Duodenal intraepithelial lymphocytosis is common in children without coeliac disease, and is not meaningfully influenced by Helicobacter pylori infection

A. Guz-Mark*, N. Zevit*,†, S. Morgenstern†,‡ & R. Shamir*†

SUMMARY

Background
Increased numbers of duodenal intraepithelial lymphocytes (IELs) characterize coeliac disease (CD) but have also been described in noncoeliacs. Controversy exists regarding an association between increased IELs and infection with Helicobacter pylori, which is commonly found in children.

Aims
To assess the relationship between H. pylori infection and duodenal IELs in a large cohort of children, with and without CD.

Methods
We reviewed gastric and duodenal biopsies of children who underwent esophagogastroduodenoscopy between January 2006 and February 2013 because of either recurrent abdominal pain (RAP) or suspected CD at Schneider Children’s Medical Center of Israel, a referral centre for Israel’s largest Health Maintenance Organization. The duodenal IEL count and H. pylori presence in antral biopsies were determined for each specimen.

Results
Children with RAP (n = 693) or CD (n = 306) were included. Among children with RAP, H. pylori was present in 33.8%. The mean IEL count in the H. pylori positive RAP group was 17.8 (±8.8)/100 enterocytes, vs. 15.8 (±8.3) in the H. pylori negative patients (P = 0.004). Increased IEL counts (≥25 IELs/100 enterocytes) were found in 15.7% of H. pylori negative, noncoeliac children. Among children with CD, there was no significant difference in IEL counts according to H. pylori status: 73.1 (±26.1) vs. 72.6 (±26.5) in H. pylori positive and negative patients respectively.

Conclusions
Our study suggests that slightly elevated duodenal intraepithelial lymphocyte counts are common in the paediatric population. Helicobacter pylori infection has no major influence on the intraepithelial lymphocyte counts in children with recurrent abdominal pain or children with coeliac disease.
INTRODUCTION
Increased numbers of duodenal intraepithelial lymphocytes (IELs) are the initial pathological hallmark of coeliac disease (CD) present prior to villous atrophy in early stages of the disease.¹ The histological finding of increased IELs and normal villous architecture is defined as a type 1 coeliac lesion according to the Marsh–Oberhuber classification,² although controversy exists as to the upper limit of normal intraepithelial lymphocytosis.¹, ³⁻⁵ Nonetheless, increased IELs have been described in numerous noncoeliac conditions including infections (viral infections, giardiasis, Helicobacter pylori), autoimmune disorders, immunodeficiencies, allergic conditions and intestinal response to medications.⁶⁻⁹ Increased IELs are also present in ~70% of patients with noncoeliac enteropathy.¹⁰ Furthermore, mild intraepithelial lymphocytosis has also been described in healthy individuals with varying prevalence in different studies.¹¹⁻¹³ Because the presence and titres of coeliac-associated antibodies are known to correlate with the extent of pathological damage,¹⁴ finding increased IELs with preserved villous architecture poses a diagnostic challenge for the clinician, especially when associated with negative or borderline coeliac serology.¹⁵

Helicobacter pylori is one of the most common human bacterial infections, estimated to infect approximately 50% of the world’s population,¹⁶ more prevalently in developing countries. Infection most often occurs during childhood.¹⁷ While the majority of cases are asymptomatic, histological evidence of chronic gastritis is present almost universally.¹⁸ Occasionally, lymphocytic gastritis may be seen in gastric epithelium and lamina propria.¹⁹ However, data concerning whether H. pylori is related to duodenal intraepithelial lymphocytosis remain equivocal. Furthermore, conflicting reports have been published concerning the influence of H. pylori on CD,²⁰⁻²² and it also remains unclear whether or not H. pylori alters the appearance of the intestinal mucosa among CD patients.

Because H. pylori infection is a prevalent infection, it is important to establish whether histological changes that are classically associated with potential CD can be influenced by its presence. The aim of this study was to assess the relationship between H. pylori infection and duodenal intraepithelial lymphocytosis in a large cohort of children, with and without CD.

