



Short Communication

Lymphoid Aggregates in Gastric Biopsies: Relationship to Other Mucosal Lesions

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Abstract. The purpose of this study is to estimate the prevalence of lymphocyte aggregates (precursor of MALT lymphomas) in gastric mucosal biopsies and to associate gastric lymphoid tissue with the age of patients, *Helicobacter*-associated gastritis and other gastric mucosal pathology. A consecutive series of gastric mucosal samples from 150 children and 256 adults were assessed for the presence of lymphoid aggregates as well as morphological characteristics, *Helicobacter pylori* status, signs of gastritis, mucosal atrophy and lymphoepithelial lesions. Fifteen selected samples with prominent lymphoid aggregates and 10 controls were examined immunohistochemically for the immunoglobulins A, G, M, lymphocytes B and T, clonality of B cell population, atypical lymphocytes and Epstein-Barr virus (EBV) antigen. There was an increase of *H. pylori* infection and mucosal lymphoid aggregates (MALT) rates in parallel with the increasing age of patients noted in the histological assessment of the mucosal samples. A close association of lymphoid aggregates with *H. pylori* infection and prominent active gastritis was found, but in adults with chronic non-active, particularly atrophic gastritis this association became weaker. No morphological and immunohistochemical signs of MALT lymphoma were present. Lymphoid aggregates in children were larger, with follicles, but less numerous and tended to be located in the intermediate and deeper parts of the gastric mucosa. Immunohistochemical studies showed an increase of IgA, IgM and lymphocytes T in the deeper part of the lamina propria in *H. pylori*-associated gastritis and lymphocyte T accumulation in the periphery of the lymphoid follicles. No evidence of monoclonality, CD31 positive lymphocytes or EBV antigen was detected. Lymphoid aggregates are related, but not exclusively, to *H. pylori* infection. Their detection rates achieve a peak in young adults with *H. pylori* infection. Lymphocytic aggregates are also present in chronic atrophic gastritis without *H. pylori* infection and may relate to autoimmune inflammatory response to other factors.

Key words: lymphoid aggregates; *Helicobacter* gastritis; immune response; children.

Introduction

Helicobacter pylori (*H. pylori*) infection is strongly associated with predominantly antral gastritis, duodenal ulcer and, to a lesser extent, with gastric ulcer disease and the intestinal type of gastric carcinoma^{2, 16, 10}. Most

recent evidence supports the involvement of *H. pylori* in the pathogenesis of gastric lymphoma of mucosa-associated lymphoid tissue (MALT) type^{3, 6, 20}. The most pathogenic strain of *H. pylori* is a strain expressing the cag A protein^{2, 16}.

Primary gastrointestinal lymphoma comprises

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a group of distinctive clinicopathological entities, most of which are not included in the lymph node-based lymphoma classification. They are predominant the B cell type, with primary gastrointestinal Hodgkin's disease practically non-existent^{3, 12}. Most low-grade B cell GIT lymphomas are of the MALT type, rather than of the lymph nodes. In the revised European-American classification of lymphoid neoplasms⁹, MALT lymphomas are classified as extranodal low-grade marginal zone B cell lymphomas. Paradoxically, most MALT lymphomas arise in the stomach, which normally contains no organized lymphoid tissue^{6, 12, 22}.

Gastric MALT lymphomas arise in MALT which is acquired as an immunological reaction to infection of the stomach by *H. pylori*. The infection triggers the acquisition of gastric MALT and may provide the ground for MALT-type lymphoma development. This concept has been supported by the histological detection of *H. pylori* in almost all gastric MALT-type lymphomas^{12, 15, 20}. Eradication of *H. pylori* resulted in the regression of most (more than 2/3) gastric low-grade MALT-type early stage lymphomas in two large studies^{1, 19}. However, over 10% of the responders may develop a recurrence of lymphoma⁵ due to resistance or different cause lymphoma. An expression of *H. pylori* gastritis and MALT reaction changes with age⁸. The influence of *H. pylori* eradication on gastric MALT is unclear. In our previous observations lymphoid aggregates failed to disappear 2 years after eradication of *H. pylori*¹³.

There is a lack of studies on lymphoid aggregates in gastric mucosa in children and on the interrelationship with other mucosal pathological lesions.

Materials and Methods

One hundred and fifty children aged between 7 and 18 years and 256 adults aged between 25 and 68 years underwent diagnostic endoscopic examination for the evaluation of symptoms referable to the proximal GIT over 3 weeks (dyspeptic symptoms with abdominal pain and/or vomiting).

