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The role of interleukin-1 beta and NF-κB as inflammatory factors in bacterial otitis externa

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KEY WORDS:
Pseudomonas aeruginosa, Otitis externa, NF-KB and IL-1β

ABSTRACT

Background: Otitis externa is one of the more common diseases in otorhinolaryngological practice and is also fre quently encountered in primary and pediatric care. It ranges in severity from a mild infection of the external audi tory canal to life-threatening malignant otitis externa.The aim of study is to provide fresh light on the population suffering from otitis externa and the relationship between Nuclear factor kappa B and IL-1 β with *P. aeruginosa* infection.

Material and method: From total patients (50) were taken two types of samples for each patient, the first included cotton swab, the second sample included five milliliters of venous blood sample, collected from Primary health care sector in balad , Balad General Hospital and private laboratory in Balad City Under the supervision of a specialist doctor. The patients who attending during (2024). Patients were interviewed directly by using an anonymous questionnaire form which included (age and sex).

Results: The current study includes 67 samples, Otitis externa = 50 , and Control = 17 . The research found that gastric patients were 41 (82%) gave positive result while the 9 (18%) were gave negative result for detecting *P. aeruginosa* by routine test (cultural and biochemiacal identification) and Vitek 2 system. In addition, this research found that the concentration of Nuclear factor kappa B and IL-1 β in *P. aeruginosa* infected patients increase significantly($P< 0.05$) than healthy control. Higher levels of NF-κB and IL-1β have been detected in otitis externa patients. NF-κB beginning contributes to the creation of IL-1β, aggravating inflammation.

Conclusion: NF-KB and IL-1Beta are cytokines that production important role in the immune system, as well as the rule of immune responses in contradiction of bacterial pathogens.

Keyword: *Pseudomonas aeruginosa*, Otitis externa, NF-KB and IL-1β

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INTRODUCTION:

Inflammation of the external ear canal, known as otitis externa (OE), can occur anywhere along the canal's length, from the tympanic membrane (TM) to the outer meatus. It is frequently accompanied with concomitant pinnal alterations. One or both ears may be affected by the condition, which can be either acute or chronic, painful, and/or itchy (1). Pseudomonas species, most frequently Pseudomonas aeruginosa, are important in the development, morbidity, and failure of treatment of swimmer's ear, a bacterial infection brought on by water that remains in the outer ear canal for an extended period of time, creating a moist environment for bacteria to remain and grow (2).

The most common swimming-related infection and diarrhea, skin rashes , swimmers ear and etc. CSOM is the most common middle ear infection characterized by recurrent ear discharges or otorrhea through a tympanic perforation from the middle ear .The incidence of CSOM in developing countries is high return to malnutrition , low socioeconomic society , poor personal hygiene , recurrent upper respiratory tract infection and lack of adequate primary health care facilities. The resistance of causative organism is very common, although the complication are less common new due to availability of broad spectrum antibiotics(2). *P. aeruginosa* is characterized with high genetic variation which is related to several infectious diseases lead to differences in their pathogenicity (3). Immune responses to *P. aeruginosa* infection include innate and adaptive immunity. The many phases of Salmonella infection are mirrored in the innate and acquired immunity, which is regulated by diverse immune cells to protect against this pathogen, with varying significance throughout discrete stages of infection. Nuclear factor kappa B (NF-KB) is the key pro-inflammatory motioning lane and is frequently activated by pro-inflammatory cytokines. The NF-KB pathway also controls the appearance of numerous genes that intercedes cell proliferation, cell differentiation, apoptosis and cancer (4). Interleukin-1β (IL-1β) is a potent pro-inflammatory cytokine that is critical for host-defence responses to contagion and injury (5). It is produced as an inactive 31 kDa precursor, termed pro-IL-1β, in response to molecular motifs passed by pathogens called ‘pathogen related molecular patterns’ (PAMPs) (6). This study aims to deliver fresh bright on the residents suffering from otitis externa and the relationship between Nuclear factor kappa B and IL-1 β with *P. aeruginosa* infection.

Materials and methods

Study area and groups

The examination was conceded out among (2024). Balad City's primary health care sector includes the Balad General Hospital and a private laboratory. Under the care of a specialist doctor. A total of 50 samples were collected from each patient, with the first being a cotton swab and the second being five milliliters of venous blood. Using disposable syringes, 3-5 ml of venous blood were taken from each person's radial vein. After that, the blood was placed in gel tubes, allowed to coagulate at room temperature, then spun for fifteen minutes at 1,500 RPM. The sera were then maintained at -20 oC until subsequent analyses were carried out, and cotton swab samples were conveyed using sterile transport swabs to the microbiology laboratory (7).

