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Estimation of Cytokines (IL-1&IL-22) in Wounds Infected with *Pseudomonas aeruginosa* in Albino Rat

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Abstract
Wound infection is described as the occurrence of microorganisms in sufficient numbers or with sufficient virulence in a wound environment to stimulate an immune system response locally, systemically, or both. The present study focused on assessing the immune response by measuring cytokine levels (IL-1, IL-22) in the serum of rats that induced wound infected with *Pseudomonas aeruginosa*. Thirty-six female Albino rats were used, divided into 3 groups GI (infected group), GII (wound without infection group), and GIII (healthy control group). An excision wound was made on the rat's back and then contaminated the wound region with a bacterial solution that contain 2.5 $\times 10^6$ CFU of *P. aeruginosa.* Following that, blood samples were taken at predetermined time periods (24 hours, 48 hours, and 72 hours) following infection to evaluate immunological response during wound infection. We found that the cytokines (IL-1) were detectable at all times (24, 48, 72) hours and observed in all studied groups while a significantly elevated level of IL-22 (0.77 ± 0.08) pg/ml was recorded in 24 hours in the infection group and then decreased at 72 hours.

Keywords: IL-1, IL-22, Wound infection, *Pseudomonas aeruginosa*

Introduction:
The skin is the body's biggest organ, accounting for 10% of the whole-body mass and protecting us from harmful elements in the environment. The epidermis, dermis, and hypodermis are the three layers of the skin, all of which are self-renewable and serve diverse functions [1]. The skin's primary role is to act as a barrier, protecting inside organs from invading microbes and UV radiation while regulating body temperature [2]. In addition, skin aids the immune system and the body's sensory detecting mechanism [3].

A wound is formed when the skin's anatomic structure and defensive functions are disrupted. One of the most common infections that contaminate wounds and causes the infection is bacteria that typically colonize wounds, and *Pseudomonas aeruginosa* has a higher prevalence in wound infection [4]. *P. aeruginosa* is a Gram-negative, aerobic, rod-shaped bacterium belonging to the Pseudomonadaceae family [5]. The optimum temperature for it is 37°C while the highest temperature for the growth is 42°C which can be considered a distinguished feature for *P. aeruginosa* from other Pseudomonas spp. It has the ability to create fluorescent pigments and is oxidase-positive; however, it does not ferment carbohydrates. As a result, colony morphology, oxidase positivity, and the presence of characteristic pigments are commonly used for identification [6]. This pathogen can infect the respiratory tract, abdomen, burns, ear infections, and wounds are the most frequent site of infection. Which was introduced

into areas devoid of normal defenses, such as when mucous membranes and skin are disrupted [7].

The cell of the immune system responds to wound infection by secreting signaling molecules such as cytokines, chemokines, and growth factors, which promote cellular cross-talk [8]. The cytokine IL-1 is well-known for its major role in inducing inflammatory responses in response to infection as a result of danger signals [9]. which warns surrounding cells to barrier disruption and attracts neutrophils to the infection site to eliminate bacteria and other pathogens. As well as stimulating fibroblasts, keratinocyte migration and proliferation rise [10]. Furthermore, the cytokine IL-22 has a potential crucial proinflammatory effect in epithelial tissues which has a role in epithelial defense and tissue response modulation during inflammatory conditions. It performs a variety of functions in cell proliferation, tissue regeneration, and cellular defense [11].

Materials and Methods:

Bacterial Sample:

This study used a stock of *Pseudomonas aeruginosa* obtained from human burn patients at the Burn Center of Al- Sadder Medical City in Al- Najaf, Iraq. Inoculated overnight culture into one bacterial culture tube with 5 ml fresh BHI broth and cultured for 18 hours at 37°C. The subculture was centrifuged (10 min, 6000 rpm). the bacterial pellet had been washed once and resuspended in 10ml of PBS solution to obtain 2.5 $\times 10^6$ CFU in bacterial suspension by measuring the optical density in a spectrophotometer at 600nm [12].