MATERIALS AND METHODS
Study design
We reviewed the medical records of patients younger than 18 years of age who underwent esophagogastroduodenoscopy (EGD) between January 2006 and February 2013 at the Institute of Gastroenterology, Nutrition and Liver Diseases at Schneider Children’s Medical Center of Israel, a referral centre for Israel’s largest Health Maintenance Organization. We included patients with a diagnosis of either recurrent abdominal pain (RAP) without identified organic cause, fitting the diagnosis of childhood functional abdominal pain according to the Rome III criteria,²³ or children with positive coeliac serology undergoing a diagnostic EGD. Patients with other diagnoses of organic illnesses, such as malignancies, inflammatory bowel disease, immunodeficiency, autoimmune diseases, food allergy and eosinophilic gastroenteropathies, were excluded. Only patients with biopsies from both stomach and duodenum were analysed, to assess H. pylori status. We also excluded a small minority of children with potential CD (defined as positive serology and normal duodenal biopsies including IELs) and children with Marsh I grading (elevated serology and increased IELs as the solitary histological finding), as their definitive clinical assignment to either the CD or non-CD groups could not be made retrospectively, and the presence of IELs in these children could not be conclusively assigned to either H. pylori or CD status.

Methods
All biopsies were formalin fixed and paraffin embedded, cut in 4-µm sections and stained with haematoxylin and eosin. Immunohistochemical staining for T-cell subsets are not routinely used for CD at our institute. We therefore compared IEL counts in 15 of the cohort’s specimens that were stained with CD3 antibodies and with haematoxylin–eosin staining, and found no significant differences between the two methods. Therefore, the evaluation of IEL count was performed on haematoxylin–eosin-stained specimens alone. For each specimen, a group of five well-oriented villi were identified, and IELs per 20 enterocytes in a villus were counted using the villous-tip method.²⁴ The average IEL count was expressed as the number of IELs per 100 enterocytes, and an increased duodenal IEL count was defined as ≥25.²⁴ An additional review of medical records of patients without CD but with increased IELs was performed to ascertain that no additional diagnoses compatible with increased IELs was present.

Statistics
Descriptive statistics were used to summarise the data. Comparisons between the groups were done by the nonparametric Mann–Whitney U-test, and categorical
variables were compared using Pearson χ² tests. Values of P < 0.05 were considered significant. All analyses were conducted using SPSS (SAS) software (IBM corp., Armonk, NY, USA).

Ethical considerations
The study was approved by the Institutional Review Board.

RESULTS
During the study period, 945 patients underwent EGD for investigation of RAP. Of these, 252 cases were excluded because of incomplete records regarding coeliac serological tests prior to EGD. From a total of 961 newly diagnosed CD patients during this period, 655 were excluded because gastric biopsies were not taken, and H. pylori status could not be determined. Also excluded from the study were 25 children with positive coeliac serology and normal histology (potential CD), and 5 children with positive coeliac serology and Marsh 1 histology (elevated IEL counts). The study design is schematically outlined in Figure 1.

**Helicobacter pylori and IEL counts in children with RAP**

Overall, 693 children with RAP were studied. Of these, 234 (33.8%) had histological evidence of *H. pylori* infection in antral biopsies, and 459 (66.2%) were *H. pylori* negative. There were no significant differences between these groups regarding age or gender distributions. Mean (±s.d.) age was 12.6 (±3.8) and 13 (±3.4) years for *H. pylori* positive and negative subjects, respectively, (P = 0.136), with 66.2% and 66.7% females in the *H. pylori* positive and negative groups respectively (P = 0.978).

The mean duodenal IEL count for the entire RAP cohort was 16.5 (±8.5) IELs/100 enterocytes (median 15, range 2–55). Among the *H. pylori* positive patients, the mean count was 17.8 (±8.8), vs. 15.8 (±8.3) in the *H. pylori* negative patients (P = 0.004), (Figure 2). The distribution of IEL counts did not significantly differ between the groups (P = 0.1) (Figure 3).

Further analysis of the group of CD-negative, *H. pylori* negative children demonstrated increased IEL counts (defined as ≥25 IELs/100 enterocytes) in 15.7% of the subjects, which segregated as follows: 84.3% had <25

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**Figure 1 | Schematic representation of the study design.**
IELs; 6.8% had 25–29 IELs; 7% had 30–39 IELs; and 2% had ≥40 IELs. The asymmetric IEL distribution is demonstrated in Figure 4.

**Helicobacter pylori and IEL count in children with CD**

We included 306 patients with CD (Figure 1); of these, 94 (30.6%) were *H. pylori* positive. *Helicobacter pylori* positive patients were slightly older than the *H. pylori* negative patients – mean (±s.d.) 9.7(±4.3) years vs. 7.8 (±4.6) years respectively (*P* = 0.001). No significant difference was found regarding gender distribution: 56% and 64% were females among *H. pylori* positive and negative patients respectively (*P* = 0.161).

We found no significant difference in IEL counts according to *H. pylori* status: 73.1(±26.1) IELs/100 enterocytes in *H. pylori* positive coeliac patients vs. 72.6 (±26.5) IELs/100 enterocytes in *H. pylori* negative coeliac patients (*P* = 0.716).

