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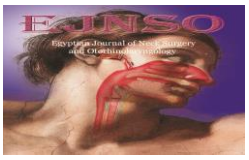

Role of cytokines and nitric oxide in chronic otitis media with effusion in adult and children

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Abstract:

Background: One of the most frequent ear infections is otitis media with effusion (OME). It appears that several inflammatory cytokines are involved in its etiology. The aim of this study is to determine the involvement of cytokines and nitric oxide (NO) in otitis media with effusion (OME) and see if there is a link between the patient's age and these cytokines.

Patients and procedures: The current study looked at the levels of IL-8, TNF-a, and nitric oxide (NO) in the middle ear fluids (MEE) of 31 adults and 24 children who had been diagnosed with OME clinically and audiologically.

Results: The amounts of IL-8, TNF–, and NO are particularly fascinating because they have traditionally been seen in high concentrations in OME. IL-8 had the highest average value of these cytokines. IL-8 concentrations were substantially greater in OME patients in the child–group compared to those in the adult–group, and mucoid-type OME was significantly higher than serous-type OME. TNF - concentrations in the OME of adult-group patients were substantially more significant than those in the child-group patients.

Conclusion: These findings suggested that these cytokines may play a role in illness etiology.

Keywords: OME, cytokines, nitric oxide.

Introduction

OME is most common in adults after a severe upper respiratory illness, such as sinusitis. Nasal discharge irritates the Eustachian tube apertures at the nasopharynx, causing Eustachian tube dysfunction, which is the primary cause of OME in adults. [1]

Patients with poor mastoid pneumatization, as measured by a mastoid size of 6 cm or less, have a greater risk of OME(2). According to Hurst, OME can also emerge as a result of severe allergies. [3]

Other causes of OME include adenoids that obstruct the Eustachian tubes or a nasopharyngeal tumour. Unilateral OME can be caused by compression or invasion of the Eustachian tube rather than physiologic dysfunction. [4]

Adult patients with middle ear fluid, on the other hand, should be treated with caution, especially if it is unilateral. Nasopharyngoscopy should be performed on all individuals who have chronic unilateral OME. [5]

Interleukin (IL)-I, IL-8, tumour necrosis factor-a (TNF-a), y-interferon, and nitric oxide are among the inflammatory cytokines found in OME. [6, 7]

OME in adults as a disease requires reconsideration because the disease's incidence is no longer so rare and is now encountered more commonly. Nasopharyngeal tumours, an age-related illness, are becoming less common. In addition, many studies found that a significant majority of cases had no identified cause. [8]

The goal of this study was to see if any inflammatory cytokines could play a role in patients with OME.

Patients and Methods:

This prospective study included 31 adults and 24 children who were diagnosed with OME clinically and audiologically and were recruited from Assuit University's otorhino-laryngology department.

The Assiut University Institutional Review Board authorized the protocol, and all enrolled patients signed a written consent form.

Clinical evaluation of the patient: The following procedures were used on the patients in this study:

Patient Assessment: Patient presenting in this study was subjected to the following:

1. Detailed history
2. Physical Examination:
3. Diagnostic testing:
   - Audiological evaluation: audiogram and tympanogram
   - Biopsy: for histopathological study for nasopharyngeal masses.

Methods of collection of middle ear effusion: All patients had tympanocentesis while under general anesthesia to collect middle ear effusion. Before tympanocentesis, the ear canal was antisepticised for one minute with 70% alcohol. The antro-inferior region of the tympanic membrane was perforated with the help of a 20 gauge spinal needle and a 1.0 mL sterilised tuberculin syringe. The middle ear fluid was suctioned into a sterile syringe and categorised into two groups based on their appearance (serous or mucoid but not purulent) (mucoid or serous groups).

Immunological assay: [9, 10, 11]

a) Interleukin-8 (IL-8) and tumour necrosis factor-alpha (TNF-alpha) concentrations were determined in OME fluid and serum.

Samples were collected into a serum separator tube and then prepared and stored. Centrifuged for 10 minutes after clot formation at 3000 x g. The samples were kept at a temperature of -20 degrees Celsius or less.