Excision Wound Model:

The procedure was performed at the animal's house in the faculty of veterinary medicine/ University of Kufa. The animals were anesthetized with an intramuscular injection with a mixture of ketamine and xylazine (20 mg/kg and 100 mg/kg, respectively) [13]. The skin of the impressed area was Shaved and creating full-thickness excisional wounds (10 mm diameter) on the dorsal region of rats models with weight (100-400g). After 24h from inducing wound, contaminated the wound region with 10 µl of bacterial suspension that might contain 2.5 $\times 10^6$ CFU of *P. aeruginosa*. A swab was taken from the wound surface after 24 hours and cultured on MacConky agar to ensure that the infection was occurring. Fallowed by blood samples collection at (24h, 48h, 72h) after infection for immunological examination.

Estimation of Interleukins:

A disposable syringe was used to collect two milliliters of blood by a cardiac puncture. For serum separation, blood samples were placed in a gel tube and centrifuged for 5 minutes at 4000 rpm. Sera were kept at -70°C for further research. The sandwich ELISA principle was employed with ELISA kits for the determination of IL-1 and IL-22 in the sera at 24, 48, and 72 hours and according to the manufacturer's instructions, the optical density (OD) is determined spectrophotometrically at a wavelength of 450 nm.

Statistical Analysis:

The data were analyzed using the ANOVA computer program, with a P value of less than 0.05 considered significant.

Results:

The Level of IL-1 in Serum of Rats in The Studied Group at Different Time Intervals:

The concentration of IL-1 in the serum at various times 24, 48, and 72 hours after a wound infection in GI revealed that the mean concentration at 24hrs was (31.37 ± 2.93) pg/ml, at 48hrs the mean was (35.33 ± 5.28) pg/ml and P value equal to 0.94, while at 72hrs was (26.96 ± 2.17) pg/ml and P value equal to 0.89. The statistical analysis showed no significant difference between different values at P value ≤ 0.05 as in Fig (1).

The concentration of IL-1 in the serum at various times 24, 48, and 72 hours after inducing wound in GII revealed that the mean concentration at 24hrs was (36.56 ± 4.75) pg/ml, at 48hrs the mean was (34.06 ± 3.47) pg/ml and P value equal to 0.99, while at 72hrs was (33.83 ± 3.42) pg/ml and P value equal to 0.99. The statistical analysis showed no significant difference between different values at P value ≤ 0.05 Fig (1).

As well as the result revealed that the mean concentration of IL-1 in the GIII was (31.78 ± 4.17) pg/ml and P >0.99. The statistical analysis showed that there was no significant difference.

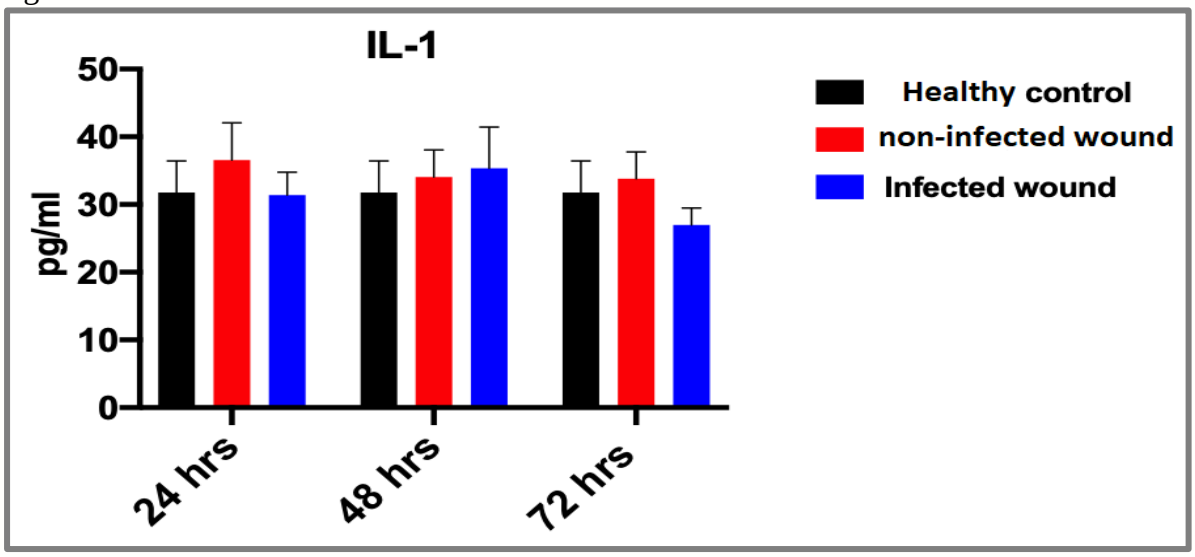

Figure (1): Serum Concentration of IL-1 Produced from GI, GII, and GIII at Different Time (24,48,72) hrs

