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Human Picornavirus and Coronavirus RNA in Nasopharynx of Children Without Concurrent Respiratory Symptoms

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The prevalence of human rhino-, entero-, and coronaviruses was investigated by RT-PCR in nasopharyngeal aspirates from 107 children without concurrent respiratory symptoms. The children were admitted to the hospital for elective surgery. The parents filled a questionnaire about the occurrence of respiratory symptoms four weeks before and two weeks after the surgery. The rate of viral detection was 45% in children with related past or recent respiratory infection whereas 20% of the samples taken from children without any related past or recent respiratory infections were positive for picornavirus RNA, $P = 0.008$. Thirty-one (29%) of the nasopharyngeal aspirates were positive for viral RNA, 18% for rhinovirus, and 11% for enterovirus RNA. Coronavirus RNA was not found in any of the children. Fifty-five percent of the children with virus-positive samples had an infection-related diagnosis. In addition, 81% of the children with virus-positive samples had had previously respiratory symptoms or there were concurrent respiratory symptoms in other family members. Only four of the 31 virus-positive samples were from children without infection-related diagnosis or recent past (or immediate future) respiratory symptoms. ***J. Med. Virol. 66:417–420, 2002.*** © 2002 Wiley-Liss, Inc.

KEY WORDS: respiratory infection; PCR; rhinovirus; enterovirus

INTRODUCTION

The reverse transcriptase polymerase chain reaction (RT-PCR) assays for respiratory viruses are more sensitive [Johnston et al., 1993; Blomqvist et al., 1999] and may detect viruses at a higher frequency than the traditional virus detection methods. Theoretically, it is possible that a positive PCR finding might also sometimes represent subclinical infection or, since RT-PCR assays detect viral RNA, remnants of past infection rather than an active current infection. However, the advantage of RT-PCR techniques for certain important respiratory viruses, namely human rhino-, entero-, and coronaviruses, cannot be questioned since for these viruses no other available practical and rapid techniques exist [Ieven and Goossens, 1997]. While nasopharyngeal colonization in healthy children with respiratory bacterial pathogens is shown by several studies [Faden et al., 1997; Faden, 1998; Bernstein, 1999], less is known of respiratory viruses in this context. Johnston et al. [1993] have shown that respiratory picornaviruses can be found in 1.4% of healthy children by virus isolation and in 12% by PCR method. There are only a few other studies reported where the presence of respiratory viruses has been demonstrated in healthy children [Horn et al., 1979; Jennings et al., 1987] and in most of the previous studies of respiratory pathogens a control population of normal children was not included.

The purpose of the present study was to examine the presence of human rhino-, entero-, and coronaviral RNA in the nasopharynx of children without concurrent respiratory symptoms and to assess the influence of preceding and forthcoming respiratory symptoms on the detection rate.

CHILDREN AND METHODS

Children

The study population comprised 107 children who had been admitted for mainly elective surgery to the Helsinki University Central Hospital from November 1999 to March 2000. Fifty-three consecutive children

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were admitted to the Department of Otorhinolaryngology for surgery because of upper respiratory tract problems and 54 to the Department of Ophthalmology for elective eye surgery. The parents completed a questionnaire regarding respiratory symptoms of the child during the preceding four weeks (cough, fever $> 38.5^{\circ}$C, sore throat, rhinitis, or acute otitis media diagnosed by a doctor and whether the child had received any antibiotics). In addition, the presence of respiratory symptoms in family members at that time was queried. The parents were also requested to send by mail a second questionnaire reporting on the same symptoms during two weeks following the surgery. The study protocol was accepted by the ethical committee of the Helsinki University Central Hospital. A written informed consent was obtained from each patient's guardian.

