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Lack of an Association between *Helicobacter* Infection and Autoimmune Hepatitis in Children

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Abstract

An association between *Helicobacter* infection and autoimmune hepatitis (AIH) in children was investigated. The prevalence of antibodies to *H. pylori* did not differ between the AIH and the control group, (22% versus 14%), and antibodies to non-gastric *Helicobacter* were not detected in either group. *H. pylori* DNA was found in two AIH liver tissues, but *Helicobacter* was not cultured from any sample.

Key words: autoimmune hepatitis, *Helicobacter*

In recent years a body of evidence suggesting a possible association between *Helicobacter* sp. infection and hepatobiliary diseases has arisen from both animal and human studies. It has been shown that persistent *Helicobacter hepaticus* infection in mice can induce chronic active hepatitis with subsequent liver tumors (Ward *et al*., 1994). Genetic material of various *Helicobacter* species has been detected in bile, gallbladder and liver tissues from patients with cholecystitis (Fox *et al.*, 1998), hepatocellular carcinoma (Nilsson *et al.*, 2001), primary biliary cirrhosis (PBC) and primary sclerosing cholangitis (PSC) (Nilsson *et al.*, 2000). All these findings have prompted us to investigate if there is any association between *Helicobacter* infection and autoimmune hepatitis (AIH).

A total of 32 children (mean age 12.8 years; range 3–18 years; 20 females, 12 males) with autoimmune hepatitis presenting for control investigation including liver biopsy were included in the study. The autoantibody profile was consistent with type I AIH in 30 children (antinuclear antibodies and/or smooth muscle antibodies), whereas the remaining two patients had type II AIH (antibodies to liver-kidney microsome type 1). From each child 2 ml of blood was taken for serology and a transcutaneous liver biopsy was obtained for histology, culturing and PCR. The control group for the comparison of seroprevalence of antibodies to *H. pylori* and non-gastric *Helicobacter* species consisted of 44 healthy children (mean age 10.3 years; range 1–18 years, 20 females, 24 males) presenting either for a routine vaccination or otherwise healthy children treated in the Department of Orthopedic Surgery.

The study was undertaken with the approval of the Ethics Committee of Children's Memorial Health Institute, and informed consent was obtained from all participants.

Antibodies to *Helicobacter pylori* and non-gastric *Helicobacter* species (*i.e. H. pullorum*, *H. bilis*, *H. hepaticus*), were detected using immunoblot assay, as described previously (Nilsson *et al.*, 1997; Kornilovs'ka *et al.*, 2002). Cultures of liver samples were performed on Wilkins Chalgren agar with 7% horse blood and Dent's selective supplement SR 147 (Oxoid) under microaerophilic conditions at 37°C for

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up to 21 days. All liver samples from AIH patients were analysed by PCR to detect *Helicobacter* sp. DNA. The DNA was extracted by use of QIAamp DNA Mini kit (Qiagen). The purified DNA was subjected to two separated PCRs with *Helicobacter* genus-specific primers (Fox *et al.*, 1998) generating 16S rRNA amplicons of 1200 or 400 bp. Amplified DNA fragments were separated by electrophoresis on 2% agarose gel, stained with ethidium bromide and visualized under UV light. The 400 bp PCR products were purified using PCR-purification kit (Qiagen) and subjected to direct sequencing of both strands with an automated DNA sequencer (ABI PRISM 310).

The prevalence of antibodies to *Helicobacter* in AIH patients and controls was compared using the chi-squared test. The prevalence of antibodies to *H. pylori* and non-gastric *Helicobacter* species did not differ significantly between AIH children and control subjects. Antibodies to *H. pylori* were found in 7 of 32 (22%) AIH patients compared with 6 of 44 (14%) controls. Antibodies against non-gastric *Helicobacter* species were not detected in any patient from either group. *Helicobacter* DNA was found in the liver samples from two children with AIH (6%). The sequencing analysis revealed that both sequences closely resembled *H. pylori*, with the similarity rates exceeding 99.7%. The two patients whose liver samples contained *H. pylori* DNA were also anti-*H. pylori* positive. None of the liver cultures showed the presence of *Helicobacter* species.

The present study revealed that the seroprevalence of antibodies to *H. pylori* did not differ significantly between children with AIH and a control group. This finding is consistent with previous observations, indicating that the frequencies of antibodies to *H. pylori* in adult AIH patients were similar to those in controls (Durazzo *et al.*, 2002; Nilsson *et al.*, 2003). On the other hand, AIH and PSC adult patients were reported to have a higher prevalence of anti-*H. pullorum*, anti-*H. bilis* and anti-*H. hepaticus* antibodies as compared to blood donors (Nilsson *et al.*, 2003), which is in conflict with the present results, where no such antibodies were detected in either AIH or control subjects. Since both, the former (Nilsson *et al.*, 2003) and the present, serological studies have been performed in the same laboratory (Department of Medical Microbiology, Lund University) using the same methods, this discrepancy cannot be attributed to methodological differences. It could, however, be related to regional variability and differences in patient populations. The Polish children participating in the study were much younger than the Swedish patients, which could influence the exposure to *Helicobacter* species.

Helicobacter DNA was found in only two AIH liver tissues, and both sequences showed high similarity to *H. pylori*. This finding is in line with the serological results, since both patients were also anti-*H. pylori* positive and did not have antibodies to other *Helicobacter* species tested. However, despite prolonged incubation, we were not able to culture *Helicobacter* sp. from any of tested samples. Similarly, with one exception (Queiroz *et al.*, 2001), all other attempts to cultivate *Helicobacter* sp. from human hepatobiliary materials have been unsuccessful, in spite of the presence of their genetic material (Fox *et al.*, 1998; Avenaud *et al.*, 2000; Silva *et al.*, 2003). The inability to cultivate *Helicobacter* in some of the former studies was suggested to be attributed to the fact that long-stored frozen samples have been used for culturing (Fox *et al.*, 1998; Silva *et al.*, 2003). In our study, however, all the samples were cultured directly after collection, and therefore sample processing was unlikely to influence the results. Detection of *H. pylori* DNA, but not culturable cells in the liver tissues of AIH patients could therefore indicate that the bacteria were either nonviable or that they were present in very low numbers or in a non-culturable coccoid form. The fact that *Helicobacter* genetic material was found only in two AIH liver samples and the seroprevalence of anti-*Helicobacter* antibodies did not differ between the AIH and the control group suggests that these bacteria are unlikely to be associated with the pathogenesis of autoimmune hepatitis in children.

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