ORIGINAL ARTICLE

Comparative analysis of IL-6 levels in post-tooth extraction inflammation among menopausal and non-menopausal: in vivo experimental

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ABSTRACT

Introduction: IL-6 acts as a biomarker of the inflammatory process. In elderly, especially women, the demand for tooth extraction treatment is quite high. A failure in the wound healing process during the menopausal period can prolong the inflammation process, thereby increasing the probability of infection, including alveolar osteitis. The menopausal process affects estrogen levels in the blood, which leads to reduced collagen formation as a key component of tissue regeneration. IL-6 levels can also increase during inflammation, especially in the chronic phase following tooth extraction. The study aims to analyze the differences in inflammatory IL-6 levels between menopausal and non-menopausal rats. **Methods**: This study applied an in vivo experimental design. A total of 30 *Rattus novergicus* were divided into two groups: a control group and a treatment group. The control group consisted of rats in which no oophorectomy was performed (non-menopausal), while the treatment group underwent ovariectomy to induce menopause. One after the procedure, tooth extraction were performed, and observations were conducted on days 3, 7, and 14 post-extractions. IL-6 levels were measured using ELISA from blood serum samples. The data were analyzed using One-Way ANOVA. **Results**: The average IL-6 levels (in ng/L) in the control (non-menopausal) group on days 3, 7, and 14 were 0.087, 0.318, and 0.247, respectively. The average IL-6 levels in the treatment (menopausal) group were 0.191, 0.452, and 0.318, respectively. The One-Way ANOVA test showed no significant difference between the groups ($p=0.799 > 0.05$). **Conclusion**: The trend of higher IL-6 levels in the menopausal group suggests a potential impact of hormonal changes on post-extraction inflammation. Although no significant difference was found, the elevated IL-6 levels in menopausal rats indicate a potential effect of estrogen deficiency on the inflammatory response following tooth extraction.

KEYWORDS

Menopause, menopausal model, interleukin-6 (IL-6), tooth extraction, inflammation.

INTRODUCTION

In general, age significantly influences the inflammatory response and the overall speed of tissue repair. Wound healing in younger individuals typically occurs more quickly and effectively than in older individuals. In older people, the wound healing process following procedures like tooth extraction can be complicated by age related systemic factors, such as anemia, ischemia, or others, which can affect the wound healing process after tooth extraction.[1] In many ways, the elderly require tooth extraction because the teeth can no longer be maintained, are loose, or have large caries. In women, there is a limitation of fertile age known as menopause.[2] Menopause occurs primarily due to a decrease in the number of primordial follicles in the ovaries. The timing of menopause is

influenced by various biological stresses, including the accumulation of free radicals, DNA damage, and metabolic chemicals. When the number of primordial follicles reaches a critical threshold, the body's hormone regulation system is disrupted.[3]

During the transition into menopause, the estrogen hormone is no longer produced by the ovaries regularly. This critical decline causes estrogen levels to decrease and can no longer inhibit the production of Follicle Stimulating Hormone (FSH) and Luteinizing Hormone (LH). The resulting imbalance leads to corpus luteum insufficiency and anovulatory menstrual cycles(the absence of ovulation of the ovum cell). once a woman experiences this phase, she is considered to have entered menopausal phase.[4]

The menopausal phase is characterized by the removal of estrogen and progesterone hormones, which initiates several systemic changes. Loss of estrogen causes unstable vasomotor control (often presenting as hot flashes) and various psychological disorders, such as anxiety and depression. It also contributes to the emergence of metabolic disorders within the body.[5] Furthermore, in the mucosa and skin, the connective tissue and blood vessels undergo atrophy, leading to reduced local blood flow. When tissue injury occurs (such as from tooth extraction), this reduced blood supply slows down the healing process.[6]

The deficiency of estrogen hormone impairs the epithelialization process during wound healing. This disruption is due to the role of estrogen, which is responsible for increasing the amount of collagen production by changing the polymerization of mucopolysaccharides and increasing the quality of hydroscopes and strengthening collagen adhesion to connective tissue.[7] Consequently, during menopause, collagen fibers, which are the basic substance for connective tissue formation, are reduced in both quality and quantity.[8] This deficit inhibits the wound epithelialization process, causing both the overall healing process and wound closure time to be prolonged.[9]

Measuring Interleuking-6 (IL-6) in post-extraction sockets offers several research benefits, as it provides a quantifiable indicator of inflammation and healing process, helps identify possible healing problems early, and allows the researchers to assess the biological effectiveness of treatments, all of which enhance clinical results in oral surgery and dentistry. IL-6 is one of the inflammatory cytokines secreted by helper cells of the immune system. It is released from adipose tissue and immune cells as a systemic response aimed at protecting the body from infection and tissue injury.[10]

Interleukin-6 (IL-6) is a multifunctional cytokine that plays a crucial role in host defense and multiple biological progress and organ systems. It is produced by various cells in the body in response to infections or tissue injuries. It is also involved in acute phase reaction of pathogenesis and various inflammatory disorders.[11] One of the examples of a tissue injury is tooth extraction. It triggers the expression of inflammatory cytokines in the jaw bone at the extraction site, including the IL-6.[12]

Previous study shows that tooth extraction leaves a visible wound on the soft tissue and a socket on the hard tissue, as IL-6 stained strongly with Hematoxylin and Eosin (H&E) analyses at the extraction site.[13] Another study shows a higher number of IL-6 levels post tooth extraction, demonstrating a direct correlation between the tissue injury and the increasing levels of the IL-6.[14] Crucially, the regulation of these pro-inflammatory cytokines like IL-6 also can be released due to estrogen deficiency.

