Natural Progression of Helicobacter pylori-Negative Gastritis with Lymphoid Follicles in Children: Prospective Evaluation and Outcome

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ABSTRACT

Background: *The association of Helicobacter pylori-negative gastritis with lymphoid follicles (LFs) in children is still unclear. Therefore, we aimed to investigate the natural history and significance of H. pylori-negative gastritis with LFs in children.*

Methods: *We identified children with histologically proven H. pylori-negative gastritis with LFs between June 2014 and January 2017. The children were invited for a follow-up examination. The clinical, endoscopic, and histological findings of the index esophagogastroduodenoscopy (EGD) were revised and compared to the follow-up findings.*

Results: *A total of 754 children underwent EGD. Among the 48 children diagnosed with H. pylori-negative gastritis, 17 (35.41%) had gastric LFs. Eight agreed to participate in the study. The mean follow-up was 25.58 ± 4.52 (range, 20.53-35.73) months. Three children still had histologic findings of chronic gastritis with LFs. Four children had resolution of the gastritis but still had LFs, and 1 patient had resolution of both the gastritis and LFs.*

Conclusion: *LFs were still present in children with H. pylori-negative gastritis after a mean follow-up of 2 years, and in some children, despite resolution of the gastritis. Therefore, this histological finding might be a non-pathological feature in children and does not need any contribution or follow-up.*

Keywords: *Gastritis, H. pylori-negative, lymphoid aggregates, lymphoid follicles*

INTRODUCTION

Gastritis is a common finding in children who undergo diagnostic esophagogastroduodenoscopy (EGD). Although various etiologies have been linked with gastritis, the focus in the last decades was on *Helicobacter pylori* infection-induced gastritis.[1] It is well documented that *H. pylori* infection is mainly acquired during childhood.[2] In recent years, the rate of *H. pylori*-associated gastritis in children has declined, particularly in developed countries.[1] Therefore, there is an increased proportion of cases with chronic gastritis without *H. pylori* infection, known as *H. pylori*-negative gastritis. The differential diagnosis of *H. pylori*-negative gastritis includes the following: lymphocytic and collagenous gastritis, autoimmune gastritis, inflammatory bowel disease (IBD), non-*H. pylori* infectious gastritis such as cytomegalovirus, Epstein–Barr virus (EBV), non-*H. pylori* Helicobacter species, and medication-associated gastritis.[3]

H. pylori-associated gastritis is a well-known risk factor for gastric adenocarcinoma through a process called the Correa cascade, in which the gastric mucosa evolves through the stages of acute gastritis, chronic gastritis, gastric atrophy, intestinal metaplasia, dysplasia, and gastric adenocarcinoma.[4] Additionally, *H. pylori* chronic gastritis is also strongly associated with the formation of lymphoid aggregates (LAs) and lymphoid follicles (LFs), with or without germinal centers, which are normally devoid of gastric mucosa. These LFs are the precursor lesions of *H. pylori*-associated mucosa-associated lymphoid tissue (MALT) lymphoma.[4,5] Recently, it was reported that LFs are also associated with *H. pylori*-negative gastritis.[5,6,7,8] However, the natural history of *H. pylori*-negative gastritis is still not fully understood. Data on the long-term evolution of this disease condition are still lacking, and the clinical consequences of LFs are still unknown. Therefore, in this study, we aimed to explore

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whether *H. pylori*-negative gastritis with LFs is a self-resolving disease or requires further follow-up.

METHODS

Study Design and Participants

This was a single-center prospective cohort study. We reviewed the data from June 1, 2014 to January 31, 2017 and identified children who were diagnosed with *H. pylori*-negative gastritis with evidence of LFs in gastric biopsies that were taken during EGD in the pediatric gastroenterology unit at Shamir Medical Center.

In this study, we included children aged 2-18 years old who had histological findings compatible with *H. pylori*-negative gastritis associated with LFs. The indications for the endoscopic examinations were iron deficiency anemia (IDA), abdominal pain, failure to thrive (FTT), vomiting, diarrhea, heartburn, and short stature. We excluded children who required urgent endoscopy during hospitalization, those with IBD or celiac disease (CD), a known past or present *H. pylori* infection, a 4-week prior treatment with antibiotics or a 2-week treatment with proton pump inhibitors (PPIs), or those who used NSAIDs. The guardians of the children who were eligible for the study, were requested to participate in this study.

Follow-up Examination

Those who consented to participate in the study were invited for a follow-up examination, provided that at least 18 months had passed from the first examination. The follow-up included filling out a questionnaire comprised of clinical characteristics and complaints, use of medications, and diet habits. Laboratory data, including anti-parietal cell antibodies, complete blood count, iron status, and vitamin B_{12} serum level were obtained, and the presence of EBV infection at the time of EGD was also confirmed. We then performed a second EGD.