Morphological, Cultural Characteristics and Vitek – 2 system for *P. aeruginosa*: this obtained by (7).

Estemation of NF-KB and IL-1 β by Enzyme-linked Immunosorbent Assay (ELISA) Protocols: this obtained by (8).

Statically analysis

The common statistical package Graph Pad Prism version 7 was used, and the data was summarised using the (Mean ±Stander Error) format (7).

Results and Discussion

Demographic study according to sex and age

For further examination at the Balad General Hospital, Balad City private laboratory, and the primary health care sector in Balad, the current study includes 50 samples from the Otitis externa patient and 17 samples from the control group. under a specialist physician's supervision. According to the study's findings, the percentage of female patients with Otitis externa was higher than that of male patients by a margin of 28 (56%) to 22 (44%), but the control group's numbers were 10 (58.8%) male and 7 (41.2%) female. Figure 1.

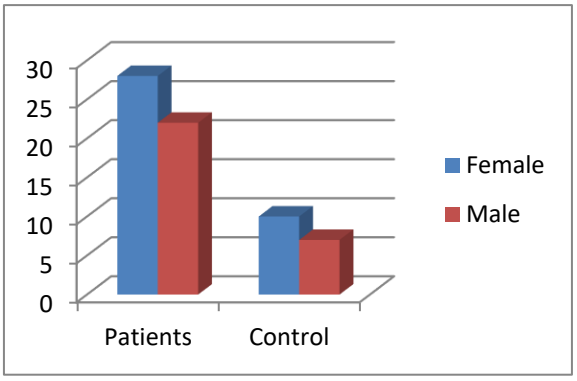
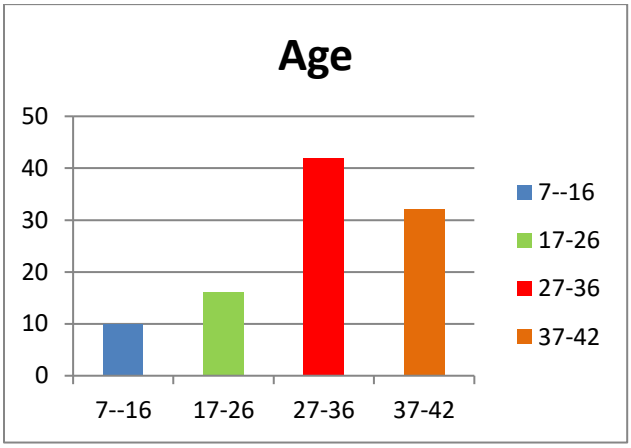


Figure (1):- Distribution of patients and control according to sex

There were a total of 21/50 (or 42% of the total patients) in the 27-36 age bracket, 16/50 (or 32% of the total patients) in the 37-42 age bracket, and the lowest frequencies in the 7-16 and 17-26 age groups, respectively, with 5/50 (or 10% of the total patients) and 8/50 (16%) patients, as shown in Figure (4-1).



Figurer (2): Distribution of the specimen according to the age groups**.**

One of the most prevalent otological conditions in head and neck surgery and otorhinolaryngology is otitis externa. In this study, the prevalence of otitis externa was 17.5%. According to study (9), otitis externa was a prevalent otological condition. These results differ from those found in other studies (10). Narrow ear canals, heavy ear wax production, high humidity in the research area, and a high incidence of recurrent otitis externa could all contribute to this high prevalence rate. Otitis externa is also frequently caused by trauma to the external canal from self-inflicted objects or scratches from cotton buds, which can lead to a superimposed infection. Because the protective ear wax layer is removed during ear cleaning, which results in infection of the external auditory canal (2), self-cleaning of the ears was the most prevalent risk factor.

This study found that the third decade (ages 27 to 36) has the highest prevalence of otitis externa. This age group's increased outside activities could be the likely cause. They are also exposed to high levels of heat, humidity, dust, and other environmental factors. Other studies (11, 12) confirmed these findings.

In this study, otitis externa affected people between the ages of 27 and 36. The

findings of this study were nearly identical to those of (9) study, which found that the age group with the highest prevalence of otitis externa was those aged 21–30 years, comprising 91 patients (27.7%), followed by those aged 31–40 years, comprising 61 patients (18.6%), and the age group with the lowest prevalence was those aged >61 years, comprising 11 patients (3.3%). In a study conducted at Sanglah General Hospital in Denpasar,(14) revealed that, of 105 patients, 72 patients (68.6%) were between the ages of 15 and 49 and had the highest prevalence of otitis externa.Twelve Even while earlier research has demonstrated that otitis externa can affect people of all ages, it may be because adults (25–65 years old) engage in more outside activities than those of other ages. Additionally, this exposes patients in this age range to high levels of heat, humidity, dust, and other pollutants(9).

