Seroepidemiology of echovirus 30 in Korean children

Joo Young Lee, Yumi Seo, Ui Yoon Choi, Jong-Hyun Kim, Jin Han Kang
Seoul, Korea

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.**

World J Pediatr 2017;13(6):611-614

Key words: **echovirus 30;**
Korea;
seroepidemiology

Author Affiliations: Department of Pediatrics, College of Medicine, The Catholic University of Korea, Seoul, Republic of Korea (Lee JY, Seo Y, Choi UY, Kim JH, Kang JH)

Corresponding Author: Ui Yoon Choi, MD, PhD, Department of Pediatrics, St. Paul's Hospital, College of Medicine, The Catholic University of Korea, 180, Wangsan-ro, Dongdaemun-gu, Seoul, 02559, Republic of Korea (Tel: +82 2 9584536; Fax: +82 2 32954861; Email: uiyoon@catholic.ac.kr)

doi: 10.1007/s12519-017-0058-x
Online First August 2017

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.[1-3] 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.[4,5] Recently, aseptic meningitis outbreaks associated with echovirus type 30 were reported in several countries, including America,[6] Greece,[7] France,[8] Italy,[9] Brazil,[10] India,[4] Korea[11-13] and China,[14] 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 epidemics, resulting in aseptic meningitis among children in the summer of 2008.[11] Echovirus 30 was the second most common serotype detected in surveillance during 1999-2011, and it was frequently observed during 2012-2014.[12,13] 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.[15] 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.[16] 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.[16]

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 and 1:2048, 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

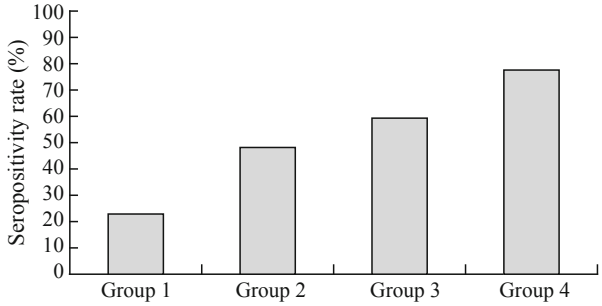

Fig. 1. Seropositivity rates of each age group.

Table. Demographic characteristics of age groups

Variables	Group 1 (3 mon-2 y)	Group 2 (3-6 y)	Group 3 (7-10 y)	Group 4 (11-15 y)	Total
Median age (y)	1.07±0.78	4.14±1.14	7.93±0.92	13.21±1.32	4.5±4.11
Male, *n* (%)	40 (57)	33 (48)	34 (60)	23 (43)	130 (52)
Female, *n* (%)	30 (43)	36 (52)	23 (40)	31 (57)	120 (48)
Total, *n* (%)	70 (100)	69 (100)	57 (100)	54 (100)	250 (100)

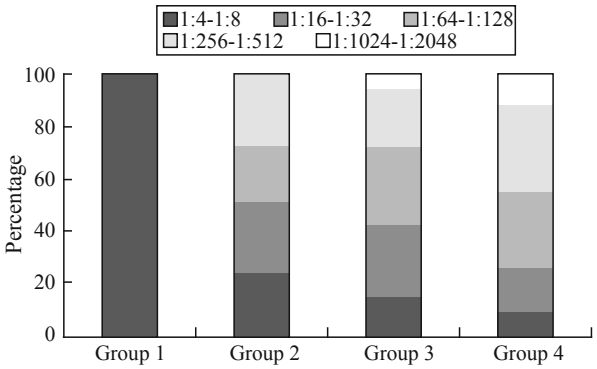

Fig. 2. Neutralizing antibody titer proportion of each age group.

and 1:8, 1:16 and 1:32, 1:64 and 1:128, 1:256 and 1:512, and 1:1024 and 1:2048, respectively (Fig. 2).