The Level of IL-22 in Serum of Rats in The Studied Group at Different Time Intervals :

The concentration of IL-22 in the serum at various times 24, 48, and 72 hours after infection in GI revealed that the mean concentration at 24hrs was (0.77 ± 0.08) pg/ml, at 48hrs the mean was (0.44 ± 0.22) pg/ml and P value equal to 0.24. The statistical analysis showed no significant difference, while at 72hrs which was (0.33 ± 0.14) pg/ml and P value equal to 0.04. The statistical analysis showed a significant difference at P value ≤ 0.05 Fig (2).

The concentration of IL-22 in the serum at various times 24, 48, and 72 hours after inducing wound in GII revealed that mean concentration at 24hrs was (0.86 ± 0.16) pg/ml , at 48hrs the mean was (0.76 ± 0.07) pg/ml and P >0.99 . The statistical analysis showed no significant difference, while at 72hrs, which was (0.40 ± 0.09) pg/ml and P value equal to 0.03. The statistical analysis showed a significant difference at P value ≤ 0.05 Fig (2).

As well as the result revealed that the mean concentration of IL-22 in GIII was (0.52 ± 0.19) pg/ml and P >0.99. The statistical analysis showed that there was no significant difference.

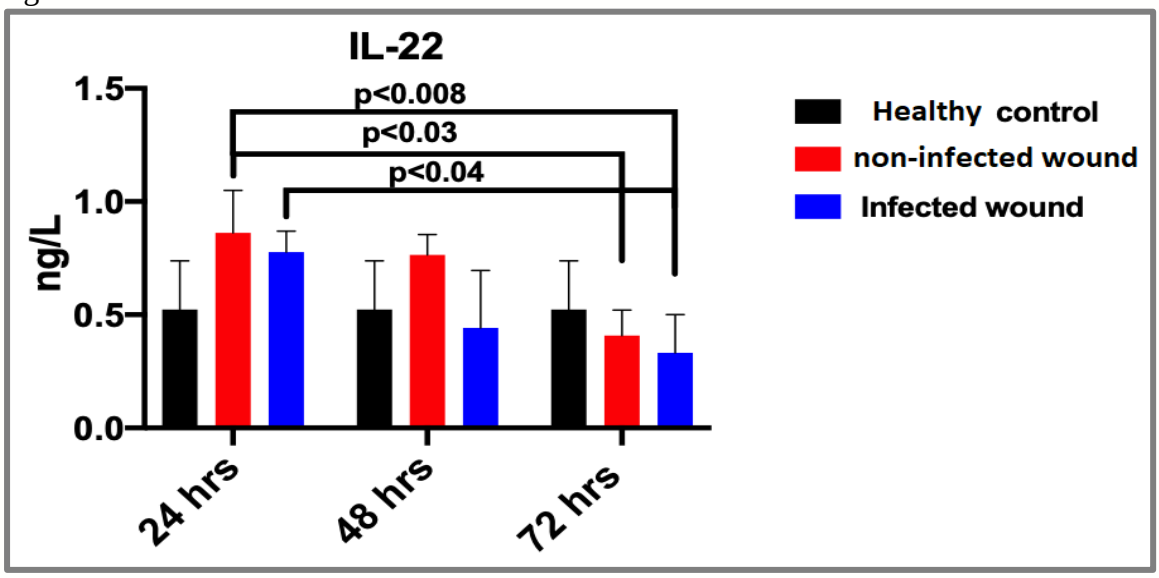

Figure (2): Serum concentration of IL-22 produced from GI, GII, and GIII at Different Time (24,48,72) hrs

Discussion:

During the comparison of the level of IL-1 concentration in the GI with those who obtained in the GII and GIII at different times lines (24, 48, 72) hour we found that there was no significant difference between these values.