In this study, otitis externa was more common in females than in males. While several research revealed considerable sex preponderance in their findings, this difference is not sufficiently large.

The study's comparison of male and female patients revealed that the female patients had the highest incidence of otitis externa. The findings of this study are consistent with studies by (15) in Iran, which found that 45.69% of victims were men and 54.31% of victims were women. At the Universitas Sumatera Utara Hospital in 2017, Sutanto's research also revealed that, out of 92 cases, the prevalence of otitis externa was higher in women (52.2%) and men (47.8%) (16) study at the ENT Polyclinic at Sanglah Hospital Denpasar, however, produced different findings. Of the 70 patients, 42 (60%) were male and 28 (40%) were female (16). In the study by (9) different findings were also discovered. Of the 392 patients, 179 (54.4%) were male and 150 (45.6%) were female, but the ratio remained at 1:12. Based on these findings, it can be concluded that otitis externa can affect people of any gender and that there is no evidence linking the two conditions.

Identification of bacteria

The initially identification of bacterial specimens depended on cultural and morphology.

Only 41 (82%) of the 50 clinical specimens were *Pseudomonas aeruginosa* isolates, whereas 9 (18%) were of other bacterial species, including *E. coli* (12%), Proteus spp. (4%) and *Klebsella spp*. (2%); the data are displayed in Figure (4-3). The results of the biochemical tests are displayed in table (4-1).

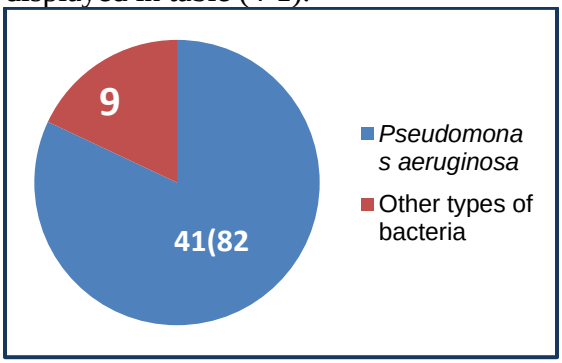


Figure (3): The percentage of bacteria

All of the isolates were oxidase and catalase positive after growing on MacConkey, blood, and nutrition agars; 84% of the isolates had pyocyanin pigment. (17) reported that when *P. aeruginosa* was cultivated in cetrimide-containing media, 80% of the organisms produced pyocyanin in 24 hours, whereas modified MacConkey agar and broth required 4-5 days. Most isolates had colonies that were mucoid and smelled like grapes. Under a microscope, rod-shaped cells with a pink tint were apparent as a result of Gram staining.

P. aeruginosa was detected in 50 ear swab samples from otitis externa patients who were infected, according to bacteriological analyses. The findings of this investigation concurred with other reports that detailed the viruses' isolation (7). The Vitek® 2 technology was used to

validate these bacteria' biochemical characteristics. Psudomonas aeruginosa is typically one of the bacterial organisms that cause otitis externa (18). Additionally, among the various bacterial causes of otitis externa, *P. aeruginosa* bacteria have been identified to be the most common agent (18).

P. aeruginosa is well-known for its significant role in long-lasting infections, particularly those involving the ear canal. Numerous enzymes that aid in tissue invasion and destruction are secreted by it, most especially elastase and alkaline protease. These proteolytic enzymes break depressed numerous cellular substrates and extracellular matrix components, allowing *P. aeruginosa* to cross the threshold the ear canal more intensely. According to investigation, *P. aeruginosa* is usually initiate as a pathogen together with *Staphylococcus aureus* in cases of ear infections, particularly chronic otitis externa (COE), which donates to the infection's perseverance and related tissue erosion (19).

Furthermore, *P. aeruginosa* effects sturdy immunological responses when it is current in infected tissues, including the release of cytokines that help inflammation, like IL-1β. In this process, the NF-κB signaling pathway must be activated. *P. aeruginosa* interrelates with Toll-like receptors (TLRs) during tissue attack, which attracts downstream signaling particles including MyD88 and eventually activates NF-κB (20). In addition to encouraging the manufacture of pro-inflammatory cytokines, this way increases chronic inflammation, which, if untreated, can cause extra tissue damage (17). Therefore, it has been demonstrated that convinced virulence factors, such as ExoU, intensify inflammatory reactions by rising IL-8 release, thus sustaining a loop of inflammation and tissue damage (21).

