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ORIGINAL ARTICLE

The Role of Chlamydia Pneumoniae and Mycoplasma Pneumoniae in the Etiology of Otitis Media with Effussion

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Objective: Aim of this study is to investigate the respiratory tract pathogens, Chlamydia pneumoniae and Mycoplasma pneumoniae that can cause otitis media with effusion (OME) by of colonization in nasopharynx and adenoid tissue.

Materials and Methods: 39 patients aged between 2 and 10 years who were submitted to myringotomy, ventilation tube insertion and adenoidectomy due to otitis media with effusion, were involved in the study. 23 patients had bilateral effusions as the rest had unilateral. Effusion and nasopharynx swab samples were obtained from patients during the surgery operation under general anesthesia. Materials were investigated by polymerase chain reaction (PCR), a molecular method for Chlamydia pneumoniae and Mycoplasma pneumoniae.

Results: Chlamydia pneumoniae was detected in effusion fluids of 7 (17.9%) patients and in nasopharynx specimens among 5 (12.8%) patients. There was only 1 (2.5%) patient that Chlamydia pneumoniae is detected where in both effusion fluid and nasopharynx swab samples. For Mycoplasma pneumoniae the effusion materials were found positive with polymerase chain reaction only in 2 (5.12 %) patients and all nasopharynx swabs were negative.

Conclusion: Chlamydia pneumoniae and Mycoplasma pneumoniae cannot be isolated with conventional methods. The new methods on isolation of bacteria within the samples of middle ear effusions help on planning new antibiotic treatment strategies.

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Introduction

Otitis media with effusion (OME) is the most frequent ear disease following acute otitis media (AOM) during childhood. It is a type of otitis media, characterized by accumulation fluid behind the tympanic membrane without the signs and symptoms of the generalized or local infection [1, 2].

The disease was named as secretory otitis media, non-suppurative otitis media, serous otitis media for years, however the term OME is accepted as the most appropriate definition due to ethiopathogenesis of the disease and the properties of the accumulated fluid. In regard to its complications such as atelectasia, cholesterol granuloma and retraction pocket formation and hearing loss its early diagnosis and appropriate treatment have major importance [3-5].

Besides the already mentioned risk factors recently, the arguments suggesting that upper respiratory tract infections (URTI) and recurring otitis media focused attention [2].

The effusion in the middle ear can be classified as acute, subacute and chronic due to the duration. Effusions persisting for more than three months following AOM should be accepted as OME [6].

The role of infection in the ethiopathogenesis of OME is still accepted to be the major factor. The studies until now could demonstrate Streptococcus pneumoniae, Haemophilus influenzae, Moraxella catarrhalis and Group A beta hemolytic streptococci as being the frequent agents being responsible of OME formation. [7]. However in 50 % of the cases microorganisms were not identified [8,9,15]. The reason is that, some bacteria causing infection in the respiratory tract cannot be isolated by routine methods. However, recently the use of polymerase chain reaction (PCR methods served for identifying microorganisms other than mentioned above [5, 10].

Chlamydia pneumoniae frequently cause respiratory tract infections is an obligatory intracellular bacteria. Mycoplasma pneumoniae lacks a cellular wall. Both were thought to play a role in the OME etiology. Because of their structure, it is not possible to isolate them in the effusion material by using conventional methods [11-14].

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We tried to bring a new sight to the etiology of OME by searching these two microorganisms using the PCR method, mainly in the nasopharynx where the bacteria mostly colonize to cause infection in the middle ear.

Materials and Methods

39 patients who were diagnosed as OME in Ankara University, Faculty of Medicine, Otorhinolaryngology Head and Neck Surgery Department during September 2006 - March 2007, involved in this study.

Their ages were ranged between 2 - 10 years. All were followed at least for 3 months as being unresponsive to the medical treatment. None of them received antibiotics within one month before the operations. The patients' nasopharynxes were evaluated by using flexible endoscopes in regard to adenoid vegetation and degree of hypertrophy.

Study was performed at the Ankara University, Faculty of Medicine, Otorhinolaryngology Head and Neck Surgery Department and Microbiology Department, in accordance with the principles of Helsinki Declaration following approval of the protocol by the Ethics Committee of Ankara University Faculty of Medicine. A written consent was signed by all parents of patients after being informed about objectives. The study includes no healthy children as a control group, since there is no material to be collected from patients without any effusion in the middle ear and for ethical reasons.

