JOURNAL OF CLINICAL MICROBIOLOGY, Jan. 2000, p. 68–70 Vol. 38, No. 1
0095-1137/00/$04.00+0

CagA Antibodies in Japanese Children with Nodular Gastritis or Peptic Ulcer Disease

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Received 26 July 1999/Returned for modification 31 August 1999/Accepted 9 October 1999

***cagA*$^+$ *Helicobacter pylori* strains have been linked to more severe gastric inflammation, peptic ulcer disease, and gastric cancer in adults, but there have been few studies of *cagA* in children. We examined the relationship between *H. pylori cagA* status and clinical status in Japanese children. Forty *H. pylori*-positive children were studied: 15 with nodular gastritis, 5 with gastric ulcers, and 20 with duodenal ulcers. *H. pylori* status was confirmed by biopsy-based tests and serum anti-*H. pylori* immunoglobulin G (IgG) antibody. As controls, 77 asymptomatic children with sera positive for anti-*H. pylori* IgG were enrolled. Levels of IgG antibodies to CagA in serum were measured by an antigen-specific enzyme-linked immunosorbent assay. In 16 patients with successful *H. pylori* eradication, posttreatment levels of CagA and *H. pylori* IgG antibodies also were studied. The CagA antibody seropositivities of asymptomatic controls (81.8%) and patients with nodular gastritis, gastric ulcers, and duodenal ulcers (80.0 to 95.0%) were not significantly different. Compared with pretreatment levels of CagA antibodies, posttreatment levels decreased progressively and significantly. We conclude that, as in Japanese adults, a high prevalence of *cagA*$^+$ *H. pylori* strains was found in Japanese children, and that there was no association with nodular gastritis or peptic ulcer disease. In the assessment of eradicative therapies, monitoring of serum anti-CagA antibodies does not appear to offer any direct benefit over monitoring of anti-*H. pylori* antibodies.**

It is widely recognized that colonization with *Helicobacter pylori* induces a persistent gastric tissue response and is an important risk factor for peptic ulcer disease and gastric cancer (4). However, the majority of *H. pylori*-positive persons are asymptomatic throughout their lifetime, and it is not known why only a subset of positive patients develop ulcer disease and cancer. Variation in clinical outcomes has been attributed to differences in bacterial strains, hosts, and environmental factors.

H. pylori strains are genetically diverse (13, 33). Although of unknown function, the cytotoxin-associated gene A (*cagA*) has been identified as a possible marker of virulence of *H. pylori* (5). Since the cytotoxin-associated gene product (CagA, 120 to 140 kDa) encoded by *cagA* is immunodominant (10, 34), a specific immune response to the CagA protein is induced as long as *H. pylori* colonization persists (6). Therefore, serum immunoglobulin G (IgG) antibodies to the CagA antigen may be a reliable marker of carriage of a *cagA*$^+$ *H. pylori* strain (10, 12) which includes the *cag* pathogenicity island (9, 35). In Western populations, *cagA*$^+$ *H. pylori* strains induce more severe gastric mucosal inflammation than *cagA* gene-negative strains (10, 15, 20) and are associated with higher risks of peptic ulcer disease (11, 12, 15) and gastric cancer (6, 16). However, there is wide geographical variation in the prevalence of *cagA*$^+$ strains (1, 29, 37) and in their genotype (28), and it is unknown whether *cagA*$^+$ strains represent a universal marker for these *H. pylori*-associated diseases. Among adults in Japan, there is no clear relationship between *cagA*$^+$ *H. pylori* strains and enhanced risk of disease (21).

Childhood is the critical period for acquisition of *H. pylori* (2, 27). As in adults, *H. pylori* appears to be associated with both a tissue response (gastritis) and duodenal ulcer in children (32). However, there have been few studies of CagA seroprevalence in children (7, 20), and its role in peptic ulcer disease has not been studied. In this study, we examined whether *H. pylori* CagA status was associated in Japanese children with nodular gastritis, which is a unique endoscopic characteristic in childhood (18, 24), and with peptic ulcer disease.

