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Statistical Analysis of Neutrophil Counts in Omega-3 Treated Post-Extraction Sockets: A Secondary Experimental Study

Inas Mayda El Zahwa[1,*]

[1]Karabük University, Karabük, Türkiye

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***Corresponding Author:**
inaselzahwa@gmail.com

Abstract
We previously reported an experimental study demonstrating that omega-3 fatty acids from milkfish (*Chanos chanos*) oil significantly lowered neutrophil counts within extraction sockets; however, the combined effect of treatment, healing time, and healing status required further evaluation. Post-extraction sockets from 32 Wistar rats were randomly assigned to four groups in a post-test-only design: control day 1, omega-3 day 1, control day 3, and omega-3 day 3. Histologically determined neutrophil counts in the granulation tissue were analyzed using two-way ANOVA to evaluate the independent effects of treatment and day. Subsequently, an exploratory dichotomization (fast vs. slow healing) was applied. The analysis revealed statistically significant main effects for both treatment ($P < 0.001$) and time ($P < 0.001$), with no significant interaction ($P=0.34$). Neutrophil counts were lowest in the omega-3 group at day 3 (mean=3.5 cells). This secondary analysis supports prior findings by demonstrating that omega-3 extract significantly reduces neutrophil infiltration. Evaluating treatment and time simultaneously provides statistical evidence that omega-3 may favorably modulate early inflammatory responses in post-extraction wound healing, although further translational studies are required.

1. Introduction

Dental extraction is a globally prevalent procedure. In Indonesia, extractions account for approximately 57.6% of all dental treatments [1]. This procedure involves the removal of a tooth from the alveolar socket for various clinical indications, such as advanced periodontal disease, severe caries, or orthodontic correction [2], [3]. Extraction inevitably causes injury to soft and hard tissues, thereby initiating the physiological cascade of wound healing.

Wound healing commences immediately and progresses through overlapping stages: hemostasis, inflammation, proliferation, and remodeling [4], [5]. During the early inflammatory stage, neutrophils and macrophages migrate to the wound site to phagocytize debris and microorganisms. Neutrophils undergo apoptosis after completing their primary defense functions, allowing macrophages to signal the resolution of inflammation [7], [8]. However, prolonged or dysregulated neutrophil infiltration can sustain inflammation and hinder the transition to the proliferative phase, potentially delaying socket healing [9], [10].

Post-extraction pharmacological interventions are often employed to promote optimal healing. Omega-3 polyunsaturated fatty acids (PUFAs), particularly eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA), have been extensively documented for their anti-inflammatory and pro-resolving properties, which include the modulation of neutrophil activity [12], [13], [26]. Previous experimental data from our group demonstrated that omega-3 extracted from *Chanos chanos* (milkfish) oil reduced neutrophil counts in Wistar rat extraction sockets on days 1 and 3 [18]. While conventional analyses indicated an anti-inflammatory effect, that initial report evaluated each time point independently without robustly estimating the combined effects of treatment and time.

To achieve a more comprehensive understanding of these dynamics, a factorial analytical approach is necessary. Therefore, this secondary study aims to statistically evaluate the magnitude of treatment and time effects on neutrophil infiltration using two-way analysis of variance (ANOVA). Additionally, we conduct an exploratory classification to determine if varied neutrophil counts can distinctively categorize faster versus slower early healing trajectories and to determine whether these variables can distinguish between faster and slower socket healing.

2. Research Method

2.1. Study design and ethical approval

This study is a secondary analysis of archival data obtained from an experimental animal study previously described by Inas et al. [18]. A post-test-only randomized controlled design was employed. The protocol was approved by the Research Ethics Committee of the Faculty of Dentistry, Islamic Sultan Agung University, Indonesia (No. 649/B.1-KEPK/SA-FKG/VIII/2015), in strict compliance with institutional guidelines for laboratory animal care.

2.2. Animals and experimental procedures

The dataset comprises 32 male Wistar rats (Rattus norvegicus), weighing 150–200 grams, randomly divided into four equal groups (n=8 per group): control day 1, omega-3 day 1, control day 3, and omega-3 day 3 [19]. Under general anesthesia (10% ketamine and xylazine), the lower left incisor was extracted. The omega-3 groups received Chanos chanos extract (54mg/1mL/200 g body weight) orally [20], while control rats received an equivalent volume of vehicle. Animals were euthanized on their respective designated days.

