The role of allergy in the etiology of otitis media with effusion; immune system and cytokines

Efüzyonlu otitis media’nın etyolojisinde alerjinin yer.; immün sistem ve sitokinler

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Objectives: Otitis media with effusion (OME) is one of the major causes of hearing loss in childhood. In this study, the role and the importance of allergy in the etiology of OME was evaluated by questioning the immune system and the cytokines.

Patients and Methods: Seventy-eight ears of 59 patients who had gone through myringotomy in our clinic between September 1999 and June 2001 with the diagnosis of OME were included in this study. The serum samples of 26 healthy volunteer children who were in the same age group and were living in the same region while having similar socio-economic properties were examined as the control group.

Results: In this study; the IFN-γ levels were significantly lower; and IL-4 and IgE levels were significantly higher in the serum samples of the patient group when compared with the control group (p<0.05). A positive correlation was found between IL-4 and IgE in the serum samples of patients (p<0.01). However, there were negative correlations between IFN-γ both with IgE and IL-4 levels, that were not statistically significant. Although negative correlations were found between IL-4 and IFN-γ, and between IFN-γ and IgE levels; a positive correlation was found between IL-4 and IgE levels in otitis media with effusion samples, that were not statistically significant.

Conclusion: T helper (Th) polarization shows that in the etiology of OME, allergy may have a role. So, in the treatment of OME allergy should be kept in mind.

Key Words: Allergy; cytokines/analysis/secretion; immunoglobulin E/blood; interleukin-4/metabolism; leukocytes, mononuclear/cytology/metabolism; otitis media with effusion/etiology/immunology; T helper, polarization; Thelper cells/metabolism.


Bulgular: Bu çalışmada, hasta grubu serum örneklerinde IFN-γ kontrol grubuna göre istatistiksel olarak düşük, IL-4 ve IgE ise kontrol grubuna göre istatistiksel olarak yüksek tespit edildi (p<0.05). Hasta serum örneklerinde IL-4 ve IgE arasında pozitif korelasyon saptandi (p<0.01). Ancak IFN-γ - IgE arasında ve IL-4 - IFN-γ arasında ise istatistiksel olarak anlamlı olmayan negatif korelasyon gözlendi. Orta kulaç efüzyon örneklerinde IL-4 ile IFN-γ arasında ve IFN-γ ile IgE arasında negatif korelasyon olmasına rağmen, IL-4 ile IgE arasında pozitif korelasyon saptandi. Fakat bu korelasyonlar istatistiksel olarak anlamlı bulunmadı.


Anahtar Sözcükler: Alerji; sitokinler/analiz/salgılanma; immünglobülün E/kan; interleükin-4/metabolizma; lökositler, mononükleer/sítoloji/metabolizma; efüzyonlu otitis media/etiology/immunology; T helper, polarization; T helper cells/metabolism.
Otitis media with effusion (OME) is a type of otitis media that is characterized by the accumulation of fluid behind the healthy tympanic membrane without creating acute symptoms and findings. The reasons underlying the development of effusion behind the healthy tympanic membrane have been discussed for many years. The data about the functional disorders of Eustachian tube, insufficient mastoid pneumatization, craniofacial abnormalities, infection, immune system problems and the complex pathologic problems caused by allergic factors are still far from being sufficient.

There are several studies attempting at verifying the presence of a relationship between allergy and otitis media. Although it has not been proven that the middle ear or the Eustachian tube (ET) are immunologic target organs, there are many evidences supporting this. The effusion accumulating in the middle ear in OME includes antigen, antibody, immunoglobulins, immune-complexes and other immunologic components. Therefore, it is possible to anticipate that the change that takes place in the mucosa is an immune-complex reaction that occurs after upper respiratory tract infections. In 1986, a study conducted on rats demonstrated that T helper (Th) cells could be allocated to two subsets as Th1 (type 1) and Th2 (type 2) according to their cytokine productions. Th1 cells activate cellular immunity by producing IL-2, IFN-γ and TNF-β. Thus, they ensure defense against viral, bacterial, fungal and protozoa infections. Th2 cells that stimulate humoral immunity produce IL-4, IL-5, IL-10 and IL-13. They also have roles in some helmintic infections with their IgE and IgG responses and trigger allergies. It is not clearly known which factors initiate cellular differentiation towards Th1 and Th2. IL-12 and IL-4 are two important cytokines that polarize the development of Th1 and Th2 cells in opposite directions. The natural immune responses in the initiation of the infection probably regulate the deviations in the predominance of Th1 and Th2 cells.

