

## Investigating the Role of Respiratory Syncytial Virus and Influenza virus in Patients with Otitis Media with Effusion

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### ABSTRACT

Otitis media (OM) is a type of inflammatory and infectious diseases and a major cause of visits to health care systems worldwide. Otitis media with effusion (OME) is accumulation of fluid with high viscosity behind the intact tympanic membrane in the middle ear whose exact etiology is unknown. The aim of this study is to investigate the role of respiratory syncytial virus and influenza in OME. This was an analytical epidemiological study conducted on 53 OME patients underwent myringotomy surgery and VT insertion. Middle ear fluid was sampled during the operation, and using PCR with specific primers, respiratory syncytial virus (RSV) and influenza were assessed. Fifty-three patients including 30 males (56.6%) and 23 women (43.4%) with mean age of  $3.98 \pm 1.70$  years were studied. The samples of 44 patients (83.0%) contained RSV and the remaining 9 patients (17.0%) showed no RSV. This difference was statistically significant ( $P: 0.001$ ). Five samples (7.5%) contained influenza virus, whereas 49 samples (92.5%) showed no influenza virus and this difference was statistically significant ( $P: 0.001$ ). Our findings showed that RSV plays an important role in the pathogenesis of the disease, whereas influenza virus does not have an important place in the future studies.

**Key words:** Otitis media with effusion, Respiratory syncytial virus, Influenza virus, Pathogenesis.

### INTRODUCTION

Otitis media (OM) is a type of inflammatory and infectious diseases that affects the middle ear and has various groups that are different regarding presentation, related complications, and treatment<sup>1</sup>. OM is divided into two categories: acute otitis media (AOM) and otitis media with effusion (OME)<sup>2-5</sup>. OME is a common childhood disease whose etiology is not quite obvious, it is created in response to chronic inflammation of the middle ear and known with continuous effusion of serous and mucous fluid with priority of mucin of high viscosity, and it does not have good drainage through the Eustachian tube (6-9). Immature functioning of immune system and dysfunction of the Eustachian tube are the most

important etiologies of OME. Other known etiologic factors include anatomy and physiology disorders, infection of the upper airways, bacterial and viral infection of the middle ear, allergy, and environmental factors such as obesity, exposure to tobacco smoke, and mechanical obstruction of nasopharyngeal by mucus adenoids<sup>3,10-16</sup>. Recent studies have highlighted the role of viruses in etiology and pathogenesis of OM especially OME. Despite the conventional administration of antibiotics for treatment of AOM, the prevalence of OME has increased by 250% in the United States from 1975 to 1990. Furthermore, although bacterial DNAs have been found in the middle ear fluid (MEF) samples of 80% OME patients, antibiotic treatments have not significantly reduced the incidence of OME.

Therefore, despite using appropriate antibiotics, middle ear effusion may occur within some weeks or months. The Polymerase Chain Reaction (PCR) assessments have shown many viruses including Respiratory Santitial Virus (RSV), adenovirus, influenza, parainfluenza, and rhinovirus in middle ear effusion<sup>17-24</sup>. Young children usually encounter upper airway infections 8 to 12 times a year where several viruses are normally associated with the disease. Entry of viruses to the middle ear can cause inflammatory response which in turn can lead to MEE. Some studies have indicated important role of RSV in the pathogenesis of OME. On the other hand, there is strong evidence that shows that respiratory viruses, known as a bacterial infection in pathogenesis of AOM, have an important etiologic role and could also affect the outcome of AOM<sup>4, 6, 17, 25-31</sup>.

Previous studies have shown that among adenovirus, rhinovirus, and influenza, RSV, the most important pathogen of OME is RSV<sup>32-36</sup>.

Therefore, investigating the role and mechanisms of actions of different viruses in pathogenesis and etiology of OME is of prime importance in the prevention and treatment of disease. Furthermore, findings of such studies can be used to develop new non drug treatment modalities<sup>37-40</sup>. Furthermore, the relatively high prevalence of the disease among children necessitates such evaluations. Serious complications of OME include hearing loss, slurred speech and learning and the subsequent complications that are great burden for societies, particularly developing countries<sup>3, 5, 41, 42</sup>.