2.3. Histological observation

As previously detailed [21], extracted sockets were fixed in 10% buffered neutral formalin, decalcified, embedded in paraffin, and sectioned at 4–6 microns. Slides were stained with hematoxylin and eosin (H&E). Neutrophil counts were quantified under light microscopy (1000x magnification) within a standardized region of interest in the granulation tissue. The average neutrophil count from granulated fields yielded a single continuous value per socket.

2.4. Data preparation for secondary analysis

Data from all 32 animals were compiled. Variables included treatment group (control vs. omega-3), time point (day 1 vs. day 3), and neutrophil count. Data management and analyses were executed using Python (Pandas, SciPy, and StatsModels).

2.5. Statistical analysis

Neutrophil counts were summarized using descriptive statistics (mean and standard deviation). A two-way ANOVA was utilized to evaluate the joint effects of treatment and day, including their interaction. Assumptions were verified using Levene's test (homogeneity of variance) and the Shapiro-Wilk test (normality of residuals). Partial eta-squared (η_p^2) was calculated to estimate effect sizes. Due to the retrospective nature of this secondary analysis and the unavailability of raw subject-level continuous covariates, exact confidence intervals for effect sizes could not be reliably generated.For exploratory analysis, neutrophil counts were dichotomized into "fast healing" (1) and "slow healing" (0) using the median value as a threshold. Because the predictor variables almost perfectly separated the dichotomous outcome (complete separation), standard maximum-likelihood logistic regression failed to converge. Established penalization methods, such as Firth's logistic regression, were not performed to avoid generating synthetic data or invalid statistical assumptions in the absence of raw primary datasets. Consequently, the classification approach is retained strictly as descriptive cross-tabulations. Statistical significance was set at $\alpha = 0.05$.

3. Result and Discussion

The descriptive analysis indicated a time-dependent decrease in average neutrophil counts across both groups, with the omega-3 cohort consistently exhibiting lower counts than the control group (Figure 1). The lowest mean neutrophil count was observed in the omega-3 group on day 3 (mean=3.5 cells).(Note: Figure 1 is retained here as per the original manuscript)The two-way ANOVA (Table 1) revealed a statistically significant main effect of treatment ($F(1,28)=1059.11$, $P<0.001$, $\eta_p^2=0.97$) and day ($F(1, 28)=173.08$, $P<0.001$, $\eta_p^2=0.86$) on neutrophil counts. The interaction between treatment and day was

not statistically significant (F(1,28)=0.94, P=0.34, η_p^2=0.03). Diagnostic checks confirmed homogeneous variances (Levene's test P=0.93) and normally distributed residuals (Shapiro-Wilk P=0.40).

