Journal of Clinical Virology 46 (2009) 234–237

Contents lists available at ScienceDirect

Journal of Clinical Virology

journal homepage: www.elsevier.com/locate/jcv

Human bocavirus and rhino-enteroviruses in childhood otitis media with effusion

Szilárd Rezes[a,*], Maria Söderlund-Venermo[b], Merja Roivainen[c], Kaisa Kemppainen[b], Zsolt Szabó[d], István Sziklai[a], Anne Pitkäranta[e]

[a] Department of Otorhinolaryngology and Head and Neck Surgery, Health Science Centre, University of Debrecen, 98 Nagyerdei krt., Debrecen, Hungary
[b] Department of Virology, Haartman Institute, University of Helsinki, 3 Haartmaninkatu, Helsinki, Finland
[c] National Institute for Health and Welfare (THL), 166 Mannerheimintie, Helsinki, Finland
[d] Department of Otorhinolaryngology, Borsod County Hospital, 72-6 Szentpéteri kapu, Miskolc, Hungary
[e] Department of Otorhinolaryngology and Head and Neck Surgery, Helsinki University Central Hospital, 4 Haartmaninkatu, Helsinki, Finland

ARTICLE INFO

Article history:
Received 1 March 2009
Received in revised form 7 August 2009
Accepted 13 August 2009

Keywords:
Human bocavirus
Rhinovirus
Enterovirus
Otitis media with effusion

ABSTRACT

Background: Viral respiratory infections play an important role in the pathogenesis of otitis media with effusion (OME) in children. The most common human rhinoviruses (HRVs) have been detected in middle ear effusions (MEE), but there is only limited data available about the closely related human enteroviruses (HEVs). The newly discovered human bocavirus (HBoV) has not, however, been identified in MEE of OME children.
Objectives: The aim of our study was to determine the presence of HBoV and HRV/HEV and the rate of coinfection in a set of MEE samples collected from OME children.
Study design: Seventy-five MEE samples from 54 children with no acute respiratory symptoms were studied with reverse transcription polymerase chain reaction (RT-PCR) for detection of HRV/HEV and quantitative PCR for detection of HBoV.
Results: Twenty-six (35%) of 75 MEE samples were positive for viral nucleic acid, 22 (29%) for HEV, 10 (13%) for HRV and 2 (3%) for HBoV. There was no statistically significant difference between mucoid and serous effusions in the rate of virus detection. Forty-three percent of bilateral cases showed a contra-lateral difference in viral finding.
Conclusions: Our results suggest that these common respiratory viruses can be associated with OME in children. Whether these viruses are causative etiologic factors of MEE persistence or merely remnants of previous infections is not known.

1. Background

The most frequently diagnosed types of otitis media in childhood are acute otitis media (AOM) and otitis media with effusion (OME). It is widely accepted and known that the most important preceding factors for AOM are respiratory viral infections.[1–3] OME is considered to be a continuation of AOM but the role of respiratory viruses in preceding or causing OME is not yet proven.[1] OME is a multifactorial disease, which is characterized by accumulation and persistence of fluid in the middle ear cavity. The precise etiopathogenesis of middle ear effusion (MEE) development remains unclear, although recently it has been shown that abnormal pressure in the middle ear cavity maintained by the Eustachian tube dysfunction, local allergic responses and unresolved bacterial and/or viral infections are important contributing factors.[4,5]

2. Objectives

A number of earlier studies have given evidence that respiratory viruses can be detected in MEE samples in children with OME. Viruses most frequently identified were rhinoviruses,[6] respiratory syncytial virus[7] and human coronavirus.[8] The most often studied respiratory viruses have been rhinoviruses, which are also the most important common cold viruses. The detection rates vary widely between 0 and 40% for rhinoviruses.[6,8,9] The positive viral findings in MEE suggest a possible role of respiratory viruses in the pathogenesis of OME. However, the presence of (rhino)viral RNA may also represent non-causative remnants of an earlier respiratory

Abbreviations: AOM, acute otitis media; OME, otitis media with effusion; HRV, human rhinovirus; HEV, human enterovirus; HBoV, human bocavirus; MEE, middle ear effusion; RT, reverse transcription; PCR, polymerase chain reaction; RNA, ribonucleic acid; DNA, deoxyribonucleic acid; PBS, phosphate buffered saline; SD, standard deviation; NCR, non-coding region; TNF, tumor necrosis factor.

* Corresponding author. Tel.: +36 203288840; fax: +36 52418189.
E-mail addresses: szrezes@gmail.hu, szrezes@freemail.hu (S. Rezes).

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doi:10.1016/j.jcv.2009.08.010

infection. A substantial proportion of respiratory infections have also been shown to be caused by enteroviruses as well as the newly discovered human bocaviruses.[10,11] Although enteroviruses replicate most prolifically in the gastrointestinal tract, there is increasing evidence to suggest that enteroviruses are also important in causing common cold.[12] Enteroviruses have not, however, been included in studies with MEE from OME children. The putative role of the newly discovered parvovirus, the human bocavirus, in OME is not yet established. In order to understand the pathogenesis of OME and its possible viral associations, more information is needed.

The purpose of the present study was to determine the presence of human bocavirus and rhino-enteroviruses, and the rate of coinfection in a set of MEE samples collected from OME children. We were especially interested in finding out whether the mucoid type shows any difference to serous MEE according to viral detection.