The conventional treatment option for OME in children with no noticeable speech and learning disabilities or hearing loss is administration of antibiotics, inhaling corticosteroid and testing pneumatic otoscopy. For hearing loss <20db, the patient remains under supervision, whereas for hearing loss > 40db or in case of any disability in learning and speech, surgery including myringotomy and tube tympanostomy is needed. When the hearing loss is ranged 21-39, it depends on effusion time (less than 3 months) and the severity of the illness<sup>3</sup>.

Considering the possible role of viruses in causing OME, and the benefits of clarifying this relationship for the prevention and treatment of this disease, and also given the importance of this disease and its serious complications, we decided to study the role of respiratory syncytial virus and influenza in OME by designing and implementing a descriptive-epidemiological study in patients with this disease that had referred to Ahvaz Imam Khomeini Hospital from April 1, 2013 to April 1, 2014 who underwent myringotomy and tube tympanostomy insertion (Ventilation Tube - VT).

## MATERIALS AND METHODS

The subjects were 53 patients with OME who referred to ENT ward of Ahvaz Imam Khomeini Hospital from 2013 to 2014 and underwent myringotomy and VT surgeries. Inclusion criteria were diagnosis of OME lasted at least for 1 month based on otoscopy, not having signs and symptoms of acute infection, and intact tympanic membrane. Given the need of patients, they underwent surgery for myringotomy and VT insertion. Middle ear fluid was sampled during the surgery by the implementers of the research. The MEE was collected by a Nelatons catheter connected to suction on one side and on the other end to micro tubes containing 1.5 ml of buffer or distilled water (the whole system had been sterilized).

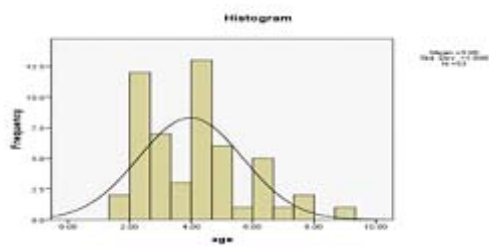
Samples collected were isolated and stored at 20 degrees Celsius and eventually were sent to virology laboratory of the medical school for the viral study. Using special kits in the laboratory RNA was extracted. Then converting RNA to cDNA was performed using Reverse Transcriptase enzyme. Then using PCR on prepared cDNAs and using specific primers, respiratory syncytial (RSV) and influenza viruses were assessed. Products of PCR were observed in agarose gel and after getting image, based on the size of the band formed in the gel, the existence or absence of specific primers in the virus was concluded. Finally, the collected data were sorted in Excel and analyzed with statistical package SPSS (version 19) using Chi square and independent T-test. To provide descriptive statistics, tables, diagrams, mean, and standard deviation were used. The significant level of 0.05 was considered for all statistical analyses of the study.

**RESULTS**

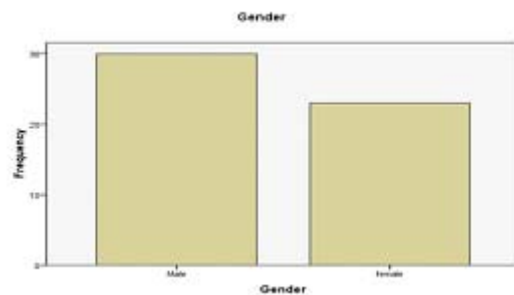
Fifty three patients with OME, with an average age of  $3.98 \pm 1.70$  years who underwent myringotomy and VT insertion entered the study. The age range of participants in the study was between 1.5 to 9 years. Thirty patients (56.6%) of the participants were male with an average age of  $4.07 \pm 1.81$  years. Twenty-three patients (43.4%) were female participants with an average age of  $3.85 \pm 1.56$  years. Male and female patients' ages undergoing myringotomy and VT did not have statistically significant differences ( $P = 0.458$ ). In addition, no statistically significant differences were observed between the participants in terms of gender, ( $P = 0.336$ ) (Figs. 1 and 2).

Studying the laboratory samples taken from middle ear fluid of patients showed that RSV existed in 44 patients (83.0%) (Fig. 3).