A compulsive background is providing by the tenacious inflammatory environment, where *P. aeruginosa* attendance fosters conditions that principal to chronic inflammation and potentially dangerous side effects as osteomyelitis and facial nerve palsy. The chronic inflammation understood in recurrent otitis media cases illustrates the serious health consequences of *P. aeruginosa* infections, creation the interaction between the host immune responses crucial and bacterium (2). These consequences stress the need of targeted interventions and efficient antimicrobial treatments to reduce the inflammatory reactions brought on by *P. aeruginosa* and protector against tissue damage in the ear channel and its environs (22).

Immunological study

Evaluation of NF-KB in patients

As shown in the Figure (4) The results of the current study showed that there was a significant decrease (P<0.0001) in NF-KB serum levels in patients with Otitis (06654 ± 0.04161) compared to control group (1.274 ± 0.1472).

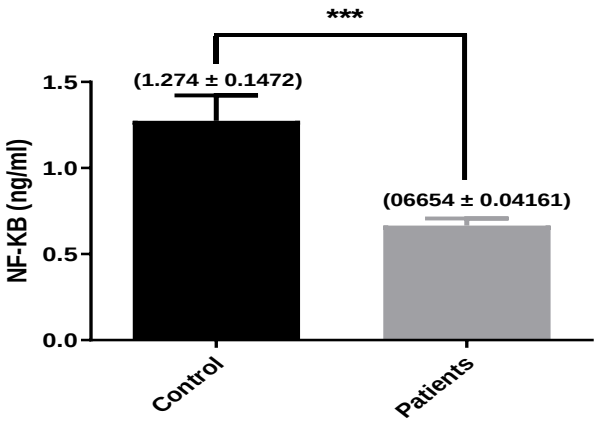


Figure (4): Serum concentration of NF-KB (ng/ml) in patients with Otitis compared to the control group.

The significance of innate immunity in normal recovery from OE (23) and the fact that canonical NF-κB activation is a major

downstream target of many innate immune receptors (24) make our gene regulation results perhaps not surprising. The dramatic regulation and kinetics of genes encoding the growth and survival factors PIK3CD and AKT1 further suggest that NF-κB plays a strong role in tissue hyperplasia. The regulation of many of the genes involved in NF-κB signaling and activation, as well as those encoding the NF-κB subunits themselves, provides strong evidence that NF-`56 yκB plays a major role during OE.

Because NF-κB plays an essential role in regulating various key inflammatory mediators (25), we sought next to determine whether the same signaling pathways also mediate the synergistic induction of TNF-α, IL-1β, and IL-8 by NTHi and TNF-α.

Evaluation of interlukin-1Beta (IL-1β)

The results of the current study also showed a decrease (P<0.0027) in serum levels of IL-1β in patients with external otitis (0.1439 ± 0.009991) compared to control group (0.2336 ± 0.03085), figure (5).

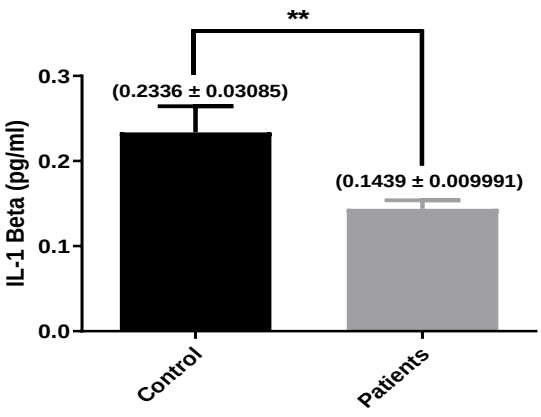


Figure (5): Serum concentration of IL-1Beta (pg./ml) in patients with Otitis compared to the control group.

Cytokines are glycoproteins, produced by inflammatory cells and epithelial cells, which modulate the immune response. Cytokines extensively conduct inter-cell communication. Inflammatory cells including neutrophils, macrophages, and lymphocytes use cytokines to coordinate all stages of the inflammatory response. Production of cytokines is conducted by a wide variety of cell types. For instance, vascular endothelial cells, neutrophils, fibroblasts, monocytes, lymphocytes, and macrophages all generate and secrete IL-1 (26, 27). The existing view is that cytokines are responsible for many of the inflammatory changes generated by pathogenic organisms during OE (26).