Preparation of the Reagents: Before using, all of the reagents were brought to room temperature. MIX Diluent Concentrate (10x): Reagent-grade water was used to dilute the MIX Diluent Concentrate (1:10).

Standard Curve: Before producing dilutions, we reconstituted the 4 ng of human IL-8 (or human TNF-alpha) standard with 4 ml of MIX Diluent to make a 1 ng/ml standard solution, and allowed it to settle for 10 minutes with gentle agitation. We made duplicate standard points by diluting the IL-8 (or TNF –alpha) standard stock solution 1:2 with an equal volume of MIX Diluent to obtain 0.5, 0.25, 0.125, 0.063, 0.031, and 0.016 ng/ml. MIX Diluent (0 ng/ml) is used as a zero standard.

-Biotinylated IL-8 (or TNF –alpha) Antibody (50x): We diluted the necessary amount of the antibody 1:50 with MIX Diluent after briefly spinning it down.

•Wash Buffer Concentrate (20x): reagent grade water was used to dilute the Wash Buffer Concentrate (1: 20).

Standard Point	**Dilution**	**[IL-8] (ng/ml) / *(TNF- alpha)***
P1	Standard (1 ng/ml)	1.000
P2	1 part P1 + 1 part MIX	0.500
P3	1 part P2 + 1 part MIX	0.250
P4	1 part P3 + 1 part MIX	0.125
P5	1 part P4 + 1 part MIX	0.063
P6	1 part P5 + 1 part MIX	0.031
P7	1 part P6 + 1 part MIX	0.016
P8	MIX Diluent	0.000

•SP Conjugate (100 x): After briefly spinning down the SP Conjugate, we diluted the conjugate 1:100 using MIX Diluent.

Procedure for the Assay:

•All reagents, working standards, and samples were prepared according to the instructions, and all reagents were brought to room temperature before use. The experiment was carried out at room temperature (20-25 C).

•In each well, 50 l of Human IL-8 (or TNF –alpha) Standard or sample was added. Incubated for two hours after covering wells with sealing tape.

•We washed it six times with 300 micro liter of Wash Buffer, inverted the plate, decanted the contents, and hit it 4-5 times on absorbent paper towel to remove the liquid completely.

•For each well, 50 micro liter of Biotinylated IL-8 antibody (or Biotinylated Human TNF-alpha antibody) was added and incubated for two hours.

•Then, as mentioned above, wash a microplate.

•Each well received 50 micro liter of Streptavidin-Peroxidase Conjugate, which was incubated for 30 minutes. We turned on the microplate reader and pre-programmed the programme.

•The microplate was washed as previously mentioned.

•For each well, 50 micro liter of Chromogen Substrate was applied and incubated for around 20 minutes. With the pipette tip, the plate was lightly tapped to achieve thorough mixing and to burst the bubbles in the well.

•Each well received 50 micro liter of Stop Solution. The colour of the sky shifted from blue to yellow

•We immediately read the absorbance with a microplate reader at a wavelength of 450 nm

Analyze the data

For each standard and sample, we determined the mean value of the duplicate readings.

•We plotted the graph using the standard concentrations on the x-axis and the associated mean 450 nm absorbance on the y-axis to construct a Standard Curve. Regression analysis employing log-log or four-parameter logistic curve-fit can be used to get the best-fit line

•Using the Standard Curve, we calculated the unknown sample concentration and multiplied it by the dilution factor

b) **Nitric oxide (NO)** was detected in both the OME fluid and the serum.

The generated nitrous acid diazotizes sulphanilamide in acid medium and in the presence of nitrite and the result is linked with N-(1-napthyl) ethylenediamine. The azo dye that

results has a strong reddish-purple colour that may be seen at 540 nm.

Assay Procedure:

	Sample /Ml	Sample blank /ml	Standard/ ml	Standard blank /Ml
Sample	0.1	0.1	-	-
R1	-	-	0.1	0.1
R2	1.0	1.0	1.0	1.0

Then mixed well, allowed to stand for 5 min, then we added:

R3	0.1	-	1.0	-

Mixed well, allowed to stand for 5 min, then we read absorbance of sample (A sample) against sample blank and of standard (A standard) against standard blank at 540 nm (520-550 nm). Linearity up to 200 µmol/L.