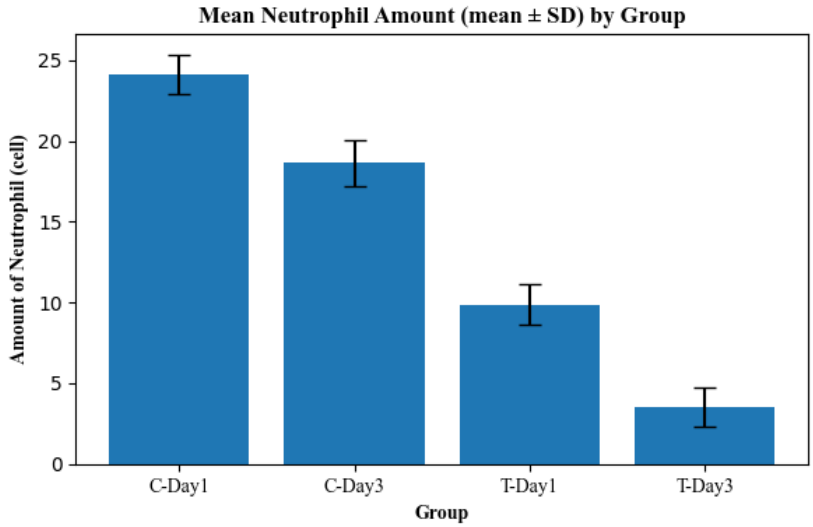

Figure 1. Mean of neutrophil count

Data analysis using Two-Way ANOVA is summarized in Table 1. The analysis revealed a significant main effect of treatment ($F(1, 28) = 1059.11$, $p < 0.001$, $\eta_p^2 = 0.97$) and day ($F(1, 28) = 173.08$, $p < 0.001$, $\eta p^2 = 0.86$) on neutrophil amounts, while the treatment by day interaction was not significant ($F(1, 28) = 0.94$, $p = 0.34$, $\eta_p^2 = 0.03$). Assumption checks confirmed the validity of the model, indicating homogeneous variances (Levene's test $p = 0.93$) and normally distributed residuals (Shapiro–Wilk $W = 0.96$, $p = 0.40$).

Table 1. Two-way Anova test result's effect of treatment, day, and interaction

Source	Sum of Squares	df	F-value
Treatment	1725.78	1.0	1059.11
Day	282.03	1.0	173.08
Treatment * Day	1.53	1.0	0.94
Residuals	45.63	28.0	-

To contextually explore healing trajectories, sockets were categorized into fast-healing and slow-healing profiles (Table 2). Fast healing was predominantly observed in the omega-3 treated groups and at the day 3 time point. Because this specific subset of aggregated data resulted in a complete separation phenomenon, predictive odds ratios could not be accurately calculated, highlighting a distinct deterministic pattern within this specific experimental cohort rather than a generalizable predictive model.

Table 2. Number and percentage of sockets classified as fast or slow healing in each group

Group	Fast Healing n (%)	Slow Healing n (%)
Control – Day 1	0 (0.0%)	8 (100.0%)
Control – Day 3	0 (0.0%)	8 (100.0%)
Omega-3 – Day 1	8 (100.0%)	0 (0.0%)
Omega-3 – Day 3	8 (100.0%)	0 (0.0%)

This secondary analysis confirms that the administration of omega-3 from *Chanos chanos* oil extract significantly reduces neutrophil infiltration in Wistar rats. The factorial statistical approach emphasizes that omega-3 effectively suppresses early inflammatory cell counts independently of time, with both factors showing substantial effect sizes. Neutrophils are critical for initial wound decontamination; however, their timely clearance is imperative to avoid chronic inflammation [22], [23]. Lower neutrophil counts in the omega-3 cohort by day 3 suggest an accelerated resolution of the inflammatory phase [24], [27].

The anti-inflammatory properties of omega-3 PUFAs involve the suppression of pro-inflammatory cytokines and the synthesis of specialized pro-resolving mediators (SPMs) [13], [26]. While the present data demonstrate massive effect sizes (η_p^2 =0.97), these results must be interpreted with caution.

Several critical limitations apply to this study. First, the small sample size (N=32) inherently increases the risk of inflation bias (often termed the "Winner's Curse"), potentially leading to an overestimation of the true effect size in the general population [28]. Second, because this study relies on secondary aggregated data, the exact computation of confidence intervals for effect sizes and the execution of penalized regression models (e.g., Firth's logistic regression) could not be performed without raw primary data, limiting the robustness of our predictive classification. Furthermore, significant translational limitations exist; the wound contraction and healing physiology in rodents differ fundamentally from human alveolar bone healing.

Therefore, while these findings suggest that omega-3 extracts may foster a favorable microenvironment for early inflammation resolution, it is premature to definitively claim direct clinical applicability. Future prospective research involving broader reparative markers, larger sample sizes, and ultimately human clinical trials is required to validate whether omega-3 interventions can serve as a reliable adjunctive therapy for post-extraction healing.

4. Conclusion

This statistical re-evaluation corroborates that omega-3 fatty acids from *Chanos chanos* extract significantly reduce early neutrophil counts in the post-extraction sockets of Wistar rats. Both treatment and healing time independently modulated inflammatory cell resolution. However, given the limitations associated with the small sample size and animal model constraints, these findings strictly suggest a potential biological mechanism that requires further translational validation before clinical application can be considered.

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