Approximately 85%, in both groups, had IEL counts higher than 40 IELs/100. Only two patients (<1% of all coeliac patients studied, all of them *H. pylori* negative) had normal IEL counts (≤25 IELs/100 enterocytes). Although no increased IEL counts were found in these two cases, they were graded Marsh 3c based on their villous atrophy according to the Marsh–Oberhuber classification, and both responded serologically and clinically to gluten-free diets. There was also no significant difference in the distribution pattern of IEL counts between the groups (*P* = 0.536).

There were small differences in the distribution of Marsh–Oberhuber scores between the groups, with a greater portion of the *H. pylori* positive patients graded Marsh 3c, as follows: among *H. pylori* positive CD patients...
– 2.1% graded as Marsh 2, 12.8% as 3a, 29.8% as 3b and 55.3% as 3c; among H. pylori negative group –16.5% as 3a, 41% as 3b, 42% as 3c, 0.5% as Marsh 4 (P = 0.034).

**DISCUSSION**

In this study, we observed a substantially high rate of increased duodenal IEL counts in non-coeliac children, namely 18% of the cases (125/693). These children had no other identified cause that may have contributed to their duodenal lymphocytosis (i.e. food allergies, inflammatory bowel disease, recognised infection with Giardia, autoimmune diseases). To eliminate potential bias of H. pylori infection, we repeated this analysis on patients with both negative coeliac serology and negative H. pylori status. \( n = 459 \). In this group of practically healthy children, 15.7\% (72/459) had IEL counts of at least 25 IELs/100 enterocytes. To the best of our knowledge, this finding in children without infection or CD has not been reported in the past.

The upper limit of normal for IEL counts has been subjected to several revisions in the past decade. Originally, an upper limit of 40 lymphocytes/100 epithelial cells was used by Marsh,\(^1\) derived from jejunal counts in adults.\(^11\) More recently, however, small adult studies have considered the upper limit of normal for duodenal IELs as 20–25 IELs/100 epithelial cells, depending on the method of counting.\(^12, 13\) Later, Corazza and Villanacci adopted an upper limit of 25 IELs in their new histological classification for coeliac lesions.\(^25\) The guidelines of the European Society for Paediatric Gastroenterology, Hepatology and Nutrition for the diagnosis of CD also stated a limit of 25 IELs/100 enterocytes to define the infiltrative lesion of CD,\(^4\) while the North American Society for Paediatric gastroenterology, Hepatology and Nutrition opted for 30 IELs in their guidelines.\(^5\) Pellegrino et al. have declared an even lower threshold of 20, to define elevated IEL counts suggesting a possibility of CD.\(^26\)

The prevalence of increased IELs in non-CD patients has also been debated. Mahadeva et al. reported a prevalence of 2.2\% for increased counts (which defined by them as above 22 IELs/100 enterocytes), in about 600 adults who underwent endoscopies for various indications.\(^27\) Recently, Shmidt et al.\(^28\) identified normal villous architecture and an increased IEL count in 4.3\% (56/1290) of their cohort of children with small intestinal biopsies available (patients with other diseases known to cause increased IELs, such as inflammatory bowel disease, were not excluded). The authors were unable to find an explanation of the increased IEL counts in 23/1290 (1.8\%) cases. The differences between that cohort and our study could be explained by the fact that Shmidt et al. evaluated IEL counts only in cases with normal architecture, and also due to their different cut-off value for increased IEL counts (>30/100 epithelial cells).

To our knowledge, our study is the first large-scale study investigating the influence of H. pylori on duodenal IEL counts. Our data demonstrate that H. pylori colonisation in the stomach has only a marginal effect on the duodenal IEL counts of non-coeliac patients – from an average of 15 IELs/100 enterocytes to 17 (H. pylori negative vs. H. pylori positive respectively). While this small difference is statistically significant, its clinical effect is negligible, especially as the counts are far lower than the IEL counts characteristic of CD, which constitutes the most important condition in the clinical differential diagnosis. Furthermore, the distribution patterns of IEL counts regarding H. pylori status showed no significant differences \( (P = 0.1) \).

