

Identification of *Chlamydia trachomatis* with Polymerase Chain Reaction in Middle Ear Fluid in Otitis Media with Effusion

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In this study, 78 middle ear aspiration samples from 59 children between the ages of 2-14 who have undergone myringotomy under general anesthesia were included. The presence of DNA genome of *Chlamydia trachomatis* was analyzed with polymerase chain reaction in the aspiration material. The relationship between the nature of the effusion fluid and the presence of *Chlamydia trachomatis* was investigated. Of the seventy-eight middle ear effusions, 26 (33.3%) were serous, 31 were (39.7%) mucoid and 21 (26.9%) were sero-mucoid in nature. *Chlamydia trachomatis* DNA genome was identified in seven (8.9%) effusion samples. Of these, three were mucoid (42.8%), three were serous (42.8%) and one was sero-mucoid (14.2%) in character. We think that during the bacteriological analyses carried out for the cases of otitis media with effusion, *Chlamydia trachomatis* should be taken into consideration both at the time of diagnosis and the treatment.

Key words: *Chlamydia trachomatis*, Otitis media with effusion (OME), Polymerase chain reaction.

OTTITIS media with effusion (OME) is a type of middle ear inflammation characterized by the accumulation of fluid behind the intact tympanic membrane without the presence of acute signs or symptoms of an infection, and it is the most common cause of hearing loss in childhood. Traditionally, middle ear effusions were considered to be sterile and any type of growth was regarded as a contamination(1). However, in recent years, around 50% of the middle ear effusions were reported to have bacterial growth(1,2). In several studies, middle ear fluid cultures of OME cases were found to be in correlation with the major bacteria profile of the acute otitis media cases(2,3).

The role of *Chlamydia* as an etiological agent in otitis media is being discussed since

1960. Some authors report isolating *Chlamydia trachomatis* in middle ear accumulates of patients with OME and AOM(4,5). However, this situation was not confirmed by others(6,7). Similar controversies also exist for *Chlamydia pneumoniae*. In few isolated cases of OME, *Chlamydia pneumoniae* is reported to have been isolated in the cultures of middle ear fluid(8,9).

Polymerase chain reaction is a technique which allows for the amplification of limited quantities of the nucleic acid of the microorganism that is being questioned and aids in its identification. This method helps us to identify the bacterial DNA present in the middle ear fluid(3).

In this study, we analyzed the middle ear aspirates of OME cases for the presence of

Chlamydia trachomatis and tried to demonstrate a possible relationship between the presence of *Chlamydia trachomatis* by PCR and the nature of the effusion material.

Subjects and Methods

Seventy eight middle ear aspiration samples from 59 children between the ages of 2-14 who have undergone myringotomy under general anesthesia in Firat University School of Medicine, Department of ENT from April 2002 to May 2003 were included in the study. Presence of the effusion for longer than three months was taken as a criterion for the presence of OME. Patients with infections, sinusitis, diabetes mellitus, and immune insufficiencies were excluded from the study.

Effusion samples were obtained from the patients under general anesthesia by using Zeiss Opmi-1 operation microscope. Outer ear canal was filled with 70% alcohol and aspiration was carried out after waiting for one minute. Following the aspiration, the anterior lower quadrant of the tympanic membrane was entered with a No. 6 cannula (if ventilation tube was to be inserted) or myringotomy was performed with an angled politzer tympanic perforator from the same quadrant. Effusion from the middle ear was directly transferred into a collector tube with a sterile single-use aspirator set (Xomed Surgical Products, Jacksonville, Florida, USA). PCR method was utilized for identification of DNA of *Chlamydia trachomatis* in middle ear effusion media.

Statistical analyses were made by using Student's *t* test, for samples by using SPSS 10.0 for Windows 98. *P* value of <0.05 was accepted as being statistically significant.

Chlamydia trachomatis DNA Identification and PCR

Middle ear effusion samples were transferred into 1.5 mL centrifuge tubes. 500 μ L K buffer (20 mM Tris pH 8.0, 150 mM NaCl, % 0.2 SDS and 10 mg/mL Pronase) containing dithiotretiol (DTT) at a concentration of 40 mM were added to the tubes. After vortexing the tubes for a minute, they were left on a rotating surface at 37°C overnight. Following this procedure, standard phenol-chloroform extraction and ethanol precipitation was carried out for total DNA isolation. Isolated DNA was diluted with 50 μ L dH₂O and digested with RNase A at a concentration of 50 μ g/ml for 30 minutes at 37°C. The samples were kept at -20°C until performing PCR(10). In this study, sterile dH₂O and *Chlamydia trachomatis* (ATCC 434) species were used as negative and positive controls, respectively, for both extraction of DNA and PCR analysis. All procedures were applied for both the samples and negative and positive controls. Ten times diluted solutions of DNA extraction product obtained from *Chlamydia trachomatis* were utilized for the specificity of the PCR method. In our experimental procedure, the highest *Chlamydia trachomatis* DNA value was found to be 8 fg (0.8 μ g/mL or 3.35 bacteria per reaction). In brief, the specificity and standardization of the PCR procedure employed in this study was found to be 3.35 bacteria.

