

Human rhinoviruses in otitis media with effusion

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Frequent viral upper respiratory tract infections (URTI) are considered to be risk factors for otitis media with effusion (OME). Atopy has also been associated with both OME and viral infections. The aim of this study was to evaluate the presence of viruses in middle ear effusions (MEE) in children 2–7 yr old with OME, and to determine risk factors for virus detection in the MEE. MEE samples, collected at the time of myringotomy from 37 children with OME were assessed. Physical examination, skin prick tests and a standardized questionnaire on OME and allergy were also performed. Viral RNA was detected by the use of reverse transcription PCR (RT-PCR). Fifteen samples (40.5%) were positive for rhinovirus (RV). One enterovirus and no other respiratory viruses were detected. Two out of five (40%), 3/7 (43%) and 10/25 (40%) were positive for RV in acute, subacute and chronic cases, respectively. Children with frequent episodes of OM, with early onset of OM (< 2 yr old), and a positive family history of allergy had a statistically increased risk of RV detection. The two groups were comparable with respect to all other parameters examined. RV is the predominant virus recovered by RT-PCR in the middle ear cavity of children with asymptomatic OME, especially those with a history of longstanding OME or repeated episodes, or children with a family history of allergy. Interactions between allergy and RV infections are likely to predispose to middle ear disease.

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Young children experience frequent mild upper respiratory tract infections (URTI) or common colds, with human rhinoviruses (RV) being the most commonly associated pathogens involved (1). URTI are considered to be significant risk factors for the induction and accumulation of the middle ear effusion (MEE). It has also been suggested that viral infections may induce immunoglobulin E (IgE) hypersensitivity (2, 3), which has been proposed as an etiological factor for otitis media with effusion (OME) (4), and that atopic individuals exhibit a defective immune response against RV infections (5). In the few studies assessing viral presence in MEE in children with OME, the most frequently detected viruses are the RV (a genus of the family Picornaviridae) (6–12).

The picture of the overall disease burden associated with RV infections has changed since the introduction of reverse transcription PCR (RT-PCR) (12). RT-PCR has been extensively used and has been proven to be a sensitive and convenient method for diagnostic and epidemiological purposes concerning RV infections (13, 14). Consequently, indirect assays for viral proteins or nucleic acid sequences documented the presence of RV, and viruses in general, in MEE in children with OME in much higher incidence as compared with culture methods (6, 9, 15).

This is a cross-sectional study aiming in evaluating the presence of viruses in MEE from children suffering from OME and undergoing myringotomy, and determining risk factors for virus implication, and in particular RV.

Patients and methods

Subjects

Children enrolled in the study were referred to the ENT department of the 'P & A Kyriakou Children's Hospital' (Athens, Greece) for symptoms related to OME. The diagnosis of OME was done by an ENT specialist by otoscopic and tympanometric evaluation, after removal of the wax. Type B indicates little or no compliance of the eardrum suggesting fluid in the middle ear space. Only children with type B tympanogram were included in the study. Adenoidal hypertrophy was also assessed.

OME was defined as the presence of MEE behind an intact tympanic membrane without signs or symptoms of acute infection. The duration of the MEE was calculated from the initial diagnosis of OME and the date of operation. Cases were characterized as acute, if their duration was less than 3 wk, subacute with duration of more than 3 wk, but less than 3 months or chronic when the duration was longer than 3 months.

Children with the following conditions were excluded: acute OM, perforations of the Tympanic Membrane (TM), craniofacial abnormalities, sensorineural hearing loss, chronic underlying diseases or under chronic pharmaceutical management (except asthma and anti-asthmatic medication).

