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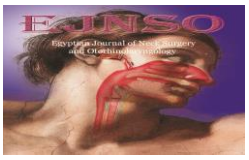

# Is serum Interleukin-6 level a risk factor in pathogenesis of otitis media with effusion in children?

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## Abstract:

**Objective:** The aim of the study was to detect the serum level of cytokine interleukin-6 (IL-6) in children with persistent Otitis media with effusion (OME)**.**

**Patients and methods:** 50 children with OME divided into 3 groups, a group I consisted of 30 children who had persistent otitis media with effusion and did not improve by medical and surgical treatment. The age range 5.8 ± 3. 4 years. Group (II) consisted of 20 children who had two or fewer OME episodes per year and improved by medical treatment and did not have a history of tubes insertion. The age range 6.3± 2.9 years. Group III consisted of 52 normal patients with similar age and sex as a control group.

**Results:** There was no significant difference between group I and group II. There was a significance difference between each group and normal patients.

**Conclusion:** The result of the present study suggesting that pro-inflammatory cytokine level Il-6 (IL-6) is significantly increased with persistent OME in children in comparison with normal patients.

**Key words:** Interleukin-6 - Otitis media – Effusion- Pathogenesis.

## *Introduction*

Otitis media with effusion (OME) is a common childhood disease characterized by the presence of fluid in the middle ear, with no symptoms and/or signs of acute inflammation. [1] OME occurs commonly during childhood, affecting 50- 90% of children at least once by 5 years of age, the presence of OME is associated with severe negative impact on child development, including hearing loss with delayed speech. [2]

The exact pathophysiology of OME is unclear, bacterial and viral infections, as well as cellular and humoral immune responses, are known to play a role. [3]

Numerous studies have confirmed the local involvement of proinflammatory cytokines in the ongoing pathological process in children with OME. [4]

A proinflammatory cytokine or an inflammatory cytokine is a type of cytokine (signaling molecule) that is excreted from immune cells and certain other cell types that promote inflammation. Inflammatory cytokines are predominately produced by helper T cells (Th) and macrophages and

involved in the up regulation of inflammatory reactions. [5]

Inflammatory cytokines play a role in initiating the inflammatory response and to regulate the host defense against pathogens mediating the innate immune response. [6]

Interleukin-6 (IL-6) seems to play an important role in the cytokine network of Otitis media. The concentrations of IL-1, IL-6, and TNF-α in middle ear effusion from children were highly correlated with each other, also discovered a significant correlation between middle ear effusion IL-6 concentrations and degree of hearing loss. Its main function is regulation of immune response, acute phase reaction, and hematopoiesis.[7] This study was to detect the serum level of cytokine (IL-6) in children with persistent OME.

## ***Patients and Methods:***

**Patients:**

This is a prospective randomized controlled trial which was conducted at Aswan university hospital included 50 children with otitis media with effusion diagnosed at ENT outpatient clinic, where 20 children (had two or fewer OME episodes per year and improved by medical treatment and did not have a history of tubes insertion), 30 cases (had persistent otitis media with effusion did not improved by medical and surgical treatment) and 52 normal children as a control group in the period from January 2017 to October 2018.

**Inclusion criteria**: patient with OME either responded [group II] or did not respond [group I] to medical treatment.

**Exclusion criteria:** Patients with Down's syndrome, immune deficiency, cleft palate or other craniofacial anomalies.

**Methods:**

**Sample preparation:**

The subjects were divided into two groups:

1. Group (I): this group consisted of 30 children who had persistent otitis media with effusion and the patients did not improve by medical treatment. The age range 5.8 ± 3.4 year .
2. Group (II): this group consisted of 20 children who had two or fewer OME episodes per year and improved by medical treatment and did not have a history of tubes insertion. The age range 6.3± 2.9 year.
3. Control group: 52 normal children with age range 6.1± 2.8 year.

The patients were subjected to the following:

- All study groups were subjected to full medical history.
- Full ENT examination.
- Audiological assessment: audiogram and tympanogram
- The study groups were subjected to serum level of IL-6.

