



Original Research Article

The Comparative Study of the Number of Adenoid Mast Cells, Serum Ige Level, and Blood Eosinophilia in Two Groups of Patients Undergone Adenoidectomy With and Without Otitis Media with Effusion

Mahmoud Shishegar¹, Mohammad Javad Ashraf² and Maryam Amiri Tehrani Zadeh^{3*}

¹Associate professor of Otolaryngology, Department of Otolaryngology, School of Medicine, Shiraz University of Medical Sciences, Shiraz, Iran

²Associate professor of Pathology, Department of Pathology, School of Medicine, Shiraz University of Medical Sciences, Shiraz, Iran

³Resident in Otolaryngology, Department of Otolaryngology, School of Medicine, Shiraz University of Medical Sciences, Shiraz, Iran

*Corresponding author

ABSTRACT

It has been reported that several factors, such as eustachian tube dysfunction, allergies, immunity, and infections, play important roles in the etiology of Otitis Media with Effusion (OME). The association between adenoid inflammation and OME has long been noted, but the exact mechanism accompanied by allergic parameters is still debated. The present study aimed to investigate the association between OME and number of adenoid mast cells, serum IgE level, and peripheral blood eosinophilia. This study was performed on 60 patients, 30 with OME as the case group and 30 with normal middle ear function as the control group. For each patient, adenoidectomy and blood sampling was done and adenoid mast cells and blood eosinophils were counted. Besides, serum IgE level was measured by enzyme-linked immunosorbent assay method. Our study results revealed no significant difference between the two groups of children with and without OME regarding adenoid mast cell, serum IgE level, and blood eosinophilia ($P>0.05$). The findings presented no significant correlation between allergic laboratory parameters and OME. Further studies with more accurate exclusion criteria and better control of the confounding factors may be a key to reach a precise conclusion about the association between allergic parameters and OME.

Keywords

Allergy,
Adenoid,
Eosinophil,
IgE,
Mast cell,
Otitis media
with effusion

Introduction

Otitis Media with Effusion (OME) refers to inflammation of the middle ear in which, some liquid is present in the middle ear space while the tympanic membrane is intact (Casselbrant *et al.*, 1985). Most clinicians

consider OME as a result of Eustachian Tube (ET) dysfunction related to the adenoid due to the proximity of adenoid to the pharyngeal opening of the ET as well as to the improvement in OME following

adenoidectomy (Gates *et al.*, 1988).

The role of adenoid in OME was suggested by both mechanical and functional obstruction of ET as well as by a source of bacterial antigens due to inadequate handling of bacteria during upper respiratory tract infection (Gates *et al.*, 1988). On the other hand, several studies have shown that the value of adenoidectomy in OME was not related to the adenoid size (Elverland *et al.*, 1980); therefore, the hypothesis of a functional role regardless of the adenoid size was insisted. Collins *et al.* (1985) suggested the “adenoid mediator release theory”. They showed that the children with fluid present in both ears had increased amounts of histamine in their adenoid tissue compared to those with no signs or symptoms of OME (Collins *et al.*, 1985). Moreover, Dennis *et al.* (1976) indicated that histamine induced vasodilation and increased vascular permeability, edema of the ET, and middle ear mucosa. The role of allergy and inflammatory changes was subsequently shown by other researches, demonstrating high levels of histamine in OME of chinchillas induced by introduction of immune complexes (Nakata *et al.*, 1992). Therefore, the association between OME and adenoid inflammation and allergy has long been noted, but the exact mechanism accompanied by other allergic parameters, such as serum IgE level and blood eosinophilia, is still debated. Hence, the present study aims to investigate the number of adenoid mast cells, serum IgE level, and peripheral blood eosinophilia in the patients with OME.

Material and Methods

Patients

This case-control study was performed on 60 patients who were admitted in ENT

department of Khalili hospital, Shiraz University of Medical Sciences from September 2013 up to March 2014. The patients were divided into two groups; 30 with OME as the case group and 30 without OME as the controls. The patients aged from 2 to 10 years old (mean±S.D: 5.6±1.5 years) and consisted of 20females and 40males. All the clinical examinations were done by the head of this research who is an otologist in the clinic.

The patients with bilateral OME by history taking, physical examination (otoscopy), and tympanometry were selected as the case group and underwent adenoidectomy plus bilateral myringotomy and Ventilation Tube (VT) insertion. The control group participants, on the other hand, were selected from the patients referring with reasons other than OME, mostly adenoidal or tonsillar hypertrophy, and had normal middle ear function through history and microscopic otoscopy. They, too, underwent adenoidectomy. The surgeries were performed under general anesthesia.