Calculation: Nitrite in sample µmol /L= (A sample ÷ A standard) × 50

Statistical analysis:

SPSS version 19, IBM, USA was used for data entry and analysis (Statistical Package for Social Science). Numbers, percentages, means, medians, and standard deviations were used to present the data. To compare qualitative variables, the Chi-square test was performed.

In the case of non-parametric data, the Mann-Whitney test was used to compare quantitative variables between two groups, and the Kruskal Wallis test was used for more than two groups. To compare quantitative factors before and after the programme, the Wilcoxon Signed Rank Test was used. The correlation between quantitative variables was measured using Spearman correlation.

When P 0.05, the P-value is considered statistically significant.

Results:

Patient characteristic: Twenty three of adult patients were males (74.2%) and 8 patient were female (25.8) with mean age ± standard deviation (SD) of 47.52 ± 17.28 years (range 18-79). while in the children group 13 were males (54.2%) and 11 were female (44.8%) with mean age ± SD of 5.10 ± 3.24 years (range 1.5-11) as shown in table (1):

Clinical picture: Patients complaint ranged from hearing loss, tinnitus, fullness sensation, crackling and vertigo (96.8%, 90.3%, 83.9%, 54.8%, 12.9%) respectively.

Nature of the effusion: The serous-type OME were significantly higher in adult-group (61.3%), whereas the percentage of mucoid-type MEEs were significantly higher in children-group than in adult-group (75%) As shown in (table 2):

Causes of OME: The possible causes of OME in adults shown in figure (1) according to their frequency:

Role of cytokines and inflammatory mediators in adult OME:

Interleukin-8 (IL-8), Tumor necrosis factor-alpha (TNF–α), Nitric oxide (NO) were measured in OME in adult as detailed in table (3).

As regard age factor: IL-8 concentrations were significantly higher in OME of child -group patients compared with those of adult -group patients. TNF-α concentration were significantly higher in OME of adult-group patients compared with those of child-group patients. NO concentrations showed no statistical significant in OME of adult -group patients compared with those of child -group patients (table 4).

TNF-α concentrations in OME showed significant positive correlation with the patients age, such that the older the patients the higher the TNF-α levels

in OME, but there were no significant correlation with age in neither IL-8 or NO (table 5).

As regard nature of the fluid: (table 6) shows that IL-8 & TNF-α concentrations in mucoid-type OME were significantly higher than that in the serious-type OME, while the concentrations of NO showed no statistical significance between mucoid-type and serous-type OME.

As regard the cause: Cytokines values in different causes of OME detailed in table 10)

Table (1): Patient characteristic

	Patients (n= 31)		Controls (n= 24)	
	No.	**%**	**No.**	**%**
Sex: Male	23	74.2	13	54.2
Female	8	25.8	11	45.8
Age: Mean ± SD	47.52 ± 17.28		5.10 ± 3.24	
Range	18.0 – 80.0		1.5 – 11.0	

Table 2: Nature of the effusion

Nature of the fluid	**Adult (n= 31)**		**Children(n=24)**		**P-value**
	No.	%	No.	%	
Mucoid	12	38.7	18	75.0	0.007*
Serous	19	61.3	6	25.0	

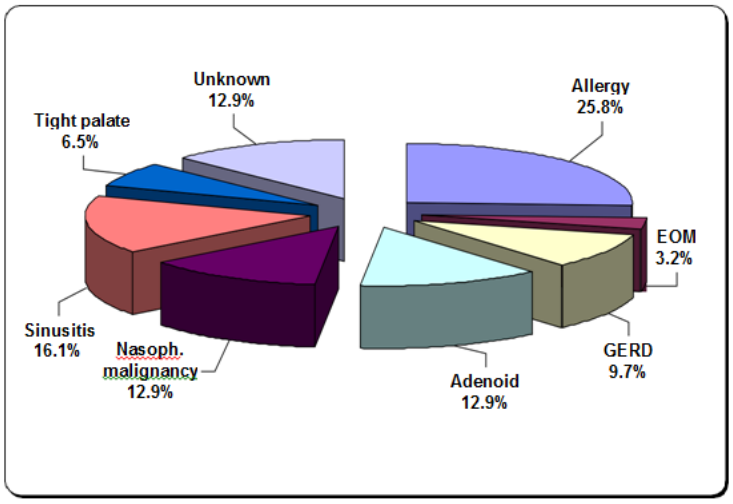

Figure 1: Causes of OME

Table (3): Cytokines in adult and children patient with OME:

	IL-8 (pg/ml)		**TNF-α** (pg/ml)		**NO** (µmol/L)	
	Mean ± SD	Median	Mean ± SD	Median	Mean±SD	Median
Adult patient	1425.48± 2209.58	280(89.0-7217.0)	145.03± 103.98	105(49.0-546.0)	54.57±38.59	31.9(11.9-130.4)
Children patient	1562.46 ± 1916.35	735(133.0-7189.0)	118.25± 143.96	84(42.0-770.0)	55.71 ± 2222.02	52.5 (23.2 - 85.0)

Table (4): Cytokines in adult & children:

		Adult	**Children**	**P-value**
IL-8: (pg/ml)	Mean ± SD	1425.48 ± 2209.58	1562.46 ± 1916.35	0.048*
	Median	280 (89.0 - 7217.0)	735 (133.0 - 7189.0)	
TNF-α: (pg/ml)	Mean ± SD	145.03 ± 103.98	118.25 ± 143.96	0.016*
	Median	105 (49.0 - 546.0)	84 (42.0 - 770.0)	
NO: (µmol/L)	Mean ± SD	54.57 ± 38.59	55.71 ± 22.02	0.481
	Median	31.9 (11.9 - 130.4)	52.5 (23.2 - 85.0)	

Table (5): Correlation of cytokines with age

	Age	
	R-value	**P-value**
IL-8	-0.261	0.070
TNF-α	0.253	0.050*
NO	-0.083	0.634

Table (6): Cytokines in relation to nature of the fluid:

	Cytokines	**Nature of the Fluid**		**P-value**
		Mucoid	**Serous**	
IL-8: (pg/ml)	Mean ± SD	3127.50 ± 2671.91	529.68 ± 1252.45	0.002*
	Median	2808.5 (140.0 - 7217.0)	224.0 (89.0 - 5670.0)	
TNF-α: (pg/ml)	Mean ± SD	179.60 ± 90.49	126.84 ± 108.21	
	Median	188.0 (77.0 - 350.0)	105.0 (49.0 - 546.0)	**0.05***
NO: (µmol/L)	Mean ± SD	56.27 ± 24.58	53.68 ± 44.86	
	Median	69.9 (19.3 - 77.7)	25.9 (11.9 - 130.4)	0.614

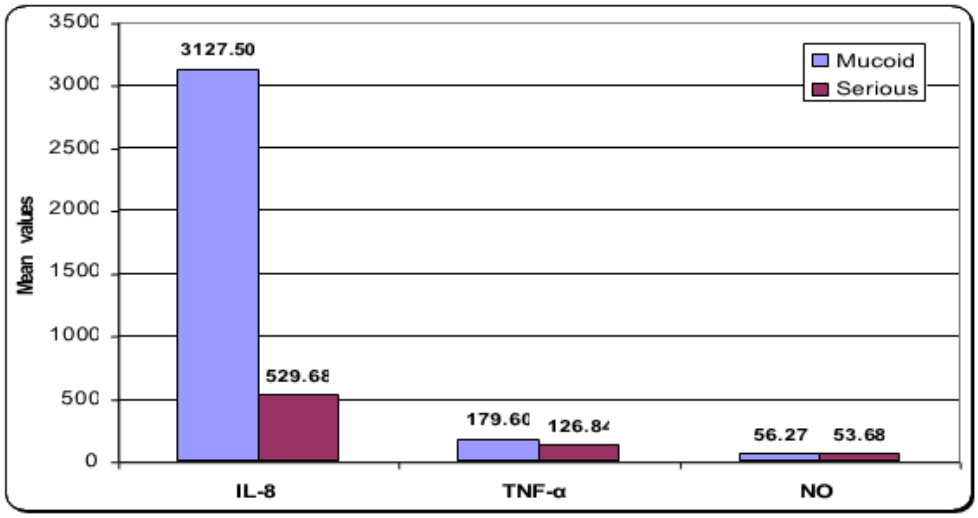

Figure (2): Cytokines in relation to nature of the fluid

Table (10): cytokines in different causes of OME:

	Adenoid	Allergy	EOM	GERD	Nasoph. Malignancy	Sinusitis	Tight palate &adenoid tissue	Unknown
IL-8:								
Mean ± SD	500.5±528.6	1653.63±2341.28	5113.0±-	104.3±13.3	1583.8±2331.0	3647.0±3174.6	182.0±59.4	287.0±148
Median Range	304.5 (133-1260)	360.5(140.0-5670.0)	5113 (5113-5113)	112 (89-112)	504 (252-5075)	4347 (266-7217)	182 (140-224)	231 (175-455)
TNF-α:								
Mean ± SD	147.8±61.5	167.50±161.37	198.0±-	126.0±30.5	108.5±60.9	205.8±108.7	94.5±14.8	81.7±35.2
Median Range	140 (84-227)	102.0 (51.0-546.0)	198 (198-198)	112 (105-161)	91 (56-196)	175 (77-350)	94.5 (84-105)	77 (49-119)
NO:								
Mean ± SD	34.2±26.5	46.50±24.86	74.8±-	50.9±51.2	61.8±49.3	39.3±27.9	107.5±12.9	87.5±59.2
Median (Range)	2 (13.9-73.1)	46.8 (18.6-74.8)	74.8 (74.8-74.8)	31.9 (12-109)	48.9 (23-127)	21.9 (15.9-77.7)	107.5 (98-117)	112 (20-130)

Discussion:

OME in adults as a disease requires reconsideration because the disease's frequency is no longer rare and is becoming increasingly common. Furthermore, many cases were reported to be of uncertain cause in prior investigations. [8, 12]

In this study, about 68% of OME in the adult group were of the serous type, while around 75% of OME in the child group were of the mucoid type. As a result, the percentage of serous-type OMEs in the adult group was significantly greater than in the child group, while the percentage of mucoid MEEs in the child group was significantly higher than in the adult group. These findings are consistent with Hotomi et al., 1994, who indicated that mucoid effusions are more common in children, whereas serous effusions are more common in adults. [13]

Children with serous effusions may have severe Eustachian tube damage, as compared to children with viscous effusions, who have a mainly inflammatory origin. [14] This can also be explained by the fact that adults have lower levels of IL-8 than children since IL-8 causes goblet cells to secrete mucin, which makes the fluid more viscous. [13]

Allergic rhinitis with or without nasal polyps (25.8%), sinusitis (16.1%), and adult-onset adenoidal hypertrophy were the most common causes in this study (12.9%). Only four patients (12.9%) had nasopharyngeal malignancy, including one case of squamous cell carcinoma and three cases of non-Hodgkin lymphoma. Other putative etiologies were GERD in 9.7% of cases, tight short palate in 6.5 percent of cases, EOM in only one instance (3.2%), and unknown etiologies in 12.9% of cases. These findings are consistent with those of Ho et al. (2008), who found that upper respiratory infection (URI) (23.0%), allergic rhinitis (18.4%), and sinusitis (17.3%) were the most common causes of isolated OME. [12]

In OME, cytokines play an important role. OME has been linked to a slew of inflammatory mediators. Cytokines, on the other hand, play a key role as initiators, mediators, and regulators of middle ear inflammation and subsequent molecular pathological processes in middle ear tissue, resulting in histopathological abnormalities in the middle ear cavity and the pathogenesis of OME.[15] OME contains several cytokines, including the pro-inflammatory cytokines IL-8 [16], IL-l, tumour necrosis factor (TNF) [17], and nitric oxide (NO). [18] These cytokines are particularly intriguing because they have traditionally been seen in high concentrations in effusions.[17] This is consistent with the findings of the current investigation, which found IL-8, TNF– alpha, and NO in all instances of OME.