Traditionally, H. pylori has been considered one of the infectious aetiologies causing increased duodenal IELs, with little quality evidence corroborating it. Previous publications have referred to an early study reporting increased IEL counts in the duodenum of patients with peptic ulcers, prior to the discovery of H. pylori.\(^29\) Memo et al.\(^30\) studied 50 H. pylori positive adults and found a higher rate of increased IELs when compared with 30 patients with non-H. pylori gastritis. The study used a very low limit of IELs (20), and only few patients were tested for CD (posing an alternative explanation of duodenal lymphocytosis). A smaller study on adolescents with type 1 diabetes \( (n = 15) \) found no effect of H. pylori infection on duodenal IELs,\(^31\) similar to the conclusions of our large-scale study. Different reports regarding an association between H. pylori eradication and subsequent decreases in duodenal IEL counts have found conflicting results.\(^32, 33\)

Our results suggest that H. pylori colonisation should not be considered a significant cause of duodenal lymphocytosis, and should not, by any means, preclude the further assessment for the possibility of CD in these patients.

Furthermore, among children with CD, we have shown that H. pylori has no clinically significant effect on the duodenal IEL counts – suggesting that H. pylori status is irrelevant to CD diagnosis. These findings are consistent with those reported by Villanacci et al., among adult population with CD.\(^21\) The only difference in our study between the two groups was a slight trend towards more advanced villous atrophy among patients with H. pylori, with greater portion of cases graded as type 3C according to Marsh–Oberhuber classification. This finding contrasts
those of both Villanacci and Aydogu, who reported higher rates of low-grade lesions among coeliac patients with \textit{H. pylori}. This discrepancy can only partly be explained by the fact that biopsies graded as Marsh 1 were not included in our analysis (as explained in the methods), as their total amount ($n = 5$) was practically negligible in our cohort. Furthermore, the pathological distribution within the range of Marsh 3a–c is debatable and known to have significant inter-observer variability, as emphasised by Corazza and Villanacci who have therefore suggested a modified method of classification.

Our study has a number of limitations. The major one is the retrospective nature of the study. However, the fact that one observer evaluated all pathological specimens limit the drawback of the retrospective design. Second, we focused on biopsies of children with RAP, with no identified organic illness, who are generally considered healthy. The possible influence of this functional disorder on duodenal pathology must be considered. However, while it would have been ideal to measure IEL counts in biopsies of completely healthy and asymptomatic children, it would also be unethical and impractical to perform. Only one study, conducted in Sweden by Walker et al., assessed duodenal IEL counts in a purely unselected population of adult volunteers. They compared biopsies of 92 adults with either irritable bowel syndrome (IBS) or functional dyspepsia – both representative of the functional gastrointestinal disorders – with 48 healthy controls. They found mast cell hyperplasia in association with IBS, and eosinophilia in association with functional dyspepsia, supporting the theory of a pro-inflammatory state of the intestinal mucosa in these disorders. However, regarding lymphocytosis, they reported only a modest increase in IEL counts in patients with IBS-constipation type, vs. patients with functional dyspepsia and controls. None of their subjects had IEL counts higher than 25/100 enterocytes, contrary to our findings, suggesting possible differences between children and adults. In contrast to Walker et al., Gargala et al. found no differences between duodenal IEL counts of \textit{H. pylori} negative, functionally dyspeptic adults and controls. One study conducted on children with functional dyspepsia also noted differences in duodenal mast cell densities but not in IEL counts. In summary, although a theoretical influence of a functional gastrointestinal disorder on the presence of intestinal inflammatory cells might be argued, available studies are insufficient at this point to support an association with duodenal IELs.

Another possible limitation may be due to our exclusion of CD patients who underwent only duodenal biopsies without gastric biopsies (in whom \textit{H. pylori} infection could not be ruled out). This may have introduced a selection bias. At our institute, biopsies from the stomach of suspected CD patients are usually taken at the discretion of the gastroenterologist, on the basis of endoscopic appearance of the gastric mucosa. As such, one can assume that the bias introduced would lead to the more severe cases of gastritis to be biopsied, which, in turn, would be associated with higher duodenal IEL counts in the \textit{H. pylori} positive CD patients due to an extension of the gastric inflammation. As this did not occur, our results suggest that the exclusion of cases without gastric biopsies had no effect on the results.

In conclusion, we demonstrated that slightly elevated duodenal IEL counts are common in the paediatric population. \textit{Helicobacter pylori} infection has no major influence on the IEL counts, neither in children with RAP nor in children with CD.

**AUTHORSHIP**

Guarantor of the article: Raanan Shamir.

Author contributions: Anat Guz Mark designed the study, acquired and analysed the data, and drafted the manuscript. Noam Zevit designed the study and revised the manuscript. Sara Morgenstern collected the data and supervised the study. Raanan Shamir conceived and designed the study, analysed the data and revised the manuscript. All authors approved the final version of the article.

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