The decrease of estrogen levels can directly lead to the increase of IL-6 production as observed during menopause or other related medical conditions. The lack of estrogen in the body is associated with increased 6 secretion of several pro-inflammatory cytokines, including IL-6, IL-1, and tumor necrosis factor (TNF).[15,16] Several studies have reported that IL-6 levels increase or tend to increase in both older individuals and post-menopausal women. This change in

the immune system, marked by the increase of IL-6 levels, is strongly linked to the estrogen deficient condition[6,17–20]

Utilizing an in vivo experimental design, the research provides controlled and quantifiable evidence regarding the differential biological mechanisms underlying post-extraction tissue recovery. The findings offer valuable insights into the complex interactions between systemic hormonal regulation and local inflammatory processes, with potential clinical implications for the optimization of postoperative care and wound management in menopausal patients.

Estrogen is essential for controlling the inflammatory response and promoting tissue healing after dental trauma, such as tooth extraction. Its anti-inflammatory properties modulate cytokine expression and immune responses within the periodontium, potentially attenuating periodontal inflammation and tissue destruction. [21] One of its primary role is controlling interleukin-6 (IL-6), a cytokine that promotes inflammation and rapidly increase following tissue damage. By keeping IL-6 within a regulated range, physiological estrogen levels aid in lowering excessive inflammation, encouraging angiogenesis, and facilitating the shift from the inflammatory phase of wound healing to the proliferative and remodeling phases.[22–24]

On the other hand, a lack of estrogen may result in chronic inflammation, delayed socket healing, and increased IL-6 expression.[25] To manage patients with hormonal imbalances, including postmenopausal women, the relationship between estrogen and IL-6 is therefore essential for comprehending the dynamics of wound healing following tooth extraction.[26,27]

The novelty of this study lies in its comparative evaluation of interleukin-6 (IL-6) levels in post-tooth extraction inflammation between menopausal and non-menopausal subjects. It represents one of the first investigations to elucidate the influence of hormonal status, particularly the estrogen deficiency associated with menopause, on IL-6–mediated inflammatory responses and oral wound healing. This study aimed to compare IL-6 levels in menopausal and non-menopausal rats with post tooth extraction treatment.

METHODS

This research was conducted at the Experimental Animal Laboratory, Faculty of Medicine, Wijaya Kusuma University, Surabaya. From a population of 30 female mice aged 2–3 months, weighing 120–200 grams and with no history of pregnancy, a random sample of 10 female mice was selected. Then they were divided into two groups: the first group, K (Control), which did not undergo oophorectomy, and the second group, P (Treatment), which underwent oophorectomy for 1 month before treatment.

Menopausal induction in experimental animals was performed using the ovariectomy method. Seven days after the adaptation period, the ovariectomy procedure was carried out.[9]The surgical procedure involved preparing equipment such as a scalpel, blade, needle holder, artery clamps, scissors, tweezers, needle, and suturing thread. the experimental animals were then weighed to determine the appropriate anesthesia dose, using Ketamine-xylazine (KX) 0.03 mg/BW and 0.04 mg/BW, administered intramuscularly in the M muscle gap.

After being anesthetized, the mice were positioned in a lateral recumbent position, then the hair in the incision area, located in the paralumbar fossa, was shaved. The incision area was then disinfected with 70% alcohol before incision. The incision was made slowly using a blade, creating 5-7 mm opening to open the abdominal muscles, followed by clamping the lower part of the oviduct using artery clamps.[17] The ovaries were then cut and checked to ensure that there was no bleeding before removing the artery clamps. The muscle layer was then sutured using chromic gut thread with a simple interrupted suture pattern.

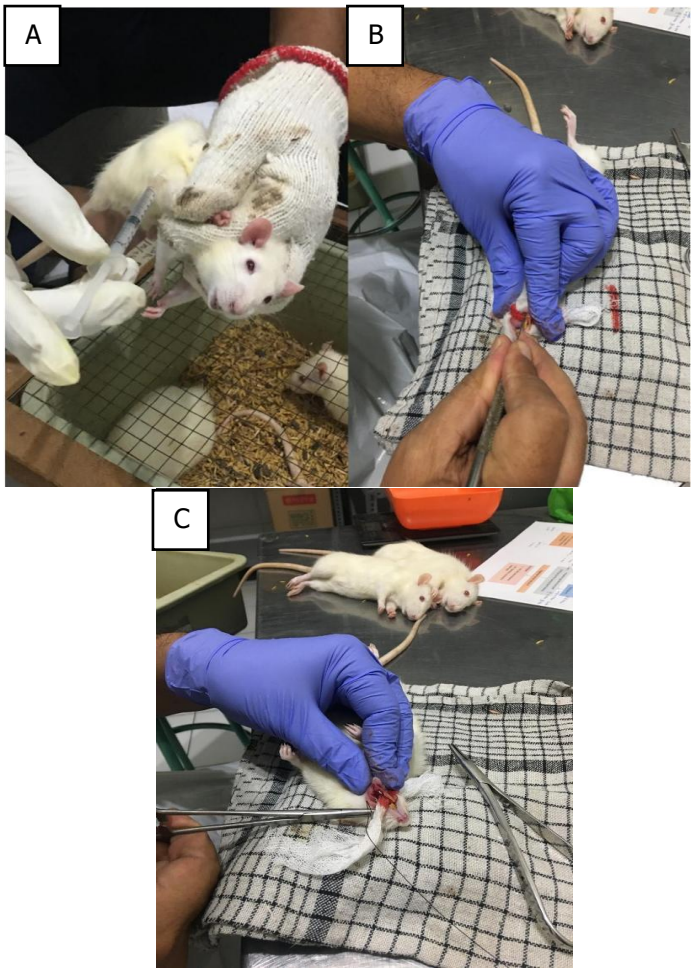

Figure 1. A. Experimental animal injected with ketamine as an anesthetic, B. Tooth extraction, C. stitching of wound from tooth extraction.