MATERIALS AND METHODS

Patients. A total of 40 *H. pylori*-positive dyspeptic patients were enrolled in this study: 20 patients with duodenal ulcer, 5 with gastric ulcer, and 15 with nodular gastritis alone (Table 1). Diagnoses were determined on the basis of findings by upper gastrointestinal endoscopy. Nodular gastritis, defined as endoscopically proven multiple nodularity in the antrum with lymphoid follicles and inflammatory cell infiltration in the lamina propria, is believed to be the major form of *H. pylori* gastritis in childhood (18, 24). The patients selected had no underlying diseases and were not taking medications, including nonsteroidal anti-inflammatory drugs. *H. pylori* status was assessed by biopsy-based tests (rapid biopsy urease test, histology, and culture) and testing for the presence of serum anti-*H. pylori* IgG antibody with a commercial enzyme-linked immunosorbent assay (ELISA) kit (HM-CAP; Enteric Products, Inc., Westbury, N.Y.). In adults, because *H. pylori* is often difficult to isolate in culture, nonculture techniques (histology, rapid biopsy urease test, serology, or urea breath test) are performed for diagnosing *H. pylori* infection (17). Our previous studies have demonstrated that compared with biopsy tests, the sensitivity of anti-*H. pylori* IgG and IgA antibodies were 88.2 and 91.2%, respectively (22). Even when *H. pylori* has not been cultured, the presence of the organism can be confirmed by a combination of these techniques. As controls, 77 asymptomatic children with positive anti-*H. pylori* IgG tests, who did not undergo endoscopy, were enrolled into this study. All sera were stored at −20°C until assay. Sixteen patients who received eradication therapy (proton pump inhibitor-based dual or triple regimens) and had successful eradication of *H. pylori* (23, 24) were studied at serial intervals. In these patients, pretreatment and posttreatment levels of *H. pylori* IgG antibodies were measured by using HM-CAP. Serum samples were taken

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TABLE 1. Characteristics of 117 study patients

Clinical status	No. of patients			Mean age (yr) ± SD (range)
	Male	Female	Total	
Gastric ulcer	2	3	5	12.2 ± 4.5 (4–16)
Duodenal ulcer	16	4	20	12.3 ± 2.6 (5–16)
Nodular gastritis	5	10	15	14.1 ± 1.4 (11–16)
Asymptomatic control	33	44	77	12.0 ± 3.5 (3–16)
Total	56	61	117	12.4 ± 3.3 (3–16)

pretreatment and at 3, 6, and 12 months after completion of eradication therapy. Informed consent was obtained from patients or their parents in all cases.

CagA antibodies. Serum anti-CagA IgG antibody levels were assayed as previously described (6). Briefly, a recombinant protein fragment of CagA (ORV220; OraVax, Inc., Cambridge, Mass.) that was purified from *Escherichia coli* cell lysates was used as an antigen and was fixed to a 96-well plate in carbonate-bicarbonate buffer. After incubation of treated wells with serum diluted 1:100, alkaline phosphatase-conjugated goat anti-human IgG (1:1,000 dilution) was added. After addition of the phosphatase substrate, absorbance was read at 405 nm. Based on results from *H. pylori*-negative controls in adults, a value of ≥0.2 ELISA unit (EU) of CagA IgG antibodies was considered to be positive (21).

Statistical methods. The difference in CagA seropositivity between asymptomatic controls and patients with each of the three clinical diagnoses was analysed by Fisher's exact test. Differences between pretreatment and posttreatment levels of CagA and differences between posttreatment levels of CagA and *H. pylori* IgG antibodies were analyzed by the paired *t* test. A value of $P < 0.05$ was regarded as statistically significant. Values were presented as means ± standard deviations.

RESULTS

CagA seropositivity. Among the *H. pylori*-positive children in this study, a high percentage in each group were seropositive for anti-CagA IgG antibodies (Table 2). There were no significant differences in seropositivity rates and levels of CagA antibodies between asymptomatic controls and patients with ulcer disease or nodular gastritis.

Effect of treatment on CagA antibody levels. All 16 patients who had successful eradication were seropositive for CagA antibodies before therapy. In these patients, the mean pretreatment level of anti-CagA antibodies was 1.0 ± 0.6 EU. Posttreatment levels were significantly decreased at 3 months (0.7 ± 0.5 EU; $P < 0.001$), at 6 months (0.6 ± 0.4 EU; $P < 0.001$), and at 12 months (0.5 ± 0.3 EU; $P < 0.01$), compared with the pretreatment levels (Fig. 1). Nevertheless, by using the threshold of 0.2 EU as the indicator of seropositivity, 8 (64%) of 11 patients studied remained seropositive even at 12 months after therapy. At 3 months posttreatment, the percent decrease in CagA antibody levels was greater than that in *H. pylori* IgG levels ($P < 0.05$).

TABLE 2. Prevalence and levels of serum anti-CagA antibody in *H. pylori*-positive controls and patients

Clinical status	No. of patients	% CagA antibody positive (95% CI)[a]	P^b	CagA level (EU)[b]	P^c
Gastric ulcer	5	80.0 (44.9–100)	1.00	1.2 ± 0.7	0.90
Duodenal ulcer	20	95.0 (85.5–100)	0.18	1.2 ± 0.9	0.87
Nodular gastritis	15	93.3 (80.7–100)	0.45	0.9 ± 0.5	0.10
Asymptomatic control	77	81.8 (73.2–90.4)		1.3 ± 0.8	

[a] CI, confidence interval.
[b] Compared with asymptomatic controls.
[c] Among children who were CagA antibody positive.

