Peripheral blood lymphocyte population in children infected with Helicobacter pylori

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Abstract

Purpose: Helicobacter pylori infection in children is associated with a chronic inflammatory process of gastric and duodenal mucosa, which may have a various clinical course ranging from asymptomatic and chronic inflammatory condition to gastric ulceration. The immune system may contribute especially to chronic gastric mucosa inflammation.

The aim of our study was to assess the levels of peripheral blood T ($CD3^+$, $CD4^+$, $CD8^+$) and B lymphocyte subpopulation ($CD19^+$) in children with Helicobacter pylori infection and to evaluate their relation to degree of antrum mucosa inflammation.

Meterial and methods: The study was performed in 32 children aged 7-18 years, hospitalized due to dyspeptic symptoms. The endoscopic examination of upper gastrointestinal tract was performed and gastric and duodenal mucosa was estimated in all patients.

The endoscopic and histological evaluation of gastric mucosa was performed according to the Sydney System [4]. The urease test (CLO-test – H. pylori) was made to estimate the severity of the infection.

Results: Moderate antrum mucosa inflammation was found in 41.2% of the examined. The highest percentage of children (58.8%) presented marked inflammation. No mild inflammation was found in children examined.

Conclusions: No correlation was found between lymphocyte levels and the degree of the inflammatory changes in antrum mucosa. The evaluation of peripheral blood lymphocytes performed in children with Helicobacter pylori infection suggests that T lymphocytes may play a predominant role in this infection.

Key words: Helicobacter pylori, peripheral blood lymphocytes, children.

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Received 29.05.2003 Accepted 27.06.2003

Introduction

Helicobacter pylori is the most important causative agent responsible for a chronic inflammatory process in duodenal and gastric mucosa. The structural components of bacterium dependent on its strain, induce the mobilization of the host's immune system to the immune response. However, its reaction varies with regard to the host's individual features and its age [1].

Most studies present the role of T lymphocytes and their assessment in blood and gastric mucosa. The data from the literature referring to adults indicate an increase in $CD4^+$ and $CD8^+$ lymphocytes in gastric mucosa of patients with chronic inflammation caused by Helicobacter pylori infection [2,3].

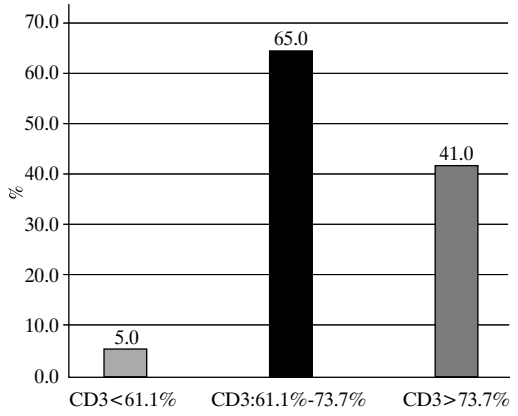
The purpose of the study was to assess the quantity of peripheral blood T ($CD3^+$, $CD4^+$, $CD8^+$) and B ($CD19^+$) lymphocyte subpopulation in children infected with Helicobacter pylori taking into consideration clinical symptoms or their absence. The results obtained were compared to the severity of gastric mucosa inflammation within antrum.

Material and methods

The study was performed in 32 children aged 7-18 years, hospitalized due to dyspeptic symptoms, in the III Department of Children's Diseases in the Medical University of Białystok. The endoscopic examination of upper gastrointestinal tract was performed and gastric and duodenal mucosa was estimated in all patients.

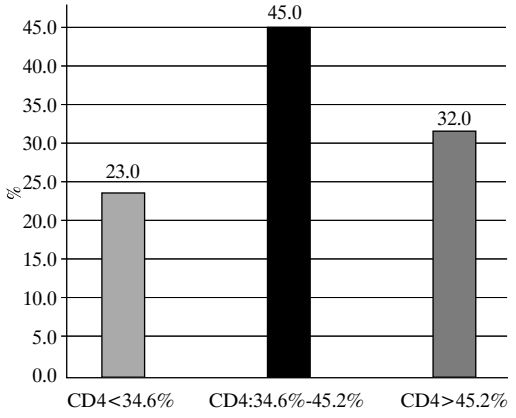
The endoscopic and histological evaluation of gastric mucosa was performed according to the Sydney System [4].