Discussion

Echovirus 30 inhabits in the gastrointestinal tract, is spread by the fecal-oral route, and is not only associated with asymptomatic infection, but a wide range of diseases, including febrile illness, respiratory infections, acute gastroenteritis and aseptic meningitis.[1-3] The Korea Centers for Disease Control and Prevention have operated a national enterovirus surveillance system since 1993 and collected samples of cerebrospinal fluid, blood, throat swabs, feces and other body fluids (saliva, urine and pericardial fluid) from patients with enterovirus-related diseases. Echovirus 30 was the second most frequently identified pathogen from 1999-2011 and one of the five most common pathogens ranked in 2012-2014. Echovirus 6 and 30 were most commonly detected among the echovirus serotypes.[12,13] Levels of neutralizing antibodies against echovirus 30 in Korean children were measured in this study. The seropositivity rate increased significantly with age and higher antibody titers were observed in higher age groups.

In a study conducted in Finland, the seropositivity rate was higher for echovirus 22 than echovirus 30, suggested that echovirus 22 infection is more common than that of echovirus 30. The seropositivity rate of echovirus 30 increased slowly in Finish people, in their thirties and forties.[17] The age-related increase in seropositivity rate was not observed for echovirus 30 in a study in Germany.[18] The prevalent serotype may vary by region. The association between environmental parameters and circulating serotypes of echovirus has not been studied; however, climatic factors including temperature, humidity and precipitation may influence survival of echovirus and contribute to differences in circulating serotypes by region. Most Korean children start to attend day care centers at age 3 and continue their activities in kindergarden, elementary school and middle school. The high child population density in educational institutions may contribute to the spread of infection, as children predominantly experience circulating echovirus 30 infections as they grow. This results in higher seropositivity rates and higher ranges of antibody titers in higher age groups. Educational institutions in Finland and Germany may not be crowded as they are in Korea. Thus, echovirus 30 may not be as abundant as it is in Korea. Children in Finland and Germany are not exposed to echovirus 30 in their childhood.

Aseptic meningitis outbreaks caused by echovirus 30, including outbreaks among high school football players reported in the USA, mainly affect children older than five years.[6] During the aseptic meningitis outbreaks in France and Bulgaria, children aged 5-14 years were mainly affected.[5,8] The echovirus 30 detection rate was highest in children aged 6-10 years, according to enterovirus surveillance in Korea from 2012 to 2014.[13] Close contact between children and the sharing of utensils may be significant risk factors. The intestinal detoxification time for children echovirus 30 has not been studied. However, echovirus 30 may exist in feces for as long as 10 weeks, as seen with enterovirus 71.[19] Avoiding infected surfaces and feces and proper hand hygiene are important measures to decrease echovirus 30 transmission.

We investigated the seropositivity of Korean children for antibodies against echovirus 30. Children aged 3 months to 2 years showed low seropositivity rates, at approximately 25%, suggesting that awareness of echovirus 30 infection should be raised for this age group.

Funding: None.

Ethical approval: The study protocol was approved by the Institutional Review Board of St. Paul's Hospital, College of Medicine, The Catholic University of Korea (PIRB-00134-005). Informed consent was obtained from parents or legal representatives of all participants.

Competing interest: There are no potential conflicts of interest to disclose.

Contributors: Choi UY coordinated and designed the study, collected samples, analyzed the data, and prepared the manuscript. Lee JY and Seo Y collected samples. Kim JH and Kang JH analyzed the data and prepared manuscript. All authors approved the final version of the manuscript.

References

1 Harvala H, Simmonds P. Human parechoviruses: biology, epidemiology and clinical significance. J Clin Virol 2009;45:1-9.
2 Esposito S, Rahamat-Langendoen J, Ascolese B, Senatore L, Castellazzi L, Niesters HG. Pediatric parechovirus infections. J Clin Virol 2014;60:84-89.

