Research Article

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Fractalkine (CX3CL1) and its receptor (CX3CR1) in children with hypertrophic adenoid and chronic otitis media with effusion
Adenoid hipertrofisi ve kronik efüzyonlu otitis mediasi olan çocuklarda Fraktalkin (CX3CL1) ve reseptörü (CX3CR1)

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Abstract

Background: Adenoid hypertrophy (AH) is one of the possible causes of chronic inflammation in the middle ear. It has been suggested that CX3CL1 and its specific receptor (CX3CR1) could be related with the pathogenesis of some inflammatory diseases. The aim of the present study was to evaluate the role of CX3CL1 and CX3CR1 in the pathogenesis of AH with chronic otitis media with effusion (COME) in children.

Materials and methods: Adenoid tissue samples were obtained from 91 pediatric patients and divided into two groups: adenoidectomy only for AH (n: 47) and adenoidectomy in conjunction with ventilation tube insertion for AH + COME (n: 44). Expression levels of CX3CL1 and CX3CR1 genes were compared.

Results: Expression levels of CX3CL1 and CX3CR1 in hypertrophic adenoid tissue were not significantly different between the AH + COME and AH only groups. Although no significant difference was detected in the expression of CX3CL1 in the adenoid samples, the expression of CX3CR1 was higher in children older than 48 months.

Conclusions: When allergy, atopy and chronic adenoiditis does not exist to obstructive adenoid hypertrophy, inflammatory fractalkine chemokine expression levels in adenoid tissue was not observed to be increased in children with COME.

Keywords: Chronic otitis media with effusion; Adenoid hypertrophy; Fractalkine; CX3CL1; CX3CR1.

Öz

Amaç: Adenoid hipertrofisi (AH), orta kulakta kronik enfalasyonun olası sebeplerinden biridir. CX3CL1 ve spesifik reseptörünün (CX3CR1) bazı inflamatur hastalıkların patogenezi ile ilişkili olabileceği ön sürtülmektedir. Bu çalışmamın amacı, CX3CL1 ve CX3CR1’in çocuklarda AH ve kronik efüzyonlu otitis media (KEOM) patogenezindeki rolünü değerlendirmektir.

Gereç ve Yöntemler: Adenoid doku örnekleri 91 pediatric hastadan alındı ve yalnız AH (n: 47) ve adenoidectomı ve CHS + COME (n: 44) gruba ayrıldı. CX3CL1 ve CX3CR1 genlerinin expression düzeyleri değerlendirildi.

Bulgular: Hipertrofik adenoid dokusundaki CX3CL1 ve CX3CR1 ekspresyon düzeyleri, yalnız AH grubu ile CHS + KEOM grubu arasında anlamlı farklılıklar göstermedi.
Adenoid örneklerinde CX3CR1 ekspresyonu 48 aydan daha büyük çocuklarda daha yüksekti, CX3CL1 ekspresyonunda ise anlamli bir fark bulunmadı.

Sonuç: Obstrüktif adenoid hipertrofisine alerji, atopi ve kronik adenoidit eşlik ettiği KEOM’li çocuklarda adenoid dokusundaki enflamatuar fraktalkin kemokininin ekspresyon düzeyinde artış olduğu gözlemlemektedir.

Anahtar Kelimeler: Kronik efüzyonlu otitis media; Adenoid hipertrofisi; Fraktalkin; CX3CL1; CX3CR1.

Introduction

Otitis media with effusion (OME) is a common childhood disorder, occurring most frequently between the ages of 6 months and 4 years without any signs and symptoms of acute otitis media (AOM) and is characterised by an accumulation of serous or mucoid fluid in the middle ear cavity [1, 2]. The disease is also called secretory otitis media, serous otitis media or “glue ear” due to the presence and features of the accumulated fluid [1]. In chronic otitis media (COME), the fluid persists for 3 months or longer in the middle ear [2]. COME may lead to hearing loss, language development disorders, poor school performance and recurrent AOM. Surgery, including interventions like myringotomy, ventilation tube insertion or adenoidectomy is usually indicated for preventing any potential damages to the eardrum [3, 4].