Figure 1. Evaluation of CD3 lymphocyte population in the course of Helicobacter pylori infection in children aged 7-18 years.



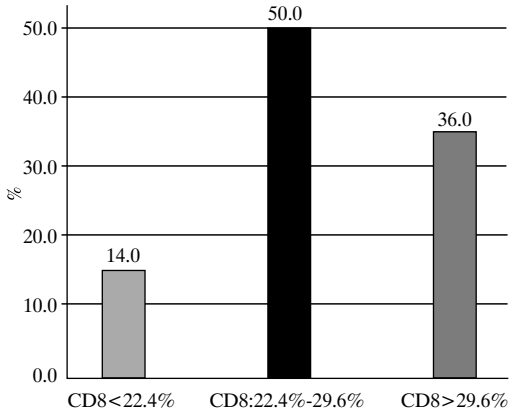
61.1%-73.3% – interval of CD3 referential values for children aged 7-18 years.

Figure 2. Evaluation of CD4 lymphocyte population in the course of Helicobacter pylori infection in children aged 7-18 years.



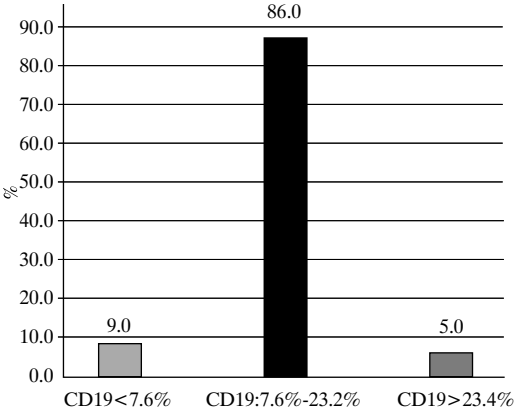
34.6%-45.2% – interval of CD4 referential values for children aged 7-18 years.

Figure 3. Evaluation of CD8 lymphocyte population in the course of Helicobacter pylori infection in children aged 7-18 years.



22.4%-29.6% – interval of CD8 referential values for children aged 7-18 years.

Figure 4. Evaluation of CD19 lymphocyte population in the course of Helicobacter pylori infection in children aged 7-18 years.



7.6%-23.2% – interval of CD19 referential values for children aged 7-18 years.

The urease test (CLO-test – H. pylori) was made to estimate the severity of the infection.

The histological assessment of antrum and corpus mocosa was carried out according to the Sydney System. It estimated the severity of Helicobacter pylori infection and the severity of antrum and corpus mucosa inflammation using hematoxylin and eosin (H-E) and modified Giemsa staining.

Peripheral blood lymphocyte subpopulations were examined with a flow cytometry instrument – EPICS XL of COULTER firm, using specific monoclonal antibodies against superficial antigen by means of triple fluorescence. The following types of peripheral blood cells were analyzed: $CD3^+$, $CD4^+$, $CD8^+$, $CD19^+$.

The values of lymphocyte populations examined in patients were compared to the referential values from the flow cytometry laboratory for the individual cells examined with regard to age intervals [5].

The study was performed in two groups of children:

1. group of 17 children (53.1%) aged 7-18 years with Helicobacter pylori – associated chronic gastric mucosa inflammation (8 girls, 9 boys; mean age – 12.9 years);

2. group of 15 children (46.9%) aged 7-15 years with dyspeptic symptoms but normal gastric mucosa and without Helicobacter pylori infection (7 girls, 8 boys; mean age – 12.0 years).

Both clinical and laboratory examinations were carried out with the consent of children's parents and approved by the Committee of Ethics at the Medical University of Białystok.

