Seroepidemiology of echovirus 30 in Korean children

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Background: Although aseptic meningitis associated with echovirus type 30 has emerged as a global public health concern, no data have been reported on children's immune status against echovirus type 30. The current study aimed to investigate the seropositivity among Korean children for antibodies against echovirus 30.

Methods: Two hundred and fifty residual serum samples were collected at St. Paul's Hospital. Individuals were categorized by age into four groups: group 1 (3 months-2 years), group 2 (3-6 years), group 3 (7-10 years) and group 4 (11-15 years). Neutralizing antibodies against echovirus 30 were measured.

Results: Seroprotective neutralizing antibodies against echovirus 30 were detected in 129 (49%) individuals. Seropositivity rates were 23%, 48%, 55% and 73% in groups 1-4, respectively. For antibody titers, 1:256-1:512 was the highest neutralizing antibody titer range in group 2, while 1:1024-1:2048 in group 3 and 4. Among the seropositive individuals in group 3 and 4, 6% and 12% had neutralizing antibody titers of 1:2048, respectively.

Conclusions: The seropositivity rate increased significantly with age. The distribution of neutralizing antibody titers varied by age group, and higher ranges of neutralizing antibody titers were observed in higher age groups. These findings suggest high susceptibility to echovirus 30 infection in children younger than 2 years old. Echovirus 30 infection in childhood may have contributed to increased neutralizing antibody titers with age.


Key words: echovirus 30; Korea; seroepidemiology

Introduction

Echovirus type 30 is a single-stranded RNA virus that belongs to the Picornaviridae family, Enterovirus genus, and Enterovirus B species. Echovirus causes a wide spectrum of diseases ranging from asymptomatic infection, nonspecific febrile illness, nonspecific rash, and upper respiratory infections to sepsis, aseptic meningitis, encephalitis and myositis, especially in neonates and children. Echovirus replicates in the gastrointestinal tract and is transmitted person-to-person mostly via the fecal-oral route, although transmission may occur through respiration. There are more than 30 serotypes of echovirus, and cases of aseptic meningitis caused by echoviruses 4, 6, 9, 11, 13 and 30 have been reported, with serotypes 6, 9 and 30 being the major pathogens of aseptic meningitis. Recently, aseptic meningitis outbreaks associated with echovirus type 30 were reported in several countries, including America, Greece, France, Italy, Brazil, India and China, and infections have especially affected children. Thus, aseptic meningitis associated with echovirus type 30, which primarily occurs in the summer and autumn in temperate climates, has emerged as a global public health concern. The Korea Centers for Disease Control and Prevention has undertaken nationwide surveillance of Enterovirus genera since 1993, and the country witnessed an echovirus type 30 epidemic, resulting in aseptic meningitis among children in the summer of 2008. Echovirus 30 was the second most common serotype detected in surveillance during 1999-2011, and it was frequently observed during 2012-2014. As no vaccine or specific preventive measure is available, maintenance immunity against echovirus 30 is essential. Cell-mediated immunity is the major protective mechanism for echovirus type 30, but neutralizing antibodies associated with humoral immunity also play a key role. No data have been reported on the immune status of children to fight echovirus type 30. This study examined the neutralizing antibody response to echovirus type 30 in Korean children. The results may be useful in the prevention and control of echovirus 30 infection.

Methods

Collection of serum samples
A total of 250 residual samples were collected from
subjects who visited St. Paul's Hospital, The Catholic University of Korea in Seoul, for medical examinations. The subjects were categorized by age into four groups: group 1 (3 months-2 years), group 2 (3-6 years), group 3 (7-10 years) and group 4 (11-15 years). Children with immunocompromised status, on immunosuppressive therapy, or with suspected viral infections were excluded. The study protocol was approved by the institutional review board of the study site (PIRB-00134-005). Informed consent for the use of residual samples was obtained from parents or legal representatives.