These results may be due to multiple factors that affect on the concentration of IL-1 which were related to tissue damage, bacterial infection, feeding quality, and exposure to stress [14]. Many previous studies report that Malnutrition promotes an increase in proinflammatory cytokine, Utariani and co-workers improved Significantly reductions in serum levels of IL-1 have been observed in the group receiving albumin infusion and those having a high casein diet [15]. Furthermore, Hughes and co-workers reported a significant reduction in IL-1β mRNA expression in peripheral leukocytes concerning LPS stimulation and explained the effect of psychological stress on wound healing, immune cells (lymphocytes, macrophages, and granulocytes) expressed receptors for numerous neurotransmitters, establishing relationships between the neurological system and the immune system. allowing the stress response to have immune-modulatory effects [16].

Measuring serum concentration of IL-22 in the GI revealed that there is no significant difference between the mean concentration at 24 hours (0.77 ± 0.08) pg/ml and at 48 hours (0.44 ± 0.22) pg/ml but there was a significant decrease in the mean concentration at 72 hours (0.33 ± 0.14) pg/ml. These results were like that obtained by Barthelemy and co-workers that investigated were the impact of exogenous IL-22 administration on the secondary bacterial invasion post influenza infection in mice. which detected the lower concentration of IL-22 at 24hr in the serum after intranasal administration and reach the lowest level at 72hr [17]. Guillon also explains the effect of *P. aeruginosa* infection on immune defenses via protease IV which is a virulence factor in *P. aeruginosa* that might damage epithelium and degrade IL-22, compromising tissue regeneration and antimicrobial defense against bacteria [18].

The result of serum concentration of IL-22 in GII showed that there is no significant difference between the mean concentration at 24 hours and 48 hours but there is a significant decrease in the mean concentration at 72 hours (0.40 ± 0.09) pg/ml. These results seem to be different from those obtained by Avitabile and co-workers who used diabetic and non-diabetic mice to evaluate the effect of IL-22 in diabetic wound healing. IL-22 reached the highest level on the third day after injury in non-diabetic wounded mice with a significantly low level of IL-22 in diabetic wounded mice. But he also showed that using recombinant mouse IL-22 as a treatment accelerated the healing process by stimulating re-epithelialization, granulation tissue development, and vascularization [19].

Conclusions:

The study concludes that the cytokine IL-22 was affected by wound infection and recorded a higher concentration in the infected group at 24 hours after infection and then decreased. While the cytokine IL-1 was detectable in all time lines (24, 48, 72) hours. And observed in an infected group as well as wound without infection and healthy control.

Ethics approval:

The experimental procedures protocols were fully approved at the request of the Research Centre's Ethics Committee and the Council on Guidelines of Kufa University to ensure that experimental animals were appropriately cared for. (Ethic no. 12917; authorization from the Animal Experimentation Ethics Agency).

References:

1. Khalili, S., Khorasani, S. N., Saadatkish, N., & Khoshakhlagh, K. (2016). Characterization of gelatin/cellulose acetate nanofibrous scaffolds: Prediction and optimization by response surface methodology and artificial neural networks. Polymer Science Series A, 58(3), 399-408. https://doi.org/10.1134/S0965545X16030093
2. Singer, A. J., & Clark, R. A. (1999). Cutaneous wound healing. New England journal of medicine, 341(10), 738-746. https://www.nejm.org/doi/full/10.1056/NEJM199909023411006
3. Böttcher-Haberzeth, S., Biedermann, T., & Reichmann, E. (2010). Tissue engineering of skin. Burns, 36(4), 450-460. https://www.sciencedirect.com/science/article/pii/S0305417909004951
4. Maharjan, N., & Mahawal, B. S. (2020). Bacteriological profile of wound infection and antibiotic susceptibility pattern of various isolates in a tertiary care center. Journal of Lumbini Medical College, 8(2), 218-224. https://doi.org/10.22502/jlmc.v8i2.367
5. Wu, M., & Li, X. (2015). Klebsiella pneumoniae and Pseudomonas aeruginosa. In Molecular medical microbiology (pp. 1547-1564). Academic Press. https://doi.org/10.1016/B978-0-12-397169-2.00087-1
6. Al-Azzawi, M. K., Makharmash, J. H., & Al-Malkey, N. K. (2020). The effect of Lactobacillus species on the Pseudomonas aeruginosa. Drug Invention Today, 14(2).
7. Migiyama, Y., Yanagihara, K., Kaku, N., Harada, Y., Yamada, K., Nagaoka, K., ... & Kohno, S. (2016). Pseudomonas aeruginosa bacteremia among immunocompetent and immunocompromised patients: relation to initial antibiotic therapy and survival. Japanese journal of infectious diseases, 69(2), 91-96. https://doi.org/10.7883/yoken.JJID.2014.573