The protocol outlined the procedure for performing tooth extraction in rats using ketamine-based anesthesia. The anterior teeth of the lower jaw were extracted. The extraction was performed with minimal luxation movement, small defects, and minimal pressure on the blood clot in the tooth socket. Considerations were made for taking observations on days 3, 7, and 14 post-teeth extraction of the teeth. Day 3 represented the acute phase of post-extraction, day 7 entered the gradation phase between the acute and chronic phases, and day 14 represented the chronic phase of the tooth extraction wound-healing process of post-teeth extraction.

This research used blood serum, the Interleukin-6 (IL-6) Kit from Bioassay Technology, Shanghai Korain Biotech Co., Ltd. Shanghai, China, was used, specifically the human IL-6 ELISA Kit with code E0135Ra and size 48T.96T, which has a sensitivity value of 0.52 ng/L, and a detection susceptibility of 0.1-40 ng/L. The kit was used for mice and time testing of 1 hour 30 minutes. The following steps were performed: the reagent was brought to room temperature before use, then 120µl of standard (48ng/L) was reconstituted with 120µl of standard diluent to produce a standard stock solution of 24ng/L. The standard was allowed to stand for 15 minutes with gentle stirring before dilution.

Duplicate standard spots were prepared by diluting standard stock solution (24ng/L) 1:2 with standard diluent to produce solutions of 12ng/L, 6ng/L, 3ng/L, and 1.5ng/L. The standard diluent served as the zero standard (0 ng/L). Implementation was carried out by washing the buffer then diluting 20 ml of Washing Buffer Concentrate 25x into deionized or distilled water. If crystals were formed in the concentrate, the solution was stirred gently until they were completely dissolved.

Reagents, standard solutions, and samples were prepared as instructed. Testing was carried out at a room temperature by determining the number of strips required for testing. The strip was inserted into the frame for use. Unused strips were stored at 2-8 °C. A total of 50 µl of standard was added. In this case, biotinylated antibodies or standard solutions were not used, because the standard solutions already contained biotinylated antibodies. Then, 40 µl of sample was added, followed by 10 µl of anti-IL-6 antibody to the sample well, and 50 µl of streptavidin-HRP was added to the standard well (not the empty control well). The plate was covered with a sealer.

The plate, covered with the sealer from the previous step was incubated for 60 minutes at 37°C. The sealer was then removed, and the plates were washed 5 times with a washing buffer. The wells were soaked with 300 µl of wash buffer for 30 seconds to 1 minute for each wash. Each well was aspirated or decanted and washed 5 times with washing buffer for automatic washing. The plate was patted dry on a paper towel or other absorbent material. A total of 50 µl of Aot substrate solution was added to each well, and 50 µl of Bot substrate solution was added to each well.

The covered plate was incubated with a new sealer for 1 minute at 37°C in the dark. Subsequently, 50 µl of stop solution was added to each well, and the blue color immediately changed to yellow. The optical density (OD value) of each well was determined immediately using a microplate reader set at 450 nm within 01 min after adding the stop solution.

RESULTS

In this study, the ELISA method was used, and data collection was carried out, grouped by days 3, 7 and 14, then analyzed statistically.

Tabel 1. IL-6 Levels Inflammation indicator control group (non-menopausal) and treatment (menopausal) day 3

Samples	Treatment	
	K	P
1	0.174	0.098
2	0.053	0.034
3	0.034	0.442
Average	0.087	0.191
Min	0.034	0.034
Max	0.174	0.442

Information:
K : The control group (non-menopausal). After 1 month, the anterior teeth were extracted, and observations were carried out on day 3 post-extraction.
P : The treatment group (menopause) underwent oophorectomy and was left for 30 days (1 month). After 1 month, the anterior teeth were extracted, and observations were carried out on day 3 post-extraction.

The table 1 above shows that the average IL6 level in the control group was 0.087 (ng/L) and in the treatment group was 0.191 (ng/L). We found that the treatment group had higher IL-6 levels than the control group. This trend can also be seen in the graphic image below:

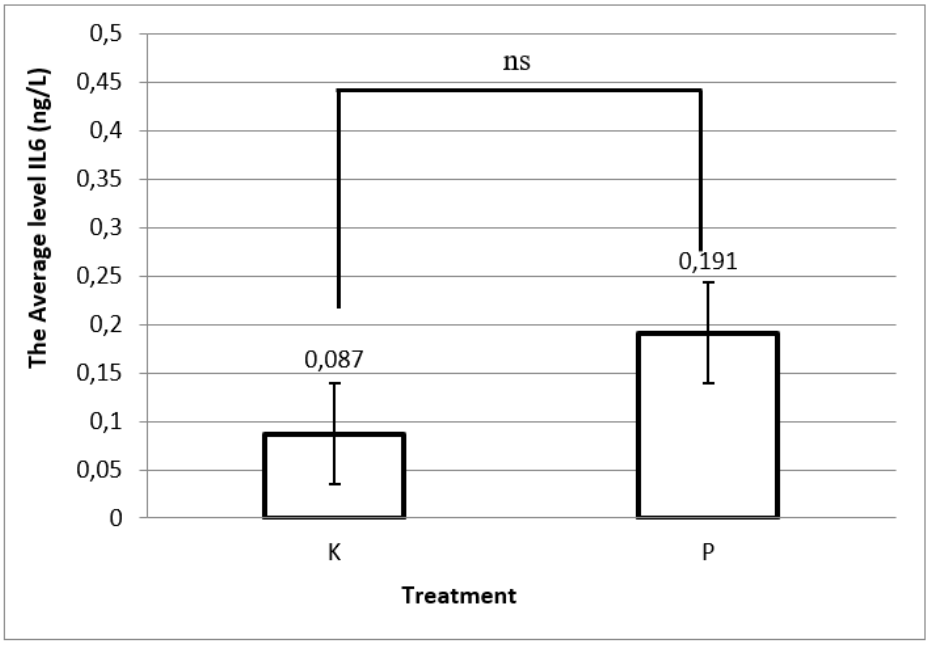

Figure 1. The average level IL-6 on the 3rd day

Tabel 2. IL-6 Levels inflammation indicator control group (non-menopausal) and treatment (menopausal) day 7

Samples	Treatment	
	K	P
1	0.155	0.974
2	0.045	0.347
3	0.755	0.034
Average	0.318	0.452
Min	0.045	0.034
Max	0.755	0.974

Information:
K : The control group (non-menopausal). After 1 month, the anterior teeth were extracted, and observations were carried out on day 7 post-tooth extraction
P : The treatment group (menopausal) underwent oophorectomy and was left for 30 days (1 month). After 1 month, the anterior teeth were extracted and observations were carried out on day 7 post-tooth extraction.