The clinical, endoscopic, and histological findings reported during the index EGD were revised and compared to those during the follow-up endoscopy.

The guardian signed an informed consent form prior to inclusion in the study. The study protocol was approved by the ethics committee of the Shamir Medical Center (Zerifin, Israel).

EGD

All EGD examinations were performed by 2 pediatric gastroenterologists using PENTAX EG27-i10 (PENTAX Medical, Germany). In addition, all patients had been fasting and were under sedation. Informed consent forms were signed by the guardians prior to endoscopy. From each patient, a total of 5 biopsy specimens were obtained for histology (2 from the body, 2 from the antrum, and 1 from the incisura) according to the new Sydney System for classification of gastritis.[9] Two more biopsy specimens (one from the antrum and one from the body) were acquired for rapid urease test (Pronto Dry; Gastrex, Paris, France).

Histologic Evaluation

Biopsy specimens were fixed in 10% formalin, embedded in paraffin, and stained with hematoxylin and eosin. All cases were examined for *H. pylori* using immunostaining (ultraView Universal DAB Detection Kit, Ventana Medical Systems, Inc., Tucson, AZ, USA). The features of gastritis were identified and graded by an experienced pathologist. Gastritis was defined by the presence of at least 5 mononuclear cells or the presence of plasma cells in the foveolae. The severity and activity of gastritis and the presence of intestinal metaplasia or gastric atrophy were recorded using the criteria for the visual analog scale set by the updated Sydney System for the classification of gastritis.[9] An LFs was defined as a dense nodular lymphocyte aggregate, with or without germinal centers (Figure 1Aand B). The number of LFs in each specimen was counted, and the measurement of the maximal LF diameter was reported. The biopsies of the index gastroscopy were unarchived and revised in the same manner by the same pathologist, and compared to biopsies taken during the follow-up EGD.

Statistical Analyses

The categorical variables were reported as number of percentages. The continuous variables were evaluated for normal distribution using a histogram and reported as median and interquartile range (IQR). The categorical variables were compared between the 2 exams using McNemar's test, and the ordinary variables using the Wilcoxon signed-rank test. *P* values of $<.05$ were considered to reflect statistically significant differences. All statistical tests were two-sided. SPSS software was used for all statistical analyses (IBM SPSS Statistics 2017, Version 25, IBM Corp, Armonk, NY, USA).

RESULTS

Patients

Between June 2014 and January 2017, a total of 754 children underwent EGD at the Pediatric Gastroenterology

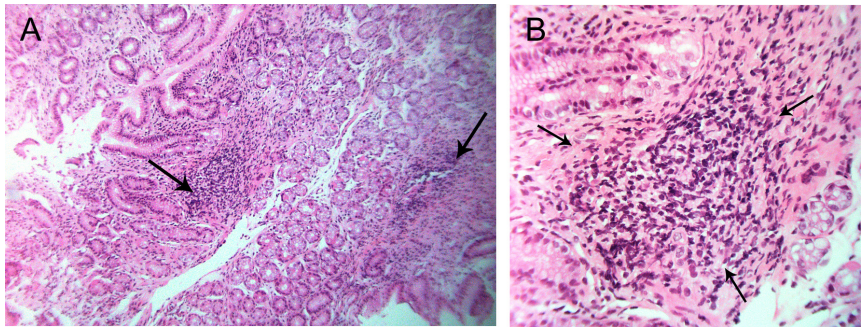

Figure 1. A, Lymphoid follicle formation (indicated by arrows) in gastric mucosa (hematoxylin and eosin (H&E) ×100). B, High-power view showing lymphoid follicles (arrows) (H&E ×400).

Service, Shamir Medical Center, Tzrifin, Israel. Forty-eight patients were diagnosed with *H. pylori*-negative gastritis. Among them, 17 patients (35.41%) had gastric LFs on histology specimens and were invited to participate in the study. Four refused to participate, one was a soldier, one was diagnosed with rhabdomyosarcoma, and one could not be reached by phone. Two patients were excluded after the second EGD: the first one was because of an *H. pylori* infection revealed by gastric biopsies and the second one was because of a diagnosis of autoimmune gastritis. Thus, 8 patients were included in the analysis. The mean follow-up period between the 2 EGD examinations was 25.58 ± 4.52 (range, 20.53-35.73) months. The demographic and clinical characteristics of the patient population are presented in Table 1. As shown in the table, the main presenting symptom at the time of each endoscopy was abdominal pain, followed by IDA, vomiting, and FTT. None of the patients were treated with PPI between the 2 EGD examinations.