The lymphokine IL-1 was first discovered to be mitogenic for mouse thymocytes. It is now understood that a wide variety of cells produce IL-1 to control immune responses (28). One of the most potent chemicals that activates osteoclasts and causes bone resorption is IL-1 . One of the clinical features that indicates the start of chronic OE is this IL-1-mediated bone loss. One of the main sources of IL-1β is neutrophils. Through a positive-feedback mechanism, IL-1 causes neutrophils to produce more IL-1 (29). The two main 17 kDa polypeptides that make up IL-1 are IL-1α and IL-1β. These two molecular species are encoded by genes on chromosome 2 (28). They bind to the same cell surface receptor and exhibit the same biological activity (29). Proteolytic cleavage of 33 kDa precursor molecules yields both IL-1α and IL-1β. While IL-1β circulates freely, IL1α functions as a membrane-associated material (30).

It has been demonstrated that IL-lβ is crucial to the pathophysiology of otitis externa (OE). Endotoxin obtained from *P. aeruginosa* induced middle ear effusion (MEE) with much greater amounts of IL-1β than controls when it was delivered into the mouse middle ear, and inoculation of IL-1β produced pathologic alterations that were comparable to those caused by endotoxin (31).

Otitis externa, also referred to as swimmer's ear, is an inflammation of the external auditory canal that is usually brought on by allergic irritants or infectious agents (fungi or bacteria). The pathophysiology of otitis externa, like other inflammatory processes, is a complicated series of immunological events that are mainly coordinated by pro-inflammatory cytokines like Interleukin-1 beta (IL-1β) and signaling molecules like Nuclear Factor kappa-light-chain-enhancer of activated B cells (NF-κB). The mechanisms behind otitis externa and its inflammatory reactions can be better understood by comprehending the interactions between these mediators. NF-κB is an important early transcription factor that responds to inflammatory stimuli, such as infections or irritants that impact the external ear. When NF-κB is triggered, it changes into the nucleus and endorses the transcription of numerous pro-inflammatory genes, such as those that yield adhesion molecules and cytokines that strengthen the inflammatory response (32). PAMPs (pathogen-associated molecular patterns) that indicate the presence of infection or bacterial lipopolysaccharides (LPS), which are often produced by Gram-negative bacteria, are instances of activates that activate NF-κB in the setting of otitis externa. By expressively boosting the immunological response in the ear canal, this activation acts as a "on switch," starting the inflammatory procedure (33).

After NF-κB is first activated, a cascade leads to amplified making of IL-1β, which is produced as an inactive ancestor called pro-IL-1β and needs both genetic transcription and enzymatic cleavage for activation. Since NF-κB drives the transcription of IL-1β, the NLRP3 inflammasome must be collected for the following processing, which is particularly important when tissue damage or severe infection occurs (34). Once the NLRP3 inflammasome is accumulated, caspase-1 is activated, which cleaves pro-IL-1β into its bioactive form, permitting it to be secreted into the extracellular environment. In otitis externa, elevated levels of IL-1β fund to the hypersensitivity and inflammatory signaling that exemplifies the condition, resulting in symptoms like pain, itching, and redness (35).

Correlation between NF-KB and IL-1β serum level according to external otitis.

When studying the correlation between the above markers, the current study found that there is a positive correlation (r= 0.6319) between NF-KB and IL-1Beta in patients with Otitis,

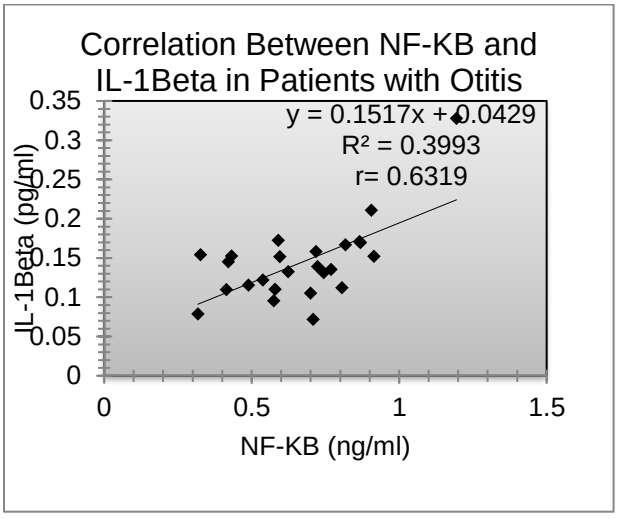


figure (6).