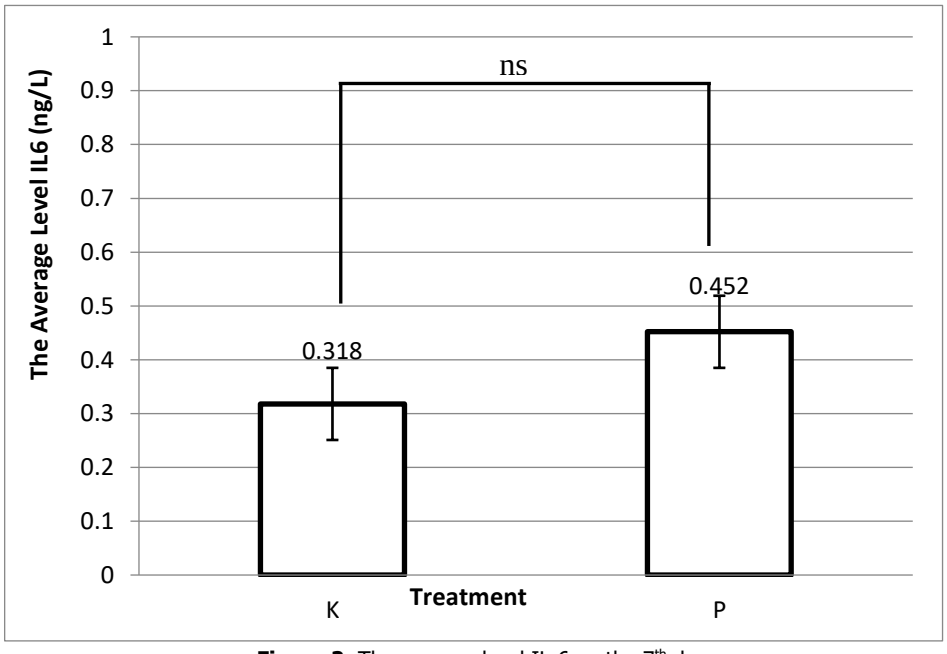

Figure 2. The average level IL-6 on the 7th day

The table 2 above shows that the average IL6 level in the control group was 0.318 (ng/L) and in the treatment group was 0.452 (ng/L), as illustrated in the figure above.

Tabel 3. IL-6 Levels inflammation indicator control group (non-menopausal) and treatment (menopausal) day 14

Samples	Treatment	
	K	P
1	0.155	0.974
2	0.045	0.347
3	0.755	0.034
Average	0.318	0.452
Min	0.045	0.034
Max	0.755	0.974

Information:
K : The control group (non-menopausal). After 1 month, the anterior teeth were extracted, and observations were carried out on day 14 post-tooth extraction.
P : The treatment group (menopausal) underwent oophorectomy, and was left for 30 days (1 month). After 1 month, the anterior teeth were extracted, and observations were carried out on day 14 post-tooth extraction.

The table 3 above shows that the average IL-6 level in the control group was 0.318 (ng/L) and in the treatment group was 0.452 (ng/L), which is illustrated in the graphic image below:

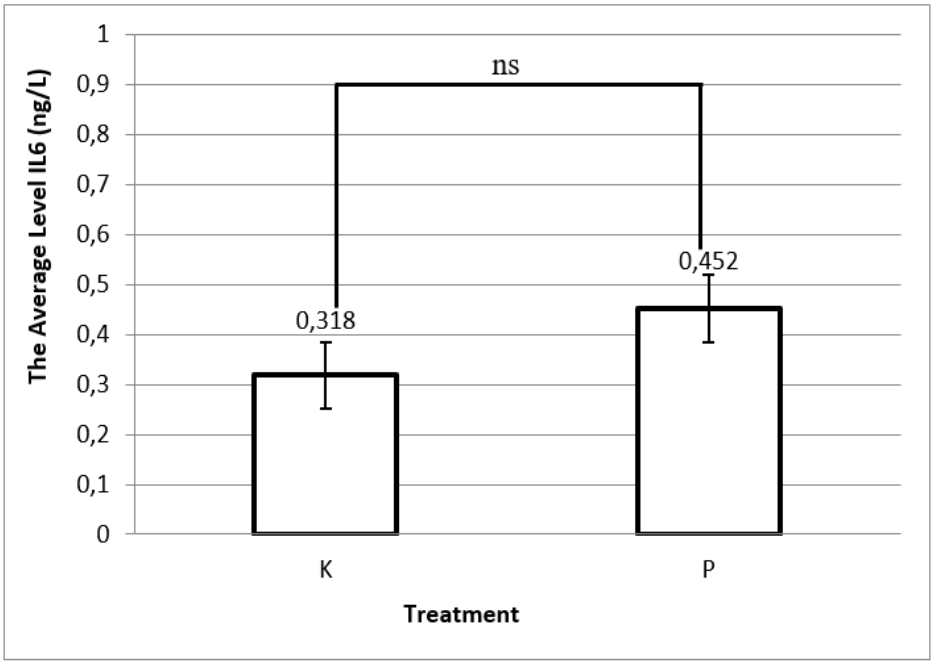

Figure 3A. The average level IL-6 on the 14th day

Tabel 4. IL-6 Levels inflammation indicator control group (non-menopausal) and treatment (menopause) days 3, 7, and 14