Th cells are divided into some subgroups as Th0, Th1 and Th2 with regards to the lymphokine types they produce. Th1 clones produce IFN-γ and IL-2, but not IL-4 and IL-5. On the other hand, Th2 cells produce IL-4, IL-5 and IL-13 yet they do not produce IFN-γ and IL-2 cytokines. Th0 clone has the cytokine profile of both Th1 and Th2 cells. Lymphokines secreted from Th1 cells activate the cellular immune system by stimulating macrophages and guiding B cells to produce IgM and IgG2. IFN-γ and IL-12 cytokines change Th polarization in favor of Th1 while IL-4 changes it in favor of Th2. Purified protein derivatives (PPD) lead to the increase and differentiation of Th2 cells in the presence of IL-4. However, IFN-γ inhibits the increase of Th2 clone in response to IL-2 and IL-4.

In this study, in the serum samples and effusion materials aspirated from middle ear of EOM patients cytokine profiles were compared in an attempt to demonstrate the Th polarization, thereby investigating the role of allergy in the pathogenesis of OME.

MATERIALS AND METHODS

78 ears of 59 patients between 4 and 8 years of age who had myringotomy under general anesthesia with the diagnosis of OME in Firat University School of Medicine Department of ENT from September 1999 to June 2001 were included in this study. Discontinuation of antibiotics two weeks prior to the operation and the presence of the effusion for more than 3 months were taken as the criteria for the diagnosis of OME. Cases with infections, sinusitis, diabetes and immune deficiency were excluded from the study.

The patient group consisted of 78 ears of 59 patients diagnosed as OME. The control group consisted of randomly selected healthy volunteer children (n=26) who were in the same age group, were living in the same region while having similar socioeconomic profiles with the approval of their families.

The effusion samples were obtained by using Zeiss Opmi-1 operation microscope under general anesthesia with the tympanosynthesis method defined by Bluestone or by myringotomy. External acoustic meatus was filled with 70% alcohol and kept for 1 minute. After the aspiration of the alcohol, tympanosynthesis was performed on the anterior-inferior part of the tympanic membrane with a 16 gauge cannula. Myringotomy was conducted by politzer tympani perforator with an angle to the anterior-inferior part of the membrane (if the ventilation tube is to be used). The effusion was directly drawn into the collector tube by using a sterile single-use aspirator set (Xomed Surgical Products,
Jacksonville, Florida USA). The effusion samples were aspirated in sterile conditions and 5 cc venous blood was drawn simultaneously and the samples were transferred to the immunology laboratory within 30 minutes. The effusion samples were 2-10 fold diluted with PBS (Phosphate buffered saline). Their dilution ratios were calculated and recorded by measuring the volumes with a micropipette. The obtained samples were stored at -20 and -70°C for IgE, IgG, IgA, IgM and cytokine analysis.

Total IgE levels in serum and middle ear effusion samples were determined with ELISA method (IgE; Int. Immuno-Diagnostics, USA). The serum samples were thawed only once after being taken from the freezer.

IFN-\(\gamma\), IL-4 levels in middle ear effusion samples and serum of the patients were determined with ELISA (ELISA, CLB, Amsterdam, Netherlands) and the results were reported as pg/ml.

IgG, IgA, IgM levels were expressed as g/L after being measured with Beckman Array 360 Systems using nephelometric methods and commercial kits (Beckman, California, USA).

Statistical analyses were made by using Student’s t test, Mann-Whitney U test and correlation analysis (spearman and multiple regression analysis) for independent samples by using SPSS 6.0 for Windows 95. P value of <0.05 was accepted as being statistically significant.

RESULTS

Seventy-eighth ears of 59 patients with OME were included in this study. Their ages ranged between 4 and 8 years (mean age 5.20±2.31); of 59 patients 22 were female and 37 were male. The control group consisted of 26 healthy children (8 male, 18 female) with ages between 4 and 9 (mean ages 6.00±0.49) years.

Of 59 patients, 44 were suffering from hearing loss, 6 had earache and 3 were suffering from fullness of ear. Effusion in the middle ear was determined in routine examination in 6 of the patients who had no symptoms. Parents of the patients that applied due to hearing loss were complaining from not having responses from their children, having irrelevant answers and from their sitting very close to TV while watching. Of 59 patients, 48 were frequently having upper respiratory tract infections and 44 of them had a history of day care or nursery. The incidence of frequent upper respiratory tract infections in healthy children of control group was 14/26 (53.8%) whereas it was 15/26 (57.6%) in children under day care or nursery. According to the histories obtained from the families, all of the patients were born with normal spontaneous birth. None of the patients had premature birth or low birth-weight. The patients had no congenital anomalies. When parents of the patients were asked for allergy it was obtained that (23.7%) 14 patients and or their parents had allergy.