Pseudomonas aeruginosa has a range of components, including lipopolysaccharides (LPS) and different surface proteins, that are critical for its interaction with the innate immune system. These components primarily target Toll-like receptors (TLRs), notably TLR4 and TLR5, present on immunological and commensal cells. TLR4 recognizes LPS, a component of bacteria's outer membrane. The accessory of LPS to TLR4 triggers a influential immunological response, triggering signaling pathways that reason inflammation. Surface proteins are those that can interact with TLR5, which

identifies flagellin, a organizational protein found in bacterial flagella. This connection also results in immunological responses (36).

This joining activates the NF-κB pathway, which in turn endorses the synthesis of numerous cytokines, including as TNF-α, IL-1β, and IL-6. This clarifies a biological process in which molecules interact to activate the NF-κB (Nuclear Factor kappa-light-chain-enhancer of activated B cells) pathway and stimulate the production of several cytokines, such as TNF-α (tumor necrosis factor-alpha), IL-1β (interleukin-1 beta), and IL-6 (interleukin-6). One important signaling system that affects inflammation, immunological responses, and cell feasibility is the NF-κB pathway. The cytokines generated by this pathway can have whichever pro- or anti-inflammatory belongings and are vital for coordinating immune responses (37).

The transcription of IL-1β be contingent on the start of the Nuclear Factor kappa-light-chain-enhancer of activated B cells (NF-κB). This paper explains how NF-κB affects the production of IL-1β, highlights the role of the NLRP3 inflammasome in converting pro-IL-1β to its active procedure, and deliberates the ensuing effects on vascular permeability and immune cell dynamics. NF-κB is a transcription factor that is indispensable to the immune response to inflammation and infection. The activation of the NF-κB pathway results in its translocation into the nucleus when it is stimulated by dissimilar pro-inflammatory cytokines, pathogen-associated molecular patterns (PAMPs), or damage-associated molecular patterns (DAMPs). NF-κB attaches itself to the promoter regions of numerous inflammatory genes, including the one that codes for pro-IL-1β, inside the nucleus. Pro-IL-1β is eventually produced as a result of this activation, but it is biologically inactive and needs additional processing to become active (38). The activation of the NLRP3 inflammasome, a multiprotein compound essential for the start of inflammatory responses, is required to transform pro-IL-1β into its active form. The NLRP3 inflammasome can be assembled in immune cells, especially macrophages, in response to a variety of stimuli, including exogenous enzymes, bacterial exotoxins, and other DAMPs (37). The first "signal" activates TLR (Toll-like receptor) signaling pathways, which in turn activate NF-κB and pro-IL-1β production. The another "signal" inductees the NLRP3 inflammasome get-together, which in turn triggers caspase-1. After being activated, caspase-1 converts pro-IL-1β into IL-1β, which is next released into the extracellular space (35). This process emphasizes how crucial the NLRP3 inflammasome is to ensuring that IL-1β is produced in a bioactive state, which intensifies the inflammatory response. IL-1β affects vascular permeability and immune cell recruitment, among other biological consequences. IL-1β increases vascular penetrability via attaching to its receptor, IL-1R, which causes endothelial cells to harvest adhesion molecules (38). Immune cells, particularly neutrophils, can more effortlessly reach the location of infection or damage because to this procedure. Furthermore, by preventing apoptosis, IL-1β increases neutrophil survival and prolongs their stay at inflammatory areas. Although this neutrophil accumulation is essential for aggressive infection, improper regulation of the inflammatory response may cause tissue damage (39).

The current study found NF-KB higher significant than IL-1Beta because NF-κB (Nuclear Factor kappa-light-chain-enhancer of activated B cells) may have developed significance than IL-1β (Interleukin-1 beta) in otitis externa due to

numerous reasons such as NF-κB is a master regulator of inflammation, regulatory the expression of multiple pro-inflammatory genes, including IL-1β and NF-κB is an upstream regulator of IL-1β, sense it controls the making of IL-1β and other pro-inflammatory cytokines.

Conclusion

In summary, NF-KB and IL-1Beta are cytokines that production important role in the immune system, as well as the rule of immune responses in contradiction of bacterial pathogens.

References

1- Macwan P, Mohanty M, Singh AK, Giri R. Pigmented fungus as a cause of chronic otitis externa: A rare case report. Journal of Integrative Medicine and Research. 2025 Jan 1;3(1):53-6.

2- Sathe N, Beech P, Croft L, Suphioglu C, Kapat A, Athan E. Pseudomonas aeruginosa: Infections and novel approaches to treatment "Knowing the enemy" the threat of *Pseudomonas aeruginosa* and exploring novel approaches to treatment. Infectious medicine. 2023 Sep 1;2(3):178-94.