Samples	Treatment					
	K3	P3	K7	P7	K14	P14
1	0.174	0.098	0.155	0.974	0.317	0.155
2	0.053	0.034	0.045	0.347	0.034	0.045
3	0.034	0.442	0.755	0.034	0.389	0.755
Average	0.087	0.191	0.318	0.452	0.247	0.318
Min	0.034	0.034	0.045	0.034	0.034	0.045
Max	0.174	0.442	0.755	0.974	0.389	0.755

Information:
K : The control group (non-menopausal). After 1 month, the anterior teeth were extracted, and observations were carried out on days 3, 7, and 14 post-tooth extraction
P : The treatment group (menopausal) underwent oophorectomy, and was left for 30 days (1 month). After 1 month, the anterior teeth were extracted and observations were made on days 3, 7, and 14 post-tooth extraction.

The Table 3 and 4 above show that the average IL6 level in the control group on the 3rd, 7th, and 14th days were 0.087, 0.318, dan 0.247(ng/L), respectively, and in the treatment group were 0.191, 0.452, dan 0.318 (ng/L), respectively. This trend can also be seen in the graphic image below:

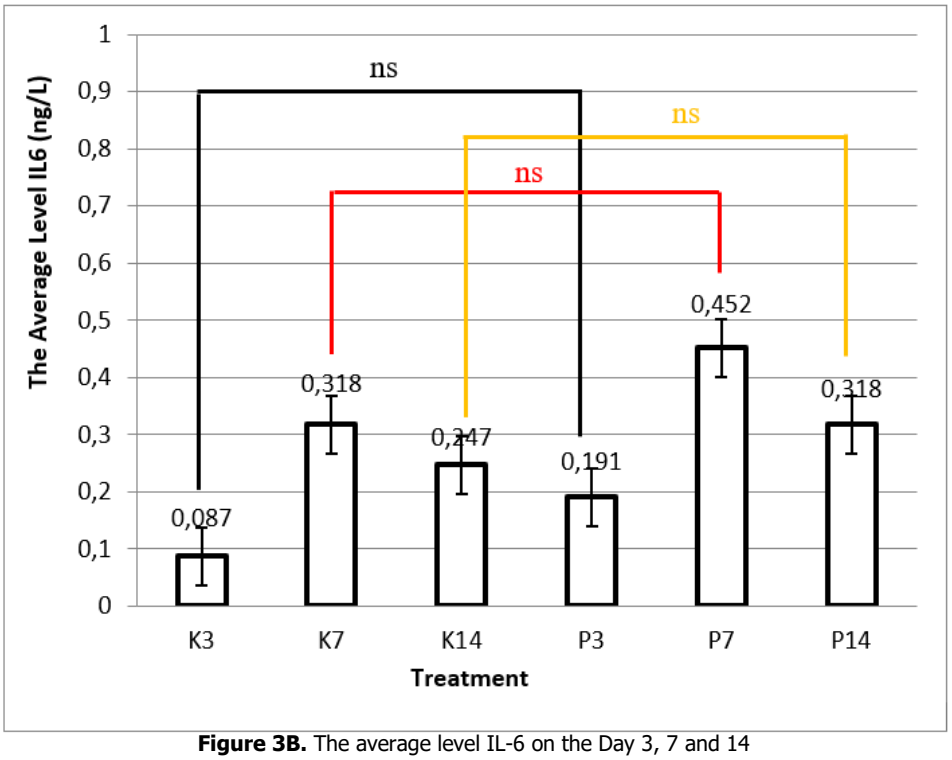

Figure 3B. The average level IL-6 on the Day 3, 7 and 14

This statistical test is needed to compare the distribution of measurement data with the standard normal distribution. For this purpose, the Shapiro-Wilk normality test was carried out with a sample size of 18, the results obtained from all data are normally distributed because the p-value is > 0.05 ~~or 5%.~~ The significance value of 0.112 > 0.05 indicates that the variance of the data is homogeneous. The One-Way ANOVA test was then used to assess the difference between groups.

The test to determine whether there is a difference between groups also used the One-Way ANOVA test. The results showed no significant difference between the control and treatment groups, with a significance value of 0.799 > 0.05. However, the average IL-6 levels in the Treatment (menopausal) group were higher than those in the Control group, as indicated by the average IL-6 value level on days 3, 7, and 14.

DISCUSSION

The results of the post-extraction observations on the 3rd day are shown in Table 1 and Figure 1, which demonstrate an increase in IL6 in the treatment group (0.191 ng/L) compared to the control group. This finding is in accordance with research by Taher and Bede(2020), which reported that the mean level of IL-6 48 hours postoperatively was significantly higher than the preoperative level. This aligns with many studies indicating that IL-6 is an early and sensitive marker of tissue injury that is particularly informative in the early postoperative period.[31]

Table 2, which present post-extraction observations on the 7th day of treatment, demonstrating IL-6 levels of 0.452 ng/L in the treatment group and 0.318 ng/L in the control group. At this time point, the treatment group exhibited a higher concentration of IL-6 than the control group. If IL-6 is considered an indicator of inflammation , a higher value in the treatment group could suggests

an increased inflammatory response, potentially indicating a negative outcome or a different healing trajectory.