After enrollment, a standardized questionnaire was completed by the study physician (FMC) regarding demographic characteristics, and OME predisposing factors. The questionnaire included information regarding date of examination, age, gender, race, number of siblings, housing conditions, exposure to tobacco smoke, daycare attendance, previous adenoidectomy, previous episodes of OM, family history of allergy and OM. Lifetime prevalence of allergy-related diagnoses (asthma, eczema, rhinitis, conjunctivitis, urticaria, anaphylaxis, food hypersensitivity) was assessed using strict clinical definitions.

The questionnaire was followed by clinical examination and performance of skin-prick tests (SPT) to seven locally relevant allergens (grass mix, weed mix, dust mite mix, olive, cat, alternaria, egg white), using the appropriate positive and negative controls. A blood sample was also obtained in which eosinophil counts were assessed.

Middle ear fluid samples were collected by myringotomy under general anesthesia from 37 children who met these criteria. In acute or subacute cases of OME, myringotomy was performed in conjunction with planned adenoidectomy. Twenty-five were males with an age range

from 2.3 to 6.7 yr (median: 4.4 yr). Adenoidectomy was also performed in 36 of the 37 children enrolled, while tonsillectomy was performed in 12 children. Tympanostomy tubes were placed in 21 of the children. The study was approved by the hospital's Ethics Committee and informed consent was obtained from parents of all participating children.

Sample collection

The collection was performed with an electronic suction by aspiration, under the control of an operating microscope device. The obtained samples were diluted with isotonic sodium chloride solution and centrifuged at 2500 *g* for 20 min. The supernatants were aliquoted and stored at -20°C, until assayed.

Virus detection

The presence of genomic material of *picornaviruses*, *influenzae viruses A and B*, *adenoviruses* and *corona viruses OC43 and 229E* in MEE samples was examined by RT-PCR as previously described (16–18). RV were distinguished from enteroviruses by digestion with the restriction enzyme *BglI* as described (13).

Reference viral strains were always included as positive controls; several negative controls (virus transport medium) were also included in each run.

Statistics

Statistical associations between RV detection in the MEE samples and possible predisposing factors were examined. Clinical manifestations and characteristics that were considered as predisposing factors included the presence of IgE sensitization; lifetime prevalence of allergy-related diagnoses, 'any allergic disease', defined as a positive clinical history of asthma, rhinitis, eczema or any combination of these; the family history of allergy and the family history of OM; the detection of elevated eosinophils (>4%) in the blood sample; the placement of tympanostomy tubes; the frequency of OM (≥ 2 episodes per year) and the presence of the first episode of OM before the age of 2 yr. The remaining demographic characteristics and OME predisposing factors, such as gender, age, season of examination, birthplace, prematurity (<36 wk), very low birth weight (VLBW), day care attendance, presence of siblings, congregation at home (persons/room), adenoidal hypertrophy or history of adenoidectomy, socioeconomic parameters (parental educa-

tion and age, urban area of residence), exposure to tobacco smoke and gestational exposure to tobacco smoke were also examined in univariate analyses.

Statistical analysis was performed using SPSS 11.5.1 (SPSS, Inc, Chicago, IL). A *p* value of <0.05 (two-tailed) was considered significant. In the case of continuous variables, statistically significant differences were determined by the *t*-test for independent samples. For categorical data, Fisher's exact test or χ^2 test was used. The crude effect of various risk factors was assessed by univariate logistic regression. The associations were measured and reported by odds ratio (OR) and their corresponding 95% confidence intervals (CI).

Results

Study population

Thirty-seven eligible children who underwent myringotomy during the period October 2002–October 2004, were enrolled. Clinical and demographic baseline characteristics of the patients are shown in Table 1. All children were from the white race, had health insurance and all but two attended day care. Samples were obtained during autumn (*n* = 24) or winter (*n* = 13). Five cases were characterized as suffering from acute OME, 7 as subacute and 25 as chronic.

Microbiologic findings and comparisons

Fifteen children (40.5%) were detected positive for RV, and one (2.7%) was found positive for the closely related *enterovirus*. No other viruses from those examined were detected.