The factors like hearing loss, ear pain, and ear discharge, symptoms of the adenoid hypertrophy, and frequent URTI, AOM, tonsillitis attacks, passive smoking, continuation to the day nursery and day care centers and presence of OME in first degree relatives were noted by applying an enquiry form to the parents of the patients.

Effusion fluid and nasopharyngeal swab samples were obtained under general anesthesia during the myringotomy procedure. Effusion fluid was aspirated into a tube containing 1 ml sterile saline solution. Nasopharyngeal swab samples were placed in Stuart carrying media (for routine examination) and to the specific 2SP carrying media for Chlamydia pneumoniae. All materials reached to the Microbiology Laboratory obeying the cold chain principles.

Besides the physical characteristics of the effusion, nasopharyngeal examination findings and degree of the adenoid hypertrophy were noted during the operation.

The samples were carried with the Stuart's medium and cultivated to 5 % sheep blood agar, Chocolate agar, MacConkey agar and Sabourraud dextrose agar and cultured at 37 °C for 24 - 48 hours. The evaluation and identification of the growing microorganisms were performed according to the colony properties, pigment formation and hemolytic activities.

DNA extraction was performed by using phenol-chloroform method to both effusion fluids and to swab samples taken to the 2SP carrying medium, and extracted DNAs were kept at minus 20 °C until PCR study would be performed.

During the PCR study, investigation for Chlamydia pneumoniae Cpn A (5' TGA CAA CTG TAG AAA TAC AGC 3'), Cpn B (5' CGC CTC TCT CCT ATA AAT 3') primers having size of 460 bp specific to the 16S rRNA gene area, and for Mycoplasma pneumoniae M.pneu S (5' AGG CTC AGG TCA ATC TGG CGT GGA 3'), M.pneu AS (5' GGA TCA AAC AGA TCG GTC ACT GGG 3') primers having size of 330 bp were used.

Amplified DNA's of bacteria were analyzed by gel electrophoresis using 2% (w/v) agarose in a Tris-Borate buffer containing ethidium bromide (0.5 microgram/ml) for detection of DNA under ultraviolet transillumination. The PCR products were confirmed by comparing the bands of DNA ladder and the gel appearances were digitally photographed.

Sample gel appearances of the C. pneumoniae and M. pneumoniae obtained with PCR method are shown in Figures 1 and 2.

Results

In this study the clinical symptoms, risk factors, seasonal characteristics and microbiologic results obtained with PCR method in 39 patients are showed in tables.

Frequency of Symptoms included in the study OME patients are shown in Table 1.

Risk factors identified in these patients for OME are shown in Table 2.

Seasonal characteristics of the OME symptoms and attacks are shown in Table 3.

The number of cases having isolated C. pneumoniae and M. pneumoniae within in the nasopharyngeal swabs with PCR method are shown in Table 4.

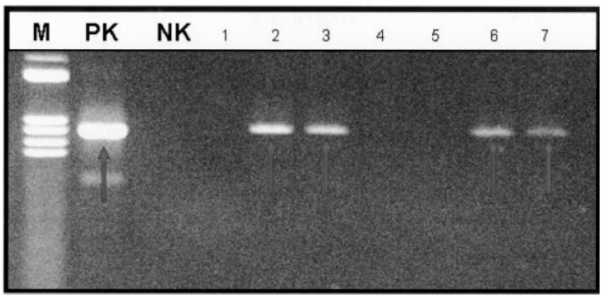

Figure 1. 4 Nasopharyngeal swabs were positive for C. pneumoniae

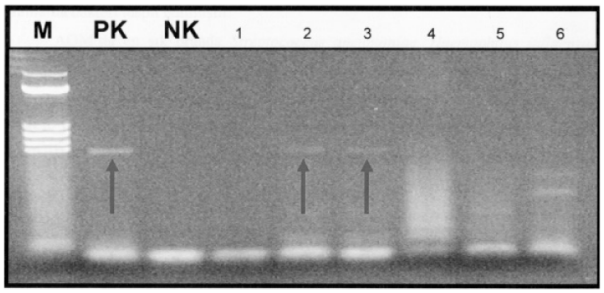

Figure 2. 2 Nasopharyngeal swabs were positive for M. pneumoniae

The patients were also grouped according to the degree of the adenoid hypertrophy, 6 patients did not have obstructive adenoid tissue whereas 7 patients had 25-50%, 18 patients had 50-75 % and 8 patients had 75-100 % obstruction of nasopharynx with their adenoid masses.

