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Studying the Serum as Well as Serous Level of IL-17 and IL-23 in Patients with Serous Otitis Media

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ABSTRACT

Serous otitis media with effusion (OME) is a middle ear inflammatory response to allergens and microbes which stimulate leukocytes to produce different inflammatory mediators after obstruction of Eustachian tube. Here, we investigated the levels of these mediators, IL-17 and IL-23, in serum and middle ear fluids of children with OME.

75 patients with otitis media and 75 age and sex-matched healthy controls were enrolled in this study. IL-17 and IL-23 levels in serous secretion of the patients and their serum levels were measured in both groups by ELISA.

Serum IL-17 levels were significantly higher in the patients than controls (p=0.001). There was no significant difference between serum IL-23 levels in patients and controls. Patients' serous levels of both cytokines of IL-17 and IL-23 were higher than those in serum according to different parameters of sex, age, and duration of the disease.

This study shows an elevated presence of IL-17 and IL-23, as pro inflammatory cytokines, in OME. These finding may represent the contribution of such cytokines in the pathogenesis of OME. Blocking such molecules may yield new non-surgical therapeutics.

Keywords: Interleukin 17; Interleukin 23; Otitis media with effusion

INTRODUCTION

Otitis media with effusion (OME) is a middle ear inflammatory disease which accompanies with hearing loss and fullness.[1] Several immuno-regulatory and allergy-associated cytokines have been identified in such effusion that are thought to promote the development of a chronic condition named serous otitis media.

Interleukin 23 (IL-23), one of such cytokines released from antigen-stimulated dendritic cells and macrophages,[2] plays a pivotal role in the establishment and maintenance of organ-specific inflammatory

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processes in which it brings the production of some other cytokines such as IL-17A, IL-6, and TNF.[3] Indeed, in vitro activation of naive T cells in the presence of IL-23 could induce the generation of a small population of IL-17-producing T cells, namely Th17 cells[3] which induce inflammatory responses, neutrophils aggregation and dealing the pathogens.[2-4] Such induction has been identified in local inflammatory sites, such as the synovial fluid of patients with Lyme arthritis and autoimmune arthritis, bullous pemphigoid, primary biliary cirrhosis and inflammatory bowel disease, suggesting concurrent involvement of both cytokines in inflammatory pathologies.[5,6]

IL-17 is a pro-inflammatory cytokine that up regulates a number of cytokines and chemokines, leading to the recruitment of neutrophils to sites of inflammation.[7,8] In terms of infection, IL-17A has been demonstrated to have a protective role against multiple microorganisms, predominantly extracellular bacteria and fungi.[9] However, IL-17A is also the classic example of a "double-edged sword" cytokine that it clearly functions as an inflammatory mediator of local pathologies such as psoriasis, rheumatoid arthritis, multiple sclerosis, inflammatory bowel disease, asthma, atopic dermatitis and systemic lupus erythematous (SLE).[10-14]

The recognition of inflammatory mediators such as IL-23 and IL-17 in middle ear effusions and their correlation with clinical parameters may allow better understanding of many complex events leading to development of permanent complications of otitis media and hopefully help to develop future interventions.

The aim of the present study was to evaluate the level of IL-17 and IL-23 in serum and middle ear effusions as well as their mutual correlation with age, sex, and duration of the disease. Understanding such concepts may elucidate some roles of these cytokines that help us to improve new nonsurgical treatments of OME.

MATERIALS AND METHODS

This study evaluated 75 cases suffering OME aged 7-12 years and 75 age and sex-matched health controls. As the routine management of OME, all patients scheduled for myringotomy after at least one month antibiotic consumption. Prior surgery or any active infection as well as inflammatory process considered as exclusion criteria. The study was approved by local ethics committee protocol (No. 9091) and written informed consent was obtained from all participants or their guardians. Furthermore, the study protocol was conformed to the ethical guidelines of the 1975 Helsinki Declaration.

Serum as well as otitis effusion samples were collected from 75 patients with OME and 75 healthy controls. Samples were taken from middle ear after myringotomy in sterile situation at operating room. There was just serum sampling in control group. The samples stored at −20°C until analysis. Concentration of IL-23 as well as IL-17 were measured using a quantitative immunoassay technique by commercially available ELISA kits (eBioscience, USA) according to the manufacturer's protocols. Samples were analyzed in duplicates and mean cytokine levels reported in pg/ml in each group.

We used a multiple logistic binary regression model with conditional backward method to survey the effective factors on OME. Using ROC curve and area under curve (AUC), we tried to determine the sensitivity and specificity of such cytokines as a diagnostic/prognostic marker of OME. All data were analyzed by SPSS 17 (Chicago, USA) software. Chi-square test for categorical data and independent t test for numerical data were used.

RESULTS

Basic and clinical characteristics of the participants are shown in Table 1. Serum IL-17 level was significantly higher in the patients than that of the controls (p=0.001). There was no significant difference between serum IL-23 levels in both groups (p=0.737) (Table 1). Middle ear effusion levels of both cytokines of IL-17 and IL-23 were higher than those in serum (p<0.001, p<0.016; respectively) according to different parameters of sex, age and duration of the disease (Table 2).

Evaluating the effect of sex, age, and serum levels of IL-17 as well as IL-23 on OME in all subjects, we found a significant effect of both cytokines of IL-17 and IL-23 on affection to such disease. Our model showed that while serum levels of IL-23 is related to decreased chance of OME affection (OR=0.9, p=0.042), serum levels of IL-17 is directly related to increased chance of affection to OME (OR=1.131,

p=0.02) (Table 3). Surveying linear correlation between serum levels of IL-23 and OME, we found a linear regression slope of 0.2 between these independent (OME) and dependent (IL-23) variables. Such slope was calculated as 3.53 between OME and serum levels of IL-17. Pearson's linear correlation coefficient between serum levels of IL-17 and IL-23 was calculated as 0.42.