The aetiology of OME is multifactorial. The disease may develop following an upper respiratory infection or after AOM due to a dysfunctional Eustachian tube and chronic inflammation. Other aetiological factors may also be involved, including environmental factors, the presence of allergic rhinitis, adenoid hypertrophy or biofilms [5, 6]. Although adenoid hypertrophy (AH) is listed among the potential causes of OME by causing a mechanical obstruction of the Eustachian tube, OME does not develop in every child with AH. Therefore, it is suggested that AH is not the cause of OME per se but the disease results from the inflammatory reaction provoked by AH via the release of the inflammatory mediators in the Eustachian tube and middle ear mucosa.

The mechanisms involved in the development of inflammation associated with hypertrophic adenoids in OME have not been clearly established, yet. Adenoids contribute to the humoral and cellular responses of the immune system, involving B and T cells, monocytes, dendritic cells and natural killer (NK) cells. The chemokine system is also well-known that the chemokines and their receptors take a critical part in coordinating the immune response in the body, including leukocyte maturation and immigration to the areas of inflammation [7].

The CX3C chemokine subclass comprises only Fractalkine (CX3CL1) and its specific receptor CX3CR1, suggested to be associated with the underlying mechanisms of several inflammatory diseases such as psoriasis, rheumatoid arthritis, atherosclerosis, HIV infection, cancer, and several other disorders [8–14]. The two isoforms of CX3CL1 are the soluble and the membrane-bound forms. While the former acts as a chemotactic factor for the T-cells, monocytes, mast cells, and NK cells; the latter is usually found in the vascular endothelial cells [8]. An increased fractalkine expression is reported in the well-innervated and vascularised organs and at the areas with increased leukocyte concentration in the body. On the other hand, it is reported that this molecule shows specificity for particular cell types [15].

The aim of the present study was to evaluate the roles of fractalkine chemokine and its specific receptor CX3CR1 in the pathogenesis of COME in children with AH.

Materials and methods

Study participants and design of the study

This was a prospective and controlled study, conducted between the dates in June 2015 and August 2016 at Baskent University Hospital in the Department of Otolaryngology, Head and Neck Surgery. Adenoid tissue samples were obtained from 91 paediatric patients assigned to two groups: Adenoid tissue samples of the patients who underwent adenoidectomy for AH (AH only group; n = 47) and that of the patients who underwent adenoidectomy and ventilation tube insertion for the comorbid presence of AH + COME (AH + COME group; n = 44).

The diagnosis of COME was made in the patients with the presence of air-fluid levels or bubbles behind the amber-coloured tympanic membrane, persisting longer than 3 months in the pneumatic otoscope examination and/or the presence of conductive hearing loss with a gap of 30 dB. Type B tympanogram was also used in making the diagnosis [16].

Nasal endoscopy was performed or lateral nasopharyngeal radiographs (if parents did not accept the endoscopy) were obtained to evaluate the size of the adenoid in all patients. The evaluations were performed by an otorhinolaryngologist. Only children with grade 3 or grade 4 AH, for example with a choanal obstruction of >75%, were included in the study. Adenoidectomies
were conducted in children, displaying the symptoms of obstruction including mouth breathing or hyponasal speech, in whom a moderate nasal obstruction due to AH was identified [17].

The parents of the children, meeting the criteria to be included in the study, gave written informed consents for participation and for the procedures in the study to be conducted. This study was approved by Baskent University’s Institutional Review Board (Project no: KA15/127) and received a financial support from Baskent University’s Research Fund. All procedures in this study were designed in accordance with the Helsinki Declaration as revised in 2008.

One parent of each child in the study completed a questionnaire, providing information about any exposure to tobacco smoke at home, histories of breastfeeding and bottle feeding, familial predisposition, number of siblings, any presence of documented allergies/allergic symptoms, frequency of upper respiratory tract infections (URTI) and frequency of AOM developing in the last 12 months, so that the multifactorial variables in the two groups could be determined. According to the information reported by the parents, the children participating in the study did not have any infection or did not use any antibiotics, antihistamines, and intranasal or systemic corticosteroids at least 2 weeks before surgery.