Neutralization assay
The microneutralization test was performed with a minor modification. Serum samples were stored at -70°C after centrifugation and inactivated at 56°C for 30 minutes before testing. Serum was diluted two-fold from 1:4 to 1:1024 in Eagle's minimum essential medium containing 0.1% bovine serum albumin. Each dilution was placed in duplicate 96-well plates. A 100 tissue culture infective dose (tissue culture infectious dose: 50) of echovirus 30 was added to each well. After incubation for 3 hours at 37°C, a rhabdomyosarcoma cell suspension was mixed with virus-inoculated dilution. The cytopathic effect was evaluated after 10 days of incubation. The highest dilution of serum that prevented the cytopathic effect was considered the antibody titer of serum. Serum samples with antibody titer dilutions ≥1:4 were regarded as seroprotective.

Statistical analysis
Percentages of subjects exhibiting seroprotectivity against echovirus 30 were calculated with a 95% confidence interval. The Chi-square test was used to compare age groups. SPSS version 20.0 software (IBM Co., Armonk, NY, USA) was used for data analysis, and P values <0.05 were regarded as statistically significant.

Results
Of the 250 subjects, 130 (52%) were male and 120 (48%) were female (male to female ratio: 1:1). The mean age of subjects was 5.5±4.11 years. The demographic characteristics of each group are presented in Table.

Overall seropositivity of echovirus 30
There were 129 (49%) total subjects who were seropositive for echovirus 30 antibodies. No significant difference was observed between genders. Among the 130 males, 66 (50.7%) were seropositive, while 63 (52.5%) of the 120 females were seropositive.

Neutralizing antibody titer distribution of subjects
Among the 129 seropositive subjects, the neutralizing antibody titers were demonstrated as follows: 22 (18%) in 1:4, 11 (9%) in 1:8, 17 (14%) in 1:16, 8 (6%) in 1:32, 14 (11%) in 1:64, 15 (12%) in 1:128, 18 (15%) in 1:256, 11 (9%) in 1:512, 5 (4%) in 1:1024, and 2 (2%) in 1:2048.

Seropositivity of echovirus 30 accordance to age group
Seropositivity rates were demonstrated as follows: 16/70 (23%) in group 1, 33/69 (48%) in group 2, 33/57 (55%) in group 3, and 41/54 (73%) in group 4 (Fig. 1). The seropositivity rate increased significantly with age (P<0.001).

Neutralizing antibody titer distribution of each age group
All seropositive subjects in group 1 presented with neutralizing antibody titers between 1:4 and 1:8. In group 2, of 33 seropositive subjects, 8 (25%), 9 (27%), 7 (21%) and 9 (27%) presented with neutralizing antibody titers between 1:4 and 1:8, 1:16 and 1:32, 1:64 and 1:128, and 1:256 and 1:512, respectively. Among the 33 seropositive subjects in group 3, 5 (16%), 9 (27%), 10 (30%), 7 (21%) and 2 (6%) revealed neutralizing antibody titers between 1:4 and 1:8, 1:16 and 1:32, 1:64 and 1:128, 1:256 and 1:512, and 1:1024, respectively. Of the 41 seropositive subjects in group 4, 4 (10%), 7 (17%), 12 (29%), 13 (32%) and 5 (12%) showed neutralizing antibody titers between 1:4

<table>
<thead>
<tr>
<th>Variables</th>
<th>Group 1 (3 mon-2 y)</th>
<th>Group 2 (3-6 y)</th>
<th>Group 3 (7-10 y)</th>
<th>Group 4 (11-15 y)</th>
<th>Total</th>
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<td>Median age (y)</td>
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<td>7.93±6.92</td>
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<td>Male, n (%)</td>
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<td>34 (60)</td>
<td>23 (40)</td>
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<td>Female, n (%)</td>
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<td>36 (52)</td>
<td>23 (40)</td>
<td>31 (57)</td>
<td>120 (48)</td>
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<td>Total, n (%)</td>
<td>70 (100)</td>
<td>69 (100)</td>
<td>57 (100)</td>
<td>54 (100)</td>
<td>250 (100)</td>
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