Iranian J Publ Health, Vol. 37, No.4, 2008, pp.109-113

Original Article

Detection of Fungal DNA in the Middle Ear Effusion of Patients Suffering from Otitis Media with Effusion

***MM Jalali [1], S Rezaie [2], A Kousha [1], F Saadat [3], R Banan [1]**

[1]ENT-HNS Research Center, Guilan University of Medical Sciences, Iran
[2]Dept. of Mycology, School of Public Health, Tehran University of Medical Sciences, Iran
[3]School of Medicine, Guilan University of Medical Sciences, Iran

(Received 25 Apr 2008; accepted 1 Dec 2008)

Abstract

Background: Otitis media with effusion (OME) is common in the children. It is proven that pathogenic bacteria as a causative agent of middle ear effusion, however the role of fungal infection in otitis media with effusion remains unclear. Therefore, this study was conducted to assess presence of fungi in OME.

Methods: From January 2005 to September 2006, a number of 62 children with proven otitis media with effusion subjected to the case series study at Amiralmomenin Hospital in Rasht City, Iran. After myringotomy, middle ear effusion was collected. In 48 patients, both ears demonstrated effusion, whereas in 14 patients, only one ear had effusion. Standard mycological culture and polymerase chain reaction (PCR) assay were performed in 110 and 79 samples, respectively.

Results: The growth of fungi was observed in 9 samples (8.8%). The result of our PCR assay showed that 23 samples (29.1%) were positive for fungal DNA.

Conclusion: Middle ear effusion from cases with OME contains fungi and it might play a role in the pathogens of OME. PCR assay is a better indicator in detection of fungus in middle ear effusion, compared with fungal culturing method. However, the estimation of its sensitivity and specificity in detection of fungal agents in these patients needs more molecular epidemiological studies.

Keywords: *Otitis media with effusion, PCR, Fungi, Iran*

Introduction

Otitis media with effusion (OME) is the most common ear disease in preschool age children and one of the most common diseases overall (1, 2). Between 20% and 50% of children will have an episode of OME between the age of 3 and 10 yr (3). OME refers to an inflammatory effusion behind an intact tympanic membrane that is not associated with acute otologic symptoms or systemic signs. The process may be classified as acute (effusion lasting up to 3 wk), subacute (up to 3 months) or chronic (more than 3 months) (1, 4-6). The chronic cases may present with a hearing impairment or secondary sequelae as tinnitus, dizziness, imbalance, speech, language and developmental delay or behavioral problems (5).

Although a number of factors leading to the development of OME have been postulated, and it is possible that in an individual case, all different mechanisms be involved (5). Eustachian tube dysfunction is considered the major etiologic factor in the development of middle ear disease. The second etiologic theory proposes an inflammatory origin to otitis (4, 7). Standard bacteriologic analysis of effusion from OME has shown the presence of various agents in some cases. Moreover, few studies have examined the presence of fungi in middle ear effusion in patients with otitis media (8).

The diagnosis of fungal infections remains a significant problem. Fungal routine diagnostic tests including culture and histopathological examinations have been limited sensitivity and specificity. PCR showed to be useful molecular techniques for detection of pathogens particularly for detection

***Corresponding author:** Tel: +98 131 2238307, Fax: +98 131 3241842, E-mail: mmjalali@gmail.com

of those with slow growing (9, 10). This assay is specific and relatively sensitive and could be applied for detection of dormant pathogens (11).
In the present study, we evaluated the role of fungus in OME by investigating their presence within middle ear effusions using fungal culture and PCR techniques in patients that underwent myringotomy and adenoidectomy.

Materials and Methods

Preparing of Samples

Samples were collected from the patients that referred to the Amiralmomenin Hospital of Guilan University of Medical Sciences from January 2005 to September 2006 and were included in the case series study. The diagnosis of OME was documented in these children clinically and paraclinically. Inclusion criteria included effusion behind tympanic membrane at least 3 months that did not resolve with routine antibiotic therapy. Also, there were tympanometric findings that support otitis media with effusion. Effusions were collected under sterile conditions using a middle ear suction trap (9). In brief, effusions were obtained by myringotomy under the general anesthesia and with the help of a Zeis microscope. At first external ear canal was filled with Betadine solution and 1 min later Betadine solution was aspirated. Then myringotomy was performed and effusion was collected with the use of an angiocath (#16) which was connected to a 20 ml syringe.
Adenoidectomy were performed in 61 cases and samples transferred for mycological culture assay. Then collected effusions were stored in an airtight container at -4° C and transferred immediately (<5 h) to the School of Public Health in Tehran University of Medical Sciences under sterile conditions.

Mycological Tests

Standard mycological culture assay was performed in 110 samples based on standard method (12). Briefly, the samples were cultured on Saboraud Glucose Agar (S) for almost 10 d at 30° C. The cultures were then investigated everyday for detection of fungal growth.