8. Strbo, N., Yin, N., & Stojadinovic, O. (2014). Innate and adaptive immune responses in wound epithelialization. Advances in wound care, 3(7), 492-501. https://doi.org/10.1089/wound.2012.0435
9. Fahey, E., & Doyle, S. L. (2019). IL-1 family cytokine regulation of vascular permeability and angiogenesis. Frontiers in Immunology, 10, 1426. https://doi.org/10.3389/fimmu.2019.01426
10. Al-Jebouri, M. M., & Al-Mahmood, B. Y. R. (2019). Estimation of Cytokines Involved in Acute-Phase Wound Infection with Reference to Residence Time of Patients in Hospitals. Modern Research in Inflammation, 8(01), 1. https://doi.org/10.4236/mri.2019.81001
11. Avitabile, S., Odorisio, T., Madonna, S., Eyerich, S., Guerra, L., Eyerich, K., ... & Cianfarani, F. (2015). Interleukin-22 promotes wound repair in diabetes by improving keratinocyte pro-healing functions. Journal of Investigative Dermatology, 135(11), 2862-2870. https://doi.org/10.1038/jid.2015.278
12. Maura, D., Bandyopadhaya, A., & Rahme, L. G. (2018). Animal models for Pseudomonas aeruginosa quorum sensing studies. In Quorum Sensing (pp. 227-241). Humana Press, New York, NY. https://doi.org/10.1007/978-1-4939-7309-5_18
13. de Moura Estevão, L. R., Cassini-Vieira, P., Leite, A. G. B., de Carvalho Bulhões, A. A. V., da Silva Barcelos, L., & Evêncio-Neto, J. (2019). Morphological evaluation of wound healing events in the excisional wound healing model in rats. Bio-protocol, 9(13). http://www.bio-protocol.org/e3285
14. Schaudinn, C., Dittmann, C., Jurisch, J., Laue, M., Günday-Türeli, N., Blume-Peytavi, U., ... & Rancan, F. (2017). Development, standardization and testing of a bacterial wound infection model based on ex vivo human skin. PLoS One, 12(11), e0186946. https://doi.org/10.1371/journal.pone.0186946
15. Utariani, A., Rahardjo, E., & Perdanakusuma, D. S. (2020). Effects of albumin infusion on serum levels of albumin, proinflammatory cytokines (TNF-α, IL-1, and IL-6), CRP, and MMP-8; tissue expression of EGRF, ERK1, ERK2, TGF-β, collagen, and MMP-8; and wound healing in Sprague Dawley rats. International journal of inflammation, 2020. https://doi.org/10.1155/2020/3254017
16. Hughes, O., MacQuhae, F., Rakosi, A., Herskovitz, I., & Kirsner, R. S. (2017). Stress and wound healing. In Stress and Skin Disorders (pp. 185-207). Springer, Cham. https://doi.org/10.1007/978-3-319-46352-0_19
17. Barthelemy, A., Sencio, V., Soulard, D., Deruyter, L., Faveeuw, C., Le Goffic, R., & Trottein, F. (2018). Interleukin-22 immunotherapy during severe influenza enhances lung tissue integrity and reduces secondary bacterial systemic invasion. Infection and immunity, 86(7), e00706-17. https://doi.org/10.1128/IAI.00706-17
18. Guillon, A., Brea, D., Morello, E., Tang, A., Jouan, Y., Ramphal, R., ... & Si-Tahar, M. (2017). Pseudomonas aeruginosa proteolytically alters the interleukin 22-dependent lung mucosal defense. *Virulence*, *8*(6), 810-820. https://doi.org/10.1080/21505594.2016.1253658
19. Avitabile, S., Odorisio, T., Madonna, S., Eyerich, S., Guerra, L., Eyerich, K., ... & Cianfarani, F. (2015). Interleukin-22 promotes wound repair in diabetes by improving keratinocyte pro-healing functions. *Journal of Investigative Dermatology*, *135*(11), 2862-2870. https://doi.org/10.1038/jid.2015.278