For the PCR stage, 50 μ L of mixture was prepared. To each tube 5 μ L of 10X reaction buffer (10 mM Tris-HCl (pH 9.0), 50 mM KCl and % 0.1 Triton X-100, Promega) 5 μ L of 25 mM MgCl₂, 4 μ L of 2 mM deoxy-nucleotide triphosphate, 10 μ L of viral DNA, 1 μ L of 5 U/mL Taq polymerase and 1 μ L each of 20 pmol concentrations of two primers: primer 1 which is specific to MOMP gene region of *C. trachomatis* (Ct.0005: 5' GAT

AGC GAG CAC AAA GAG AGC TAA 3') and primer 2 (Ct. 06: 5' TTC ACA TCT GTT TGC AAA ACA CGG TCG AAA ACA AAG 3') and 23 μ L of sterile dH₂O were added(10). Following 30 cycles of one minute at 94°C, one minute at 55°C and one minute at 72°C, amplification was terminated by waiting ten minutes at 72°C. The obtained PCR products were run on 3% agarose gel containing ethidium bromide and were visualized under UV light and bands of 281 base pairs (BP) were observed as expected.

Results

All patients were between the ages of 2-14 (mean 5.20 ± 2.31); there were 22 girls and 37 boys, adding to a total of 59 children and 78 ears. The most common complaint as expressed by the parents was hearing loss. Of 59 children, 44 had hearing loss, six had ear pain and three had fullness of the ear upon admission. The remaining six patients who did not report any complaints were identified during routine ENT examinations.

Of the seventy-eight middle ear effusions, 26 (33.3%) were serous, 31 were (39.7%) mucoid and 21 (26.9) were sero-mucoid in character. *Chlamydia trachomatis* DNA genome was identified in seven (8.9%) effusion samples. Of these, three were mucoid (42.8%), three were serous (42.8) and one was sero-mucoid (14.2%) in character. There was no correlation of statistical significance between the character of the middle ear effusion and the presence of *Chlamydia trachomatis* ($p > 0.05$).

Discussion

Chlamydia is an obligatory intracellular parasite of 0.2-1.5 μ m diameter; depending on its developmental phase within the cell it might present different appearances. *Chlamydia trachomatis* is a specific human parasite and the causative agent for trachoma, inclu-

sion conjunctivitis, urethritis and proctitis etc.(11). Identification of *Chlamydia trachomatis* in infants as a respiratory pathogen has resulted in focusing the attention to the possible relationship between other respiratory diseases of the childhood and *Chlamydia trachomatis*. If mother is infected with *Chlamydia trachomatis* while giving birth, then the child acquires the infection from the mother. The transmission rate from an infected mother is around 50%. In 30-40% of these children, nasopharyngeal infections develop and only 10% develop pneumonia afterwards.

The data pertaining to the role of *Chlamydia trachomatis* in infants with otitis media was first collected by Tipple, *et al.*(5) by isolating *Chlamydia trachomatis* in middle ear effusions of few children with Chlamydial pneumonia. However, we are not certain whether these children have AOM or not. Schachter, *et al.*(12) reported that children born to *Chlamydia* positive mothers did not demonstrate any significant difference in the incidence of AOM when compared to controls. They thus concluded that AOM was not a common complication of infantile chlamydial infection.

The possible relationship between OME and *Chlamydia trachomatis* has been the topic of many research studies. Of 44 patients with OME, Zhang, *et al.*(13) identified *Chlamydia trachomatis* DNA in middle ear aspirates of 14 patients with PCR technique. Zhang and colleagues(13) reported that in 14 out of 44 EOM patients, DNA of *Chlamydia trachomatis* examined by PCR in the middle ear aspiration samples was positive. In other studies, no relationship was reported between OME and *Chlamydia trachomatis*. Hammerschlag, *et al.*(7) stated that they could not grow *Chlamydia trachomatis* in any of the middle ear aspirates obtained from

Key Message

- Identification of *Chlamydia trachomatis* in 8.9% of the middle ear aspirate samples demonstrates that this microorganism should be taken into consideration in the treatment of Otitis media with effusion.

68 children with OME. In our study of the 78 middle ear effusion samples obtained, we were able to identify *Chlamydia trachomatis* DNA genome in seven samples (8.9%).