In our research, we discovered that IL-8 had the highest mean value of these cytokines in adults, with a mean value of 1425.48 (2209.58) pg/ml. This is in agreement with Johnson et al. (1997), who said that of the tested pro-inflammatory cytokines, IL-8 had the highest amount (up to 1793 pg/ ml of total protein). [19]

We discovered that IL-8 concentrations in OME of child–group patients (1562.46-1916.35 pg/ml) were considerably higher than those in adult–group patients (425.48 - 2209.58 pg/ml). This is consistent with Ali and El-Sherif (2004), who found that IL-8 concentrations in MEEs of child-group patients were considerably greater than those in adult-group patients. [20]

According to Hotomi (1994), the mean level of IL-8 in MEEs of children was 616.7 ± 211.0 pg/mgTP, while it was 197 ± 66.7 pg/mgTP in MEEs of adults.[13]

In the current investigation, mucoid-type OME had considerably higher IL-8 concentrations than serious-type OME. Goblet cells release IL-8 in response to TNF- and IL-1 activation, which explains this. Thus, goblet cells may be a source of IL-8 in the middle ear during the inflammatory process, and the high level of IL-8 seen in OME could be linked to the higher number of goblet cells found in OME. [21]

IL-8 causes the goblet cells to secrete both MUC5AC and MUC5B mucins, as well as an increase in the viscosity of the cell culture media. Due to increased IL-8 content in OME of the child-group, the percentage of serous-type OME was substantially higher in the adult-group than in the child-group, but the percentage of mucoid MEEs was

significantly higher in the child-group than in the adult-group.

According to Johnson et al. (1997), OME has revealed a high level (67 pg/mg) of TNF in effusions, which is consistent with our findings [19] that show considerable TNF concentrations in both the adult (145.03±103.98 pg/ml) and juvenile (118.25 ±143.96 pg/ml) groups.

According to Yellon et al. (1992), the macrophage appears to create more TNF [17] as the patient's age increases. This is consistent with our findings, which reveal that TNF concentrations in OME of adult-group patients were considerably greater than those in child-group patients. In this study, there was also a link between patient age and TNF, with the older the patients, the higher the TNF level in OME.

In cultured human airway epithelial cells, TNF- has been demonstrated to cause mucus hypersecretion.[22] TNF-enhanced MUC5AC secretion in all experimental settings, according to Smirnova et al. (2000). [23] The increase in TNF-induced mucin secretion was first noticed after one hour and lasted for the next 24 hours. This is consistent with our findings, which reveal that TNF levels in mucoid-type OME were substantially greater than in serious-type OME. TNF-induced MUC5AC hyper-secretion, on the other hand, is a time-dependent, dose-restricted, and cell-growth related phenomena, according to Smirnova et al. (2000). [23]

TNF- alpha (200 mg/ml) did not cause mucin hypersecretion, according to the researchers. This lack of reaction to a high concentration of TNF could be explained by the down regulation of TNF- receptors and the production of mucus gel on the cell surface, which could prevent TNF and TNF- receptor interactions on the cell surface, reducing TNF- driven mucin hypersecretion. This could also explain why some adult patients with serous effusions that aren't mucoid have high TNF levels.

It's worth noting that TNF has a different effect on mucin release in goblet cells than IL-8. TNF- stimulates mucin secretion in a short-term (up to 36 hours) and quick (peak response 7 hours after exposure) manner. The effect of IL-8 on mucin secretion was the most delayed (peak response 72 hours after exposure) and extended (up to 5 days). As a result, IL-8 may help to maintain chronic OME by causing extended mucin secretion from the enlarged goblet cell population. The presence of IL-8 in many kinds of chronic otitis media supports this theory. [24] According to Pospiech et al. (2000), IL-8 can extend the inflammatory process in the middle ear, therefore long-term treatment and observation of children with high IL-8 levels in MEE may be necessary. [25]

The mediator of cell damage, monocyte chemotaxis, and connective tissue proliferation has all been proven to be significant roles for nitric acid (NO) in inflammation. [26] NO levels were high in both the adult (54.57 38.59 mol/L) and children (55.71 22.02 mol/L) groups in our study. This is in line with Fischer et al. (1995), who claimed that large levels of NO are present during OME. [27]