Table 3B, demonstrating observations on the 14th day post-extraction, presents IL6 levels of 0.318 ng/L in the treatment group, and 0.247 ng/L in the control group. These results indicate that both groups exhibited very low IL-6 levels at 14 days post-extraction, with the treatment group showing a slightly higher level. Studies by Sulaeman et.al, 2020, report that IL-6 levels are generally highest during the first few days post-extraction (estimation 24 hours to 7 days) and gradually decrease as the wound heals. By day 14, the IL-6 levels typically return toward baseline.[32]

After tooth extraction on the 3rd, 7th and 14th days there was pro-inflammatory activity in the treatment group and in the group that underwent removal of the ovaries by the ovariectomy method (menopause), which caused the healing process to slow down or become delayed. The aging process affects the wound healing process. In older people, it takes longer than in younger people. This is caused by the obstruction of blood supply due to atherosclerosis, cell degeneration, tissue atrophy and decreased immune system function, which results in the tissue regeneration process not being able to take place normally.[28] Because the menopausal event in women occurs physiologically in line with the aging process, it can be concluded that slow wound healing is a combination of these two factors.[29]

In order to observe the inflammatory response under menopausal conditions, IL-6 levels were measured on the 3rd day, 7th day, and 14th day after tooth extraction in mice in both the control and treatment groups. As shown in Table 4, there were clear differences on days 3, 7 and 14, with the treatment group showing higher IL-6 levels on day 7 compared to days 3 and 14. Previous oral health research in menopausal age groups, in which the data were analyzed using a non-parametric correlation test, demonstrated that women who had experienced menopause for 5-10 years had fewer teeth and were more likely to experience irreversible periodontal disease (2.65 ± 0.35). It was concluded that menopausal women generally had a poor oral health status.[24]

The finding suppors the observation that on day 7 (IL-6: 452 ng/L), wound healing reached its peak, as a significant inflammatory response occurred with the emergence of macrophages followed by T lymphocytes. The presence of macrophages and T lymphocytes is essential in normal wound healing conditions. Macrophages are both a major source and target of IL-6. Meanwhile T-lymphocytes are directed by IL-6 toward inflammatory Th17 responses while suppressing regulatory T cells. Together, IL-6 links innate immunity (macrophages) with adaptive immunity (T cells), making it a central cytokine in inflammation and wound healing.[30]

Based on the Anova test, which shows no significant difference between the control and treatment groups (sig. 0.799 > 0.05), the menopausal group has the higher IL-6 levels than the control group on days 3, 7 and 14. A previous study (Parmasari et al., 2018) on wound healing with swelling in two groups, the menopausal and non-menopausal groups, found a significant difference between them on days 3 and 5 after tooth extraction. Tooth extraction can cause tissue injury, which leads to an increase in the expression of pro-inflammatory cytokines at the extraction site, including IL-6.[15]

Our findings show no significant difference for average IL-6 levels three days after tooth extraction between treatment group or the post-menopause group and the control group with no menopause treatment. In essence, while post-menopausal women might have higher general inflammatory markers, the localized and acute inflammatory process following an extraction appears comparable to the control group at the three-day mark in the described study.[35]

Even after seven days and fourteen days, IL-6 levels did not exhibit any significant difference between the two groups. In this study, tooth extraction did not cause significant changes in IL-6 levels between the control and post-

menopausal groups. Notably, there was an increase in IL-6 levels on day 7 after extraction compared to day 3 in both groups, indicating increased level of IL-6 expression after the extraction. By day 14 after extraction, the IL-6 levels had decreased compared on days 3 and 7. The increase of IL-6 levels indicates that there was tissue injury caused by the tooth extraction.

Studies have found that up to 7 days after the tissue damage from tooth extraction, IL-6 levels have temporarily increased, and after 10 days it starts to decrease, indicating that the inflammatory condition has already resolved. Our findings also show consistent results with Indrawati et al. (2019). In a study about post-tooth extraction with sponge amnion treatment, Indirawati et al.[14] reported an increase in IL-6 in the control group, with highest level of IL-6 post tooth extraction. In contrast, the treatment group showed a decrease of IL-6 levels, demonstrating that tissue injury caused by the post tooth extraction increases the IL-6 levels.

However, it should be noted that this finding shows higher levels of IL-6 in post-menopause group on days 3, 7, and 14 compared to the control group. A previous study by Cioffi et al. found a higher concentration of IL-6 in post-menopausal women, correlating with changes in serum hormone levels. Kim et al. reported that IL-6 levels are significantly higher in post-menopausal women compared to the pre-menopausal women in healthy non-obese women. [18] reported higher levels of IL-6 in late perimenopausal women. This research found that the IL-6 levels were higher among post-menopausal women compared to those of reproductive age, both with coronary artery disease (CAD).[19]

This research also revealed that the immune system balance was disturbed in ovariectomized mice, shown by the increased levels of IL-1β, IL-6, and TNF-α. Increased mRNA and protein levels of these cytokines were observed in serum as well as in specific tissues such as the liver, adipose tissue, aorta, and hippocampus of ovariectomized (OVX) mice. This occurs because the lack of ovarian hormones after ovariectomy removes inhibition of immune responses and sensitizes the system to inflammatory challenges, thereby raising the basal inflammatory state.[33] The lack of estrogen induces an increase in pro-inflammatory cytokines. The higher level of IL-6 in post-menopausal group may occur due to the estrogen deficiency, as estrogen can inhibit the production of IL-6.[28] Our findings in Figure 3 are consistent with their previous studies, highlighting that the estrogen deficiency, as observed in menopause conditions, affects pro-inflammatory cytokines such as IL-6[29]

The loss of estrogen's anti-inflammatory properties and increased bone turnover after menopause should theoretically increase IL-6. However, biological variability, a small sample size, measurement limitations, or stronger confounding factors may obscure the effect and all lead to a lack of statistical significance. Therefore, the absence of a significant difference does not necessarily imply a lack of biological relevance. The tendency may need to be revealed by larger, more controlled research or through stratification.

The limitations of this study include the use of a limited sample size; therefore, further research should use a larger sample size to better control the homogeneity of the study. Additionally, the inclusion criteria for determining menopausal mice did not involve checking estrogen levels beforehand, because previous studies indicated that a reliable menopausal period is typically achieved 30 days after oophorectomy. In future studies, it is better to measure estrogen level to confirm menopausal status more accurately.