Regarding the duration of OME, positive samples were found in 10/25 (40%), 3/7 (43%) and 2/5 (40%) among chronic, subacute and

acute cases. There were no differences in demographic characteristics between subjects with or without virological detection (Table 1).

A statistically increased risk of RV detection in MEE was associated with a history of two or more reported episodes of OM per year of the child's life (OR = 10.6, CL: 2.17–52.14; *p* = 0.004), and a first episode of OM before the age of 2 yr (OR = 5.3, CL: 1.16–24.67; *p* = 0.03). A fourfold increased risk for RV detection was found among children with a family history of allergy (OR = 4.4, CL: 1.03–18.63; *p* = 0.05). No statistical difference was found with regard to IgE sensitization, diagnosis of asthma or rhinitis, history of eczema, food and drug reactions, anaphylaxis, elevated eosinophils and family history of OM. Tympanostomy tube placement was also not found to be related with RV detection in the middle ear (Table 2).

Discussion

Detection of RV in MEE of children suffering from OME using viral culture was first reported in 1990, with an incidence of 8% in subacute cases of OME (6). Later Pitkaranta et al. (15), applying RT-PCR, found RV in 19% of the MEE of children with asymptomatic longstanding OME. Our study confirms the high rates of RV detection in the MEE, not only in chronic or subacute OME, but also in acute cases. It is also of interest that RV RNA was found with a frequency of approximately 40%, irrespectively

Table 2. Univariate logistic regression analysis of risk factors for rhinovirus (RV) detection in the middle ear effusions (MEE) of patients with otitis media with effusion (OME)

	RV (+) n (%)	RV (–) n (%)	Odds ratio (p)	95% confidence interval
Episodes of OM (≥2 per year)	10 (71)	4 (19)	10.6 (0.004)	2.17–52.14
First episode of otitis before the age of 2 yr	8 (57)	4 (20)	5.3 (0.03)	1.16–24.67
Immunoglobulin E sensitization	3 (20)	5 (23)	0.85 (0.84)	0.17–4.26
Any allergic disease	8 (53)	8 (36)	2 (0.31)	0.53–7.6
Asthma	6 (40)	7 (32)	1.43 (0.61)	0.36–5.61
Eczema	2 (13)	3 (14)	0.97 (0.98)	0.14–6.67
Rhinitis	3 (20)	4 (18)	1.13 (0.89)	0.21–5.95
Conjunctivitis	4 (27)	4 (18)	1.16 (0.54)	0.34–7.91
Urticaria	6 (40)	7 (32)	1.43 (0.61)	0.36–5.61
Food reactions	3 (20)	2 (9)	2.5 (0.35)	0.36–17.17
Drug reactions	3 (20)	2 (9)	1.43 (0.61)	0.36–5.61
Anaphylaxis	0 (0)	1 (4.5)	–*	–*
Family history of allergy	10 (71)	8 (36)	4.4 (0.05)	1.03–18.63
Family history of OM	7 (50)	10 (45.5)	1.2 (0.79)	0.31–4.59
Eosinophilia (>4%)	2 (17)	4 (21)	0.75 (0.76)	0.12–4.89
Tympanostomy tube placement	7 (47)	14 (67)	0.4 (0.23)	0.11–1.71

* , OR, 95% CI cannot be calculated because of 0 RV positives. Values in bold are statistically significant.