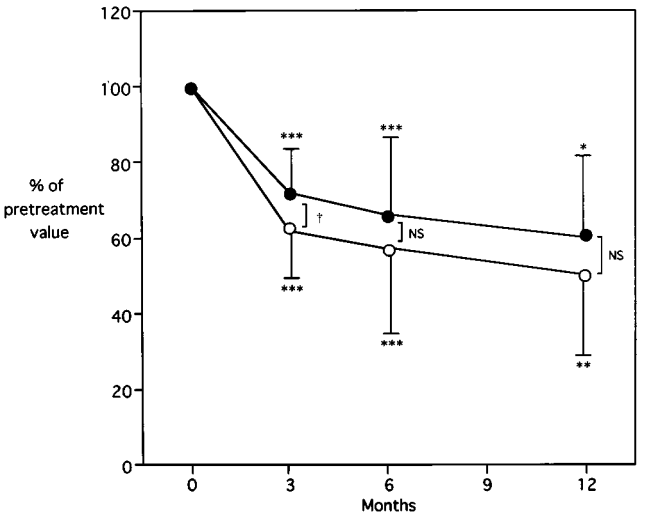

FIG. 1. Levels of serum anti-CagA (open circles) and anti-*H. pylori* IgG antibodies (solid circles) after eradication therapy in 16 patients. Mean posttreatment levels at specified follow-up times are expressed as percentages of pretreatment levels. Error bars, standard deviations. *, $P < 0.05$ versus pretreatment level; **, $P < 0.01$; ***, $P < 0.001$. NS, not significant. †, $P < 0.05$, comparing anti-*H. pylori* and anti-CagA antibodies.

DISCUSSION

In Western countries, patients with duodenal ulcer are more likely to be colonized by *cagA*$^+$ *H. pylori* strains than patients with gastritis alone (10, 11, 15). In the West, *H. pylori* strains that are *cagA*$^+$ also are associated with more-substantial gastric tissue involvement with increased neutrophil infiltration, in adults (10, 15) and in children (20). Similarly, in gastric mucosa from persons colonized with *cagA*$^+$ strains, increased gastric epithelial cell interleukin-8 (IL-8) mRNA (38) and IL-8 secretion (14, 31) have been observed. However, similarly close correlations have not been universally observed (19). In addition, among Swedish children and adolescents, there was no significant correlation between degree of gastric inflammation and *H. pylori* cytotoxin production (8).

In Western patients, CagA seropositivity has been shown to be higher in those who have atrophic gastritis, which is a precursor to gastric cancer (3, 26). Although *cagA*$^+$ strains may play a role in the development of gastric cancer, they are neither necessary nor sufficient for this process (6). In Japan, high CagA seropositivity rates have been observed in asymptomatic adults, reinforcing the insufficiency of carriage of a *cagA*$^+$ strain for gastric cancer development (21). CagA seroprevalence varies geographically (1, 29, 37). The frequency of *cagA*$^+$ strains observed in asymptomatic children has ranged from 76% in Mexico (7) to 40% in France (20). In Japan, *cagA*$^+$ *H. pylori* strains are common in persons of all ages. Thus, available evidence from this and previous studies (1, 28, 29, 37) suggests that *cagA*$^+$ strains are not significant as disease-specific pathogenetic markers.

Allelic differences within *cagA* that distinguish Western and East Asian *cagA*$^+$ *H. pylori* strains have been reported (28, 36). These differences may reflect variation in other parts of the *cag* pathogenicity island, which has been considered to encode potential virulence-related genes. It is possible that Asian *cagA*$^+$ *H. pylori* strains do not induce the accentuated tissue damage caused by Western *cagA*$^+$ strains. In any event, both host and bacterial factors should be considered in order to understand the pathogenesis of *H. pylori*-associated diseases.

As a noninvasive assay, determination of serum CagA IgG

antibody levels may be useful in the assessment of eradicative therapy, as is evaluation of *H. pylori* IgG antibody levels (25, 30). In this study, levels of serum CagA IgG antibodies decreased significantly after eradication of *H. pylori* using antimicrobial therapy. However, most successfully treated patients remained CagA seropositive for months during the follow-up period. Similarly, seroconversion to negativity for *H. pylori* IgG and IgA antibodies requires about 12 months after successful eradication (22). Thus, the humoral immune responses, not only to *H. pylori* group antigens but also to the CagA protein, do not quickly disappear after the organism is eliminated. Assessing anti-CagA antibodies as a marker for eradication does not appear to offer any direct benefit over use of anti-*H. pylori* antibodies alone.

ACKNOWLEDGMENTS

We thank Takuji Fujisawa (Kurume, Japan), Hitoshi Tajiri (Osaka, Japan), Mutsuko Konno (Sapporo, Japan), and Shun-ichi Maisawa (Morioka, Japan) for providing the sera of patients and controls.

This work was supported in part by the Medical Research Service of the Department of Veterans Affairs.

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