

B (CD20⁺) lymphocytes in the antrum mucosa of children with *Helicobacter pylori* infection

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Abstract

The aim of the study was to estimate the expression of CD20⁺ lymphocytes in the antrum mucosa in children, infected with *Helicobacter pylori* and after bacteria eradication.

Biopsy specimens of gastric mucosa were the specimens, collected from 59 *H. pylori*-positive patients (Group I), 29 patients after *H. pylori* infection (Group II) and 18 *H. pylori*-negative children (Group III). The collected specimens were assessed for infection and inflammation and the expression of CD20⁺ lymphocytes was estimated, using mice monoclonal antibodies. The expression of CD20⁺ lymphocytes in the inflammatory infiltrate of the antrum mucosa correlated with the severity of gastritis, found in children with *Helicobacter pylori* infection and was the highest in comparison with the group of children after *H. pylori* eradication.

Key words: B lymphocytes, gastric mucosa, *Helicobacter pylori*, children.

Introduction

A chronic inflammatory response is characterized by damage to the epithelium and the infiltrate of mononuclear cells, including T and B-lymphocytes, plasmatic cells, macrophages or eosinophils [1]. The lymphocytes, accumulated in the pyloric mucosa, form lymphatic follicles in a significant percentage of children infected with *H. pylori* (50-100%). Lymphoidal texture in the gastric mucosa, found mainly in patients at the developmental age, is responsible for the granulation in the gastric mucosa, described in the endoscopic examination [2, 3].

The aim of the study was to evaluate the expression of CD20⁺ lymphocytes in the antrum mucosa of children, infected with *H. pylori* and after its eradication, and to find a correlation between the expression of CD20⁺ and the inflammation of the gastric mucosa. Material and methods Gastric mucosal biopsy specimens, collected from the following three groups of individuals, were studied by light microscopy: (I) 59 children (29 girls and 30 boys; the age range: 2-19) with chronic active inflammation of the gastric mucosa with *H. pylori* infection with positive IgG antibody anti- *H. pylori*; (II) 29 children (14 girls and 15 boys; the age range: 3-19) after *H. pylori* infection without timely colonization and without active inflammation of the gastric mucosa with maintained positive IgG antibody anti- *H. pylori*. (III) 18 children (12 girls and 6 boys; the age range: 5-17) with gastrointestinal disease, without *H. pylori* infection, with correct level IgG antibody anti- *H. pylori*. In all the examined children, endoscopic examinations and histopathological studies of the stomach were performed. Obtained sample sections were diagnosed, according to the Sydney System [4]. Urease test was performed in the course of endoscopy in all the children. The specimens were fixed in 10% buffered formalin. They were processed, oriented on edge, embedded in paraffin, cut in sequential 5- μ m sections, and stained by H+E for the evaluation of inflammation and by Giemsa to identify *H. pylori* bacteria (according to Gary's) [5]. All the biopsy specimens from each of the study groups were stained by an immunohistochemical method for the evaluation of CD20⁺ lymphocytes in the mucosa. ABC method was used, according to commercial protocol. The number of CD20⁺ lymphocytes in the mucosa was counted in discrete areas, measuring 0.785 mm², each by using a light microscope. All the counts were performed at magnification of 200x. The numbers of positively stained cells were presented as the mean values per 1 mm² of the analysed gastric section area. All the CD20⁺ lymphocyte counts were performed by a single observer (I. K.), who was unaware of either the *Helicobacter pylori* status or of the subject's clinical group.

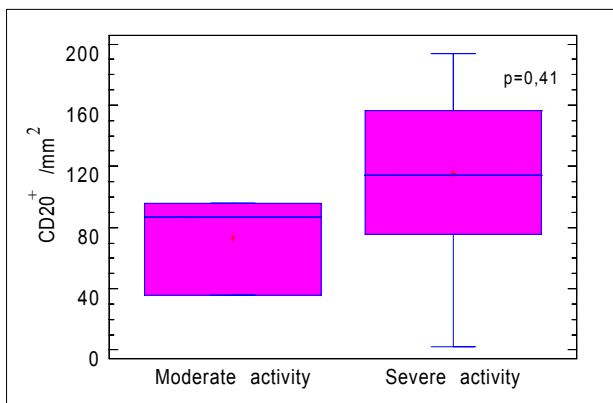
The analysis of the preparations and their photographic documentation were performed with an Olympus Bx50 light micro-

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Table 1. CD20⁺ lymphocytes in the antrum mucosa in children.