3. Study design

3.1. Children

The study population comprised 54 children (32 boys), median age 5.4 years, range 2.8–9.8 years with OME who had been admitted for elective surgery (adenoidectomy or tonsilloadenoidectomy), either to the Department of Otorhinolaryngology and Head and Neck Surgery of the University of Debrecen or to the Borsod County Hospital of Miskolc from November 2006 to April 2007. All children were free of acute respiratory infections at least during the preceding 3 weeks and at the time of surgery. All children had conductive hearing loss documented by pure tone audiometry and type B tympanograms by tympanometry for a minimum of 4 weeks prior to operation. The middle ear effusion showed unresponsiveness to conservative treatment with antibiotics and nasal decongestants for 7–14 days after the first diagnosis of MEE. The study protocol was accepted by the Institutional Ethics Committee of the Health and Science Centre of the University of Debrecen. A written informed consent was obtained from each child's parents.

3.2. Sample collection

Middle ear fluids were obtained under general anaesthesia. After mechanical cleaning of the external auditory canal a myringotomy was performed on the anterior part of the tympanic membrane and specimens of MEE were aspirated with a gentle suction to a sterile glass tip. Samples were characterized as serous or mucoid effusions based on the viscosity of the fluid. Samples were rinsed out from the collector with 1 ml of PBS into sterile tubes, tightly capped, frozen immediately and stored at −70 °C for 2–28 weeks before processing. All samples were then transported on dry ice to the University of Helsinki in Finland for virological analysis. Middle ear fluid specimens were analyzed at the National Public Health Institute in Helsinki for rhino- and enterovirus RNA and at the Department of Virology of the Haartman Institute of the University of Helsinki for bocavirus DNA.

3.3. RT-PCR method for picornavirus (HRV, HEV) detection

Extraction of viral RNA from MEE samples was performed with a commercial RNA isolation procedure (QIAamp, QIAGEN GmbH, Hilden, Germany). Reverse transcription PCR was carried out by the method described previously by Blomqvist et al.[13] and Suvilehto et al.[14] The primers were targeted to highly conserved 5′NCR sequences shared by rhino- and enteroviruses and therefore the differentiation of rhinoviruses from enteroviruses was carried out by a liquid-phase hybridisation assay with Europium-labelled oligonucleotide probes (Wallac Oy, Turku, Finland, one for rhinovirus and the other for enterovirus).[15] Quantification of lanthanide fluorescence was performed in a time-resolved manner. The cut-off value of positive samples for both europium-labelled probes was the mean of the negative controls plus 5 times the SD of the mean.

Table 1
Number of positive samples for respiratory virus nucleic acid in two groups of samples (serous and mucoid) based on the type of effusion in 54 children with otitis media with effusion. Differences between these two groups were statistically non-significant with Fisher's Exact Test (level of significance: $P \leq 0.05$).

	Total ($n = 75$)	Serous ($n = 36$)	Mucoid ($n = 39$)
Positive samples	26 (34.7%)	10 (27.8%)	16 (41.0%)
Single virus	19 (25.3%)	8 (22.2%)	11 (28.2%)
Dual virus	6 (8.0%)	1 (2.8%)	5 (12.8%)
Triple virus	1 (1.3%)	1 (2.8%)	0 (0.0%)
Rhinovirus positive	10 (13.3%)	2 (5.6%)	8 (20.5%)
Enterovirus positive	22 (29.3%)	9 (25.0%)	13 (33.3%)
Bocavirus positive	2 (2.7%)	2 (5.6%)	0 (0.0%)

3.4. Quantitative PCR for bocavirus DNA detection

DNA was purified from 200 μl fluid of serous or 20 mg glue of mucoid middle ear effusion sample with the QIAamp DNA Mini Kit (QIAGEN GmbH, Hilden, Germany) and eluted in 200 μl of distilled water, of which 5 μl was used in a total reaction volume of 25 μl. The TaqMan universal PCR master mix (PE Applied Biosystems) was used and the PCR, amplifying a part of the NP1 gene[16] was performed as described before by Kantola et al.[17] using the Stratagene Mx3005P thermal cycler. A positive quantifiable result was obtained with down to 10 copies/μl of the HBoV ST2-containing plasmid.[18] To avoid contamination, samples and PCR mixtures were prepared under laminar flow hoods in separate rooms. Water was used as negative control.

4. Results

4.1. Virus positive middle ear effusion samples

Altogether, 26 (34.7%) of the total 75 MEE samples were positive for viral nucleic acid (Table 1). The two HBoV-positive samples had 4.4 HBoV-genome copies/ml in mucoid effusion and 596 copies/ml in serous effusion, respectively. A sole virus species was detected in 19 of the 26 virus positive samples; enterovirus in 15 (78.9%), rhinovirus in 3 (15.8%) and bocavirus in 1 (5.3%) sample. There were 6 cases of dual (rhino and enterovirus in each) and one case of triple viral infection detected.

4.2. Middle ear effusion types and virus positivity

According to the type of the effusion, the samples were divided into two groups: mucoid and serous effusions (Table 1). In the mucoid MEE group, the proportion of virus positive samples (except for bocavirus) was higher than in the serous group, but there was no statistically significant difference between these two groups with Fisher's Exact Test.

4.3. Bilateral otitis media cases

Twenty-one (39%) children had a bilateral disease while 33 (61%) children had MEE only in one ear. Virus detection showed identical results for both ears in 12 (57%) of the 21 children who had a bilateral disease (Table 2). There were no children with bilateral OME with a different virus detected in the other ear.