We discovered that there was no statistical difference in NO concentrations between mucoid-type and serous-type OME, or between adult and child groups. This is in contrast to Austin et al. (1997), who demonstrated the role of NO as mucus secretion mediators in OME. [28] Others have demonstrated that inhibiting NO can change the outcome of an experimental otitis media model. Ball et al. (1996), for example, discovered that inhibiting NOS with N-nitro-L-arginine methyl ester (L-NAME) reduced middle ear

effusion in rats from 2 to 6 hours after endotoxin injection. [29] During otitis media, Rose et al. (1997) discovered that inhibiting NO lowered mucin formation. [30]

As a result, inhibiting NO generation could be a unique method to treating otitis media with effusion.

Conclusion:

Because IL-8 causes mucin secretion from goblet cells, which makes the fluid more viscous, more than 60% of the cases in the adult group were of the serous type, whereas nearly 75% of the cases in the child group were of the mucoid type.

The most common aetiology in adults were rhinogenic (approximately 40% of cases), with nasopharyngeal cancer being verified in only around 13% of cases. As a result, NPC is less common in adult patients with solitary OME, and nasopharyngeal biopsy is not required in these instances; however, thorough monitoring with repeated fibro-optic examination is recommended.

IL-8, TNF– alpha, and NO are particularly intriguing because they have traditionally been seen in high concentrations in OME. IL-8 had the highest average value of these cytokines.

IL-8 concentrations were substantially greater in OME patients in the child–group compared to those in the adult–group, and mucoid-type OME was significantly higher than serous-type OME.

There is a link between patient age and TNF-, with the older patients having greater TNF- levels in OME.

NO levels were high in both the adult and kid groups, implying that blocking NO could change the course of OME.

Conflicts of interest: None of the authors have any conflicts of interest to declare.

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References:

1. Thrasher RD and Allen GC. Middle ear, otitis media with effusion. eMedicine. November 2007.
2. Sade J. The nasopharynx, Eustachian tube and otitis media. J. LaryngolOtol. 1994,108, 95–100.
3. Hurst DS. Association of otitis media and allergy as demonstrated by intradermal skin testing and eosinophil cationic protein levels in both middle ear effusion and mucosal biopsies. Laryngoscope. 1996; 106: 1128-37
4. Kew J, King AD, Leung SF, Tong MC, Ku PK, Wong KK et al .middle ear effusion after radiotherapy. Correlation with pre-radiotherapy nasopharyngeal tumor patterns. American journal of otology; 2000, 21: 782-5.
5. Williamson IG, Dunleavey J, Bain J, et al. The natural history of otitis media with effusion—a three-year study of the incidence and prevalence of abnormal tympanograms in four South West Hampshire infant and first schools. J LaryngolOtol , 1994, Nov; 108(11):930-4.
6. Lin, a., Huang, W., Jiang, H., and Wang, J. Nitric oxide and cytokines in otitis media with effusion. Zhonghua. Er.Bi. Yan.Hou.Ke. Za.Zhi, 2000, 35 (1):23-5.
7. Kariya, S., Okano, M., Aoji, K., Kosaka, M., Chikumoto, E., Hattori, H., Yuen, K., Nishioka, S., Nishioka, K., and Nishizaki, K. Role of macrophage migration inhibitory factor in otitis media with effusion in adults. Clin.Diagn.Lab. Immunol.,

