

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