CONCLUSION

The menopausal group's tendency toward elevated IL-6 levels raises the possibility that hormonal changes may have an effect on post-extraction inflammation, since estrogen aids in the production of collagen fibers, which enhance hygroscopic quality and fortify collagen's adhesion to connective tissue.

The greater levels of IL-6 in menopausal rats suggest that estrogen deprivation may have an impact on inflammation following extraction. This study therefore indicates that clinicians should exercise greater caution when performing tooth extraction in menopausal female patients, as complications such as chronic inflammation, prolonged bleeding, and delayed wound healing may occur.

This research implies that in menopause, in which the production of estrogen hormone naturally diminishes after tooth extraction, there is also a decrease in the synthesis of new tissue, including collagen and fibrinogen, which may prolong or postpone wound healing. Another important point is that, although the risk is not as common as it is in women of reproductive age, preventing secondary infections is crucial because it helps reduce inflammation and accelerate the healing process.

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REFERENCES

1. Yuan Y, Xu B, Yang J, Wu M. Effects of platelet-rich fibrin on post-extraction wound healing and wound pain: A meta-analysis. Int. Wound J. 2024;21(2):e14654. https://doi.org/10.1111/iwj.14654.
2. Sjuhada Oki A, Amalia N, Tantiana. Wound healing acceleration in inflammation phase of post-tooth extraction after aerobic and anaerobic exercise. Science & Sports. 2020 Jun 1;35(3):168.e1-168.e6. https://doi.org/10.1016/j.scispo.2019.06.001
3. Tyagi T, Alarab M, Leong Y, Lye S, Shynlova O. Local oestrogen therapy modulates extracellular matrix and immune response in the vaginal tissue of post-menopausal women with severe pelvic organ prolapse. J. Cell. Mol. Med. 2019 Apr;23(4):2907–19. https://doi.org/10.1111/jcmm.14199
4. Alzoubi EE. Oral Manifestations of Menopause. Journal of Dental Health, Oral Disorders & Therapy. 2017;7. https://doi.org/10.15406/jdhodt.2017.07.00247
5. El Khoudary SR, Chen X, Qi M, et al. The Relation Between Systemic Inflammation and the Menopause Transition: The Study of Women's Health Across the Nation. J Clin Endocrinol Metab. 2025;110(11):e3566-e3576. https://doi.org/10.1210/clinem/dgaf175
6. Mohammadi S. Association of Inflammatory Markers and Growth Factors with Radiographically Assessed Wound Healing of Extraction Sockets. Theses & Dissertations.2023;754. https://digitalcommons.unmc.edu/etd/754
7. Saad Mohammed Sulaiman1, Ahmad Orkhan Hasan1 NAN. Role of TNF alpha and IL-6 in Inflammatory Process after Tooth Extract in Children Under 12 Years. 2020;14:1072–1075. https://doi.org/10.37506/ijfmt.v14i2.3049
8. Bertone-Johnson ER, Manson JE, Purdue-Smithe AC, Hankinson SE, Rosner BA, Whitcomb BW. A prospective study of inflammatory biomarker levels and risk of early menopause. Menopause. 2019;26(1):32–8. https://doi.org/10.1097/gme.0000000000001162
9. Anand P, Hardt D, Mccloskey J. The Santa Cruz sluicing data set. Language. 2021 Jan 1;97:e68–88. https://doi.org/10.1353/lan.2021.0009
10. Sherwood L. Human Physiology: From Cells to Systems [Internet]. Cengage Learning; 2015. https://books.google.co.id/books?id=8WVvCgAAQBAJ
11. Aliyu M, Zohora FT, Anka AU, Ali K, Maleknia S, Saffarioun M, et al. Interleukin-6 cytokine: An overview of the immune regulation, immune dysregulation, and therapeutic approach. International immunopharmacology. 2022 ;111:109130. https://doi.org/10.1016/j.intimp.2022.109130
12. Soma T, Iwasaki R, Sato Y, Kobayashi T, Nakamura S, Kaneko Y, et al. Tooth extraction in mice administered zoledronate increases inflammatory cytokine levels and promotes osteonecrosis of the jaw. J Bone Miner Metab. 2021 May;39(3):372–84. https://doi.org/10.1007/s00774-020-01174-2
13. Nishio C, Rompré P, Moldovan F. Effect of exogenous retinoic acid on tooth movement and periodontium healing following tooth extraction in a rat model. Orthodontics & craniofacial research. 2017 Jun;20 Suppl 1:77–82. https://doi.org/10.1111/ocr.12151
14. Indrawati DW, Munadziroh E, Sulisetyawati TIB, El Fadhlallah PM. Sponge amnion potential in post tooth extraction wound healing by interleukin-6 and bone morphogenetic protein-2 expression analysis: An animal study. Dent Research J. 2019;16(5):283–8. . https://doi.org/10.4103/1735-3327.266089
15. Tu SJ, Wang SP, Cheng FC, Chen YJ. Extraction of gray-scale intensity distributions from micro computed