PCR Analysis

PCR analysis was performed for 79 samples using specific pan-fungal primers on isolated DNA molecules from middle ear effusion (MEE) samples. Briefly, High molecular weight DNA was isolated from MEE samples by DNP^{TM} DNA isolation kit (fermentase, Germany). PCR analysis of genomic DNA obtained from MEE samples were performed according to a standard protocol (13) using synthetic oligonucleotide pan-fungal primers including: PAN-S1; 5′- tcc gta ggt gaa cct gcg g -3′, as forward and PAN-As1; 5′- tcc tcc gct tat tga tat gc -3′, as reverse primers. The PCR reaction was performed in a volume of 50 µl containing 20 ρM of each mentioned primers, 20 mM $(NH4)_2$ SO_4, 75 mM Tris-HCl (pH. 8.8), 1 mM $MgCl_2$, 0.2 mM dNTP mix, 1.2 Units of thermo stable DNA polymerase (Advance Biotechnologies, UK), and 1 µl of template (genomic DNA). The PCR reaction parameters were 30s at 95°C, followed by 35 cycle of denaturation at 48 °C for 60s, annealing at 72° C for 90s. PCR products were analyzed by ethidium bromide staining after electrophoresis in on 1% agarose gel. Samples were dried for 2 h and exposed overnight, then visualized under UV light**.**

Results

A total of 110 aspiration samples from 62 children aging between 1 and 14 yr (mean age, 6.74± 2.3) were collected. Thirty seven of the subjects were girls and 25 were boys. In 48 patients, both ears demonstrated effusion, whereas in 14 patients, only one ear had effusion. Standard mycological culture assay indicated the growth of different fungal species in 9 samples (8.18%). These species were *Penicillium* spp., *Aspergillus fumigatus* and *A. flavus*, with 5, 3 and 1 isolates, respectively.
Because of the quantity of MEE samples, PCR analysis was performed only for 79 cases of them. According to our PCR analysis, 23 samples (29.1%) were positive for fungal DNA. Positive and nega-

tive results in mycological culture or PCR assay were depicted in Table 1.

Table 1: Results of fungal culture and PCR assay from 79 samples of MEE

PCR / Culture	Negative	Positive	Total
Negative	56	17	73
Positive	-	6	6
Total	56	23	79

Discussion

The purpose of our study was to investigate the relations of fungal infection in the children with OME. Using PCR assay, we found fungal DNA in 29.1% of samples. The results of this study are consistent with findings from other studies. Results of a case control study of children aged 6-12 indicated that mold, dampness in the home and flooding were associated with otitis media (8). Also fungal DNA was present in 34% of 29 cases with otitis media with effusion (9).

Recent techniques for identification of bacterial and viral antigens facilitate to determine infectious agents. In 1995, Post et al. reported that 77.3% of their patients were PCR positive for one or more of organisms. Although, the role of anaerobic bacteria in chronic MEE remains unclear, they have been isolated in 0-10% of chronic MEE samples (14). In addition, the role of viruses as primary or co-pathogenic organisms in otitis media has gained increasing attention (7, 15, 16).

Cases in which fungi have been implicated in acute otitis media include patients with prior systemic disease due to organism or complicated by granulation tissue. Mycological infections of the mastoid and middle ear including *Candida* and *Aspergillus* species were identified in patients with chronic suppurative otitis media and with human immunodeficiency syndrome (17).

Using PCR, the number of episodes of otitis that are now categorized as no growth may decrease (2). Culture of middle ear effusions yield positive results for only 20-30% of patients, by PCR, up to 75% of middle ear effusions are positive for pathogenic bacteria (15). Perhaps other pathogens such as fungi are causes of other cases of OME.

In the present study, the PCR protocol has been performed for detection of fungal agents in MEE patients which showed to be able to detect more positive cases than fungal culture. Using mycologic culture, we indicated fungal species in 8.18% of samples. Comparison to PCR results, it has not enough sensitivity for detection viable organisms present in otitis media, possibly because pathogens are not free floating in middle ear effusion that would be required for successful cultures. This has been shown by Post who demonstrated the presence of bacterial aggregates attached to the middle ear mucosa as a bacterial film (18). Therefore negative fungal cultures may either the true absence of viable organisms, the failure of fungal culture itself or the unavailability of fungi because of fungal biofilm. In conclusion, 56 samples (70.9%) were totally negative and 6 cases (7.59%) were totally positive in both PCR protocols as well as fungal culture. However, in 17 cases (21.5 %) PCR protocol was the only positive detector for fungal agents. PCR and culture yielded concordant results in 62/79 effusion samples, Cohen's kappa coefficient equaled 0.33.

It might be argued that fungal DNA detected in the present study was left from an earlier infection and PCR assay relies on the detection of genetic material regardless of the viability of the agents (19). But it can be considered that an immune reaction might cause damage to the mucosa, thereby predisposing an individual for secondary bacterial infection. Although there isn't enough evidence for this notion that middle ear mucosa serves as the target organ for allergy (16). In conclusion, compare with fungal culturing method, we suggest the PCR assay as a better detector of fungal colonization in MEE patients. However, the estimation of its sensitivity and specificity in detection of fungal agents in MEE patients needs more molecular epidemiological studies with more detected samples.