Experimental Groups	CD20 ⁺ lymphocytes in the antrum mucosa /mm ²								
	Number of patients (N)	Result (min)	Result (max)	Arithmetic mean (x)	Median value (ME)	Moda	Standard deviation (SD)	Low quartile	High quartile
Group I	23	2	194	109,9	96	72	51,4	76	146
Group II	14	0	79	20,6	13	0	25,8	2	25
Group III	5	0	43	17,4	18	-	17,6	2	24

Figure 1. Correlation between the expression of CD20⁺ lymphocytes and the activity degree of antrum mucosa inflammation in children with *Helicobacter pylori* infection (Group I)

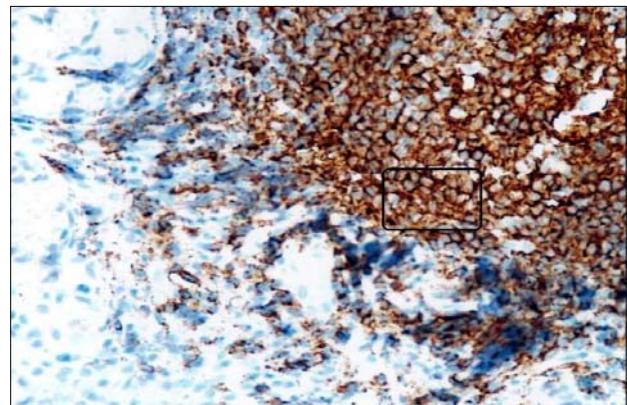
scope, with a video circuit and a Pentium 120 PC computer with Lucia G (Nikon) software for microscope image analysis. CD20⁺ lymphocytes density was compared between the groups of studied subjects, using Student's "t" test and Chi². Statistical calculations included the arithmetic mean, standard deviation (SD), the median value (ME), the minimal result (min) and the maximal result (max).

Both clinical examinations and all the laboratory tests were performed in the children, following previous unrestrained consents of either their parents or of legal protectors and an approval of the Ethical Commission at the Medical University of Białystok.

Results

When evaluating antrum gastritis, according to the Sydney System in Group I with *H. pylori* infection, severe inflammation was reported in 45.8% of the examined children; moderate inflammation in 52% and mild inflammation in 1.7% of the patients. In Group II after *H. pylori* eradication, moderate inflammation was observed in 6.9% of the examined children; mild inflammation in 13.8% and 79.3% of children had normal gastric mucosa. In controls, mild inflammation was reported in 15.4% of children and 79.3% had normal gastric mucosa.

The performed histopathological assessment of the antrum gastric mucosa showed that 28.8% of the children with active *H.*

Photo 1. Antrum mucosa. Immunohistochemical reaction with CD20⁺. Magnification 200x.

pylori infection had lymphatic follicles. In children after *H. pylori* eradication, lymphatic follicles were present in 20.7% of them.

A quantitative assessment of CD20⁺ lymphocytes in the antrum mucosa (the mean number of cells per 1 mm² of specimen area), performed by immunohistochemical staining, revealed a significant increase in their expression in children with *H. pylori* infection (109.9 cells/mm²), in comparison with children after eradication (20.6 cells/mm²) and in the controls (17.4 cells/mm²) Table 1 and Photo 1.

The expression of CD20⁺ increased with the severity of inflammation in the gastric antrum. The expression of CD20⁺ lymphocytes in moderate inflammation of the antrum mucosa equalled 86.7 cells/mm² in the children with *H. pylori* infection. In case of severe inflammation of the antrum mucosa, the expression of CD20⁺ lymphocytes was 127.77 cells/mm² (Fig. 1).

Discussion

The quantitative analysis of CD20⁺ lymphocytes in the antrum mucosa showed their five-fold higher expression in children with ongoing *H. pylori* infection than in those after past infection, which may prove a significant role of the humoral response in the elimination of *H. pylori* infection in the examined group. The expression of CD20⁺ lymphocytes correlated with the severity of inflammatory process in the antrum.

Elitsur et al. [6] compared the composition of immunocompetent cells in the lymphatic nodules in children and in the MALT type lymphoma in adults, using the immunohistochemical techniques. T lymphocytes were observed only in the lymphatic follicles of children. No such lymphocytes were found in the MALT - type lymphoma in adults. Apart from CD20⁺ markers, no significant differences were observed between B lymphocytes in the lymphatic follicles of children and B - cell lymphomas of the MALT - type in adults. Krenska - Wiącek et al. [7] evaluated B-lymphocytes with CD19⁺ marker in blood serum from children with *Helicobacter pylori* - related gastritis. The authors found a higher percentage of B CD19⁺ lymphocytes in children with infection than in healthy children and a significant decrease in the percentage of these lymphocytes after eradication.

Our study proved a higher expression of CD20⁺ lymphocytes in the inflammatory infiltrate of the antrum mucosa and a correlation between their expression and the severity of the inflammation in children with *H. pylori* infection, in comparison with children after bacteria eradication. This confirms a role of B-lymphocytes in the complex pathogenic mechanisms of inflamed gastric mucosa in children *H. pylori* infected.

Conclusion

The expression of CD20⁺ lymphocytes, assessed in the antrum mucosa of children with *H. pylori* infection, increased with the severity of inflammatory process and differed statistically significantly with regards to the inflammation grade.

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