The patients who presented with cleft palate, craniofacial abnormalities such as Down syndrome, TreacherColins, unilateral OME, allergic rhinitis, atopy, asthma, any significant comorbidities, and active upper respiratory infection at the time of sampling were excluded from the study. Also, in order to eliminate the effects of the confounding variables, the two groups were matched regarding age, gender, and time of sampling (seasonality).

All the patients' parents signed written informed consents for participation in the study and completed the demographic information questionnaires. The study protocol was approved by the Ethics Committee of Shiraz University of Medical Sciences.

Sample collection and measurements

At the time of routine pre-operation sampling from peripheral blood, 3-4 cc of the patients' blood were collected for IgE level evaluation and blood smear preparation.

In order to measure serum IgE level, the patients' blood clot samples were centrifuged to collect their sera. The sera were then kept at -4°C until all the 60 patients' samples were ready to be analyzed using Enzyme-Linked Immunosorbent Assay (ELISA) method.

Peripheral blood eosinophils were counted on the smears prepared from 1 cc of the patients' blood samples, transferred into oxalate, and stained with eosin using the Romanowsky method.

The adenoid tissues were collected after curettage of nasopharynx in the operating room, kept in 10% formaldehyde (fixation), and sent to our histopathology laboratory in the School of Medicine, Shiraz University of Medical Sciences. The tissues were handled in the routine method and were embedded in paraffin. Then, 5 µm sections from the entire adenoid tissues were obtained. Afterwards, all the sections were stained with toluidine blue for histopathological examination. Toluidine blue stained the mast cell cytoplasmic granules metachromatically purple.

Eosinophil and mast cell counting was done on various regions of each specimen lam at ×400 magnification (high power field). The total number of eosinophils and mast cells in 10 high power fields was counted manually and the results were recorded as the mean number of cells per high power field for each specimen.

All the histopathological examinations were done by a pathologist who is one of the authors of this study, too and was blinded to whether the specimen belonged to the case or the control group.

Statistical analysis

All the statistical analyses were performed using the SPSS statistical software (v.19). Independent samples t-test and One-way ANOVA were used for analyzing the variables. $P < 0.05$ was considered as statistically significant.

Result and Discussion

This study was conducted on two groups of 30 patients. The adenoid mast cells count in high power fields was compared in the two groups. According to the results, the mean count for both groups was 1.3 cells per field ($P=0.34$). The two groups were also compared regarding the eosinophils count in high power fields of the peripheral blood smear. The mean eosinophils count was 1.2 and 1.3 cells per field in the case and the control group, respectively and the difference was not statistically significant ($P=0.6$). The mean serum IgE level was 143 µg/dl in the case and 92 µg/dl in the control group, but the difference was not statistically significant ($P=0.45$) (Table 1). In order to determine whether the patients in the two groups were fully matched regarding age and gender and the effect of these confounding variables was eliminated, we analyzed the three items separately between the two sexes. The same results were obtained. Also, Pearson's correlation coefficient was used to assess the association between the three variables and the patients' age. The results demonstrated no significant correlations in this regard, so that the confounding error of age and sex was ruled out.

Adenoiditis has been considered as a prospective place of allergic inflammation. Several studies have emphasized that viral infections and allergic rhinitis played roles in the pathogenesis of OME (Settipane, 1999; Skoner, 2000).

The association between OME and adenoid inflammation and allergy has long been illustrated, but the precise allergic mechanism has not been well identified. The “adenoid mediator theory” was suggested by Collins *et al.* (1985) who reported high amounts of histamine in the adenoid tissue in the children with fluid present in both ears in comparison to those with no signs or symptoms of OME. Thus, an immediate-type hypersensitivity reaction could activate their degranulation with release of histamine and other allergic mediators (Ulualp *et al.*, 1999; Abdullah *et al.*, 2006). Furthermore, Papatziarnos *et al.* (2006) described other immune-phenotypical features of adenoids of atopic children: increased numbers of IgE+ and FcεRI+ cells as well as presence of IgE+ plasma cells supposed to be derived from local differentiation. Cassano *et al.* (2003) indicated no correlations between allergic rhinitis and adenoid hypertrophy, whereas a previous study reported opposite conclusions, demonstrating sensitization to mold allergen as a risk factor for adenoid hypertrophy in the children with allergic rhinitis (Huang and Giannoni, 2001; Mattila *et al.*, 2003).