Patients were excluded from the study if they had a documented history of immunodeficiency disorders; any inflammatory, autoimmune, malignancy or haematological disorders; ciliary dyskinesia, cystic fibrosis, allergy; previous ear surgery or adenoidectomy; or congenital malformations such as cleft palate. The patients allergy; previous ear surgery or adenoidectomy; or congenital malformations such as cleft palate. The patients were included in the study if they had preoperative com-

Statistical analysis

SPSS for Windows version 22.0 was used for analysis (SPSS Inc., Chicago, IL, USA). The conformity of the data to a normal distribution was tested with the Shapiro-Wilk test if the sample size was >50 and with the Kolmogorov-Smirnov test if it was ≤50. The normally distributed quantitative variables were expressed as mean ± standard deviation and median. The maximum and minimum
(max–min) values were used for non-normal distributions and frequency tables were used for qualitative variables.

The Independent Samples T-test was used for the statistical comparison of two groups of normally distributed data. The Mann-Whitney U test was used to compare whether there is a difference between the dependent variables of two independent groups. The relationship between two categorical variables was assessed using the Chi-square test. A value of $p < 0.05$ was considered statistically significant in all statistical evaluations.

**Results**

The study included a total of 91 paediatric patients, comprising 31 females and 60 males. The “AH + COME” group included 44 subjects, comprising 13 females and 31 males with a mean age of 49.45 ± 15.91 months. The 47 subjects in the “AH only” group comprised 18 females and 29 males with a mean age of 55.26 ± 15.91 months. No significant difference was found between the “AH + COME” group and the “AH only” group in terms of age ($p = 0.085$) or gender ($p = 0.507$).

Expression levels of fractalkine (CX3CL1) chemokine and its receptor (CX3CR1) in hypertrophic adenoid samples were not significantly different between the “AH + COME” and “AH only” groups ($p = 0.714$ for CX3CL1 and $p = 0.218$ for CX3CR1) (Table 1).

The number of AOM episodes in the last 12 months was found to be statistically significantly higher in the “COME + AH” group ($n ± SD$ values: COME + AH = 3.43 ± 2.05 vs. AH only = 1.15 ± 1.25; $p = 0.000$). No statistically significant difference was determined between the groups in respect to the frequency of upper respiratory tract infections in the last 12 months ($n ± SD$ values: COME + AH = 6.14 ± 2.455 vs AH only = 5.89 ± 2.035; $p = 0.608$).

No statistically significant difference was determined between the groups in respect to the average counts of WBC, MPV, NEUT, LYM, EOS, and in respect to NLR ($p > 0.05$) (Table 2).

Breastfeeding and bottle feeding durations were categorized into 3 periods of <6 months, 6 months to

| Table 1: Results of relative expression levels of CX3CL1 and CX3CR1 between AH only and AH + COME groups. |
|---------------------------------|---------------------------------|---------------------------------|---------------------------------|
| Relative expression levels ($2^{-\Delta \Delta CT}$) | **CX3CL1** | **CX3CR1** |
| AH only n (47) | AH + COME n (44) | AH only n (38) | AH + COME n (39) |
| Mean | 0.0273470 | 0.0292964 | 0.0062379 | 0.0107559 |
| Standard deviation | 0.02218365 | 0.02815620 | 0.0107559 | 0.01805944 |
| p-Value | 0.714 | 0.218 |

Expression levels of fractalkine (CX3CL1) chemokine and its receptor (CX3CR1) in hypertrophic adenoid samples were not significantly different between the AH + COME and AH only groups.