Patients presenting focal gastric mucosal lesions such as ulcers, tumors, etc. were excluded from the study. From the remaining patients 6 biopsy samples were taken: 2 from the antral of the greater curve, 2 from the angulus and 2 from the corpus mucosa. Samples were fixed in buffered formalin for less than 24 h, routinely processed to paraffin blocks, cut in 4µm sections and stained with HE, periodic acid Schiff for mucin secretion, Giemsa stain for *Helicobacter* micro-

Table 1. Lymphocytic aggregates and other mucosal lesions in antral biopsies

	Adults (n=254)	Children (n=150)
Lymphoid aggregates total rate	53	21.5 (14*)
<i>H. pylori</i> infection overall rate	44	30.5 (25*)
in cases with lymphoid aggregates	66 (91**)	75 (94**)
Lymphoid aggregates in <i>H. pylori</i> gastritis	28.8	50(66*)
Atrophy/intestinal metaplasia overall rate	25	2
in cases with lymphoid aggregates	50	2

*In children younger than 11 years.

**In cases with active or/and high grade gastritis.

Table 2. Morphological characteristics of lymphoid aggregates in gastric mucosa

Lymphoid aggregates	Adults	Children	Student's <i>t</i> -test
Size*	2.2	2.5	p<0.05
Quantity**	2.2	1.8	p<0.05
Presence of follicles***	0.5	0.9	p<0.05
Localisation			
superficial	2.6%	0	
intermediate	40%	45%	
deep	48%	50%	

* 1 – 110–800 µm, 2 – 800–1100 µm, 3 – over 1100 µm.

** Scale 1–3: small, medium or large.

*** 0 – absence, 1 – present.

organisms and Masson's trichrome for fibrous tissue and assessed in a light microscope. The biopsy material was examined for the presence and severity of *H. pylori* infection, grade and activity of inflammation and the presence of atrophy, fibrosis and intestinal metaplasia (IM). For a classification of the grade and activity of gastritis we used the Sydney system¹⁷. All lesions relevant for this study are listed in Table 1. Lymphoid aggregates with and without germinal centers were counted, their largest diameters were measured and recorded using the scale¹¹: 110–800 µm = 1; 800–1100 µm = 2, and over 1100 µm = 3, and collated into Table 2. In addition, the localisation of aggregates (mucosal superficial or intermediate in the lamina propria, or deeply seated submucosal included) are recorded as well (Table 2). All morphological readings were double-checked by two pathologists (authors). Student's *t*-test was used for the statistical evaluation of results.

Fifteen cases of *H. pylori*-associated chronic gastritis with particularly prominent lymphoid aggregates

were selected for immunohistochemical examination by PAP technique for the immunoglobulins A, G, M, lymphocytes B (CD20 antibody) together with clonality of B cell population (kappa and lambda antibodies), lymphocytes T (CD3 and CD8 antibodies), atypical lymphocytes and vascular endothelium (CD31 antibody) and for the presence of Epstein-Barr virus (EBV) antibody.

All antibodies were produced and supplied by the Dako Company.

Results

H. pylori infection rates and frequencies of lymphoid aggregates in gastric mucosa increased in parallel with the age of the patients up to 45 years and decreased in older patients.

In routine gastric biopsy specimens collected from 254 adult patients with non-ulcer dyspepsia, lymphoid aggregates with or without follicle formation were present in 53% of all endoscopic material. Within the gastric biopsy material collected from 150 children, lymphoid aggregates were present in 21.5% of the material. Children younger than 11 years showed these lesions in only 14% of cases. Overall rates of *H. pylori* infection of gastric mucosa showed an increasing tendency with age: 25% in children below of 11 years, 30.5% in children between 11 and 18 years and 48% in adults aged between 25–45 years (Table 1). The rate of *H. pylori* infection in patients over 45 years was 38%.

There is a close relationship between lymphoid aggregates and *H. pylori* infection, particularly in younger patients. The size of lymphocytic aggregates in children and, to a lesser extent, in adults correlates with the grade and activity of chronic gastritis and histological identification of *H. pylori*. *H. pylori* infection reaches 100% in cases with lymphoid aggregates associated with chronic active gastritis. There is also a correlation between atrophic gastritis and lymphoid aggregates. Non-active atrophic gastritis with or without IM is twice as common in the presence of lymphoid aggregates as in the overall histological sections, irrespective of *H. pylori* identification. *H. pylori* microorganisms in the cases with signs of atrophy were not present, despite the presence of lymphoid aggregates. Non-active gastritis with signs of atrophy and without *H. pylori* infection were noted almost exclusively in patients over 45 years.