The tympanic membrane was opaque, dark and collapsed in 45 patients. It was in onion membrane appearance, slightly collapsed and its vascularity was increased in 10 patients. However, it was in frosted glass appearance and collapsed in 4 patients. There was postnasal mucoid drainage in the examination of nasopharynx in 32 patients. In the nose examination of these patients, mucoid secretion was determined in the middle ear. The comparison of serum immunoglobulin and cytokine levels of the control group and the patient group with OME is given in Table I.

When the correlations between IL-4, IFN-\(\gamma\) and IgE were examined, a positive correlation was found between IL-4 (pg/dl) and IgE (kIU/L) (rs=0.735, p<0.01) (Fig. 1). However; there were negative correlations between IFN-\(\gamma\) (pg/dl) and IgE (kIU/L) and between IL-4 (pg/dl) and IFN-\(\gamma\) (pg/dl). But these correlations were not statistically significant.

A negative correlation between IL-4 - IFN-\(\gamma\), IFN-\(\gamma\) - IgE; and a positive correlation between IL-4 and IgE were found in all middle ear effusion samples. But these correlations were not statistically significant.

DISCUSSION

Many agents known to have inflammatory potentials were determined as a consequence of biochemical and immunologic studies made in animal models and middle ear effusions in OME cases. Determination of mediators such as immunoglobulins, cytokines and kallikrein-kinine system in effusion, supported that the inflammation occurred as a consequence of the interaction between these medi-
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Whether this inflammation in middle ear is a part of the systemic immune response or middle ear acts as an organ that has the ability to form an independent immune response are the questions to be replied.

There are many studies suggesting that there is a relationship between allergy and otitis media. Although it has not been demonstrated that middle ear or ET was an immunologic target organ, there are many evidence on this subject. The effusion collected from middle ear in OME includes antigen, antibody, immunoglobulins, immune-complexes and other immunologic elements. Therefore, it is possible to think that the changes that occur in the mucosa can be an immune-complex reaction formed after upper respiratory tract infection.

ET obstruction can also be related to allergy. Osur et al. reported that 60% of children with seasonal allergic rhinitis had ET obstruction. They suggested that the inflammation of ET mucosa due to allergens caused proximal blockage and ET dysfunction and that the secondary negative middle ear pressure resulted in liquid transudation into the middle ear.

The allergic reaction can be local, general or combined in various parts of the organism. T-lymphocytes are the conductors of the allergic reaction. The allergen is taken by the cells that would present the antigen according to its route of entry and the sensitive organ. This dendritic cell can be an alveolar macrophage, an M cell in a Payer plaque, a B lymphocyte or a Langerhans cell in the skin. The antigen is to be processed in this cell and presented to helper/inducer CD4+ T lymphocytes, on the surface of the antigen presenting cell and near MHC Class II molecules, by secreting IL-1 and with the help of other co-signals. They are identified by CD3/TCR complex, IL-2 receptors are also stimulated and IL-2 is secreted. Three different group functions were determined when helper T lymphocytes (Th) were multiplying. Th0 cells are responsible from providing the balance; Th1s are responsible from cellular cytotoxicity and Th2s from the management of IgE synthesis. The differentiation in favor of Th2 is predominant in allergic people. A medium genetically poor from IL-12 and rich in IL-4 was thought to be responsible from this. Th2 cells also increase the synthesis of IL-4, IL-5, IL-6, IL-13 and IgE whereas Th1 cells make inhibitory effects by IFN-γ and IL-2.

<table>
<thead>
<tr>
<th>Serum</th>
<th>Patient group (n=59)</th>
<th>Control group (n=26)</th>
<th>p value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ig A (g/L)</td>
<td>1.871±0.880</td>
<td>1.877±0.601</td>
<td>&gt;0.05</td>
</tr>
<tr>
<td>Ig G (g/L)</td>
<td>15.094±4.532</td>
<td>9.606±2.112</td>
<td>&lt;0.05</td>
</tr>
<tr>
<td>Ig M (g/L)</td>
<td>1.107±0.443</td>
<td>1.824±0.742</td>
<td>&lt;0.05</td>
</tr>
<tr>
<td>Ig E (kiU/L)</td>
<td>103.060±67.799</td>
<td>43.835±41.554</td>
<td>&lt;0.05</td>
</tr>
<tr>
<td>IL-4 (pg/ml)</td>
<td>2.516±6.067</td>
<td>0.339±0.272</td>
<td>&lt;0.05</td>
</tr>
<tr>
<td>IFN-γ (pg/ml)</td>
<td>1.439±0.2</td>
<td>6.417±2.799</td>
<td>&lt;0.05</td>
</tr>
</tbody>
</table>

Fig. 1 - The relationship between serum IgE and IL-4 levels in OME cases.
The main actors of this study are IFN-γ and IL-4 related mechanisms which are the cytokines that reflect the polarization of Th1 and Th2 cells. When the relationships between IFN-γ, IL-4 cytokines and total IgE were examined, a positive correlation was found between IL-4 and IgE in serum samples of 59 patients that had OME (p<0.01). A negative correlation was found between IFN-γ - IgE and between IL-4 - IFN-γ. But this correlation was not statistically significant.

