Seroprevalence of Enteric and Nonenteric Adenoviruses in Bangladesh

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Single serum samples obtained from infants between 0 and 24 months of age admitted to a diarrheal disease hospital in Bangladesh were tested for the presence of adenovirus-specific immunoglobulin G (IgG) and IgA antibodies by using enzyme immunoassay and neutralizing antibodies to adenovirus types 2, 40, and 41. IgG antibodies were more prevalent than IgA antibodies, and neutralizing activity to enteric adenovirus was found in serum samples from 50% of infants who had reached 2 years of age.

Enteric adenoviruses (EADV) have been reported to be the second most commonly detected viral agents causing diarrhea in infants (1, 2, 7, 9). Seroprevalence studies of EADV have previously been undertaken by two groups using serum neutralization tests (6, 8). A study of seroprevalence of EADV in samples from Kuwait, Gambia, Hong Kong, New Zealand, the United Kingdom, and Guatemala indicated a widespread presence of EADV. Where a good number of sera were collected, around 50% of sera from children less than 4 years of age had neutralizing activity to EADV (6). A study conducted in Japan reported neutralizing antibody activity to peak in children between the ages of 37 to 48 months (8).

The aim of this study was to examine the seroprevalence of EADV and nonenteric adenovirus (ADV) in Bangladeshi children less than 2 years of age.

Serum specimens were collected between February 1990 and May 1991 as residues from samples collected from patients admitted to the Clinical Research Centre of the International Centre for Diarrhoeal Disease Research, Bangladesh, for treatment of diarrhea. Sera were stored at −20°C.

ADV type 2 (ADV2) was propagated in HeLa cells, and ADV40 and ADV41 were propagated in Graham G293 cells (3). Cell lysates (virus infected and mock inoculated) were centrifuged at 8,800 × g for 30 min at 4°C (and used for serum neutralization tests) or further centrifuged at 100,000 × g for 90 min at 4°C, suspended in minimal volume of phosphate-buffered saline (PBS) (pH 7.2), and stored at −20°C for use in enzyme immunoassays (EIAs).

An EIA was used to detect anti-adenoviral immunoglobulin A (IgA) and IgG, in which ADV40 or ADV2 antigen and matching cellular antigens were diluted in 0.06 M carbonate-bicarbonate buffer (pH 9.6), added to microtiter plates (Nunc immunoplate; Nunc, Roskilde, Denmark), and incubated overnight at 4°C for detection of IgA and IgG, respectively. Serum samples were added in duplicate in double dilutions starting at 1/100 in 2% (wt/vol) skim milk powder in PBS with 0.05% (vol/vol) Tween 20, and sera diluted at 1/100 were added to cellular-antigen-coated wells. Plates were incubated for 3 h at room temperature, and then horseradish peroxidase-conjugated anti-human IgA or IgG (Silenus Laboratories, Melbourne, Australia) diluted in 2% skim milk powder-PBS-Tween 20 was added and plates were incubated for 1 h at 37°C. Substrate containing 3,3',5,5'-tetramethylenediamine was used, and the optical density at 450 nm was measured. The serum dilution showing an optical density at 450 nm of at least twice that of the cellular-antigen-coated well was regarded as the end point.

Acute- and convalescent-phase serum pairs from patients with known EADV infections were not available; therefore, specificity of the EIAs was determined by using competitive blocking of patients’ sera at dilutions of 1/100. Rabbit hyperimmune serum (raised against fluorocarbon-extracted stool ADV40, concentrated by ultracentrifugation) and free ADV40 antigen competed with serum antibodies, and no blocking was found with preimmune rabbit serum.

Neutralizing antibodies were estimated by a fluorescent focus neutralization assay. Sera at dilutions of 1/200 and 1/2,000 were added to six wells of a sterile microtiter plate, after which ADV40, ADV41, and ADV2 preparations at dilutions resulting in approximately 600 fluorescing cells per well were added to pairs of wells. Plates were incubated at 37°C and 5% CO2 for 1 h, and then the virus-serum mixture was added to wells containing washed monolayers of H407 cells (4) and incubated at 37°C and 5% CO2 for 48 h. Cells were fixed with 70% (vol/vol) acetone, air dried, and stained with a rabbit hyperimmune serum to ADV diluted in PBS and subsequently with fluorescein isothiocyanate-conjugated anti-rabbit IgG F(ab′)2 (Silenus). Antibody titer was expressed as the serum dilution giving 50% reduction in the number of fluorescing cells in virus control wells.