Lymphoid aggregates in children were localised in the deeper parts of the gastric mucosa, were larger, with frequent follicles and germinal centers, but less numerous compared to the morphological characteristics of MALT in older patients (Table 2). Differences in the size and distribution of lymphoid aggregates in gastric

mucosa in various grades of chronic gastritis and in different age groups were related to the histological identification of the microorganism, the degree and the activity of the chronic gastritis. The localisation of lymphoid follicles with germinal centers in deeper parts of the gastric mucosa in children is commonly related with intensely active inflammatory infiltrate in the superficial lamina propria. The smaller, more numerous and superficially localised aggregates of lymphoid tissue are more common in adults. The presence of lymphoid aggregates in the mucosa of the gastric corpus is related to the grade and activity of the gastritis. *H. pylori*-associated gastritis was present in 80% of the corpus mucosal samples with lymphoid aggregates.

The distribution of IgA, IgG and IgM-secreting cells and lymphocytes T was different in *H. pylori*-associated gastritis when compared to controls. Infected mucosa showed IgA and IgM-secreting cells and lymphocytes T in the intermediate and deeper parts of the lamina propria and this was related with the density of inflammatory infiltrate. No monoclonal cellular infiltrates were noted. Lymphocytes T were accumulated in the periphery of lymphoid follicles and in the intraepithelial lymphocytic infiltrate, while CD20-positive cells were mainly accumulated within lymphoid aggregates. CD31 antibody indicated a marked increase of vascularity in the lamina propria of cases with signs of atrophic lesions. There was no evidence of CD31-positive atypical lymphocytes and the reaction with EBV antibody was negative in all tested mucosal samples.

Discussion

According to previous reports, lymphoid aggregates in the gastric mucosa are a specific feature of *H. pylori*-associated gastritis and represent the immune response of the mucosa to *H. pylori*, partially as a result of autoimmune processes^{12, 18, 21, 22}. The detection rates of *H. pylori* infection in the gastric mucosa with lymphoid aggregates varies from 27 to 100% in different works^{6, 11, 21} and depend on the type and extent of gastric mucosal sampling, methods of *H. pylori* detection, etc. In the present work, in “routine” gastric mucosal biopsy samples using standard histological methods, the detection rates of *H. pylori* are 30.5% in children and 44% in adults, are related to the grade and activity of *H. pylori*-associated gastritis and reach 100% in high grade active gastritis. The size of lymphocytic aggregates in chronic gastritis correlates with the histological presence of *H. pylori*, particularly in children. This finding was noted previously in chronic gastritis of adults by others¹¹. Differences in size, quantity and

localisation of lymphoid aggregates in the gastric mucosa in children when compared to mucosal lesions in adults can be explained by a more active mucosal response to more recently acquired *H. pylori* infection and a different immune response in children. Smaller, superficially localised lymphoid aggregates indicate a more chronic response to infection or a different etiological factor.

According to our observations, persistent lymphoid aggregates can be present in „burned out” gastritis and in chronic atrophic gastritis with or without intestinal metaplasia when *H. pylori* in histological examination is usually negative and indicate a possibility of other than infective etiological factors. Moreover, lymphoid aggregates in antral mucosa are present in 50% of patients with pernicious anemia⁴, when *H. pylori* infection is consistently negative. In our previous work¹³, lymphoid aggregates persisted after *H. pylori* eradication within the 2 years of endoscopical follow-up.

In our present study we did not find lymphocytic gastritis, other possible precursor lesions for MALT lymphoma^{7, 14} or lesions indicative of an early stage of MALT lymphomas, such as monoclonal B cell population, lymphoepithelial lesions or atypical lymphocytes. Immunohistochemical examination confirms previous findings^{1, 3, 13} and indicates a predominant lymphocyte T participation in chronic response with autosensitisation of T cells in *H. pylori* gastritis. Signs of EBV infection, another possible infective cause of lymphoid tissue proliferation, or malignant transformation of lymphoid infiltrate in our study were negative.

In conclusion, lymphoid aggregates are related, but not exclusively, with *H. pylori* infection and their rates in *H. pylori*-infected and non-infected mucosa change with age, which indicate an autoimmune response for *H. pylori* infection and possible response to other causes. No relationship to EBV infection nor transformation to MALT lymphoma were found.

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