Table 1. Demographic and Clinical Characteristics of the Patients

	First Gastroscopy, *n* (%)	Second Gastroscopy, *n* (%)
Female, n (%)	5 (62.5)	
Age, mean ± SD (range)	10.74 ± 5.25 (5.33-16.86)	12.88 ± 5.19 (7.39-18.93)
BMI, mean ± SD (range)	17.90 ± 4 (13.51-26.67)	19.21 ± 4.79 (14.28-27.34)†
Clinical characteristics		
IDA	1 (12.5)	0
Abdominal pain	5 (62.5)	5 (62.5)
FTT	1 (12.5)	0
Vomiting	1 (12.5)	1 (12.5)
Diarrhea	0	0
Heartburn	0	1 (12.5)
Short stature	0	0

†*n* = 7
SD, standard deviation; BMI, body mass index; IDA, iron deficiency anemia; FTT, failure to thrive.

Endoscopic and Histological Findings of Index Biopsies

H. pylori-negative chronic gastritis with LFs was reconfirmed histologically in the gastric tissue specimens of the index EGD. The gastritis was limited only to the antrum in 3 out of 8 (37.5%) patients, to the body in 1 (12.5%), and pangastritis in 4 (50%) (Figure 2). Only 1 patient had chronic active gastritis. There was no evidence of atrophy or intestinal metaplasia in any of the specimens. None of the 8 patients had endoscopic findings of gastritis during the index endoscopy.

Endoscopic and Histological Findings of Follow-up Biopsies

Out of the 8 patients with chronic gastritis with LFs, 3 still had histologic findings of chronic gastritis. Figure 2 summarizes the percentage of patients with antral gastritis,

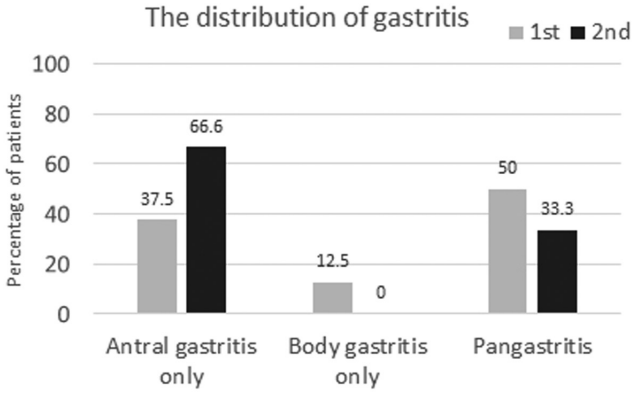

Figure 2. Topographical patterns of *Helicobacter pylori*-negative gastritis.

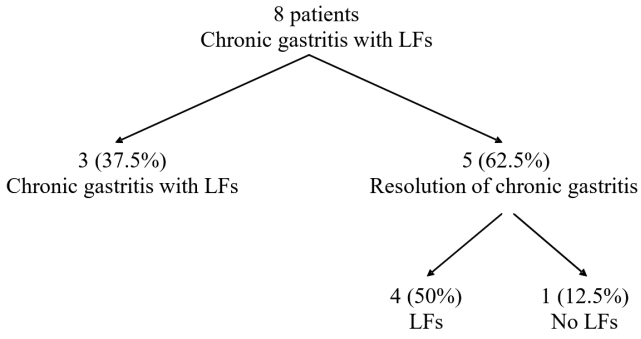

Figure 3. The presence of gastritis and lymphoid follicles in the follow-up examination of children with gastritis and lymphoid follicles. LFs, Lymphoid follicles.

corporal gastritis, and pangastritis. Totally 2 out of the 3 (66.6%) had antral gastritis and 1 (33.3%) had pangastritis (Figure 2). All the patients with gastritis still had LFs. There was neither gastric atrophy nor intestinal metaplasia. The remaining 5 patients no longer had inflammation, but 4 of them still had LFs. Thus, only 1 patient had resolution of both the gastritis and LFs (Figure 3). The mean measured diameter of the LFs was found to be 155 (range: 50-500) μm. The endoscopic findings of gastritis during the follow-up EGD were reported in 1 patient.

DISCUSSION

Our study demonstrates that the presence of LFs associated with *H. pylori*-negative gastritis is a persisting condition in pediatric patients, even after a mean period of a 2-year follow-up.