Samples

Nasopharyngeal aspirate samples were collected in the surgery room just after induction of anesthesia. One milliliter sterile physiological saline was administered into the nose and nasopharyngeal aspirate samples were obtained with a sterile suction catheter (Juhn Tym-Tap Middle Ear Fluid Collector). The catheter was inserted through a nostril to a depth of 4 to 8 cm, followed by gentle application of suction with an electric suction device. The nasopharyngeal aspirate was collected automatically into a small tube attached to the suction catheter. Immediately after suction, the tube was removed from the catheter, tightly capped, and stored at -70°C until processed for RT-PCR.

RT-PCR

Extraction of viral RNA from nasopharyngeal aspirates was carried out using a commercial RNA isolation procedure (RNeasy®, Qiagen GmbH, Hilden, Germany). RT-PCR was carried out by methods published previously with minor modifications [Blomqvist et al., 1999; Nokso-Koivisto et al., 2000]. The primers and probes for human rhinovirus and human coronaviruses 229E and OC43 have been published previously [Pitkäranta et al., 1997; Blomqvist et al., 1999; Nokso-Koivisto et al., 2000]. The primers used for rhinovirus RT-PCR also amplify a region of the 5′ non-coding region of the human enterovirus genome. For coronavirus analysis, two sets of primer pairs complementary to the nucleocapsid protein genes of human coronaviruses OC43 and 229E, respectively, were used in a single tube.

The rhinovirus and coronavirus PCR products were detected as described previously by using microtiter plate hybridization with some modifications [Blomqvist et al., 1999; Nokso-Koivisto et al., 2000]. The probes were labeled with fluorescent lanthanides [Heinonen et al., 1997] and the results scored as described in detail elsewhere (S. Blomqvist et al. unpublished data). The probe for the enterovirus group was 5′-AAGTAGTCGGTTCCGC-3′. Both cDNA synthesis and PCR tests for all samples were carried out in two parallel wells followed by hybridization step in three parallel wells for both PCR assays.

Interpretation of Results. Positive controls were rhinovirus type 2, coxsackievirus type A16, human coronavirus 229E (all provided by American Type Culture Collection, Rockville, MD), and human coronavirus OC43 (provided by Kathryn V. Holmes, University of Colorado, Denver, CO). At each step several negative controls were included. The results were expressed as counts per second. The cutoff value of positive samples for the europium labeled probes was ten times the mean of all the negative controls [Halonen et al., 1995], and for the samarium labeled probes the mean of all the negative controls plus five times the standard deviation of the mean, modified from Heinonen et al. [1997]. A positive result in all six wells was required to score a specimen positive.

RESULTS

The study comprised 48 girls and 59 boys and the median age of the children was 3.1 years (range 1 month to 16.7 years). The parents of 96 children returned the preoperative questionnaire. Altogether 63 (66%) children had some respiratory symptoms during the four weeks before the sample collection, and out of those one half had symptoms during one week before the sample collection. Thirty three children had been totally free of respiratory symptoms. Eleven (10%) children had received antibiotic treatment during the four weeks before the surgery and 21 (20%) families reported respiratory symptoms in other members of the family at the time of the sample collection. The second questionnaire concerning postoperative symptoms was returned by 72 families. Out of those, 26 (36%) reported that the study children had some respiratory symptoms during two weeks after the surgery. The families of 35 children did not return the postoperative form. There was no difference between these children and the rest of the study children in median age, diagnosis, or preoperative symptoms.

Altogether, 31 (29%) nasopharyngeal aspirate samples were positive for viral RNA, 19 (18%) being positive for rhinovirus and 12 (11%) for enterovirus (Table I). None of the samples was positive for the human coronaviruses OC43 and 229E. Dual viral infections were not detected. According to the main diagnosis, the children were divided into two groups, to children who had an infection-related diagnosis (e.g., recurrent otitis, sinusitis, respiratory infections) or diagnosis not related to infection (mainly congenital eye diseases). Seventeen out of 31 virus positive samples (55%) were found in the 38 children with an infection-related diagnosis resulting in a positivity rate of 45%. Among children with diagnosis not related to infections, 20% of the samples were virus positive (Table I). Children with diagnosis not related to infections had statistically significantly less virus-positive samples than children