2003, 10(3):417-22.
8. Finkelstein Y, Ophir D, Talmi YP, et al. Adult-onset otitis media with effu-sion. Arch Otolaryngol Head Neck Surg 1994, 120:517–527.
9. Jeremy H et al. Am J Hum Genet. 2001, 69(2): 413–419.
10. Nanki T et al. j. Immunol.2001, 167(9): 5381-5.
11. Taylor PC. Mol. Biotechnol. 2001, 19(2): 153-68
12. Ho KY, Lee KW, Chai CY, Kuo WR, et al. Early recognition of nasopharyngeal cancer in adults with only otitis media with effusion. J Otolaryngol Head Neck Surg; 2008, 37:362–365.
13. Hotomi M., Samukawa T. and Yamanaka N. Inter-leukin 8 in otitis media with effusion. Acta Otolaryngol. (Stokh.) 1994,114, 406–409.
14. Mogi G., Chaen T. and Tomonaga K. Influence of nasal allergic reactions on the clearance of middle ear effusions. Arch. Otolaryngol. Head Neck Surg. 1990,116, 331–334.
15. Smimova, M.G, Birchall, J .P, and Pearson, J .P. In vitro study of IL-8 and goblet cells: possible role of IL-8 in the aetiology of otitis media with effusion. Acta Otolaryngol.2002, 122: 146-52
16. Maxwell K.S., Fitzgerald J.E., Burleson J.A. et al. Interleukin 8 expression in otitis media. Laryngoscope 1994,104, 989–995.
17. Yellon, R. F., Leonard, G., Marucha, P., Sidman, J., Carpenter, R., Burleson, J., Carlson, J., and Kreutzer, D. Demonestration of interleukin-6 in middle ear effusion. Arch. Otolaryngol. Head Neck Surg., 1992, 118 (7): 745-8.
18. John, E.O., Russell, P.T., Nam, B.H., Jinn, T.H.,and Jung,T.T. Concentration of nitric oxide metabolites in middle ear effusion. Int. lPediatr. Otorhinolaryngol. 2001, 60(1):55-8.
19. Johnson, M., Leonard, G., and Kreutzer, D.L. Murine model of interleukin-8 induced otitis media. Laryngoscope, 1997, 107: 1405-8.
20. Ali A. and El-Sherif A. Role of cytokines and nitric oxide in otitis media with effusion. AAMJ, 2004, Vol. 3, N. 2.
21. Hill J, Hutton DA, Green GG, Birchall JP, Pearson JP. Culture of human middle ear mucosal explants, mucin production. Clin Otolaryngol; 1992, 17: 491 – 6.
22. Levine SJ, Larivée P, Logun C, Angus CW, Ognibene FP, Shelhamer JH. Tumor necrosis factor-alpha induces mucin hypersecretion and MUC-2 gene expression by human airway epithelial cells.Am J Respir Cell Mol Biol. 1995 Feb;12(2):196-204.
23. Smirnova MG, Birchall JP, Pearson JP. TNF-Alpha in the regulation of MUC5AC secrection. CYTOKINE, 2000, 12, 11 (November), pp 1732–1736.
24. Smirnova MG, Kiselev SL, Birchall JP. Up-regulation of mucin secretion in HT29-MTX cells by pro-inflammatory cytokines TNF- α and IL-6. Eur Cytokine Netw ; 2001, 12: 119 – 25.
25. Pospiech L1, Jaworska M, Kubacka M. Soluble L-selectin and interleukin-8 in otitis media with effusion. Auris Nasus Larynx. 2000, Jul; 27(3):213-7.
26. Kuo PC and Schroeder RA. The emerging multifaceted roles of nitric oxide. Ann Surg; 1995, 221:220 – 235.
27. Fischer BM1, Krunkosky TM, Wright DT, Dolan-O'Keefe M, Adler KB. Tumor necrosis factor-alpha (TNF-alpha) stimulates mucin

secretion and gene expression in airway epithelium in vitro. Chest. 1995 Mar; 107(3 Suppl):133S-135S.
28. Austin S. Rose, Jiri Prazma, Scott H. Randell, Henry C. Baggetr, Andrew P. LaneANE, and Harold C. Pillsbury. Otolaryngol Head Neck Surg; 1997, 116:308-16.
29. Ball, S.S., Prazma, J., Dais, D., Rosbe, K. W., Pillsbury, H.C. Nitric oxide: a mediator of endotoxin-induced middle ear effusions. Laryngoscope, 1996, 106(8):1021-7.
30. Rose, A.S., Prazma, J., Randell, S. H., Baggett, H. C., Lane, A. P., and Pillsbury,H.nC. Nitric oxide mediates mucin secretion in endotoxin-induced otitis media with effusion. Otolaryng. Head neck surg., 1997, 116 (3):308-16 .