Prevalence of CagA and VacA Antibodies in Children with Helicobacter pylori-Associated Peptic Ulcer Compared to Prevalence in Pediatric Patients with Active or Nonactive Chronic Gastritis

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Helicobacter pylori is associated nowadays with different digestive diseases, such as gastritis, gastric and duodenal ulcer, and mucosa-associated lymphoid tissue lymphoma, and is considered to be a risk factor for the development of gastric cancer (16). The reasons for developing one or another disease are not well understood and several factors are possibly involved (1).

Some virulence factors in H. pylori clinical isolates (such as CagA or VacA) have been proposed as related to more severe gastric diseases in adults (4, 18), although some reports indicate that a high prevalence of cagA gene is found irrespective of the disease developed (5, 13, 15). Little information exists as to the prevalence of infection by CagA- and VacA-positive bacteria among asymptomatic or symptomatic children suffering different levels of lesions (6). Overall, very few data exist on the prevalence of these virulence markers in children with duodenal or gastric ulcer (10).

The aim of this study was to determine the antibody response to six different antigens in pediatric patients infected with H. pylori who had a peptic ulcer (PU) (gastric or duodenal), compared with the response in patients who had nonactive chronic gastritis (NACG) or active chronic gastritis (ACG).

A total of 117 H. pylori-positive children submitted to gastroscopy due to exhibiting different clinical symptoms were enrolled in a prospective study to test two different eradication therapies from November 1996 to April 1999. The ethics committee of each hospital approved the study. A total of 56 patients had NACG (age, 3 to 17 years; mean age, 9.7 ±3.4; 58.3% males), 36 patients had ACG (age, 3 to 18 years; mean age, 10 ± 3.2 years; 57% males), 36 patients had PU (age, 4 to 18 years; mean age, 10.2 ± 4.1; 64% males; 17 with duodenal, 7 with gastric, and 1 with both duodenal and gastric ulcers). Serum from each patient was taken at the time of the endoscopy and stored at −20°C until used. H. pylori infection was determined by culture or histology as soon as possible after the endoscopy. The antibody response to specific antigens (19.5, 26.5, 30, 35, 89, and 116 kDa) was determined by immunoblot (Helicoblot 2.0; Genelabs Diagnostics, Singapore) following the manufacturer’s recommendations and previously described methodology (6, 19). A serum sample was considered H. pylori positive by immunoblot analysis if it was positive for any one band at 116 kDa (CagA), 89 kDa (VacA), or 35 kDa or any two bands from among the 30-, 26.5-, and 19.5-kDa antigens (6, 19). A lineal-trend chi square was applied to the statistical study (level of statistical significance, P < 0.05). Odds ratios for seropositivity against CagA, VacA, CagA+VacA, and CagA+VacA+35-kDa antigen in the groups of ACG and PU, referred to the NACG status, were calculated.

Western blot was positive in 107 of the 117 H. pylori-positive children: 91% in the NACG group, 97.1% in the ACG group, and 84.6% in the PU group. These differences were not statistically significant. The global percentages of patients with serological responses against the 19.5-, 26.5-, 30-, 35-, and 89-kDa (VacA) and 116-kDa (CagA) antigens were 47, 85.4, 80.3, 40.1, 30.7, and 44.4%, respectively. Mitchell et al. (14) studied the antibody responses to the same six antigens and found that the 26.5-, 30-, and 116-kDa antigens had the most prevalent responses (81.5, 79.6, and 76% of children, respectively). In contrast, antibody responses to the 19.5-, 35-, and 89-kDa

| Patient group | No. of patients | % of patient group with antibody response at kDa* |
|---------------|----------------|-----------------------------------------------|
|              |                | 19.5 | 26.5 | 30 | 35 | 89 | 116 |
| NACG          | 56             | 50   | 87.5 | 76.8 | 39.3 | 23.2 | 39.2 |
| ACG           | 36             | 50   | 91.6 | 91.6 | 47.2 | 33.3 | 44.4 |
| PU            | 25             | 36   | 72   | 72   | 32   | 44   | 56  |

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antigens occurred in 55.5, 24, and 37% of children. In our study, antibodies against 26.5- and 30-kDa antigens were also the most prevalent, although no high prevalence of CagA was found.