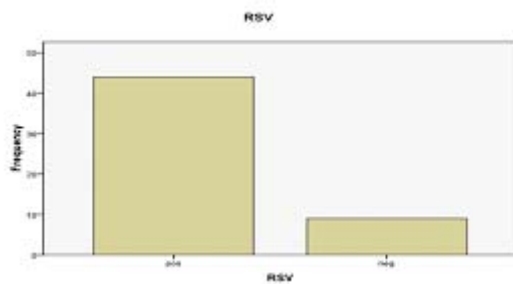
RSV patients included 25 males (56.8%) and 19 females (43.2%). There was no significant difference ( $P = 0.944$ ) among the patients of RSV, in terms of gender. Also between age and risk of RSV no statistically significant difference was found ( $P = 0.118$ ). Moreover, there was no statistically significant differences ( $P = 0.118$ ) between the age of patients and suffering from RSV. Thus, it was concluded that age is not associated with infection with RSV in patients with OME (Fig. 4).



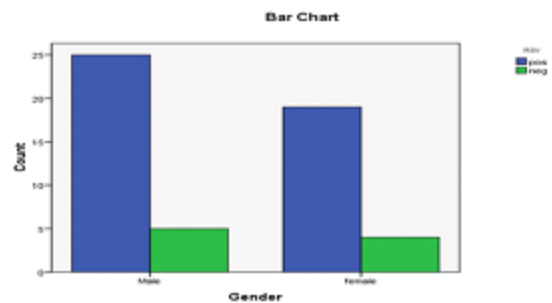
**Fig. 1: Age histogram of the study participants**



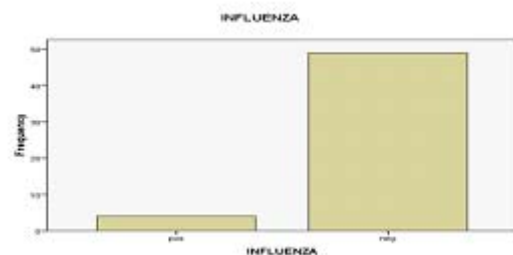
**Fig. 2: Gender of the participants in the study**



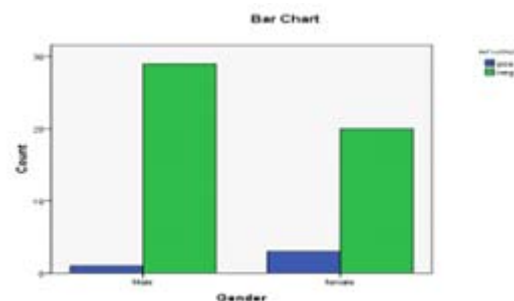
**Fig. 3: Prevalence of RSV in the study population**



**Fig. 4: Frequency of RSV in both genders**



**Fig. 5: Frequency of influenza virus in the population studied**



**Fig. 6: The frequency of influenza virus in both genders**

In PCR tests conducted on samples taken from patients, a total of 4 patients (7.5%) had influenza viruses and in a sample of 49 patients (92.5%) influenza virus was not found. This difference was statistically significant ( $P = 0.0001$ ) indicating that influenza virus has no role in the creation of OME (Fig. 5).

One patients with influenza virus was a man (25.0%) and 3 (75.0%) were women, although the number of women was 3 times more than men, there is no significant correlation between the flu and gender ( $P = 0.185$ ). No statistically significant correlation was seen between age and incidence of flu viruses ( $P = 0.448$ ), thus age and gender of patients with OME have no relation with the extent of risk of these patients to suffer influenza virus (Fig. 6).

In all four cases with influenza virus, RSV was also observed, but no significant correlation was found between the incidence of influenza and RSV ( $P = 0.347$ ). Therefore, containing the influenza virus has no relation to the risk of RSV infection (Fig. 7).

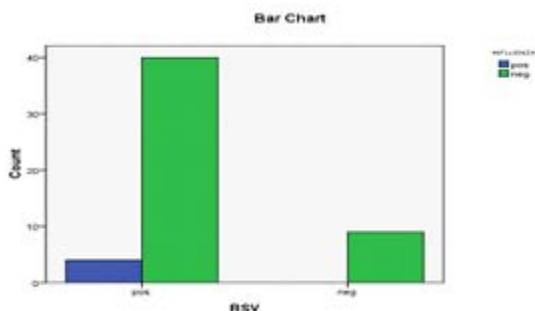


Fig. 7. RSV connection with the influenza virus

## DISCUSSION

The present study was aimed to investigate the role of influenza and RSV viruses in pathogenesis of OME. The average age of children with OME undergoing myringotomy and VT included in the study was  $3.98 \pm 1.70$  years, which is almost in the middle of typical age of incidence of otitis media<sup>4</sup>. Moreover, in this study, there was no statistically significant difference between gender and age of suffering in both genders indicating that in the specified age range, gender cannot be considered as an etiologic factor for the development of OME.