Hammerschlag, *et al.*(7) reported that *Chlamydia trachomatis* related pneumonia would be a very rare instance in infants above six months of age. In our study, the average age of the patients was 5.20 ± 2.31 years. We think that other than transmission through infected mothers, insects and non-chlorinated swimming pools might be other possible routes. Also, a postoperative specific treatment was applied to the patients detected DNA genome of *Chlamydia trachomatis* in their effusion liquid. No recurrence of effusion was found while following the patients detected positively DNA genome of *Chlamydia trachomatis* in an average period of 10 months. Nowadays, there are studies comparing the character of the effusion and the growth rates of the bacteria in the culture. In such studies, bacterial growth rates of 29-80% are reported for purulent effusions and 25-43% for mucoid effusions(14).

The difficulty in obtaining material for culture in cases of OME brings about the possibility of empirical treatment. In empirical treatment, the important thing is to know the underlying microbiology of the pathology. Concerning the involved microorganisms (*H. influenzae*, *S. pneumoniae*, *B. catarrhalis*) OME has similarities with acute otitis media(3,15). Thus, most of OME patients have at least used ampicillin group of antibiotics once. Ampicillin has limited *in vitro*

effects against *Chlamydia trachomatis*.

As PCR detects DNA from both live and dead bacteria, it can also detect DNA from a previous attack of AOM. Yet, in a research conducted on chinchilla models Post, *et al.*(3) have demonstrated bacterial DNA being eliminated from middle ear effusion samples two days after bacterial death. These findings demonstrate that the bacterial DNA that we have detected in middle ear effusion samples were probably originating from live bacteria and was not due to a previous attack of AOM. If these bacteria are alive, the reason behind their not being grown in culture media is the low number of colony forming units (low CFU). In middle ear effusion samples obtained from patients with OME, the measurement of bacteria were reported as 10^4 CFU or below(16). This number might be due to the bactericidal effects of the antibiotics used prior to surgery. Moreover, in contrast to AOM, OME has a subclinical presentation.

Klimek, *et al.*(17) demonstrated that in patients with chronic OME, administration of amoxicillin resulted in levels of $6.2 \mu\text{g/mL}$ in middle ear effusion samples. Imagining that the antibiotics should at least be administered for a minimum of ten days, they concluded that this high level of antibiotics resulted in lowered number of live bacteria. We as well think that the reason behind not being able to grow *Chlamydia trachomatis* in middle ear aspirate cultures, is the low number of live bacteria due to the utilized antibiotics.

The most important obstacle in detecting microbial DNA in body fluids is the presence

of heme or urea which both act like polymerase inhibitors(18). This might cause false negative results. Isolation of DNA from non-purified samples might eliminate these polymerase inhibitors. However, a small part of target DNA might be lost during this procedure(19). In our study, we did all our PCR assays after the isolation of DNA in order to minimize the number of false negativities.

Contributors: EK was responsible for planning and design of the study, partially analysing the data, writing and editing of the manuscript. YB and AO studies *Chlamydia trachomatis* in middle ear fluid in otitis media with effusion. IK was responsible for planning and design of the study. TK was responsible for planning and/or design of the study, writing and/or editing of the manuscript. SY was responsible for planning and/or design of the study, and HCA was involved in writing and editing of the manuscript.

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Evaluation of the Use of DMSA in Culture Positive UTI and Culture Negative Acute Pyelonephritis

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This prospective study was done to assess the frequency of acute pyelonephritis (APN) in febrile children with positive urine culture as documented by Tc^{99m} DMSA scintigraphy (DMSA) and the frequency of vesicoureteric reflux (VUR) in these children. Secondly, to determine the frequency of APN, in febrile children with supportive evidence for UTI but with negative urine culture, as documented by DMSA and frequency of VUR in them. Thirdly to stress the utility of DMSA to diagnose APN in urine culture negative febrile children and to suggest DMSA as a clinical tool in evaluation of fever of unknown origin (FUO). This study included 42 children with positive urine culture and 26 children with negative urine culture who had supportive evidence of UTI as determined by the predetermined criteria and diagnosed to have APN by DMSA. All of them had ultrasonogram (USG), DMSA and voiding cystourethrogram (VCU). They were followed up for a minimum period of 6 months. Out of the 42 children with positive urine culture 92.9% had features of APN in the DMSA of whom 82.1% had vesicoureteric reflux (VUR). The DMSA was abnormal in 26 children with negative urine culture, of whom 65.4% had VUR. Ultrasound suggestive of parenchymal change was observed in 47.6% in the culture positive group and 65.4% in the culture negative group. In conclusion, it is suggested, that DMSA is a useful investigation for the diagnosis of APN in febrile UTI. DMSA is indicated in febrile children with negative urine culture but with supportive evidence of UTI and in FUO. An abnormal DMSA is a strong indication for work up for VUR.

Key words: *Acute pyelonephritis, DMSA, Fever of unknown origin, Scintigraphy, Urinary tract infection, Vesicoureteric reflux.*

URINARY tract infection (UTI) is a frequent bacterial infection in children and urine culture is a 'confirmatory gold standard' for the diagnosis. A positive urine culture indicates infection anywhere in the urinary tract. It is not synonymous with acute