

**How to Cite:**

Al-Awade, A. R. M., Al-Saadi, M. A. K., & Kadhem, U. H. (2022). Cytokine profile associated with fungal infections in patients with otitis media. *International Journal of Health Sciences*, 6(S9), 2666–2672. <https://doi.org/10.53730/ijhs.v6nS9.13007>

# Cytokine profile associated with fungal infections in patients with otitis media

**Anas R. M. Al-Awade**

Department of Microbiology, College of Medicine, University of Babylon, Babylon, Hilla, Iraq.

Corresponding author email: [anasalawade1984@gmail.com](mailto:anasalawade1984@gmail.com)

**Mohammed A.K Al-Saadi**

Department of Microbiology, College of Medicine, University of Babylon, Babylon, Hilla, Iraq.

**Uday Hussein Kadhem**

Department of Microbiology, College of Medicine, University of Babylon, Babylon, Hilla, Iraq.

**Abstract**---The current study includes a collection of (90) middle ear swabs samples from patients with middle ear infection who attended at the ENT unit at Al- Hilla Teaching Hospital and Imam Sadiq Hospital in Babylon city during the period from October 2021 to February 2022. The blood samples were collected from all patients as well as (40) apparently healthy control. The fungal isolates were identified according to cultural, biochemical tests, confirmed by VITEK 2 system compact. The results revealed that 15:90 were fungal positive culture. The most common fungal pathogen is *Candida parapsilosis* (10:15). Cytokine profile for patients revealed that IFN- $\gamma$  and CTLA-4 increase in patients as compared to control versus IL-10 increases in control than patients.

**Keywords**---otitis media, ELISA, IFN- $\gamma$ .

**Introduction**

Otitis media is a group of diseases in the middle ear, which can be divided into two major diagnostic groups: acute otitis media (AOM) and otitis media with effusion (OME) [1]. Otitis media (OM) is an inflammation of the middle ear with acute otitis media (AOM) being an acute inflammation of the middle ear. OM continues to be one of the most common childhood infections and is a major cause of morbidity in children. The pathogenesis of OM is multifactorial, involving

the adaptive and native immune system, Eustachian tube dysfunction, viral and bacterial load, genetic and environmental factors [2].

Cytokines play a central role as initiators, mediators and regulators of middle ear inflammation and subsequent molecular-pathological processes in middle ear tissues, leading to histopathological changes in the middle ear cavity and the pathogenesis of OME [3]. Interleukin-10 (IL-10) plays an important role in regulating the inflammatory response by inhibiting the release of immune mediators, phagocytosis, and the presentation of antigens to T-cells and macrophages [4, 5].

Fungi can either be the primary pathogen or be superimposed on bacterial infections or can be secondary pathogen in previously perforated tympanic membrane. It is mainly characterized by pruritus, otalgia, aural fullness, hearing impairment and tinnitus. Various predisposing factors have been proposed for fungal ear infection, including immunocompromised host, steroid usage, trauma, swimming, ear picking, use of headwear, use of oils, instrumentation of ear, fungal infection elsewhere in the body like dermatomycosis and malnutrition in children [6]. Wide spectrum of fungal agents such as *Aspergillus*, *Penicillium*, *Mucor*, *Rhizopus*, *Scopulariopsis*, *Absidia* and *Candida* are involved, species of *Aspergillus* and *Candida* being the most common etiological agents. *Aspergillus* spp. and *Candida* spp. as the predominant fungi [6, 7].

This study aimed to show the cytokine profile in patients with otitis media and the role of immune anergic factor CTLA-4 in the immune response against fungal infections associated with OM.

## **Materials and Methods**

### **Patients and Samples collection**

The patient group included 90 patient with otitis media, collected from both sexes (male 57 and female 33) with age range (1-80 years). They were suffering from otitis media in ENT Department of AL-Hilla Teaching Hospital and Imam Sadiq Hospital in Babylon city after clinically diagnosed by consultant physician during the period from October 2021 to February 2022. Thirty (30) healthy as control samples which are apparently healthy patients age marched groups for each 15 male and 15 female.

The blood samples and smears were taken from infected area by sterilized cotton swab, and then samples had been inoculated on Sabouraud's Dextrose Agar for fungal at 25°C for 5-7 days.