In 2 patients pathogen bacteria H. influenzae and group B beta hemolytic streptococcus were isolated by conventional cultures in nasopharyngeal samples as the other patients had normal bacterial flora.

Effusions were obtained bilaterally from 16 and unilateral from 23 cases. 51 (%82) of the effusion material had mucoid and 11 (% 17.7) had serous character. Chlamydia pneumoniae was identified by PCR method in the effusion fluid of 7 (17.9 %) and in nasopharyngeal swab material of 5 (12.8 %) of these patients. Of these 7 patients on whom Chlamydia pneumoniae was identified, 4 had bilateral and 3 had bilateral OME.

Of the 7 patients on whose effusion fluid C. pneumoniae was identified, only 1 had C. pneumoniae in the nasopharyngeal swab. Within the nasopharyngeal swabs of the remaining 6 cases, C. pneumoniae could not be isolated. In other words, of the 5 cases with nasopharyngeal swab positive, C. pneumoniae could be identified in the effusion fluid of only 1 (2.5 %). In this situation, C. pneumoniae could be shown in only one of the patients of the study both in middle ear effusion fluid and also in nasopharyngeal swab with PCR.

Table 1. Frequency of Symptoms included in the study OME patients

Symptoms	Number of Case	%
Hearing Loss	28	71.8
Otalgie	14	35.9
Otorrhea	3	7.7
Nasal Obstruction	28	71.8

Table 2. Risk factors identified in these patients for OME

Risk Factors	Number of Case	%
Frequent attack of AOM	9	23.1
Frequent Upper Respiratory Tract Infection	20	1.3
Frequent tonsillitis attacks	3	7.7
Passive smoking	16	41.0
Day-care centers	9	23.1
Family OME story	6	15.4
Adenoidectomy story	10	25.6
İnsertion ventilation tube story	9	23.1

Table 3. Seasonal characteristics of the OME symptoms and attacks

Seasonal Characteristics	Number of Case	%
Summer	1	2.6
Winter	33	84.6
All Year	5	12.8

Table 4. Number of (%) positive C.pneumoniae and M. pneumoniae identified in the nasopharyngeal swabs with PCR method in 39 patients

Bacteria	Number (%) of positive Effusion fluid sample	Number (%) of positive Nasopharyngeal swab samlple
C. pneumoniae	7 (% 17.9)	5 (%12.8)
M. pneumoniae	None	2 (% 5.1)

Mycoplasma pneumoniae could not be identified in the effusion fluid with PCR in none of the patient included in the study and Mycoplasma pneumoniae identified in nasopharyngeal swab samples of 2 patients (5.1 %) with PCR.

Discussion

The ethiopathogenesis of the OME, is still in debate. Therefore, there is no consensus also, on the treatment of the disease, as it is considered to have multi-factorial pathogenesis. Priorly, it was thought that OME was

sterile and developed because of Eustachian dysfunction or allergy. Later studies supported infections to be responsible in the etiology and the rate of identifying bacteria in the effusion fluid raised up to 52 %.

The most frequent agents of acute otitis media (S. pneumoniae, H. influenzae, M. catarrhalis, S.aureus) are also identified in OME fluid, but in less and different rates [13, 15]. Recently by inserting specific methods into the test batteries, it has been possible to identify the microorganisms in higher rates. For example, Palmu, et all, isolated S. pneumoniae 27 % in standard cultures and this rate increased to 47.1 % with PCR method [16].

In some cases, bacteria could not be identified although having clinical signs of infection and surgery such as adenoidectomy, myringotomy and ventilation tube placement are applied following unsucceeding with medical treatment. This encouraged the physicians to focus on trying to identify and isolate the bacteria that are hard to grow in laboratory conditions (e.g. C. pneumoniae and M. pneumoniae).

C. pneumoniae is an obligatory intracellular bacteria and cause especially in closed, crowded groups of all age groups with mild and asymptomatic respiratory tract infections [17]. It is shown to be responsible for lower and upper (tonsillitis, pharyngitis, otitis media, sinusitis etc) respiratory tract infections in different rates in the studies [12,18]. It is said that 80 - 90 % of the C. pneumoniae infections go on without pneumonia, only with upper respiratory tract infection findings like pharyngitis. So, C. pneumoniae became an important issue to be searched for the OME etiology, as it cannot be grown with the conventional culture methods and as URTI and middle ear infections go together.