| Table 2: Results of risk factors. |
|---------------------------------|---------------------------------|---------------------------------|---------------------------------|
| Gender (n) | AH only n (47) | AH + COME n (44) | p-Value |
| Male | 29 | 31 | 0.507 |
| Female | 18 | 13 |
| Age (month, mean ± SD) | 55.26 ± 15.90 | 49.45 ± 15.84 | 0.085 |
| AOM episodes (n ± SD)* | 1.15 ± 1.25 | 3.43 ± 2.05 | 0.000 |
| URTI episodes (n ± SD) | 5.89 ± 2.035 | 6.14 ± 2.455 | 0.608 |
| Tobacco smoke exposure (n) | | | |
| Yes | 10 | 5 | 0.203 |
| No | 37 | 39 |
| School attendance (n) | | | |
| Yes | 37 | 34 | 0.807 |
| No | 10 | 10 |
| NLR | 1.2886 ± 1.09905 | 0.9566 ± 0.43861 | 0.065 |

The number of AOM episodes in the last year was found to be statistically significantly higher in the COME + AH group while other risk factors were similar.
1 year, and >1 year. A similar distribution was observed in both groups in terms of their mode feeding during infancy (p > 0.05). There were no significant differences between the groups with respect to the number of siblings (p = 0.704), the presence of tobacco smoke exposure (p = 0.203), and school attendance (p = 0.807).

Of the 44 patients in the “COME + AH” group, mucoid fluid was detected in 33 (75%) and serous fluid in 11 (25%) patients after myringotomy. There was not a statistically significant difference between the expression levels of CX3CL1 and CX3CR1 in the hypertrophic adenoid tissue. The type of the effusion was not statistically different between the CX3CL1 and CX3CR1 groups (p = 0.881 for CX3CL1; p = 0.248 for CX3CR1). The following factors were found to be not associated with age; including the history of AOM and URTI episodes; the mean WBC, MPV, NEUT, LYM, and EOS counts, and NLR levels in determining the characteristics of the effusion (p > 0.05).

The study patients were divided into two groups according to age as ≤48 months and >48 months. There were no statistically significant differences between the expression levels of CX3CL1 (p = 0.346) and CX3CR1 (p = 0.053) in the “AH + COME” group by age categories. No significant difference was detected in the expression of CX3CL1 in the adenoid tissue samples, whereas, the expression of CX3CR1 was significantly higher in children older than 48 months (p = 0.016). No significant difference was detected between the two groups with respect to the average WBC, MPV, NEUT, LYM, and EOS counts and NLR levels.

**Discussion**

OME is a major disease of the childhood period, leading to hearing impairments in children most commonly [19]. Recent studies have demonstrated the significant role of AH as a salient risk factor for COME. Several hypotheses were suggested to explain the associations between AH and the development of OME, investigating direct and indirect mechanisms such as obstruction, dysfunction of the Eustachian tube, chronic bacterial or viral infections, and impaired mucociliary clearance [20, 21].

Some authors, including Wright et al. [22], have supported the role of obstruction and stated that the adenoid may compress or obstruct the Eustachian tube lumen, causing negative pressure and a subsequent development of OME. However, the incidences of OME in children with AH have been shown to vary from 35% to 69.1% in different studies [23, 24]. Because every child with AH does not develop COME, it is suggested that inflammatory reactions in the hypertrophic adenoid tissue may both affect the Eustachian tube functions and take part in inducing an inflammation in the middle ear.

Studies have demonstrated that inflammatory chemokines, in the adenoid tissues and the middle ear fluid are involved in the pathogenesis of OME [25]. For example, one study has shown lower quantities of CXCL4 [26] and another has reported increased mRNA levels of interleukins (IL-1β, IL-6, IL-8) and TNF-α in middle ear effusion (MEE) [27]. Another chemokine, the monocyte chemotactic protein-1, which is responsible for the monocytes to migrate and accumulate at the chronic inflammation site, has been found at higher levels in MEE [28]. Histamine levels and mast cell numbers have also found to be at high levels in the adenoids of children with OME, suggesting a possible association of Eustachian tube obstruction with histamine release from the adenoids. Similarly, increased quantities of IL-7R expressing T-lymphocytes (CD127 and CD132) were reported in hypertrophic adenoids with OME and associated with irregularities in the immunological responses [29].

