Helicobacter pylori and TT virus prevalence in Japanese children

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Background. The major transmission route of Helicobacter pylori, oral-oral or fecal-oral, remains to be established. TT virus (TTV), a recently discovered microbe that is prevalent in healthy persons, is believed to be mainly transmitted by nonparenteral routes. The purpose of this study was to test the hypothesis that these two microorganisms have a common mode of transmission. Methods. We investigated the seroprevalence of H. pylori and TTV in a cross-sectional study of 454 healthy Japanese children from birth to age 15 years, living in five different geographic areas. Determination of H. pylori status was based on the presence of specific serum IgG and IgA antibodies, determined using enzyme immunoassays. TTV DNA was detected and the titer was determined using semiquantitative polymerase chain reaction with heminested primers. Results. The overall prevalences of H. pylori and TTV were 12.2% and 21.6%, respectively. An age-related increase of prevalence was shown for H. pylori (P < 0.001), but not for TTV (P = 0.23). Titers of TTV DNA significantly decreased with age (P = 0.02). There were significant geographic differences in TTV prevalence (P < 0.001), but not in H. pylori seroprevalence (P = 0.33). There was no true correlation between the prevalence of these two organisms (r coefficient = −0.02 and P = 0.66). Conclusions. Although Japanese children frequently acquire both H. pylori and TTV, especially in early childhood, their acquisition appears to be independent.

Key words: Helicobacter pylori, TT virus, transmission, child

Introduction

Helicobacter pylori, Gram-negative spiral bacteria, are commonly detected in the human stomach, and play an important role in the pathogenesis of peptic ulcer disease and gastric cancer in adults.12 H. pylori is also associated with antral gastritis and duodenal ulcer in childhood.4,14 Most acquisition of H. pylori occurs in childhood.4,6 Because humans are the primary reservoir of the organism, person-to-person transmission appears most likely.7 Because H. pylori has been detected in dental plaque,8 in vomitus,9 and in stools,10,11 oral-oral, vomitus-oral, or fecal-oral transmission is possible. While the oral-oral route does not appear to be important for transmission in adults, it could be important in childhood.12 The major transmission route remains to be established.

A novel, nonenveloped human virus, named TT virus (TTV), was isolated from the serum of a patient with posttransfusion hepatitis of unknown etiology in 1997.13 The genome of TTV is a circular single-stranded DNA molecule of approximately 3.8 kilobases.14,15 Due to its genomic structure and physicochemical properties, TTV has been tentatively placed within the Circoviridae family or in a novel virus family, the Circinoviridae.4 Its genomic sequence shows an extremely wide range of divergence,16 and at least 30 genotypes and five major phylogenetic groups (groups 1–5) have been identified.17 TTV has been detected in adult patients with non-B, non-C chronic hepatitis or with fulminant hepatitis, and in patients with hemophilia who were treated with blood products.18 TTV can be parenterally transmitted, via transfusion or intravenous drug use.15 However, TTV carriage is frequent in the normal population and in healthy blood donors.13,18,19 Because it is shed into feces,20 it may also be transmitted by the fecal-oral route: the infectivity of TTV in feces has recently been demonstrated in a chimpanzee transmission study.21 The existence of bloodborne and fecal-oral transmis-
sion could be responsible for its worldwide occurrence in human populations.\textsuperscript{19}

We hypothesized that \textit{H. pylori} and TTV may have a common route of transmission, possibly fecal-oral. A recent study found no difference in TTV prevalence between adult ulcer patients with or without \textit{H. pylori} infection.\textsuperscript{22} However, in that study, the number of patients was relatively small and the prevalences of both organisms were high, and so it is difficult to reach a definitive conclusion. To test our hypothesis, we examined the prevalences of \textit{H. pylori} and TTV in Japanese children from five different geographic areas.

**Patients and methods**

**Patients**

One hospital in each of five Japanese cities participated in this study. Each city had a population of more than 200,000 and the cities are distributed from north to south in Japan. In total, 454 children from birth to age 15 years who visited the outpatient clinics were enrolled in this study (Table 1). Diagnoses included acute illnesses (in 384 children), such as upper respiratory tract infections; and chronic illnesses (in 70 children), including neurologic, endocrine, or renal diseases. None of the children tested had either prior transfusion of blood or blood products, acute or chronic liver disease, prior surgery, or peptic ulcer disease. Serum samples were frozen at $-20^\circ$C and stored until tested. Informed consent for this study was obtained from parents of all patients.