The results obtained were analyzed statistically. Statistical comparisons were done with Kołmogorov-Smirnov test. A p value <0.05 was considered to be significant. The results were presented in a graphic form.

Results

The evaluation of peripheral blood $CD3^+$ lymphocyte T population was performed using specific monoclonal antibod-

Figure 5. Evaluation of CD3 lymphocyte population in blood serum of children Helicobacter pylori infected and the severity of antrum mucosa inflammation.

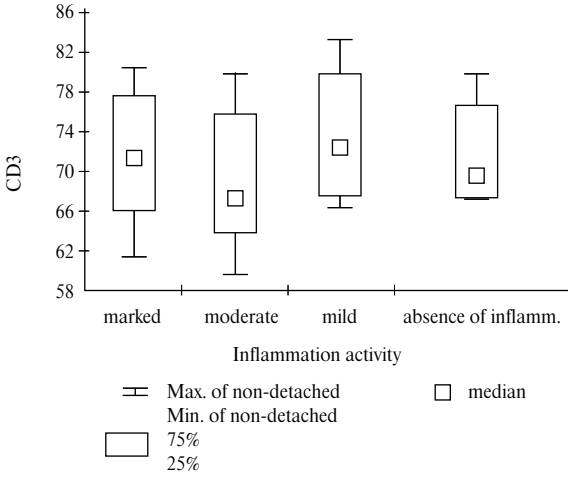


Figure 7. CD8 lymphocytes in blood serum of children infected with Helicobacter pylori and severity of antrum mucosa inflammation.

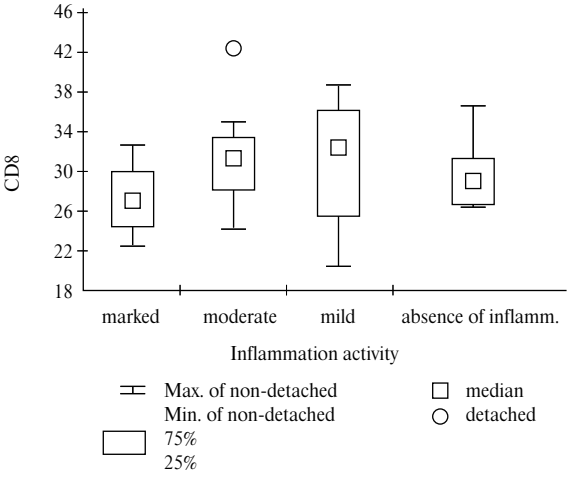


Figure 6. CD4 lymphocytes in blood serum of children infected with Helicobacter pylori and the severity of antrum mucosa inflammation.

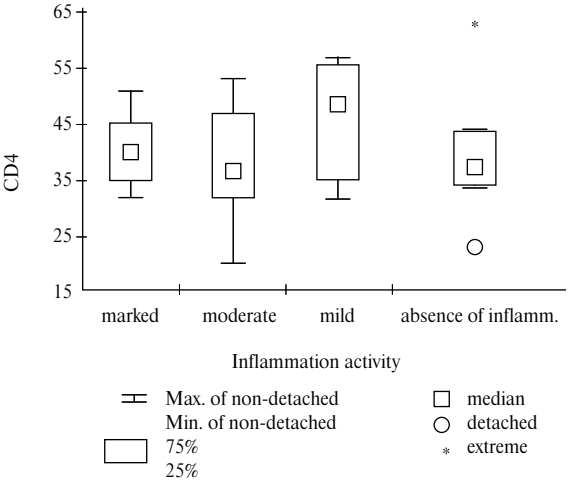
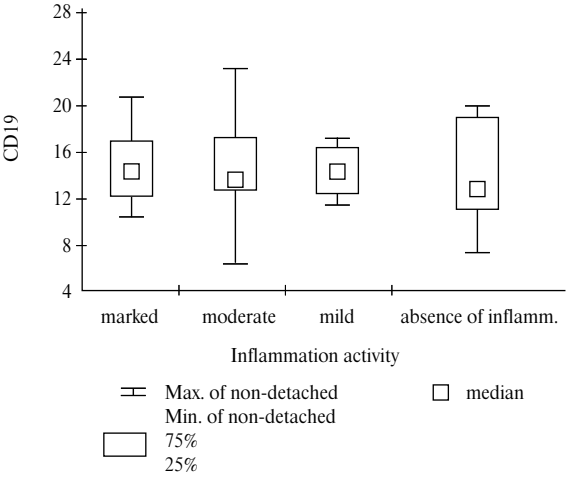