Table 1. Demographic characteristics and otitis media with effusion (OME) predisposing factors in children with rhinovirus (RV) positive (+) and negative (–) specimens. Numbers are mean (range) for continuous variable and number (percentage) for categorical variable

Factor	Total	RV (+)	RV (–)	<i>p</i>
Gender (male) (%)	25 (68)	11 (73)	14 (64)	0.72
Age (years)	4.4 (2–7)	4.2 (2–6)	4.5 (3–7)	0.59
Season of examination (winter) (%)	13 (35)	7 (47)	6 (27)	0.3
Day care attendance (%)	35 (95)	13 (87)	22 (100)	0.16
Siblings (%)	25 (68)	11 (73)	14 (64)	0.72
Congregation at home (persons/room)	0.9 (0.5–1.5)	0.9 (0.6–1.5)	0.8 (0.5–1.3)	0.14
Adenoidal hypertrophy (%)	36 (97)	14 (93)	22 (100)	0.41
History of adenoidectomy (%)	1 (3)	1 (7)	–	0.41
Residence (urban) (%)	20 (54)	8 (53)	12 (54.5)	0.94
Exposure to tobacco smoke (%)	10 (27)	5 (33)	5 (23)	0.71

of duration, not unlike the frequency detected in common colds.

However, we should point out that we did not exclude children with recent symptoms of URTI, as our aim was to evaluate the overall burden of viral infections in asymptomatic OME. The detected RV RNA in the MEE in children with subacute or chronic OME may represent either residual non-infectious RNA from a previously cleared infection, or persistent infection. Although Moyse et al. (9) recovered RSV RNA in 15% of mucosal cells from children with chronic OME, Pitkaranta et al. (19) did not detect RV RNA in middle ear biopsies from children with longstanding OME. Moreover, in acute OM or in the turbinates of patients of common cold, RV RNA has been detected with a frequency of up to 41% (20–23). RV is also the most prevalent pathogen during URTI and rhinitis, with an incidence approximately of 40% (24). Consequently, asymptomatic infection cannot be ruled out, more so in cases with co-existent adenoidal hypertrophy, such as herein. On the other hand, there are no control samples from the upper respiratory system or blood to assess the frequency of RV in asymptomatic patients.

In a recent study, RV has been found in around 40% of adenoids from children undergoing adenoidectomy, far from a URTI, while children with OME were more often positive for RV than those with dry ears (25). These results support the view that RV, after harboring the nasopharynx and the adenoids, or as we recently showed, after circulating in the blood stream (26), may play a role in the persistence of OME by entering via the eustachian tube, and by causing intermittent middle ear infections, inducing and maintaining OME.

RV is the major cause of acute asthma exacerbations that follow common colds (26). It has also been shown that RV after reaching and replicating in epithelial cells of the lower airways are able to activate these cells to produce pro-inflammatory mediators (27), such as cytokines and chemokines, and up-regulate co-stimulatory molecules in human bronchial epithelial cells (28). It is, therefore, possible that similar events may take place in the middle ear cavity, which is lined by respiratory epithelium of the same origin as the one that is found in the tracheobronchial tract, leading to the development and/or persistence of OME.

The lack of association between asthma, or any other atopic condition, and RV presence in this study could be attributed to the possibility that atopy is a strong predisposing factor for

chronic OME, regardless of specific viral presence, or it may simply be due to low statistical power.

We also found that children positive to RV had a history of early onset of OM and more frequent episodes of OM. Early onset and several prior episodes of OM have been found to increase the risk of OME (29). These associations may indicate that such children are more susceptible to RV infections, leading to early and frequent OM episodes. It is also possible that the children in which the virus was detected are exposed to other factors that increase the risk of RV infections and common colds.

However, it should be noted that there was no difference between children in which RV was detected or not, with respect to most known risk factors for viral infections, such as age, gender, seasonal distribution, home congregation, child-care attendance, number of siblings, adenoidal pathology or smoking in the house.

Family history of allergy, but not family history of OM, was found to statistically increase the risk of viral detection. Prior epidemiological studies have associated family history of allergy with viral respiratory tract infections in early childhood (30, 31). It is possible that allergy predisposition may increase the risk of RV infections, and their common complication, OM.

In conclusion, RV is quite frequently present in the middle ear of children with OME, especially in the ones with an early presentation and/or repeated episodes. Whether this increased frequency is etiologically associated with OME pathogenesis will require prospective investigations.

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