3 Wolthers KC, Benschop KS, Schinkel J, Molenkamp R, Bergevoet RM, Spijkerman IJ, et al. Human parechoviruses as an important viral cause of sepsislike illness and meningitis in young children. Clin Infect Dis 2008;47:358-363.
4 Kumar A, Shukla D, Kumar R, Idris MZ, Jauhari P, Srivastava S, et al. Molecular identification of enteroviruses associated with aseptic meningitis in children from India. Arch Virol 2013;158:211-215.
5 Mladenova Z, Buttinelli G, Dikova A, Stoyanova A, Troyancheva M, Komitova R, et al. Aseptic meningitis outbreak caused by echovirus 30 in two regions in Bulgaria, May-August 2012. Epidemiol Infect 2014;142:2159-2165.
6 Croker C, Civen R, Keough K, Ngo V, Marutani A, Schwartz B, et al. Aseptic meningitis outbreak associated with echovirus 30 among high school football players-Los Angeles County, California, 2014. MMWR Morb Mortal Wkly Rep 2015;63:1228.
7 Mantadakis E, Pogka V, Voulgari-Kokota A, Tsouvala E, Emmanouil M, Kremastinou J, et al. Echovirus 30 outbreak associated with a high meningitis attack rate in Thrace, Greece. Pediatr Infect Dis J 2013;32:914-916.
8 Chomel JJ, Antona D, Thouvenot D, Lina B. Three ECHOvirus serotypes responsible for outbreak of aseptic meningitis in Rhône-Alpes region, France. Eur J Clin Microbiol Infect Dis 2003;22:191-193.
9 Milia MG, Cerutti F, Gregori G, Burdino E, Allice T, Ruggiero T, et al. Recent outbreak of aseptic meningitis in Italy due to Echovirus 30 and phylogenetic relationship with other European circulating strains. J Clin Virol 2013;58:579-583.
10 dos Santos GP, da Costa EV, Tavares FN, da Costa LJ, da Silva EE. Genetic diversity of Echovirus 30 involved in aseptic meningitis cases in Brazil (1998-2008). J Med Virol 2011;83:2164-2171.
11 Park IS, Lee HS, Choi SH, Kim HJ, Hwang SY, Cheon DS, et al. Epidemiology and clinical characteristics of enterovirus infections in children: a single center analysis from 2006 to 2010. Korean J Pediatr Infect Dis 2013;20:81-88. [In Korean]
12 Hyeon JY, Hwang S, Kim H, Song J, Ahn J, Kang B, et al. Accuracy of diagnostic methods and surveillance sensitivity for human enterovirus, South Korea, 1999-2011. Emerg Infect Dis 2013;19:1268-1275.
13 Lee YP, Hyun JY, Korea Centers for Disease Control and Prevention (KCDC). Laboratory-based enterovirus surveillancein in Korea, 2012-2013. PHWR Public Health Wkly Rep 2014;7:421-424. [In Korean]
14 Xiao H, Guan D, Chen R, Chen P, Monagin C, Li W, et al. Molecular characterization of echovirus 30-associated outbreak of aseptic meningitis in Guangdong in 2012. Virol J 2013;10:263.
15 Juhela S, Hyöty H, Lönnrot M, Roivainen M, Simell O, Ilonen J. Enterovirus infections and enterovirus specific T-cell responses in infancy. J Med Virol 1998;54:226-232.
16 Rabenau H, Weber B. Evaluation of a new automated microneutralization assay for the quantitative detection of neutralizing antibodies against enteroviruses. Zentralbl Bakteriol 1994;280:534-539.
17 Joki-Korpela P, Hyypiä T. Diagnosis and epidemiology of echovirus 22 infections. Clin Infect Dis 1998;27:129-136.
18 Weber B, Rabenau H, Cinatl J, Maass G, Doerr HW. Quantitative detection of neutralizing antibodies against polioviruses and non-polio enteroviruses (NPEV) using an automated microneutralization assay: a seroepidemiologic survey. Zentralbl Bakteriol 1994;280:540-549.
19 Teng S, Wei Y, Zhao SY, Lin XY, Shao QM, Wang J. Intestinal detoxification time of hand-foot-and-mouth disease in children with EV71 infection and the related factors. World J Pediatr 2015;11:380-385.

Received June 22, 2016
Accepted after revision September 30, 2016