3- De Sousa T, Hébraud M, Dapkevicius ML, Maltez L, Pereira JE, Capita R, Alonso-Calleja C, Igrejas G, Poeta P. Genomic and Metabolic Characteristics of the Pathogenicity in Pseudomonas aeruginosa. International journal of molecular sciences. 2021 Nov 29;22(23):12892.

4- Cohen TS, Parker D, Prince A. *Pseudomonas aeruginosa* host immune evasion. InPseudomonas: Volume 7: New Aspects of Pseudomonas Biology 2014 Dec 14 (pp. 3-23). Dordrecht: Springer Netherlands.

5- Al-Qahtani AA, Alhamlan FS, Al-Qahtani AA. Pro-inflammatory and anti-inflammatory interleukins in infectious diseases: A comprehensive review. Tropical medicine and infectious disease. 2024 Jan 4;9(1):13.

6- Quan, W.,. The role and mechanism of innate immune molecules in Alzheimer's disease and cerebrovascular disorders. 2021.

7- Al-Sadawi AA, Al-Ammar M. Association between Genotypic and Phenotypic Formation of Biofilm in *Pseudomonas aeruginosa* Isolated from Diabetic Foot Patients. HIV Nursing. 2022;22(2):199-203.

8- Alquraishi ZH, Alabbasy AJ, Alsadawi AA. Genotype and phenotype detection of E. coli isolated from children suffering from urinary tract infection. Journal of Global Pharmacy Technology. 2018 Jan;10(1):38-45.

9- Adegbiji WA, Aremu SK, Olatoke F, Olajuyin AO, Ogundipe KO. Epidemiology of otitis externa in developing country. International Journal of Recent Scientific Research. 2017;8(6):18023-7.

10- Jamal A, Alsabea A, Tarakmeh M, Safar A. Etiology, diagnosis, complications, and management of acute otitis media in children. Cureus. 2022 Aug 15;14(8).

11- Gul N. The Role of Cortical Mastoidectomy in Tympanoplasty for Persistent or Intermittent Discharging Chronic Suppurative Otitis Media: A Randomized Controlled Study. International Journal of Life Sciences, Biotechnology and Pharma Research. 2025 March .14(3).

12- Samarei R, Roshani A, Shokrani S. Headache as a sign of ear, nose, and throat diseases: evidence from a specialized clinic. Health Science Monitor. 2024 Jan 10;3(1):69-76.

13- Salim QM, Adnan A, Adriztina I, Winanto ID. Prevalence and Profile of Patients with Otitis Externa at the Universitas Sumatera Utara Hospital

in Medan in 2020-2021. Buletin Farmatera. 2023 Jan 30;8(1):73-83.
14- Triastuti I, Sudipta IM, Sutanegara SWD. Prevalensi penyakit otitis eksterna di RSUP Sanglah Denpasar Periode Januari - Desember 2014. E-Jurnal Med. 2018;7(6):1–4.
15- Kiakojuri K, Omran SM, Jalili B, Hajiahmadi M, Bagheri M, Shahandashti EF, Rajabnia R. Bacterial otitis externa in patients attending an ENT clinic in Babol, North of Iran. Jundishapur journal of microbiology. 2016 Feb 13;9(2):e23093.
16- Tanaya PWD, Asthuta AR, Saputra KAD SI. Prevalensi Kasus Otitis Eksterna Berdasarkan Usia, Jenis Kelamin Dan Diabetes Melitus Di Poliklinik Tht Rsup Sanglah Denpasar Tahun 2018. J Med Udayana. 2020;9(1):22–7.
17- Alquraishi ZH, Al-Kraety IA, Alsadawi AA. Bacteriological Study of *Pseudomonas aeruginosa* Isolated from Tonsillitis Patients. International Journal of Drug Delivery Technology. 2019;9(3):477-80.
18- Mohammed BQ, Abdullah AH, Rayshan AR. Molecular identification of several *Pseudomonas aeruginosa* virulence genes. J. Anim. Health Prod. 2024;12(s1):67-74.
19- van den Berg, B., Chang, J., Duizendstra, A., Jansen, R., Gutierrez, A.J.P. and Zhao, T., 2014. The effect of probiotics on skin.
20- Gugliandolo E, Fusco R, Ginestra G, D'amico R, Bisignano C, Mandalari G, Cuzzocrea S, Di Paola R. Involvement of TLR4 and PPAR-α receptors in host response and NLRP3 inflammasome activation, against pulmonary infection with pseudomonas aeruginosa. Shock. 2019 Feb 1;51(2):221-7.
21- Bagayoko S. Study of the role of inflammatory cell death in the regulation of pulmonary bacterial infections: Identification of novel factors involved in pulmonary pathology mediated by the Exotoxin U of P. aeruginosa. 2022.
22- Ruffin M, Brochiero E. Repair process impairment by *Pseudomonas aeruginosa* in epithelial tissues: major features and potential therapeutic avenues. Frontiers in cellular and infection microbiology. 2019 May 31;9:182.
23- Zhang G, Ghosh S. Toll-like receptor–mediated NF-κB activation: a phylogenetically conserved paradigm in innate immunity. The Journal of clinical investigation. 2001 Jan 1;107(1):13-9.
24- Dev A, Iyer S, Razani B, Cheng G. NF-κB and innate immunity. NF-kB in Health and Disease. 2010 Sep 17:115-43.
25- Ma B, Hottiger MO. Crosstalk between Wnt/β-Catenin and NF-κB signaling pathway during inflammation. Frontiers in immunology. 2016 Sep 22;7:378.
26- Ryan F, Francos-Quijorna I, Hernández-Mir G, Aquino C, Schlapbach R, Bradbury EJ, David S. Tlr4 deletion modulates cytokine and extracellular matrix expression in chronic spinal cord injury, leading to improved secondary damage and functional recovery. Journal of Neuroscience. 2024 Feb 7;44(6).
27- Georgiev GP. *Chemokine orchestration of leukocyte-skin interactions* (Doctoral dissertation, University of Leeds).2021.
28- Santarlasci V, Cosmi L, Maggi L, Liotta F, Annunziato F. IL-1 and T helper immune responses. Frontiers in immunology. 2013 Jul 15;4:182.