Acknowledgements

The authors gratefully acknowledge the contributions of the staff and physicians of the Amiralmomenin Hospital and Molecular Biology of Mycology Department who have participated in this study. This investigation was supported by a grant from Guilan University of Medical Sciences, Rasht, Iran.

The authors declare that they have no conflict of interests.

References

1. Probst R, Grever G, Iro H (2006). *Basic otorhinolaryngology a step by step learning guide*. 1st ed. Georg Thieme Verlag, p. 240.
2. Klein JO, Tos M, Hussl B, Naunton RF, Ohyama M, van Cauwenberge PB (1989). Recent advances in otitis media. Definition and classification. *Ann Otol Rhinol Laryngol Suppl*, 139: 10.
3. Browning GG (1997). Aetiopathology of inflammatory conditions of the external and middle ear. In: *Scott- Brown's otolaryngology*. Ed, Booth JB. 6th ed, Butterworth- Heinemann International Edition, pp. 3/3/10-14.
4. Coates H, Thornton R, Langlands J, Filion P, Keil AD, et al. (2008). The role of chronic infection in children with otitis media with effusion: evidence for intracellular persistence of bacteria. *Otolaryngol Head Neck Surg,* 138(6): 778-81.
5. Roberts JE, Rosenfeld RM, Zeisel SA (2004). Otitis media and speech and language: a meta-analysis of prospective studies. *Pediatrics,* 113(3 Pt 1): e238-48.
6. Di Francesco R, Paulucci B, Nery C, Bento RF (2008). Craniofacial morphology and otitis media with effusion in children. *Int J Pediatr Otorhinolaryngol*, 72(8): 1151-8. Epub 2008 Jun 2.
7. Moyse E, Lyon M, Cordier G, Mornex JF, Collet L, Froehlich P (2000). Viral RNA in middle ear mucosa and exudates in patients with chronic otitis media with effusion. *Arch Otolaryngol Head Neck Surg,* 126(9): 1105-10.
8. Yang CY, Cheng MF, Tsai SS, Hung CF, Lai TC, Hwang KC (1999). Effects of indoor environmental factors on risk for acute otitis media in a subtropical area. *Journal of Toxicology and Environmental Health Part A*, 56: 111–19.
9. Kim EJ, Catten MD, Lalwani AK (2002). Detection of fungal DNA in effusion associated with acute and serous otitis media. *Laryngoscope*, 112(11): 2037-41.
10. Catten Md, Murr AH, Goldstein JA, Mhatre AN, Lalwani AK (2001). Detection of fungi in the nasal mucosa using polymerase chain reaction. *Laryngoscope*, 111: 399-403.
11. Shin EJ, Guertler N, Kim E, Lalwani AK (2003). Screening of middle ear effusion for the common sinus pathogen Bipolaris. *Eur Arch Otorhinolaryngol*, 260: 78-80.
12. de Hoog G, Guarro J, Gene J, Figueras M (2000). *Atlas of clinical fungi*. 2nd ed. Centralbureau for Schimmelcultures, Utrecht, The Netherlands, and University Rovira i Virgili, Reus, Spain.
13. Rezaie S, Ban J, Mildner M, Poitschek C, Brna C, Tschachler E (2000). Characterization of a cDNA clone, encoding a 70 kDa heat shock protein from the dermatophyte pathogen Trichophyton rubrum. *Gene*, 241(1): 27-33.
14. Bluestone CD, Klein JO (2001). *Otitis media in infants and children*. 3rd ed. WB Saunders Company, pp. 79-94.
15. Chantzi FM, Papadopoulos NG, Bairamis T, Tsiakou M, Bournousouzis N, Constantopoulos AG, et al. (2006). Human rhinoviruses in otitis media with effusion. *Pediatr Allergy Immunol,* 17(7): 514-8.
16. de Ru JA, Grote JJ (2004). Otitis media with effusion: disease or defense? A review of the literature. *International Jour-*

nal of Pediatric Otolaryngology, 66:331-39.

17. Bluestone CD, Klein JO (2003). Otitis media and eustachian tube dysfunction. In: *Pediatric Otolaryngology*. Eds, Bluestone CD, Stool SE, Alper CM, Arjmand EM, Casselbrant ML, Dohar JE, Yellon RF,eds. 4th ed. WB Saunders Company, pp. 553-54.
18. Post JC (2001). Direct evidence of bacterial biofilms in otitis media. *Laryngoscope*, 111: 2083-94.
19. Gok U, Bulut Y, Keles E, Yaclin S, Doymaz MZ (2001). Bacteriological and PCR analysis of clinical material aspirated from otitis media with effusions. *International Journal of Pediatric Otolaryngology*, 60: 49-54.