Figure 8. CD19 lymphocytes in blood serum of Helicobacter pylori infected children and severity of antrum mucosa inflammation.



ies against superficial antigen by means of triple fluorescence. It proved an increase in the percentage of these cells above the upper limit of referential value interval for the age in 41.0% of the examined. In 5% of children infected, the values of $CD3^+$ were below the lower limit of referential value (*Fig. 1*).

The evaluation of $CD4^+$ lymphocyte T population in the course of Helicobacter pylori indicated the values below the interval of referential values for the age of 7-18 years in 23% of children included in the study. The values of $CD4^+$ were above the upper referential value for this age group in 32% of the examined (*Fig. 2*).

In our study, the assessment of peripheral blood $CD8^+$ lymphocyte T population showed the values of these cells within the referential value interval in 59% of cases children infected with Helicobacter pylori and above the upper limit of norms accepted for this age in 36% of the examined (*Fig. 3*).

The evaluation of peripheral blood B lymphocytes ($CD19^+$) proved that their values were included in the referential value interval in 86% of children examined; they were below – in 9%, and above the age norms accepted – in 5,0% of the examined cases (*Fig. 4*).

The histological evaluation of gastric mucosa biopsies performed in children with infected Helicobacter pylori according to the Sydney System, made it possible to estimate the severity of mucosa inflammation. Moderate antrum mucosa inflammation was found in 41.2% of thesaces. The highest percentage of children (58.8%) presented marked inflammation. No mild inflammation was found in examined children. Moderate antrum mucosa inflammation measured by the number of infiltrating granulocytes was observed in 52.9% of cases, whereas marked inflammation was found in 47.1% of children Helicobacter pylori infected.

The analysis of percentage values of peripheral blood T and B lymphocyte subpopulations proved no statistically significant differences in comparison with the severity of gastric antrum mucosa inflammation in children Helicobacter pylori infected.

The following figures present the results obtained in our study: *Fig. 5, 6, 7, 8*.

Discussion

Antigen-specific and non-specific response of the immune system caused by Helicobacter pylori antigen stimulation leads to gastric and duodenal mucosa inflammation. This response may promote the infection elimination, but may also contribute to gastric mucosa damage.

In people with infected Helicobacter pylori, specific IgG and IgA antibodies in gastric secretion prove that gastric mucosa takes part in a immune response [6]. Helicobacter pylori colonization in the layer of mucus or under the epithelial cells of the stomach is accompanied by immunologically competent cells. Hence, it is not completely known how a cell response influences the bacteria colonization. Gunasekaran et al. [6] described the increase in the quantity of $CD4^+$ and $CD8^+$ within mucosa follicles and $CD8^+$ in gastric epithelium during Helicobacter pylori infection.

D'Elios et al. [7] proved that in patients with chronic non-ulceric gastric or duodenal inflammation, cytokines of Th1 profile were balanced by Th2 cytokin production, which might be crucial in inducing a protective response in such patients.

Bamford [8] showed that the profile of cytokines produced by $CD4^+$ phenotype lymphocytes isolated from gastric mucosa and exposed in vitro to urease during 48 hours was the same as in patients infected and not infected with Helicobacter pylori. Th1 type cytokines were found in both groups.