### **Immunological method**

The following ELISA kits, IL -10, IFN- $\gamma$ , and CTLA-4 (from Bio-Techne, China), were used in this study and the results were calculated depending on the optical density reading for each standard and samples optical density, Then, the standard curve was plotted by the mean OD value for each standard on the Y-axis

against the concentration on the X-axis and draw a best fit curve through the points on the graph.

### Results and Discussion

The fungi infection in otitis media patients than others which mixed (fungi with bacterial). Yeast (*Candida parapsilosis*), *Malassezia furfur*, and *Aspergillus niger* were isolated from OM in (10:15) 66.66% , 4:15(26 .66), and 1:15( 6.66%) respectively. The result agreement with [8] who expressed that *Candida parapsilosis* is the most common fungal pathogen in OM infection.

### IFN- $\gamma$ in fungal infection of the otitis media

The human interferon gamma (IFN- $\gamma$ ) concentration was measured in serum of patients and control. The results of this test was showed a high significant differences in patients with a fungal infection of the otitis media  $61.93 \pm 2.5$  a mg/l comparison with control  $75.03 \pm 5.5$  bc mg/l. This result might show that the infection are mostly at acute stage due to increase of the IFN- $\gamma$  levels. This result might be show that the fungi causes of otitis media in more induced of IFN- $\gamma$  than other causes such as bacterial and unknown causes. This result was compatible with [9].

Interferon- $\gamma$  (IFN- $\gamma$ ), granulocyte-macrophage colony-stimulating factor (GM-CSF) and granulocyte colony-stimulating factor (G-CSF) activate microbial killing by polymorphonuclear leukocytes (PMNs), and IFN- $\gamma$ , GM-CSF and macrophage colony-stimulating factor (M-CSF) increase killing by monocytes and macrophages [10].

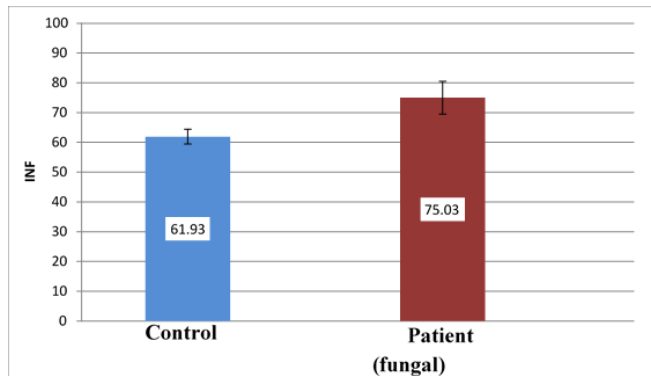


Figure (1): Concentration of IFN- $\gamma$  mg/dl in Otitis Media patients according to the fungal infection and control group

Table (1): Concentration of IL 10, IFN- $\gamma$  and CTLA-4 mg/dl in Otitis Media Patients (fungal) and Control

Groups	IFN- $\gamma$	IL-10	CTLA-4
	Mean $\pm$ S.E		
Control	61.93 $\pm$ 2.5 <sup>a</sup>	43.07 $\pm$ 1.9 <sup>b</sup>	34.03 $\pm$ 1.7 <sup>a</sup>
Patient (fungal)	75.03 $\pm$ 5.5 <sup>b</sup>	27.82 $\pm$ 1.8 <sup>a</sup>	43.79 $\pm$ 2.2 <sup>b</sup>

Different letters refer on significant differences.

### IL-10 in fungal infection of the otitis media

The human Interleukin 10 (IL-10) concentration was measured in serum of patients and control. The results of this test was showed a high significant differences in patients with a fungal infection of the otitis media 27.82 $\pm$ 1.8 a mg/l comparison with control 43.07 $\pm$ 1.9b mg/l, This result might show the humoral immunity activity as which induce secretion IL-10 as defense response of immune system against pathogens. This result was compatible with several studies [11].