Patient blood samples (5cc) were taken from veins in the antecubital fossa. 2 cc of the sample was taken on EDETA vacutainer tube & the remaining 3 cc was taken on Serum separator tubes (SST). The EDETA sample is sent directly to the lab for estimation of HbA1c concentration. The serum sample is allowed to clot for 30 minutes at room temperature before centrifugation for 15 minutes at approximately 1000 x g. Serum was removed and stored at -20° C until assayed .

**Determination of serum IL6:**

**Principle of the assay :**

The R&D Systems, Inc IL-6 ELISA Kit (USA) is an enzyme immunoassay developed for rapid detection of IL-6.

The assay employs the quantitative sandwich enzyme immunoassay technique (**Peter Perlmann and Eva Engvall, 1971**).

A monoclonal antibody specific for IL6- has been pre-coated onto a microplate. Standards and samples are pipetted into the wells and any IL6 present is bound by the immobilized antibody. After washing away any unbound substances, an enzyme-linked polyclonal antibody specific for IL6 is added to the wells. Following a wash to remove any unbound antibody-enzyme reagent, a substrate solution is added to the wells and colour develops in proportion to the amount of IL6 bound in the initial step. The colour development is stopped and the intensity of the colour is measured.

**Reagent preparation :**

All reagents and samples were brought to room temperature before use.

***Substrate Solution:*** Colour Reagents A and B were mixed together in equal volumes within 15 minutes of use. Protected from light. 200 µL of the resultant mixture was required per well .

***IL-6 Standard:*** Was reconstituted with 1.3 mL of deionized water. This reconstitution produced a stock solution of 300pg/mL. The standards allowed to site for a minimum of 15 minutes with repeated gentle agitation prior to making dilutions .

***In a polypropylene tube:*** We Pipetted 667 µL of Calibrator Diluent into the 300 pg/mL tube. Pipetted 500 µL of the appropriate Calibrator Diluent into the remaining tubes. The stock solution was used to produce a dilution series (fig. 1 above). Each tube mixed thoroughly before the next transfer. The 300 pg/mL standard serves as the high standard. The appropriate Calibrator Diluent serves as the zero standard (0 pg/mL).

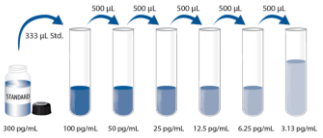


Fig.1.

***Assay procedure :***

All reagents and samples were brought to room temperature before use .

1. We add 100 µL of Assay Diluent RD1W to each well.
2. We add 100 µL of Standard, control, or sample per well. Covered with the adhesive strip provided. Incubated for 2 hours at room temperature .
3. We aspirated each well and washed it, repeating the process three times for a total of four washes. Washed by filling each well with Wash Buffer (400 µL) using an auto-washer. Complete removal of liquid at each step was essential to good performance. After the last wash, we removed any remaining Wash Buffer by aspirating. We inverted the plate and blotted it against clean paper towels .
4. We add 200 µL of IL6 Conjugate to each well. Incubated for 1 hour at room temperature .
5. We repeat the aspirating/wash as in step 3 .
6. We add 200µL of Substrate Solution to each well. Protected from light. Incubated for 20 minutes at room temperature .
7. We add 50 µL of Stop Solution to each well .
8. We determined the optical density of each using a microplate reader set to 450 nm.

*Results were estimated from standard curve produced by plotting the optical density for the standards versus the concentration of the standards and draw the best curve. The data was linearized by using log / log paper and

regression analysis was applied to the log transformation.

## ***Statistical analysis:***

Data were analyzed using Statistical Program for Social Science (SPSS) version 15.0. Quantitative data were expressed as mean± standard deviation (SD).

The following tests were done:

- **Independent-samples t-test of significance** was used when comparing between two means.
- **Probability (P-value)**
  - P-value <0.05 was considered significant.
  - P-value <0.001 was considered as highly significant.
  - P-value >0.05 was considered insignificant.