When prevalence according to the gastric disease was studied, 39.2% of the NACG group, 44.4% of the ACG group, and 56% of the PU group had an anti-CagA response (no statistically significant differences) and 23.2% of the NACG group, 33.3% of the ACG group, and 44% of the PU group had an anti-VacA response (P = 0.056) (Table 1). Among the patient groups, 21.4% of NACG, 30.6% of ACG, and 44% of PU had a simultaneous response to CagA and VacA (P < 0.05), and 10.7% of NACG, 22.2% of ACG, and 32% of PU had a simultaneous response to CagA, VacA, and the 35-kDa protein (P < 0.05).

The odds ratios for seropositivity against CagA, VacA, and CagA + VacA simultaneously, according to the level of gastric lesion (related to NACG), are shown in Table 2. A patient with PU showed (in relation to patients who had only NACG) a probability to have a positive response to CagA of 1.97, to VacA of 2.6, to CagA and VacA simultaneously of 2.88, and to CagA, VacA, and 35-kDa antigen simultaneously of 3.92.

Pediatric data have estimated the prevalence of H. pylori cagA strains or CagA serum antibody in symptomatic children to be between 33 and 80% (3, 6, 9, 11, 12, 14, 17). Moreover, the simultaneous presence of anti-VacA and anti-p35 antibodies predicts with good sensitivity a predisposition to ulcers (2).

According to our data, when using a lineal-trend chi square we found a higher level of seroprevalence to simultaneous CagA and VacA and to simultaneous CagA, VacA, and 35-kDa protein among patients with PU compared to patients with NACG. However, for diagnostic purposes these serologic markers have no clinical usefulness due to the low level of sensitivity and specificity.

The sensitivity, specificity, positive predictive value, and negative predictive value of the responses to CagA, VacA, and 35-kDa antibodies as an indirect diagnostic method to identify children with ulcers are shown in Table 3. Detection of CagA showed a sensitivity of 56% and specificity of 59% to detect children with ulcers. VacA detection showed 44% sensitivity and 73% specificity. Detection of both CagA and VacA showed 44% sensitivity and 75% specificity to detect children with ulcers, and detection of CagA, VacA, and the 35-kDa antigen simultaneously showed 32% sensitivity and 85% specificity.

The antibody response against a specific antigen changes with the age of the patient; however, the highest response in all age groups is against the 26.5-kDa and 30-kDa antigens. The youngest group shows a lower percentage of CagA, VacA, and 35-kDa antigen response and the highest response to 19.5-kDa antigen. Response to 19.5-kDa antigen decreases with age, while response to 35-kDa antigen, VacA, and CagA increases with age.

Screening dyspeptic patients for gastroscopy in primary care with anti-CagA instead of anti-H. pylori antibodies has been shown not to be useful by some authors (8). Currently, no means exist to distinguish children infected with H. pylori who will have a severe outcome later in life from those who will not. Due to the strong correlation between CagA-positive serology and severe gastric lesions found by some authors, they suggest that CagA antibody detection by serology could be useful to target children for antimicrobial therapy. However, according to our results, CagA antibody detection was not useful to differentiate between patients suffering from ulcer and gastritis.

Table 2: Odds ratios for seropositivity against CagA, VacA, CagA+VacA, and CagA+VacA+35-kDa antigen, according to the level of gastric lesion (related to NACG)

| Patient group | No. of patients | CagA (P = 0.174) | VacA (P = 0.056) | CagA+VacA (P = 0.039) | CagA+VacA+35kDa (P = 0.0193) |
|--------------|----------------|-----------------|-----------------|----------------------|-----------------------------|
| NACG         | 56             | 22 1            | 13 1            | 12 1.00              | 6 1                         |
| ACG          | 36             | 16 1.24         | 12 1.65         | 11 1.61              | 8 2.38                      |
| PU           | 25             | 14 1.97         | 11 2.60         | 11 2.88              | 8 3.92                      |

Note: *Seropositivity in children with ACG or PU compared to those with NACG, determined by a lineal-trend chi-square test. Pos, positive; OR, odds ratio.

Table 3: Sensitivity, specificity, positive predictive value, and negative predictive value of the different markers as an indirect diagnostic method to identify children with ulcers

| Marker          | Sensitivity | Specificity | PPV  | NPV  |
|-----------------|-------------|-------------|------|------|
| CagA            | 56          | 59          | 27   | 83   |
| VacA            | 44          | 73          | 30   | 83   |
| CagA+VacA       | 44          | 75          | 32   | 83   |
| CagA+VacA+35kDa | 32          | 85          | 36   | 82   |

Note: *PPV, positive predictive value; NPV, negative predictive value.

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