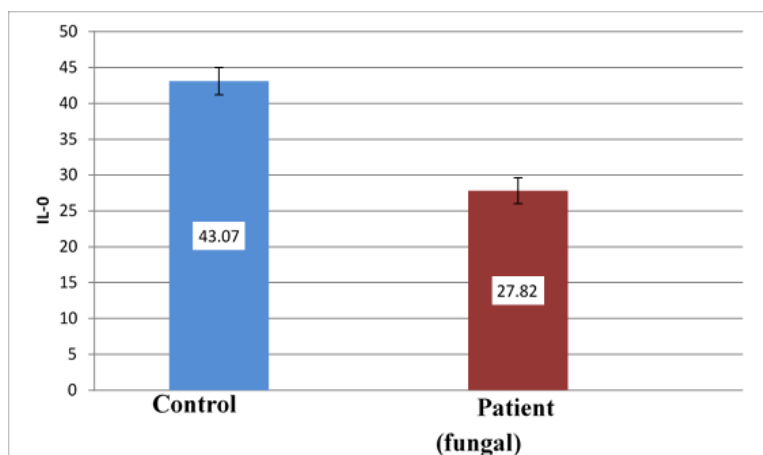


Figure (2): Concentration of IL-10 mg/dl in Otitis Media patients according to the fungal infection and control group

### CTLA-4 in fungal infection of the otitis media

The human Cytotoxic T lymphocyte Associated Antigen 4 (CTLA-4) concentration was measured in serum of patients and control. The results of this test was showed a high significant differences in patients with a fungal infection of the otitis media 43.79 $\pm$ 2.2 b mg/l comparison with control 34.03 $\pm$ 1.7 a mg/l, CTLA-4, a surface receptor of immunoglobulins [12], T-cell activation inhibitor Co-receptor cytotoxic T-cell lymphocyte antigen-4 (CTLA-4; CD 152) is a key T-cell proliferation and expansion inhibitor, it has damping effect on the activation mechanism and terminates T-cell responses. As such, T-cell tolerance and autoimmunity are necessary to regulate [13].

CTLA-4, the first scientifically targeted immune control point receptor, is found exclusively on T cells where the amplitude of the early stage of T cell activation is

primarily controlled [14]. CTLA-4, a surface receptive immunoglobulin cell, is a T-cell activation inhibitor [15]. It is expressed predominantly on naive T cells after activation [16].

CTLA-4 is a high affinity CD28 homologue for B7- 1/2. Although the interaction of the CD 28:B7- 1/2 serves as a co-simulator for T-cell propagation and activation, CTLA-4:B7- 1/2 binding, it is also a co-inhibitory signal to stop early T-cell activation, leading to inhibition of T-cell co-stimulation [17,18] provides a negative feedback loop upon activation of T-cells. CTLA-4 inside the intracellular vesicles is also rapidly transferred to the immunological synapse after T-cell activation[19]. CTLA-4 is stabilized with the CD80/CD86 binding in the immune synapse, allowing the CD28 binding to be collected and inhibited. CTLA-4 restricts CD28 signalization downstream, inhibits the pathway PI3K and AKT [20]. CTLA-4 also removes CD28 ligands CD80/86 from adjacent cells by transendocytosis *in vivo*, like APCs, besides inhibiting T-cell activation [21].

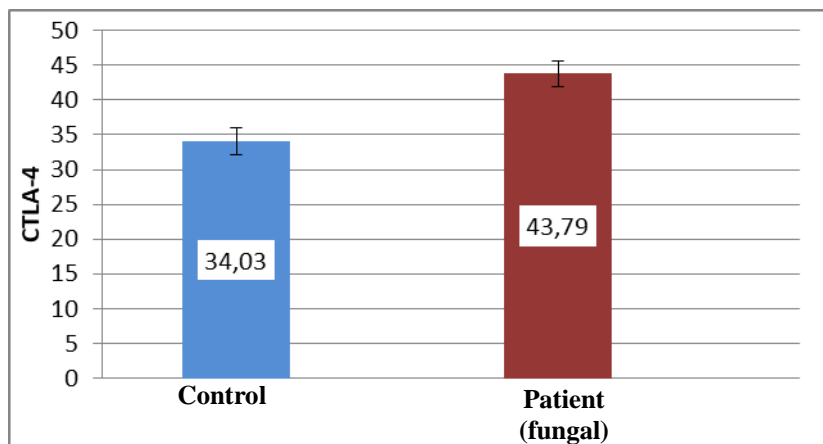


Figure (3): Concentration of CTLA-4 mg/dl in Otitis Media patients according to the fungal infection and control group

### Conclusion

The findings of this study show that the concentration of immune anergic factor CTLA-4 was high in patients than healthy indicating the immune suppression status in patient that increases the possibility for microbial infections.