Up to now, few studies have reported an increased number of adenoid mast cells in the patients with OME. A study that evaluated mast cell ultrastructure in the adenoids of the children with and without OME demonstrated no increase in the rate of degranulation in the former group (Drake-Lee *et al.*, 1994). On the contrary, Kiroglu *et al.* (1998), using several techniques (light microscopy, immunocytochemistry, and

electron microscopy), described an increased number of lymphocytes, mast cells, macrophages, dendritic cells, and M cells in adenoid tissues from the patients with OME that seemed to be related to the presence of infectious foci. Also, a cross-sectional, prospective study performed by Abdullah *et al.* (2006) showed that the number of adenoid mast cells in adenoid specimens was significantly greater in the children with OME compared to those without OME (P=0.000). They suggested that inflammation might play a role in OME. Alles *et al.* (2001) studied 209 children referred to ‘Glue ear/Allergy’ clinic with a history of chronic or recurrent OME. The results of blood tests revealed eosinophilia in 40% and raised serum IgE in 28% of the cases. A number of the former studies have tried to evaluate probable relationships between IgE-mediated allergy and OME (Irander *et al.*, 1993; Caffarelli *et al.*, 1998; Mion *et al.*, 2003; Aydogan *et al.*, 2004). In the study by Amirzargar *et al.* (2003), IgE concentration of serum and middle ear contents was 3 folds higher in the OME patients compared to the normal controls and an allergic background was found in the OME patients.

Chantzi *et al.* (2006) conducted a study on 88 children (1–7 years old) with OME and 80 matched controls with no history of OME who had referred for reasons other than OME, mostly adenoidal or tonsillar hypertrophy and rhinosinusitis. They measured specific IgE by skin-prick tests and/or fluorezyme immunoassay. They also eliminated the possible confounders by excluding the children with acute otitis media, perforations of the tympanic membrane, craniofacial abnormalities, sensorineural hearing loss, chronic underlying diseases, and chronic pharmaceutical management (except for asthma and antiasthmatic medication). Finally, they

concluded that “IgE sensitization and respiratory allergy symptoms are independent risk factors for the development of OME, suggesting that both immunological and mechanical pathways may contribute to the development of the disease.” Yet, not including objective measures for allergic disease diagnosis was the main limitation of their study (Chantzi *et al.*, 2006). Furthermore, the study was performed from October 2002 to May 2004 and seasonal allergies might have impacted the final results.

In the present study, we attempted to eliminate the possible confounding factors, including cleft palate abnormality, craniofacial abnormalities such as Down syndrome, Treacher Colins, unilateral OME, allergic rhinitis, atopy, asthma, any significant comorbidities, and active upper respiratory infection at the time of sampling. Besides, the two groups were fully matched with respect to age, gender, and time of sampling (seasonality). Our results revealed no significant difference between the case and the control group regarding the adenoid mast cells count in high power fields (P=0.34). Although serum IgE level was higher in the case group, the difference was not statistically significant (P=0.45). Also, no significant difference was observed between the two groups concerning the peripheral blood smear eosinophilia.

Our specific knowledge about the mechanism linking adenoids, allergy, and otitis media is still very limited, and epidemiological data are still quite controversial (Marseglia *et al.*, 2009). However, none of the studies conducted on the issue completely adjusted the effects of the possible confounders on the relationships between allergic mechanisms and OME. To the best of our knowledge, none of the previous studies that investigated the mechanisms related to adenoids, allergy, and otitis media has pointed to eliminating the allergic patients with active signs and symptoms or asthmatic patients. Besides, there is a different group of allergy triggers that are closely tied to particular seasons (Myszkowska *et al.*, 2002), while the time of sampling was not considered in the previous studies although otitis media alters following seasonality.

In conclusion, with respect to prior studies, we concluded that finding a correlation between OME and allergic parameters or far beyond that, considering allergy as a causative factor for OME, is yet to be deeply investigated. Larger sample size, more accurate exclusion criteria, and better control of confounding factors may be a key to reach a precise conclusion.

Table 1 Comparison of serum IgE level, number of mast cells, and blood eosinophils in the case and control groups

	Patients (n)	Control group (mean±S.D)	Case group (mean±S.D)	P-value
Serum IgE level (µg/dl)	30	92.4 ± 131.7	143 ± 139	0.45
Mast cells per hpf	30	1.3 ± 1.2	1.3 ± 0.8	0.34
Blood eosinophils per hpf	30	1.3 ± 2.9	1.2 ± 1.3	0.6

Acknowledgment

The present article was extracted from the thesis written by Maryam Amiri Tehrani Zadeh M.D and was financially supported by Shiraz University of Medical sciences grants No 4516-91/01/01.