29- Ruscitti P, Cipriani P, Carubbi F, Liakouli V, Zazzeroni F, Di Benedetto P, Berardicurti O, Alesse E, Giacomelli R. The role of IL-1β in the bone loss during rheumatic diseases. Mediators of inflammation. 2015;2015(1):782382.

30- Afonina IS, Müller C, Martin SJ, Beyaert R. Proteolytic processing of interleukin-1 family cytokines: variations on a common theme. Immunity. 2015 Jun 16;42(6):991-1004.

31- Schousboe LP, Ovesen T, Eckhardt L, Rasmussen LM, Pedersen CB. How does endotoxin trigger inflammation in otitis media with effusion?. The Laryngoscope. 2001 Feb;111(2):297-300.

32- Stapes IS. Commonly referred to as swimmer's ear and synonymous with otitis externa (OE), acute otitis externa (AOE) is inflammation to the external auditory canal. This condition is. Fast Facts Handbook for Pediatric Primary Care: A Guide for Nurse Practitioners and Physician Assistants. 2020 Sep 14:20.

33- Lipska J, Hamerska J, Hamerska L, Bocianiak B, Antczak J, Kajka A, Fenrych U, Wojtczak K, Skupińska O, Ruta D. Swimmer's ear: Prevention, Diagnosis, Treatment, and Management Strategies for Athletes. Quality in Sport. 2024 Jul 29;18:53330-.

34- Wang P, Qian H, Xiao M, Lv J. Role of signal transduction pathways in IL-1β-induced apoptosis: Pathological and therapeutic aspects. Immunity, Inflammation and Disease. 2023 Jan;11(1):e762.

35- Gupta P, Barthwal MK. IL-1 β genesis: the art of regulating the regulator. Cellular & Molecular Immunology. 2018 Nov;15(11):998-1000.

36- Pier GB. *Pseudomonas aeruginosa* lipopolysaccharide: a major virulence factor, initiator of inflammation and target for effective immunity. International journal of medical microbiology. 2007 Sep 10;297(5):277-95.

37- Rabaan AA, Al-Ahmed SH, Muhammad J, Khan A, Sule AA, Tirupathi R, Mutair AA, Alhumaid S, Al-Omari A, Dhawan M, Tiwari R. Role of inflammatory cytokines in COVID-19 patients: a review on molecular mechanisms, immune functions, immunopathology and immunomodulatory drugs to counter cytokine storm. Vaccines. 2021 Apr 29;9(5):436.

38- Balde A, Benjakul S, Nazeer RA. A review on NLRP3 inflammasome modulation by animal venom proteins/peptides: mechanisms and therapeutic insights. Inflammopharmacology. 2025 Feb 11:1-9.

39- Fox S, Leitch AE, Duffin R, Haslett C, Rossi AG. Neutrophil apoptosis: relevance to the innate immune response and inflammatory disease. Journal of innate immunity. 2010 Feb 11;2(3):216-27.