### References

1. Abzug, M.J. and T.J. Walsh, Interferon- $\gamma$  and colony-stimulating factors as adjuvant therapy for refractory fungal infections in children. *The Pediatric infectious disease journal*, 2004. 23(8): p. 769-773.
2. Ahn, J.J., et al., Experimental infection with *Listeria monocytogenes* as a model for studying host interferon- $\gamma$  responses. *JoVE (Journal of Visualized Experiments)*, 2016(117): p. e54554.

3. Arijit, D., A Study on Incidence and Types of Ootomycosis in Patients Diagnosed with Chronic Suppurative Otitis Media. 2017, PSG Institute of Medical Sciences and Research, Coimbatore.
4. de Araújo, N.D., et al., Translating unconventional T cells and their roles in leukemia antitumor immunity. *Journal of Immunology Research*, 2021. 2021.
5. Dhanda, S.K., P. Vir, and G.P. Raghava, Designing of interferon-gamma inducing MHC class-II binders. *Biology direct*, 2013. 8(1): p. 1-15.
6. Dovedi, S.J., et al., Design and Efficacy of a Monovalent Bispecific PD-1/CTLA4 Antibody That Enhances CTLA4 Blockade on PD-1+ Activated T Cells Modulated CTLA4 Inhibition via Preferential Binding to PD-1. *Cancer Discovery*, 2021. 11(5): p. 1100-1117.
7. Felce, J.H., et al., Single-molecule, super-resolution, and functional analysis of G protein-coupled receptor behavior within the T cell immunological synapse. *Frontiers in cell and developmental biology*, 2021. 8: p. 608484.
8. Juyal, D., et al., Significance of fungal flora in chronic suppurative otitis media. *Annals of Tropical Medicine and Public Health*, 2014. 7(2): p. 120.
9. Lecocq, Q., et al., The next-generation immune checkpoint LAG-3 and its therapeutic potential in oncology: Third time's a charm. *International Journal of Molecular Sciences*, 2020. 22(1): p. 75.
10. Mpakali, A. and E. Stratikos, The role of antigen processing and presentation in cancer and the efficacy of immune checkpoint inhibitor immunotherapy. *Cancers*, 2021. 13(1): p. 134.
11. Pardoll, D., *Cancer Immunotherapy with Vaccines and Checkpoint Blockade*, in *The Molecular Basis of Cancer: Fourth Edition*. 2014, Elsevier Inc. p. 709-738. e8.
12. Ray, R., et al., Prevalence of fungal infection in chronic otitis media-A study at a tertiary care hospital in Eastern India. *Int J Curr Microbiol App Sci*, 2015. 4(3): p. 684-90.
13. Rye, M.S., J.M. Blackwell, and S.E. Jamieson, Genetic susceptibility to otitis media in childhood. *The Laryngoscope*, 2012. 122(3): p. 665-675.
14. Sabat, R., et al., Biology of interleukin-10. *Cytokine & growth factor reviews*, 2010. 21(5): p. 331-344.
15. Schneider, H. and C.E. Rudd, Diverse mechanisms regulate the surface expression of immunotherapeutic target ctla-4. *Frontiers in immunology*, 2014. 5: p. 619.
16. Skotnicka, B. and E. Hassmann, Proinflammatory and immunoregulatory cytokines in the middle ear effusions. *International journal of pediatric otorhinolaryngology*, 2008. 72(1): p. 13-17.
17. Smirnova, M.G., et al., Role of the pro-inflammatory cytokines tumor necrosis factor-alpha, interleukin-1beta, interleukin-6 and interleukin-8 in the pathogenesis of the otitis media with effusion. *European cytokine network*, 2002. 13(2): p. 161-72.
18. Thorsen, S.U., et al., Maternal and cord blood 25-hydroxyvitamin D3 are associated with increased cord blood and naive and activated regulatory t cells: the barwon infant study. *The Journal of Immunology*, 2021. 206(4): p. 874-882.
19. Unsdgaard, J.V., et al., Deep metric learning for otitis media classification. *Medical Image Analysis*, 2021; 71:102034.

20. Vella, J.P. and A. Mehboob, *The Immunobiology of Transplant Rejection and Acceptance*, in *Transplantation Surgery*. 2021, Springer. p. 51-63.
21. Wu, J., et al., Local induction of bladder Th1 responses to combat urinary tract infections. *Proceedings of the National Academy of Sciences*, 2021. 118(10): p. e2026461118.