In this study, IFN-γ levels were significantly lower but IL-4 levels were significantly higher in serum samples of the patient group compared with the control group (p<0.05). The presence of a statistically significant correlation between IL-4 and IgE in serum samples of patients was demonstrated in vivo in this study (p<0.01). A positive correlation between IL-4 and IgE, a negative correlation between IFN-γ and IgE levels were determined in a total of 78 middle ear effusion samples (p>0.05). It is known that the production and the secretion of IFN-γ by Th1 lymphocyte subgroup have important roles in the predominance of Th1 cells with regards to their number and activity. However, IL-4 is secreted by the Th2 lymphocyte subgroup and it stimulates the proliferation and activity of Th2 cells.[19]

The decrease in IFN-γ production after the stimulus with antigen in mononuclear cell cultures obtained from chord blood of newborns was demonstrated as a risk factor in approximately 33% of the allergic patients in a six-year section study.[15] Normal IFN-γ production but increased IL-4 in children with nutrient hypersensitivity and specific IgE were determined in another study.[16] Atopic asthma is a disease including inflammatory response of respiratory system and with increases in Th2 cytokines and especially in IL-4 and IL-5.[17,18]

Furthermore; serum IgG and IgE levels were higher but IgM levels were lower in the patient group compared with the control group (p<0.05). The higher IgE serum concentrations determined in the patients with OME compared with the control group support the change in Th1/Th2 balance in favor of Th2 in these patients as well as the role of allergy in OME etiology. In addition, this difference in serum IgG concentrations demonstrates that the immune response became more mature.

The close relation between OME and allergy observed in this study may raise a question regarding what cause the hypersensitivity. Studies showed that respiratory viral infections in the early childhood is an important the potential for enhanced allergic sensitization. Data from animal models provide support for the concept that enhanced allergic sensitization caused by increased uptake of allergen during infection may play a critical role, as well as T-cell-mediated immune responses to viral infection.[19] The story of frequently having upper respiratory tract infections occurred in 81.3% of our study group patients support the relation between OME and viral infections. We think that viral infection in the early childhood could promote allergic sensitization, thus allergy may become an important risk factor in OME etiology.

The T cells specific for allergens in atopic patients generally belong to Th2 subgroup and they produce IL-4 and IL-3 that stimulate IgE production by B cells and have roles in IgE controlled allergic diseases.[20] Many studies have shown that medical treatment could be obtained by regulating these cytokines with triggering cytokine levels or cytokine antagonists.[21,22] IFN-γ therapy was found to be effective in atopic dermatitis.[23] But this treatment does not have established clinical protocols at the moment. In a study, IFN-γ therapy was recommended to patients that had eosinophil ratios of less than 9% and serum IgE levels of less than 1500 IU/ml.[21] Jung et al.[23] demonstrated that the decreased IFN-γ production in cultures prepared from T cells in atopic patients did not have an intrinsic or genetic basis. It was proven that the return of IL-2 and IFN-γ production to normal could improve the degenerated Th1 response on precursor T cell levels. With the use of recombinant IFN-γ in long-term therapy in patients with atopic dermatitis, an important decrease was observed in the parameters demonstrating clinical severity.[22] A H1 blocker, terfana
dine, was demonstrated to specifically inhibit the production of Th2 type cytokines (IL-4, IL-5) in human peripheral T cells. But it had not inhibited the Th1 cytokines such as IL-2 and IFN-γ.[24] Testa et al.[24] determined a clear-cut clinical improvement in patients with allergic rhinitis with the use of H2 blockers. They explained this case with the view that H2 blockers increased the IFN-γ levels, but decreased the IL-4 and total IgE levels. Further studies to be conducted will help in planning new treatment protocols by giving priority to the molecular basis of allergic diseases.
Recent studies has reported that frequently experienced upper respiratory tract infections causes increased allergic sensitizations. It is evident that frequently recurrent upper respiratory tract infection complaints are among the most common complaint of patients with the childhood disease of OME. Results from this study arised a question that whether an interaction like an allergic reaction that is localised to the medial ear secondary to the recurrent upper respiratory tract infections is happening. In this patients, both resistance to the medical treatment and repeated endication of ear tube placement supports this hypothesis. We think that the determined dominance of Th2 presence in serum samples of the patients deserves further investigation to determine whether this is a systemic reflection of a reaction localized to the medial ear or the local affects of a systemic reaction.

REFERENCES