The significance of LFs/LAs in pediatric patients was mainly investigated in cases of *H. pylori*-positive gastritis. In this setup, the eradication of *H. pylori* may resolve gastritis and prevent the progression to long-term complications such as MALT lymphoma. Fichman et al. reported in a retrospective study that despite the eradication of *H. pylori*, LFs persisted in 50% of the patients, suggesting that LFs might be a normal histological finding in the stomach of pediatric patients.[10]

The Updated Sydney System indicated that the presence of LFs in *H. pylori*-negative gastritis suggests that the organism has been overlooked.[9] Our patients were without previous *H. pylori* diagnosis, and we confirmed the absence of *H. pylori* infection twice using immunostaining. Only 1 out of the 10 children with *H. pylori*-negative gastritis during the index biopsies had detectable organisms in the second EGD, indicating a missed infection in the index EGD. This finding corresponds to the previously described literature, in which a small percentage (<10%) of patients with *H. pylori*-negative gastritis were likely to have a missed infection (false negative).[11] Moreover, the recent use of PPIs or antibiotics,[4] a common cause for false *H. pylori*-negative gastritis, was included in the exclusion criteria in our study. None of our patients smoked tobacco or drank alcohol, the other risk factors for *H. pylori*-negative gastritis.[12] Finally, children with other causes of *H. pylori*-negative gastritis, such as IBD and CD, were also excluded from the study.

The rate of LFs among *H. pylori*-negative gastritis in our population was found to be 35.41%. This rate varies in different studies: 51.3% in children in Israel,[8] 47% in the northern region of the United Arab Emirates,[6] 14% in Tunis,[13] and 4.1% in Houston, USA.[7] We demonstrated that LFs were the dominant histological finding even without the presence of gastritis, as 7 out of the 8 followed-up children still had LFs present in their gastric specimens after a mean follow-up period of 2 years (Figure 3). Okamura et al. found that the size of the LFs in nodular gastritis and in atrophic gastritis was greater than that observed in the normal mucosa.[14] However, we could not demonstrate such a correlation between the presence of gastritis and the size of LFs in the follow-up EGD. None of our patients had atrophy Operative Link on Gastritis Assessment (OLGA) stage 0 or intestinal metaplasia in the follow-up EGD. In addition, the 155-μm (range: 50-500) mean diameter of the LFs in our study was smaller than that of nodular and atrophic gastritis.

Data in the literature indicate that *H. pylori*-negative gastritis is a distinct condition that merits further investigation.[11] The causes and complications of this disease are still not fully investigated,[1] and the importance of LFs and/or LAs in children is still under debate. Moreover, there are limited data regarding the endoscopic and histological outcomes of *H. pylori*-negative gastritis with LFs in children. To our knowledge, our study is the first to prospectively examine these outcomes. Of the 8 children with *H. pylori*-negative gastritis with LFs, only 3 still had chronic gastritis with LFs, and 4 children still had LFs but the gastritis was resolved, and 1 patient had resolution of both the gastritis and LFs. No complications such as atrophy or intestinal metaplasia were observed.

Carpentieri et al. designed a study to evaluate the significance of gastric LFs and LAs in children with and without *H. pylori* infection.[15] Although there are no exact definitions of LFs and LAs in the literature, LAs have been

defined those not having germinal centers that occasionally may be found in children's normal gastric mucosa. In contrast, LFs present with germinal centers in mucosa-associated gastritis. Among 605 antrum biopsies, histologic gastritis was diagnosed in 80 biopsies, while the presence of LFs with gastritis had the strongest correlation with *H. pylori* (R = 0.5, $P < .00001$). Conversely, LAs with gastritis had no significant correlation with *H. pylori*. Moreover, an examination of the biopsies without gastritis revealed a higher frequency of LAs (65/525) compared to LFs (2/525). They concluded that LFs should be distinguished from LAs that are not significantly associated with *H. pylori* infection and may be a component of the normal gastric lymphoid tissue.[15]

This study has some limitations. First, the power of the study was limited due to the low number of guardians who provided informed consent for a second EGD. Second, our study was conducted at a single center, which limits the generalization of our findings to other institutions or populations. Third, we did not perform a serology test, whose sensitivity does not decrease even when there is a low bacterial load in the stomach, and we relied on the patient's medical history to rule out previous *H. pylori* infection. Finally, we defined LFs as a dense nodular lymphocyte aggregates with or without germinal centers. There is a lack of consistency in the literature with regard to the definitions of LFs and LAs. Some defined LFs as LAs with a germinal center[5,15,16] but in other studies, there is no distinct differentiation between LFs and LAs.[10,14,17,18]

CONCLUSION

As LFs were a dominant histological finding in children with *H. pylori*-negative gastritis even after a mean follow-up of 2 years and in some children after the resolution of gastritis, we assume that this histological finding might be a non-pathological feature in children. Therefore, we believe that endoscopic and histological follow-up is not mandatory. Further prospective studies on a large pediatric population are required to clarify this matter.

Ethics Committee Approval: Ethics committee approval was received for this study from the ethics committee of Shamir Medical Center (IRB 0128-16-ASF).

Informed Consent: Informed consent was obtained from all patients.

Peer Review: Externally peer-reviewed.

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Conflict of Interest: The authors have no conflict of interest to declare.

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