tomography imaging for femoral cortical bone differentiation between low-magnesium and normal diets in a laboratory mouse model. Scientific Reports. 2019;9(1):1–11. https://doi.org/10.1038/s41598-019-44610-8
16. Cheng CH, Chen LR, Chen KH. Osteoporosis Due to Hormone Imbalance: An Overview of the Effects of Estrogen Deficiency and Glucocorticoid Overuse on Bone Turnover. Int. J. Mol. Sci. 2022 ;23(3). https://doi.org/10.3390/ijms23031376
17. Andersen PL, Vermette P. Intracellular insulin quantification by cell-ELISA. Experimental cell research. 2016 Sep;347(1):14–23. https://doi.org/10.1016/j.yexcr.2016.06.014
18. Metcalf CA, Johnson RL, Freeman EW, Sammel MD, Epperson CN. Influences of the menopause transition and adverse childhood experiences on peripheral basal inflammatory markers. Brain, behavior, & immunity - health. 2021;15:100280. https://doi.org/10.1016/j.bbih.2021.100280
19. Rao YQ, Li J, Wang WJ. Effects of Gengnianchun on learning and memory ability, neurotransmitter, cytokines, and leptin in ovariectomized rats. Int. J. Clin. Exp. Med. 2015;8(6):8648–60. https://pmc.ncbi.nlm.nih.gov/articles/PMC4538054/
20. Tunheim EG, Skallevold HE, Rokaya D. Role of hormones in bone remodeling in the craniofacial complex: A review. JOBCR. 2023;13(2):210–7. https://doi.org/10.1016/j.jobcr.2023.01.009
21. Palanisamy S. The impact of estrogen on periodontal tissue integrity and inflammation-a mini review. Frontiers in dental medicine. 2025;6:1455755. https://doi.org/10.3389/fdmed.2025.1455755
22. Pernoud LE, Gardiner PA, Fraser SD, Dillon-Rossiter K, Dean MM, Schaumberg MA. A systematic review and meta-analysis investigating differences in chronic inflammation and adiposity before and after menopause. Maturitas. 2024;190:108119. https://doi.org/10.1016/j.maturitas.2024.108119
23. Tartibian B, FitzGerald LZ, Azadpour N, Maleki BH. A randomized controlled study examining the effect of exercise on inflammatory cytokine levels in post-menopausal women. Post Reproductive Health. 2015;21(1):9–15. https://doi.org/10.1177/2053369114565708
24. Fischer V, Kalbitz M, Müller-Graf F, Gebhard F, Ignatius A, Liedert A, et al. Influence of menopause on inflammatory cytokines during murine and human bone fracture healing. Int. J. Mol. Sci. 2018;19(7). https://doi.org/10.3390/ijms19072070
25. Chen L, Cheng S, Sun K, Wang J, Liu X, Zhao Y, et al. Changes in macrophage and inflammatory cytokine expressions during fracture healing in an ovariectomized mice model. BMC Musculoskeletal Disorders 2021;22(1):494. https://doi.org/10.1186/s12891-021-04360-z
26. De Maeyer RPH, Sikora J, Bracken O V, Shih B, Lloyd AF, Peckham H, et al. Age-Associated Inflammatory Monocytes Are Increased in Menopausal Females and Reversed by Hormone Replacement Therapy. Aging Cell. 2025;e70249. https://doi.org/10.1111/acel.70249
27. Haffner-Luntzer M, Fischer V, Prystaz K, Liedert A, Ignatius A. The inflammatory phase of fracture healing is influenced by oestrogen status in mice. Eur. J. Med. Res. 2017;22(1):23. https://doi.org/10.1186/s40001-017-0264-y
28. Imaculada de Queiroz Rodrigues M, Ohana de Lima Martins J, Silva PG de B, Carlos Ferreira Júnior AE, Quezado Lima Verde ME, Sousa FB, et al. Tocilizumab, a Potent Interleukin-6 Receptor Inhibitor, Decreases Bone Resorption and Increases the Rate of Bacterial Infection After Tooth Extraction in Rats. Journal of oral and maxillofacial surgery: JOMS. 2020 Dec;78(12):2138–46. https://doi.org/10.1016/j.joms.2020.08.012
29. Habbab KM, D'Aiuto F, Habbab MA, Porter SR. Molecular markers relevant to myocardial injury following dental extraction in patients with or without coronary artery disease. BDJ open. 2019;5:9. https://doi.org/10.1038/s41405-019-0018-8
30. Amin MF, Meidyawati R, Djamil MS, Latief BS. Inflammatory cytokine serum levels in sockets following extraction of teeth with apical periodontitis. JIDMR. 2019;12(1):129–32. http://www.jidmr.com/journal/wp-content/uploads/2019/04/22-19411-ED-Benny-S.-Latief.pdf
31. Taher, H.A. and Bede, S.Y., 2020. Evaluation of the Serum Level of Interleukin-6 in Patients Undergoing Surgical Removal of Impacted Mandibular Third Molars. JRMDS, 8(1), pp.56-60. https://www.jrmds.in/articles/evaluation-of-the-serum-level-of-interleukin6-in-patients-undergoing-surgical-removal-of-impacted-mandibular-third-molars 45468.html#:~:text=Discussion,extraction%20of%20impacted%20third%20molars.
32. Sulaiman, S.M., Hasan, A.O. and Naji, N.A., 2020. Role of TNF alpha and IL-6 in Inflammatory Process after Tooth Extract in Children Under 12 Years. IJFMT, 14(2). https://10.37506/ijfmt.v14i2.3049
33. Baeza, I., De Castro, N.M., Arranz, L., Fdez-Tresguerres, J. and De la Fuente, M., 2011. Ovariectomy causes immunosenescence and oxi-inflamm-ageing in peritoneal leukocytes of aged female mice similar to that in aged males. Biogerontology, 12(3), pp.227-238. https://link.springer.com/article/10.1007/s10522-010-9317-0
34. López Carriches, C., Martínez González, J.M. and Donado Rodríguez, M., 2006. Variations of interleukin-6 after surgical removal of lower third molars. https://dialnet.unirioja.es/servlet/articulo?codigo=5864962
35. Huang, X., Ni, B., Li, Q., Liu, M., Zhao, M., Zhang, Y., Shi, X. and Wang, W., 2024. Association between postmenopausal osteoporosis and IL-6, TNF-α: a systematic review and a meta-analysis. Combinatorial Chemistry & High Throughput Screening, 27(15), pp.2260-2266. https://DOI:10.2174/0113862073262645231121025911