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The humoral immuneresponse to *Helicobacter pylori* infection in children with gastrointestinal symptoms

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Abstract

The prevalence of *Helicobacter pylori* is high in Eastern Europe. The purpose of this study was to estimate the prevalence of *H. pylori* in symptomatic Lithuanian children and to identify the infection by clinicopathological and serological analyses.

One hundred sixteen symptomatic children (age 8–16) with gastritis and duodenal ulcer were included. Biopsies were histologically assessed according to the Sydney-System. Serum IgG antibodies against *H. pylori* were detected by an enzyme-linked immunosorbent assay (ELISA), using low molecular mass antigen. The western blot technique was used to detect serum antibodies against the cytotoxin-associated protein (CagA) using whole cell antigen.

Histologically the prevalence of *H. pylori* infection was 79% and not influenced by demographic factors. Mucosal inflammation and atrophy were associated with a *H. pylori* infection. Intestinal metaplasia was found in eight children, suggesting early *H. pylori* acquisition in life.

Increased levels of IgG antibodies were detected in 57% of children. The prevalence of IgG antibodies was significantly higher in patients with duodenal ulcer compared to children with gastritis. Forty-four (67%) *H. pylori*-seropositive children had antibodies against CagA. Low molecular weight-ELISA and whole cell-western blot results were significantly associated with histopathology, the presence of duodenal ulcer and the CagA status. A high number of false seronegative cases were due to poor immunological responses in children and poor locally validated tests.

The prevalence of *H. pylori* infection in Lithuanian children is higher compared to Western Europe. The infection is acquired in early life. Diagnosing *H. pylori* infection, serology is helpful, but endoscopy/histology remains as gold standard.

Keywords: *Helicobacter pylori*; IgG antibodies; Infection; ELISA; Immunoblot; Children; Lithuania

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1. Introduction

In recent years epidemiological data regarding the prevalence of *H. pylori* infection in different age groups and populations came from all over the world.

The etiologic importance of *H. pylori* infection in the development of various gastrointestinal diseases including neoplastic changes in the upper gastrointestinal tract is widely accepted and the infection is mostly acquired in early childhood [1], and if untreated, the infection remains in the gastric mucosa for life [2]. The acquisition rate of *H. pylori* infection varies greatly between populations and is much higher in developing compared to industrialized countries [3–5].

Similar to those data obtained from studies on *H. pylori* in adults, children in developing countries show a higher prevalence of *H. pylori* infection [6–8] compared with paediatric patients in developed countries [9]. The prevalence of *H. pylori* infection in children varies and appears to be mostly dependent on the socioeconomic status. The knowledge about geographic differences of *H. pylori* strains is limited. Especially in certain populations in developing countries where the *H. pylori* infection still lack a good documentation [9].

To diagnose and characterise the infection in children some problems exist. Using non-invasive tests would make it much easier to screen these populations. Unfortunately, such tests are not available everywhere and appear often less accurate than in adults [10]. Obviously, due to the improvement of economic status in many Eastern European countries, the prevalence of *H. pylori* gets closer to those found in developed countries [11]. Studies from Lithuania have shown that more than 60% of young adult population and more than 80% of people older than 40 years old are infected with *H. pylori*. However, the prevalence of *H. pylori* in children is unknown.

The purpose of our study was to determine the prevalence of *H. pylori* infection in Lithuanian schoolchildren and to characterise the infection based on clinicopathological data and IgG antibody levels to *H. pylori* using well-standardized ELISA and western blot techniques.

2. Material and methods

The study was approved by the Ethics Committee of the Kaunas University of Medicine. Informed consent to participate in the study was obtained from the children and from their parents.

2.1. Subjects and endoscopy

One hundred sixteen children with upper gastrointestinal symptoms were consecutively admitted to endoscopy at the Kaunas University of Medicine, performed in a standardised manner by one single experienced endoscopist. Endoscopic findings were recorded according to the updated Sydney-System [12] and two antral biopsies and one biopsy from corpus were taken for histology and rapid urease test.

2.2. Histology

One biopsy from antrum (lesser curvature, 2 cm from pylorus) and one biopsy from corpus (lesser curvature, 4 cm from angular incisure) were routinely paraffin processed. Sections were stained with hematoxylin–eosin, Giemsa and periodic acid Schiff (PAS) staining. Morphology, inflammation of the antrum and corpus, and *Helicobacter*-like organisms (HLO) were all evaluated by the same experienced pathologist according to the updated Sydney-System [12].

2.3. Rapid urease test

One antral biopsy (angular incisure) was used for rapid urease test (RUT®, Poland) according to the manufacture's procedure.

2.4. Detection of serum IgG antibodies against H. pylori

2.4.1. Serum samples

Blood samples were obtained from all patients by venous puncture. Serum was separated by centrifugation and stored at −20 °C until use.

2.4.2. LMW-ELISA

Serum samples were tested for the presence of antibodies against *H. pylori* by ELISA, using a low molecular mass (LMW) antigen (mainly 15–35 kDa) prepared by filtration, as described previously [13]. Briefly, microtiter plates were coated with 100 μl LMW antigen preparation (10 μg ml^{-1}) diluted 1:10 in phosphate buffer saline (PBS), pH 7.4, and incubated overnight. Plates were washed and serum diluted 1:100 was added to the plates and each sample was tested in triplicate and positive and negative controls were included. The plates were washed and 100-μl horseradish-peroxidase-conjugated rabbit antibodies against human IgG diluted 1:2000 were added to each well. The chromogenic reaction was developed with orthophenylediamine (4 mg OPD in 10 ml citric acid buffer and 15 μl 30% H_2O_2 was added just before use). The chromogenic reaction was stopped with 250 μl H_2SO_4. The absorbance was read in a spectrophotometer at 492 nm. The test values were corrected for day-to-day and plate-to-plate variation by dilutions of control sera. The results were expressed as ELISA units (EU). The cut-off levels of positive and negative results have been estimated in a