The main aim of this present study was to investigate the expression levels of fractalkine and its specific receptor in hypertrophic adenoid tissues to gain insight into the involvement of this unique inflammatory chemokine in the development of OME. To the best of our knowledge, this is the first study to have focused on fractalkine particularly among the chemokines associated with OME. Fractalkine (CX3CL1) and its receptor CX3CR1 display both adhesive and chemotactic functions in regulating the migration of leukocytes to the site of inflammation. High levels of expressed fractalkine and its receptor are known to be related to the pathogenesis of some inflammatory diseases including atherosclerosis [11], psoriasis [13], rheumatoid arthritis [14], and periodontal diseases [30]. However, the current study did not yield similar results as the levels of expressed CX3CL1 and CX3CR1 in hypertrophic adenoid tissues were not different between the “AH + COME” and “AH only” groups.

According to the currently revised clinical practice guidelines on OME [3], adenoidectomy is found to be as beneficial as the insertion of tympanostomy tubes in children undergoing surgery for OME at the age of 4 and older. For children younger than 4 years old, only the insertion of tympanostomy tubes is recommended. Adenoidectomy for the treatment of OME was found to be related to shorter periods of effusion persistence in the middle ear, reduced need for repeated surgeries, and lower failure rates. Based on these recommendations in the guidelines and the reports in the literature,
we categorized the adenoidectomy specimens into two groups, namely <4 years and ≥4 years, in order to determine whether the expression levels of fractalkine in the adenoid tissue vary by age. We found that the expression levels of CX3CR1 in the adenoid samples were higher in children older than 4 years while CX3CL1 expression levels were similar in the patients both <4 years and ≥4 years old.

Age might be a key risk factor with regards to the development of OME because of it is directly correlated with the time of angulation of the Eustachian tube and significantly correlated with the stages of immune system development [6, 31]. However, the comparison of the study parameters by age did not show any statistically significant differences in this present study.

Similar to the previously reported studies [6, 31], the majority of patients in both groups were males but there was not a significant gender difference between the two groups. However, there are also studies in the literature reporting a female preponderance [32].

OME may occur during or after a URTI due to either a Eustachian tube dysfunction spontaneously or as a result of AOM [2]. Similar to the study by Alho et al., it was found in the current study that children with a history of higher frequencies of AOM in the previous 12 months were more likely to develop OME. This was the only risk factor in our study demonstrating a statistically significant difference between the two groups.

It has been highlighted in some studies [31] that children with a longer breastfeeding duration have a lower risk of developing OME but in the current study and in several previously conducted other studies [33], no relationship has been found between the duration of breastfeeding and OME. In the current study, no increased risk was determined between bottle feeding and COME, which is consistent with the findings of other studies.

Although one of the most studied risk factors for OME is the exposure to the cigarette smoke at home, no statistically significant association of the passive exposure to the cigarette smoke with the development of OME was found in this present study, which was a similar finding to those of other studies [7, 34].

As in other studies, no significant differences were determined between the study groups with respect of the number of siblings in the current study [6]. The rates of attendance to daycare or school were 77% in the “AH+COME” group and 78% in the “AH only” group. Although the majority of the participating children attended a daycare centre or school, there was not a significant difference between the two groups in terms of these risk factors.

Some studies have suggested that PLR and NLR may be the beneficial predictors of prognosis and recurrence in infectious and non-infectious inflammatory pathologies. NLR is also reported to be used as a marker of subclinical inflammation [35, 36]. Boztepe et al. [34] showed that the preoperative NLR and PLR levels could be used to predict the viscosity of MEE. In the current study, NLR was associated with neither OME development nor MEE viscosity. Therefore, it might be concluded that pre-inflammatory markers including NLR would not be beneficial to predict local inflammation in MEE.

This is the first study suggesting the hypothesis that fractalkine in adenoid tissues may be associated with the presence of middle ear effusion. In conclusion, in the absence of any allergies, atopic clinical conditions, and chronic adenoiditis leading to an obstructive adenoid hypertrophy, the inflammatory chemokine fractalkine doesn’t take part in the development of OME. Further studies are warranted to determine the role of inflammatory pathways, in which fractalkine is involved in association with the development of OME independent of the adenoid volume.

Conflict of interest: There is no conflict of interest between the authors.

References