P values given were calculated by using the chi-square test.

By testing serum samples for IgG and IgA antibodies by

<table>
<thead>
<tr>
<th>Age group (mo)</th>
<th>No. of serum samples tested</th>
<th>No. of antibody* positive serum samples also:</th>
<th>No. of antibody- negative serum samples also:</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Neut positive</td>
<td>Neut negative</td>
</tr>
<tr>
<td>0–6</td>
<td>99</td>
<td>50</td>
<td>26</td>
</tr>
<tr>
<td>7–12</td>
<td>64</td>
<td>23</td>
<td>11</td>
</tr>
<tr>
<td>13–24</td>
<td>55</td>
<td>41</td>
<td>7</td>
</tr>
</tbody>
</table>

* Antibody refers to IgG and/or IgA specific for ADV. Neut refers to neutralizing antibody to at least one ADV serotype.

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using EIA, titers of either isotype were detected among 76.8, 54.7, and 87.3% of infants in the 0- to 6-, 7- to 12-, and 13- to 24-month age groups, respectively (Table 1). The median titers for IgG were 1/500, 1/200, and 1/800 for infants in the 0- to 6-, 7- to 12-, and 13- to 24-month age groups, respectively, compared with titers of less than 100, less than 100, and 1/400 for IgA antibodies (data not shown).

As indicated by the sera determined to be antibody positive by EIA, the proportion of children positive for neutralizing antibodies increased with age, as 65.8, 65.7, and 85.4% of serum samples from infants in the 0- to 6-, 7- to 12-, and 13- to 24-month age groups, respectively, were positive (Table 1) and neutralizing activity to enteric serotypes significantly increased between the 7- to 12- and the 13- to 24-month age groups (24 to 58%, \( P < 0.01 \), Table 2). For all sera tested, geometric mean titers of neutralizing antibodies (negatives were assigned a titer of 100) to ADV2, ADV40, and ADV41 were 138, 179, and 188; 146, 159, and 168; and 159, 308, and 390, respectively, for 0- to 6-, 7- to 12-, and 13- to 24-month age groups, respectively.

Among the sera that were negative for specific IgG and IgA antibodies, between 20 and 30% had neutralizing activity; 13 of 15 (86.7%) of all sera negative by EIA had neutralizing antibodies to enteric serotypes, and 5 of 15 (33%) had neutralizing antibodies to ADV2.

This investigation attempted to examine the neutralizing activity against enteric serotypes in sera from infants in the age range in which most EADV infections have been reported to occur (1, 5, 7, 9).

A significant increase of positivity for antibodies by EIA (54.5 to 87.3%, \( P < 0.001 \)) in infants in the age groups of 7 to 12 and 13 to 24 months was found, suggesting that a large proportion of infants more than 12 months of age are infected by ADVs resulting in antibody production. The increase was also observed for EADV-specific antibodies, as demonstrated by the proportion of sera positive for neutralizing antibodies (Table 2) and the increase in geometric mean titers.

In a Japanese population, Shinozaki et al. (8) found neutralizing activity among 53% of children older than 4 years of age only. Kidd et al. (6) found neutralizing titers in 50% of serum samples from infants 25 to 48 months of age and 100% from children 4 years of age or older. The early high prevalence found in this study may be a result of testing sera from children that presented with symptoms of diarrhea. Alternatively, Bangladeshi children may be infected with EADVs earlier in life than children from developed countries.

Neutralizing activity against EADV in IgG- and IgA-negative sera was observed in about 20% of serum samples and may be attributed to the presence of neutralizing IgM antibodies or greater sensitivity of the neutralization test. No trend of reactivity to a particular serotype(s) was observed among those IgG- and IgA-negative sera.

Our findings show that EADV infections occur in Bangladeshi children less than 2 years of age, as demonstrated by presence of specific antibodies in sera.

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