**Assay of anti-\textit{H. pylori} antibodies**

Serum titers of IgG and IgA antibodies to \textit{H. pylori} were measured using commercial enzyme immunoassay (EIA) kits (HM-CAP and PP-CAP, respectively; Enteric Products, New York, NY, USA). For both assays, EIA values (EV) of more than 2.2, 1.8–2.2, and less than 1.8 were defined as positive, indeterminate, and negative, respectively.\textsuperscript{23} Children who had positive results on either the IgG or the IgA assay were judged to be colonized with \textit{H. pylori}, and those in whom both IgG and IgA measurements were negative were judged to be \textit{H. pylori}-negative. If both measurements were indeterminate, or if one was indeterminate and the other was negative, the child was excluded from the study of \textit{H. pylori} seroprevalence. In infants aged less than 6 months, only IgA measurements were used, because IgG antibodies probably reflect maternal antibodies from \textit{H. pylori}-positive mothers.\textsuperscript{24}

**Detection of TTV DNA**

Nucleic acids, extracted from 50µl of serum by the High Pure Viral Nucleic Acid Kit (Roche Diagnostics, Mannheim, Germany), were resuspended in 40µl of distilled water (dH$_2$O), and 20µl of this DNA solution was examined for the presence of TTV DNA. Polymerase chain reaction (PCR) with primers based on the sequence of a coding region (N22 PCR), which detects TTV group 1, including the prototype TTV strain,\textsuperscript{15,20} was used for the detection of TTV DNA in the present study. Briefly, the first-round PCR was performed with sense primer NG059 (5’-ACAGACAGAGGAGAAGGCAACATG-3’) and antisense primer NG063 (5’-CTGGCATTCTACCATTCTCTCAATG-3’) for 35 cycles (94°C for 30s; 60°C for 45s; 72°C for 45s [plus 7min in the last cycle]). The second-round PCR was performed with sense primer NG061 (5’-GGCAACATGYTRTGGATAGACTGG-3’) and antisense primer NG063 for 25 cycles under the same conditions.\textsuperscript{15,20} To estimate the relative titer of TTV DNA viral load in serum, serial tenfold dilutions of the extracted nucleic acids in dH$_2$O, supplemented with 20µl/ml glycogen (Roche Diagnostics) were examined as described above. The highest dilution testing positive was defined as the viral load, corresponding to approximately 10$^4$, 10$^5$, 10$^6$, or 10$^7$ DNA copies/25µl serum.

**Table 1. Prevalence of \textit{Helicobacter pylori} and TT virus among 454 children in five cities in Japan**

<table>
<thead>
<tr>
<th>Area</th>
<th>Number of children</th>
<th>% Male</th>
<th>Age, in years (mean ± SD)</th>
<th>Prevalence (%)*</th>
</tr>
</thead>
<tbody>
<tr>
<td>Hachinohe</td>
<td>76</td>
<td>47.9</td>
<td>6.7 ± 5.4</td>
<td>10.0</td>
</tr>
<tr>
<td>Sendai</td>
<td>80</td>
<td>50.0</td>
<td>6.1 ± 5.3</td>
<td>14.5</td>
</tr>
<tr>
<td>Ohmiya</td>
<td>99</td>
<td>55.6</td>
<td>5.2 ± 4.1</td>
<td>7.4</td>
</tr>
<tr>
<td>Wakayama</td>
<td>105</td>
<td>52.4</td>
<td>6.1 ± 4.8</td>
<td>12.2</td>
</tr>
<tr>
<td>Kurume</td>
<td>94</td>
<td>44.7</td>
<td>6.5 ± 4.6</td>
<td>17.0</td>
</tr>
<tr>
<td>Total</td>
<td>454</td>
<td>50.3</td>
<td>6.1 ± 4.8</td>
<td>12.2</td>
</tr>
</tbody>
</table>

*P = 0.33 and P < 0.001 for geographical differences in \textit{H. pylori} and TT virus prevalence, respectively

*426 Children were studied