Falk et al[19], examined the throat swab and effusion samples of 26 children having otitis media with PCR and determined C. pneumoniae DNA at 6 out of the 9 children with acute otitis media and 10 out of 17 children with OME. In this study, bacteria could not be isolated with cell culture in the effusion fluids in 4 children diagnosed as persistent OME and in one case, bacterial DNA that was determined with PCR in effusion sample again diagnosed as persistent OME

Many studies were performed to understand the role of C. pneumoniae in otitis media with effusion. For example, Storgard et all determined C. pneumoniae DNA in 5 cases out of their 53 cases whereas Jero et all reported that they could not determine the DNA of C. pneumoniae in none of their samples in this study with 123 cases [20,21].

Besides various factors, laboratory methods are thought to play a role at the background, in the studies investigating the relation between respiratory tract diseases and OME, determining different results about C. pneumoniae [14].

In this study C. pneumoniae is identified in middle ear aspiration material of 7 patients (17.9 %) by PCR method. C. pneumoniae is also isolated in nasopharynx swabs of 5 patients (12.8 %) by PCR method, but there was only 1 patient (2.5 %) whom C. pneumoniae is determinate in both effusion fluid and nasopharyngeal swabs.

This may be due to capture of the patients who have URTI and middle ear infection of C. pneumoniae together, at different stages of the disease as nasopharynx is the only relation area of both ears. This idea may be interpreted as; C. pneumoniae causes persistent infections and the microorganisms at the nasopharynx infect both ears during acute URTI, but after the infection at the nasopharynx healed, the persistent infection at the middle ear due to these bacteria continues.

Mycoplasmas'' role in the OME etiology like C. pneumoniae, which are seen frequently in upper and lower respiratory tract infections and hard to diagnose with the conventional methods, could not be understood exactly yet [14]. This bacteria was reported in low rates in this performed studies. For example Storgaard et all, determined M. pneumoniae in only one case's nasopharynx and middle ear out of their 150 cases' effusion material with PCR whereas Diamond reported that they could not isolate Mycoplasma in their 102 effusion culture [22,23].

In our study we did not determine M. pneumoniae in effusion fluid as M. pneumoniae is seen with PCR only in 2 patients nasopharyngeal swab.

In the studies performed with the thought that adenoid tissue is acting as a reservoir for pathogen bacteria are triggering development of otitis, it is determined that more respiratory pathogen lives in the nasopharynx of the patients with chronic OME [24, 25]. So, although nasopharyngeal culture is thought to be an alternative to the culturing with myringotomy for investigation of the pathogen before, later, nasopharyngeal cultures thought to be non-specific and cannot lead for the treatment from clinical point of view. When the nasopharyngeal and middle ear cultures are compared, nasopharynx culture was found to be sensitive but not specific for the

determination of middle ear diseases [26,27].

The concept that adenoid tissue acts as a reservoir for C. pneumoniae and M. pneumoniae and can cause recurrent upper respiratory tract infection (URTI) should be investigated. C. pneumoniae, because of its structural and antigenic properties, and also its decreasing effect on apoptosis and ciliary movement, thought to cause adenoid hypertrophy [26,28-30] In our study Haemophilus influenzae and Group B beta hemolytic streptococcus cultured as pathogen bacteria only in two cases with conventional techniques in our study. At the other 37 patients, normal throat bacterial flora was determined. When nasopharyngeal swab is examined with PCR, C.pneumoniae was found in 5 (12.8 %) of 39 cases and M. pneumoniae was determined in 2 cases.

Due to the results of our study it is possible to conclude that, PCR methods can assist for further identification of microorganisms as was evident in 17.9 % of the effusion material and 12.8 % of the nasopharyngeal swabs. However as having simultaneously positive isolation in only one patient, it is possible to speculate that there is an important relation between OME and C. pneumoniae; and C. pneumoniae can persist in the tissues. A similar relationship could not be postulated for M. pneumoniae. But, as both of these bacteria can lead to many chronic or recurrent, treatment resistant infections, they may also have an important role in the etiology of OME. To investigate such a relation, longer studies performed on more patients are needed. Another important aspect of C. pneumoniae and M. pneumoniae are related with their resistance to penicillin. If these bacteria are shown to play an active role at the development of AOM and OME, leading to the chronic course of the disease and causing resistance to treatment, then, the antibiotic of first choice should be changed. So, especially on the recurrent cases and in cases with resistance to antibiotic, C. pneumoniae and M. pneumoniae, which cannot be diagnosed with routine methods, should be thought and treatment may be needed to change.

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