In our study, the assessment of $CD3^+$ lymphocytes indicated an increase in the percentage of these cells above the upper limit of the referential value interval for the age in 41% of children examined. The values of $CD3^+$ were below the lower limit of the referential value interval for the age only in 5% of children infected with Helicobacter pylori.

The assessment of $CD4^+$ lymphocytes in the course of Helicobacter pylori infection showed the values below the interval of referential values in 23% children aged 7-18 years.

According to Crabtree, $CD8^+$ and $CD22^+$ cells are the main lymphocyte subpopulation among the lymphocytes isolated from gastric mucosa in patients infected with Helicobacter pylori infection. Though, it is not known whether they have a cytotoxic or suppressive function [9].

In our examinations, the values of $CD8^+$ lymphocytes within the referential value interval were found in 50% of children Helicobacter pylori infected and above the upper limit of the age norms – in 36% of the cases.

The results of our study indicate that peripheral blood T lymphocytes as well as $CD4^+$ and $CD8^+$ cell subpopulation were enhanced in 1/3 of patients.

The cytometric examination carried out by Quiding-Jarbrink [10] showed that there was an increase in the production of $CD4^+$ and $CD8^+$ cell subpopulations in Helicobacter pylori infected patients with ulcerative duodenal inflammation and in asymptomatic ones. Patients with Helicobacter pylori infection manifested increased IFN gamma levels [7]. This and other cytokines produced in response to T lymphocytes may be responsible for the intensified secretion of hydrochloric acid in the stomach and lead to duodenal ulceration [10].

In other study, the humoral and cellular reactions were estimated in the group of adult patients with chronic gastric mucosa inflammation and Helicobacter pylori infection and the uninfected group but stimulated with the extract of Helicobacter pylori antigens. The similar reactions were observed in both groups, but a significantly higher level of IFN gamma was reported in patients stimulated with the extract of Helicobacter pylori antigens [11].

Additionally, Th1 cells were proved to recognize specifically Helicobacter pylori antigens. The predominance of Th1 type response is not profitable for the host and the infection with highly virulent Helicobacter pylori bacterium strains together with such factors as individual genetic characteristics may lead to chronic gastric mucosa inflammation and other diseases [11].

A proper immune response which would cause the shift of cytokine profile to Th2 response leading to the initiation of the protective response of the immune system against specific Helicobacter pylori antigens, would contribute to lessening the tissue damage of the host.

The values of B lymphocytes ($CD19^+$) were within the interval of referential values in 86% of the examined cases and their increase was found in 5% of our patients.

The assessment of peripheral blood lymphocytes in children with Helicobacter pylori infection seems to confirm a dominant role of T cell in this infection.

Helicobacter pylori infection – associated inflammatory changes in gastric mucosa may persist for a few to more than ten years manifesting themselves in neutrophil infiltration in the stage. The size of leukocyte infiltration correlates with the severity and activation of gastric mucosa inflammation within antrum and corpus [12].

The results of morphological examination of gastric mucosa proved that inflammatory changes in chronic gastric mucosa inflammation differed with regard to the severity of inflammation.

The analysis of the percentage values of peripheral blood lymphocyte subpopulations with regard to the severity of the inflammatory process in the stomach in the course of Helicobacter pylori proved no statistically significant differences for these lymphocytes. The results obtained were presented in figures.

Our earlier assessment of corpus and antrum mucus cells using monoclonal antibodies showed the increase in $CD8^+$ and $CD20^+$ lymphocytes in patients Helicobacter pylori infected [12].

Hatz et al. indicated a high percentage (95%) of T cells ($CD3^+$) in the mucosa of patients with Helicobacter pylori infection. $CD4^+$ cells predominated in the lamina propria and $CD8^+$ lymphocytes constituted 75-80% of intraepithelial lymphocytes (IEL). B cells ($CD22^+$) were rarely found and lymphoid follicles were absent, whereas in our study, lymphoid follicles were reported on average in more than half of patients infected with Helicobacter pylori. The results of our study are in agreement with other authors' findings [13-15].

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