References

- Abdullah, B., S. Hassan, D. Sidek, and Jaafar, H. 2006. Adenoid mast cells and their role in the pathogenesis of otitis media with effusion. *J. Laryngol. Otol.*, 120: 556–560.
- Alles, R., Parikh, A., Hawk, L., Darby, Y., Romero, J.N., Scadding, G. 2001. The prevalence of atopic disorders in children with chronic otitis media with effusion. *Pediatr. Allergy Immunol.*, 12: 102–106.
- Amirzargar, A., Fegghi, S., Nicknam, M., Saki, N. 2003. Immunological aspects of secretory otitis media in Iranian children, immunoglobulin and complement concentration in serum and glue. *IJAAI*, 2: 25–29.
- Aydogan, B., Kiroglu, M., Altintas, D., Yilmaz, M., Yorgancilar, E., Tuncer, U. 2004. The role of food allergy in otitis media with effusion. *Otolaryngol. Head Neck Surg.*, 130: 747–750.
- Caffarelli, C., Savini, E., Giordano, S., Gianlupi, G., Cavagni, G. 1998. Atopy in children with otitis media with effusion. *Clin. Exp. Allergy*, 28: 591–596.
- Cassano, P., Gheraldi, M., Cassano, M., Fiorella, M., Fiorella, R. 2003. Adenoid tissue rhinopharyngeal obstruction grading based on fiberoendoscopic findings: a novel approach to therapeutic management. *Int. J. Pediatr. Otorhinol. Laryngol.*, 67: 1303–1309.
- Casselbrant, M.L., *et al.* 1985. Otitis media with effusion in preschool children. *Laryngoscope*, 95: 428–436.
- Chantzi, F., *et al.* 2006. IgE sensitization, respiratory allergy symptoms, and heritability independently increase the risk of otitis media with effusion. *Allergy*, 61: 332–336.
- Collins, M., Church, M., Bakhshi, K., Osborne, J. 1985. Adenoid histamine and its possible relationship to secretory otitis media. *J. Laryngol. Otol.*, 99: 685–691.
- Dennis, R.G., Whitmire, R.N., Jackson, R.T. 1976. Action of inflammatory mediators on middle ear mucosa: a method for measuring permeability and swelling. *Arch. Otolaryngol.*, 102: 420–424.
- Drake-Lee, A., Price, J., Varley, R. 1994. Mast cell ultrastructure in the adenoids of children with and without secretory otitis media. *J. Laryngol. Otol.*, 108: 1058–1063.
- Elverland, H., Mair, O., Haugeto, Schroder, K. 1980. Influence of adenoid hypertrophy on secretory otitis media. *Otol. Rhinol. Laryngol.*, 90: 7–11.
- Gates, G.A., Avery, C.A., Prihoda, T.J. 1988. Effect of adenoidectomy upon children with chronic otitis media with effusion. *Laryngoscope*, 98: 58–63.
- Huang, S.W., Giannoni, C. 2001. The risk of adenoid hypertrophy in children with allergic rhinitis. *Allergy Asthma Immunol.*, 87: 350–355.
- Irander, K., Borres, M., Bjorksten, B. 1993. Middle ear diseases in relation to atopy and nasal metachromatic cells in infancy. *Int. J. Pediatr. Otorhinol. Laryngol.*, 26: 1–9.
- Kiroglu, M.M., *et al.* 1998. Adenoids and otitis media with effusion: a morphological study. *Am. J. Otolaryngol.*, 19: 244–250.

- Marseglia, G.L., *et al.* 2009. Role of adenoids and adenoiditis in children with allergy and otitis media. *Curr. Allergy Asthma Rep.*, 9: 460–464.
- Mattila, P.S., Joki-Erkkila, V.-P., Kilpi, T., Jokinen, J., Herva, E., Puhakka, H. 2003. Prevention of otitis media by adenoidectomy in children younger than 2 years. *Arch. Otolaryngol. Head Neck Surg.*, 129: 163–168.
- Mion, O., De Mello, J.F., Lessa, M.M., Goto, E.Y., Miniti, A. 2003. The role of rhinitis in chronic otitis media. *Otolaryngol. Head Neck Surg.*, 128: 27–31.
- Myszkowska, D., Stepalska, D., Obtulowicz, K., Porebski, G. 2002. The relationship between airborne pollen and fungal spore concentrations and seasonal pollen allergy symptoms in Cracow in 1997–1999. *Aerobiologia*, 18: 153–161.
- Nakata, J., Suzuki, M., Kawauchi, H., Mogi, G. 1992. Experimental otitis media with effusion induced by middle ear effusion. *Laryngoscope*, 102: 1037–1042.
- Papatziamos, G., van Hage-Hamsten, M., Lundahl, J., Hemlin, C. 2006. IgE-positive plasma cells are present in adenoids of atopic children. *Acta. otolaryngologica*, 126: 180–185.
- Settipane, R.A. 1999. Complications of allergic rhinitis. In: *Allergy and Asthma Proceedings*, OceanSide Publications, Inc., Pp. 209–213.
- Skoner, D.P. 2000. Complications of allergic rhinitis. *J. Allergy Clin. Immunol.*, 105: 605–609.
- Ulualp, S.O., Sahin, D., Yilmaz, N., Anadol, V., Peker, O., Gursan, O. 1999. Increased adenoid mast cells in patients with otitis media with effusion. *Int. J. Pediatr. Otorhinol. Aryngol.*, 49: 107–114.