In this study, RSV was detected in 83% of patients with otitis media and the difference was statistically significant. This shows that RSV can be considered as an etiologic factor in the development of OME. In this study, patients were not evaluated for the presence of pathogenic bacteria that is as a disadvantage of the study that makes arguing RSV's being etiologic or not impossible. It is not known, first, whether the samples containing this virus were infected by pathogenic bacteria or whether RSV can cause illness in the absence of the pathogenic bacteria or virus alone cannot be an agent of infection in otitis media.

Since in the course of several studies it is shown that RSV has a prominent role in the pathogenesis of OME<sup>14, 30, 31, 35, 43</sup>, for finding a clearer link between respiratory syncytial virus and otitis media, more investigations with more population and also reviewing other pathogenic factors such as bacteria in the samples taken from patients in OME are essential in the future. Especially, since in other studies conducted in the field of RSV in OME, conflicting results have been obtained. For example, in the study conducted by Monobe *et al.* (2003), 73% of samples (39 people) have been infected with RSV<sup>32</sup>. While in the study by Maseda E *et al.* (2000), none of the 32 children with otitis media had RSV<sup>33</sup>. Interstitial cases have also been reported for existence of RSV in MEE of children with OME compared to previous studies. In the study by Shaw CB *et al.* and Pitkaranta A *et al.* and Okamoto *et al.*, respectively, RSV is seen in 9.9% and 6.26% and 75% of patients (34, 36, 44). In the study by Abu Sitteh *et al.* conducted in 2008 on 30 patients with OME by PCR method, 30% of cases showed respiratory syncytial virus<sup>34</sup>.

In the study by Terho and colleagues, out of 456 patients with AOM, 41% were infected by viruses at airways out of whom, only 65 people were infected with RSV, and finally in MEE samples 48 patients (74%), had respiratory syncytial virus. It was shown that effective vaccines against infection of the upper airways caused by RSV can reduce the incidence of AOM<sup>45</sup>. In a study by Shun and colleagues carried out to check the presence of RSV in the AOM through checking the existence of RSV antigen, it was concluded that RSV can be a

direct cause for AOM at least in the early stages of infection with the virus<sup>46</sup>. Since OME can be considered as a continuation of AOM and in up to 50% of the cases middle ear effusion occurs directly after a period of acute infection<sup>47</sup>, RSV can be a cause of OME. Despite this evidence, and also because we know that the pathogenesis of inflammatory diseases is more complex than that can be expressed in a simple causal relationship<sup>47</sup>, we cannot simply ignore the role of RSV in developing OME until that role of this pathogen is well-lit.

In this study, 7.5% of the patients with OME were infected by influenza virus that is statistically significant indicating the influenza virus does not have an etiologic role in the development of otitis media. This finding is consistent with the findings of Monobe *et al.* that reported 5.1% of the patients were positive for influenza virus<sup>32</sup>. In the study by Abu Sitteh *et al.*, none of the 30 patients with otitis media, were infected with influenza virus<sup>34</sup>. Also in the study by Terho and colleagues, of 456 patients with acute otitis media, only 24 cases had respiratory infection with influenza virus from which in less than half of the cases (10 patients, 42%) influenza had reached virus middle ear fluid<sup>45</sup>.

Findings of our study in line with previous similar studies demonstrate that the flu virus has no important role in the pathogenesis of OME. However, as vaccination against influenza virus

reduced the incidence of AOM (48-50), conducting further studies is necessary on the role of this virus in the pathogenesis of OME.

## CONCLUSION

The results of this study showed that RS virus has an important role in the pathogenesis of OME, whereas the flu virus did not show a significant role. The findings of this study can be used to design and develop ways to prevent, treat, and manage this disease. To investigate the role of RS virus at different stages of the disease, more controlled studies are needed.

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## Authors' Contributions

All authors had equal role in the designing, conducting, statistical analysis, and manuscript composing.

## Conflict of Interest

The authors have no conflicts of interests.

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