TABLE I. Diagnosis and Preceding Respiratory Symptoms Associated With Occurrence of Picornaviral RNA in 107 Study Children

	Number of children with specimen revealing			Percent of children with a virus positive specimen (%)
	Rhinovirus	Enterovirus	No virus	
Infection related diagnosis	9	8	21	45
Diagnosis not related to infection	10	4	55	20
Preoperative interview				
No symptoms in child nor in family member[a]	4	1	25	17
Symptoms in child and/or in family member[a]	11	10	45	32
No data	4	1	6	
Total	19	12	76	29

[a]Symptoms during four weeks preceding the sample collection.

with infection related diagnosis ($P = 0.008$, Chi-Square Test).

Altogether 81% (21/26) of the children with preoperative data available and a sample positive for viral RNA had respiratory symptoms prior to the operation or there were concurrent symptoms in other family members (Table I). Five children had a virus-positive specimen without concurrent respiratory symptoms during preceding four weeks.

Most of the children did not have any respiratory symptoms during two weeks after the surgery. Only one out of the 22 virus positive children who also returned the second questionnaire did not have any of the predisposing factors (respiratory symptoms before or after the sample collection or concurrent symptoms in other family members).

DISCUSSION

It was shown in this study that rhino- and enteroviral RNA can be detected by sensitive RT-PCR method in the nasopharynx of almost one third of children without significant concurrent respiratory symptoms. However, when viral findings were linked with past or future respiratory symptoms, the viral detection rate in "healthy children" was only 5%.

Respiratory viruses have been found in the nasopharynx of asymptomatic patients also with other methods such as virus culture. With conventional methods the prevalence of respiratory viruses has been approximately 3% [Horn et al., 1979; Hudgel et al., 1979; Jennings et al., 1987]. While in one study 12% of asymptomatic children were reported to be positive for rhinovirus in nasal aspirates by PCR [Johnston et al., 1993], we are unaware of comparable studies concerning human entero- and coronaviruses. In the current study, the specimens were positive for enteroviruses in 11% of children, although only one child was enterovirus positive without preceding respiratory symptoms. Fecal excretion of enteroviruses is known to continue for several weeks after the acute phase symptoms, but how long nasopharyngeal secretion can continue, is not known.

Respiratory coronaviruses have been difficult to diagnose and most diagnoses of respiratory coronavirus infections have been made earlier by using serology. Human coronaviruses appear to be responsible for a minor proportion of infections at least in small children [Nokso-Koivisto et al., 2000]. Although in the present study children were older, human coronavirus 229E and OC43 RNAs were not found in the nasopharyngeal aspirates by RT-PCR from any of the children. Because coronavirus infections occur mostly during late spring, the lack of positive findings can also reflect the epidemiological situation in the population.

In the Finnish Otitis Media Cohort Study, where children were followed from 2 to 24 months of age, 29% of all nasopharyngeal aspirate samples collected during upper respiratory infection were positive for rhinoviruses [Vesa et al., 2001] and rhinoviruses were found to be clearly the most common respiratory viruses in children. In the present study, rhinoviral RNA was found in 18% of nasopharyngeal aspirates and one fifth of these positive samples were from children without preceding respiratory symptoms. In experimental rhinovirus infections of adults, rhinovirus shedding has shown to continue at low levels for up to 3 weeks [Hendley and Gwaltney, 1988]. Whether this is true with children and also with natural infections is not known. In one earlier study, it has been shown that adults shed fewer viruses than children [Monto, 1994].

Detection of human rhino- and enterovirus RNA by RT-PCR (or by any method) in the nasopharynx must be interpreted cautiously because their presence alone does not establish causality of the concurrent illness. However, in the present study it was shown that in most cases viral RNA detection can be linked to past (or future) respiratory symptoms. Further studies, where the duration of respiratory virus colonization in the nasopharynx and stability of viral RNA can be evaluated, are still needed.

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