## ***Results:***

Fifty children were enrolled in this study, 40 were females and 10 were males. They were divided into two groups: group I: (30) children had otitis media with effusion (23 females and 7 males) and group II: 20 children (17 females and 3 males). Control group 52 children 34 females and 18 males.

Table (1): Comparison between group I and group II as regard IL-6.

| Variables \ Groups | | Group I (N = 30) | Group II (N = 20) | p-value |
|---|---|---|---|---|
| IL-6 | Mean | 48.6 | 33.2 | 0.1 |
| | ±SD | 42.1 | 17.6 | |

This table shows no statistically significant difference (p-value > 0.05) between the two groups regard IL-6.

Table (2): Comparison between group I and normal group as regard IL-6.

| **Variables \ Groups** | | **Group I (N = 30)** | **Normal (N = 52)** | **P-value** |
|---|---|---|---|---|
| **IL-6** | **Mean** | 48. 6 | 7.5 | **< 0.001*** |
| | **±SD** | 42.1 | 1.8 | |

* P-value < 0.001 is considered highly significant.

This table shows highly statistically significant difference (p-value < 0.001) between group I and normal group as regard IL-6.

Table (3): Comparison between Group II and normal group as regard IL-6.

| **Variables \ Groups** | | **Group II (N = 20)** | **Normal (N = 52)** | **P-value** |
|---|---|---|---|---|
| **IL-6** | **Mean** | 33. 2 | 7.5 | **< 0.001*** |
| | **±SD** | 17.6 | 1.8 | |

*P-value < 0.001 is considered highly significant

This table shows highly statistically significant difference (p-value < 0.001) between group II and normal group as regard IL-6.

## ***Discussion :***

OME is one of the most common diseases in pediatric practice and causes a detrimental effect on healthcare throughout the world, reducing quality of life in affected children. [8]

Among many factors which cause OME, it seems that allergic processes mediated by cytokines play a role. [9]

Many previous studies have demonstrated a predominance of Th-2 mediators in OME of atopic children. [10]

A study conducted by **Zielnik et al.,** [11] reported that an elevated level of IL-6 in the middle ear mucosa of atopic children with persistent OME. **Smironova et al 2005** [12] showed that the presence of cytokines including IL-6, identified in the otitis media was responsible for chronic inflammation

of the middle ear and chronic OME. One of the few studies evaluating systemic cytokines in this condition was reported by **Johnston et al 2013**. and showed a systemic response consistent with a TH-2-type response. This study compared cytokine levels (IL-6, INF-γ) in children with and without chronic or recurrent OM. Although on univariate analysis, children with OME had increased levels of serum IL-6 and INF-γ compared with children without OME. In current study, we found that serum levels of IL-6 were insignificantly increase in children with persistent OME in comparison with those control OME (P=5.8). This is not similar to findings of previous studies. [13]

In study of **Ho Yun Lee 2013** [14] showed that the levels of expression of all the cytokines tested, IL-6, -8, -10, and -12; IFN-γ; and TNF-α did not differ significantly between otitis-prone and non-otitis-prone groups. Effusion in the middle ear is a chronic state, with inflammation occurring for at least three months.

In study of **Xie et al., 2011** [15] used enzyme-linked immunosorbant assay (ELISA), to measure the levels of IL-6 in OME from patients with OME to study the role of cytokines in the pathogenesis of the disease. Significant levels of IL-6 (> 62.5 ng/L) were found in 19 (86.36%) of 22 MEEs. The mean (+/- s) levels of IL-6 were 507.68 +/-.

It revealed that the shorter the course, the higher the concentration of IL-6 in MEE. These findings suggested that during the early stages of OME, IL-6 might participate in the defensive reaction of organism, resulting in an excessive inflammatory reaction with a potential for pathological changes. It is concluded that the immunological mechanisms probably play a significant role in the pathogenesis of OME.

## ***Conclusion:***

The result of present study suggesting that pro-inflammatory cytokine level Il-6 is significantly increase with persistent OME in children.

**Conflict of interest:** The authors declare no competing interests.

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