Using ROC curve and area under curve (AUC), we tried to determine the sensitivity and specificity of IL-17 and IL-23 as diagnostic markers of OME. We found AUC of IL-17 and IL-23 as 0.614 and 0.466, respectively (data not shown). Therefore, such parameters do not have a power as strong as to predict the diagnosis of the disease.

Table 1. Basic and clinical characteristics of healthy subjects and patients with serous otitis media

		Patients (n=75)	Control (n=75)	*p* value
Age (years)	<6	28	36	0.187
	≥6	47	39	
Sex	Male	51	45	0.307
	Female	24	30	
Duration of disease (months)	<12	51	-	-
	≥6	24	-	
Otitis serous (mean±SD)(pg/mL)	IL-17	12.12±8.8	-	-
	IL-23	6.11±5.1	-	-
Serum (mean±SD) (pg/mL)	IL-17	8.5±7.1	5.15±3.2	0.001
	IL-23	4.1±4.5	3.9±3.1	0.737

Table 2. Serous and serum levels of IL-17 and IL-23 according to different parameters in patients with serous otitis media

Cytokine	Variable		Otitis serous (mean±SD) (pg/ml)	Serum (mean±SD) (pg/ml)	*p* value
IL-17	Age (years)	<6	10.7±7.5	7.05±6.4	<0.001
		≥6	12.9±9.5	9.37±7.5	<0.001
	Sex	Male	13.35±9.1	9.3±7.39	<0.001
		Female	9.5±7.9	6.77±6.4	<0.001
	Duration of disease (months)	<12	8.2±7.04	5.84±5.9	<0.001
		≥6	2.37±6.35	14.16±6.25	<0.001
IL-23	Age (years)	<6	5.96±4.5	3.71±3.74	<0.001
		≥6	6.2±5.4	4.32±4.92	<0.001
	Sex	Male	6.32±5.09	4.2±4.65	<0.001
		Female	5.66±5.2	3.9±4.2	<0.004
	Duration of disease (months)	<12	4.19±3.71	2.54±2.87	<0.001
		≥6	10.2±5.31	7.4±5.5	<0.016

Table 3. Multiple binary logistic regression model evaluating the effect of different parameters on otitis media in all subjects

		B	S.E.	Wald	df	Sig.	Exp(B)
Step 1(a)	Serum IL-17	0.123	0.040	9.616	1	0.002	1.131
	Serum IL-23	-0.106	0.052	4.131	1	0.042	0.900
	Sex	-0.069	0.314	0.048	1	0.827	0.934
	Age	-0.536	0.328	2.674	1	0.102	0.585

DISCUSSION

Our study showed significant higher serous levels of IL-23 and IL-7 than their serum levels of the OME patients. Furthermore, we showed an increased serum level of IL-17 in patients compared to that in healthy controls. Considering that IL-23/IL-17 pathway has a remarkable role in chronic inflammatory states and internal immunity against pathogens,[4] we may conclude Th17 pathway involvement in pathophysiology of OME.[15] Although there was a significant increase of serous levels of IL-23 compared to their serum levels in OME patients, there was no significant increase of serum levels of IL-23 in OME patients than those in healthy controls. Such finding may reflect the idea that IL-23 could act locally with no any systemic presentation. However, in the case of IL-17, while it exerts its pro inflammatory effects locally, it is produced systemically and regulates different kinds of cells including macrophages, fibroblasts, epithelial cells, and endothelial cells to recruit immune effector cells to site of inflammation.[12,16,17]

In accordance with the view that IL-23 is necessary for the production of IL-17, we detected a remarkable up regulation of IL-23 expression in middle ear effusion of the patients regardless of different factors of sex, age, and duration of the disease. The pathophysiologic relevance of the IL-23/IL-17 axis in inflammatory conditions is highlighted by clinical effectiveness of antibodies targeting IL-17 such as Ustekinumab,[18] secukinumab,[19-21] ixekizumab,[19-21] brodalumab,[19-21] and IL-23 such as guselkumab and tildrakizumab[22] in treating some inflammatory diseases such as psoriasis.[17] The hope is that future studies, inhibiting this pathway, evaluate a permanent treatment for OME and reduce some aggressive therapies (like tympanostomy) indicated for refractory OMEs.

Regression model used in our study showed a significant effect of serum levels of both IL-17 and IL-23 on affection to OME. Comparing such effects by linear correlation analysis, we found that the main effect is induced by serum levels of IL-17 (not IL-23). Although a paradoxical positive and negative effect on OME was seen by such cytokines in our regression model, Pearson's linear correlation coefficient between serum levels of IL-17 and IL-23 showed that the changes of such cytokines are unidirectional with different slopes. It means that both cytokines have actually a positive but different effect to induce OME.

According to our knowledge, our work is the first in an evaluation of those cytokines in OME. Therefore, we did not find any opposing results.

Our study had some limitations. First, serial samples were not performed to monitor the changes of IL-17 and IL-23 in different complicated clinical conditions of OME. This limitation allowed just a cross-sectional analysis of cytokine profile of only limited robustness. Second, we did not compare serous samples of both groups. Such sampling was impossible from the healthy participants.

This study brought up the possible role of IL23/IL17 pathway in pathogenesis of the OME. Considering such role may guide us to set up new therapeutics in nonsurgical treatment of OME.

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The authors have no conflicts of interest to declare.

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