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RESEARCH ARTICLE

An Ex-Vivo Study of Photobiomodulation Effects on Hematological and Inflammatory Markers in Breast Cancer Patients

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Abstract

Introduction: Photobiomodulation with low-level laser therapy has been demonstrated immunomodulatory and anti-inflammatory effects in various clinical settings. However, its systemic effects on hematological parameters in breast carcinoma patients remain underexplored. Objective: To evaluate the impact of LLLT on immune and inflammatory blood markers in breast carcinoma patients, with a focus on fluence-dependent responses based on varying exposure durations. **Materials and Methods:** One hundred samples of venous blood from breast carcinoma patients were randomly assigned to either a non-irradiated control group or one of the experimental groups that were treated with a laser. Experimental group samples were subjected ex vivo to an 810 nm, 500 mW near-infrared laser for 1, 1.5, 2, and 2.5 minutes, corresponding to energy densities of 30, 45, 60, and 75 J/cm². We compared important hematological measurements total white blood cell (WBC), lymphocyte (LYM), granulocyte percentage (GRA %), hemoglobin content (HGB), and platelet (PLT) and assessed means between groups by one-way ANOVA and suitable post hoc tests. **Results:** LLLT resulted in a significant decrease in WBC, LYM, HGB, and PLT values across all irradiated samples compared to controls. In contrast, GRA% showed a consistent and significant increase in all laser-treated groups. No dose-response trend was observed among the laser durations, suggesting a threshold biological effect. **Conclusion:** Ex vivo LLLT alters key hematological indices in blood from breast carcinoma patients, marked by immune cell redistribution and mild erythrocyte suppression. These outcomes support the potential of LLLT to moderate inflammatory in addition to immune responses, warranting further investigation in in vivo and clinical settings.

Keywords: Low-Level Laser Therapy- Breast Cancer- Hematological Parameters- Immunomodulation

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Introduction

Breast cancer is the most common woman's cancer and is diagnosed in about 2.3 million new cases and over 685,000 deaths every year [1]. Regardless of technological development in early detection, surgical techniques, targeted therapies, and adjuvant treatment regimens, breast cancer remains a significant burden to public health due to metastasis, immunosuppression, and systemic inflammation that are often associated with the disease as well as its treatments [2]. Increased interest exists, therefore, in the development of adjunctive, non-invasive treatments that would complement conventional treatments with reduced side effects and improved quality of life.

Low-level laser therapy (LLLT), or photobiomodulation therapy (PBMT), is an efficacious, multifaceted, biophysical modality of therapy with applications from wound healing to musculoskeletal disorders, to the management of neuropathic pain, to chemoprevention or radiotherapy-induced mucositis prevention [3, 4]. LLLT refers to the use of red or near-infrared light of low power densities to modify biological activity without inducing thermal damage [5]. At the cellular level, LLLT is thought to act primarily by photons being absorbed by cytochrome c oxidase in the mitochondrial electron transport chain, thereby raising ATP production, modulating ROS levels, and exciting transcription factors for inflammation and immunomodulation [3, 6].

The anti-inflammatory and immunomodulatory effects of LLLT have been extensively demonstrated

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through in vivo as well as in vitro studies. It has been found to modulate leukocyte migration, enhance phagocytic activity, and inhibit the release of pro-inflammatory cytokines, including TNF-α and IL-6 [7]. All these actions demonstrate that LLLT can play an integral role not just in relieving symptoms but also in modifying the tumor microenvironment and systemic immune status of cancer patients.

In the clinical setting, photobiomodulation therapy has become an increasingly accepted supportive intervention in the field of oncology, particularly to prevent and manage oral mucositis in patients receiving chemotherapy or radiotherapy, as confirmed by the Multinational Association of Supportive Care in Cancer (MASCC) in addition to the International Society of Oral Oncology (ISOO) [8]. Other reports support its efficacy in treating breast cancer–related lymphedema, as well as reducing patients' radiation dermatitis, pain, and tissue recovery [9]. Widespread integration of PBM therapy into oncology practice remains limited for many reasons, including the absence of standardized treatment guidelines related to wavelength, fluence, and time of exposure, as there has yet to be conclusive evidence from clinical trials in bigger institutions on safety and systemic immunological effects gains associated with long-term PBM treatment. Another factor is the concern that PBM treatment may also inadvertently stimulate tumor proliferation under inappropriate dosing circumstances, requiring more rigorous mechanistic and clinical studies [10].

However, while tissue repair and inflammatory effects of LLLT have been well documented in a local manner, the systemic effects of LLLT of particular interest to hematological markers of immune and inflammatory status are underinvestigated in cancer patients.

Critical hematological parameters white blood cells (WBC), lymphocytes (LYM), granulocytes (GRA), hemoglobin (HGB), and platelets (PLT) are important indicators of immune competence, systemic inflammation, and disruption of tumor-associated blood cell production [11, 12]. These markers are not only followed regularly during cancer therapy but also provide prognostic information. For instance, elevation in neutrophil-to-lymphocyte ratio (NLR) has been observed to portend poor survival in breast cancer and can signal a tumor-promoting inflammatory milieu [13, 14]. Alterations in platelet counts are also connected with tumor growth and metastatic potential by platelet growth factors and immune evasion [15].

The purpose of this research is to investigate the hematologic and immunologic effects of LLLT on patients with breast cancer through the measurement of WBC, LYM, GRA, HGB, and PLT in various laser exposure times. Through proper statistical analysis and correlating it with laser dose, we hope to identify whether LLLT has systemic immunomodulatory effects that are clinically significant.

Materials and Methods

Sample Collection

All procedures were carried out in accordance with ethical standards set by institutions and international ethical standards, and approved by the Institutional Review Board (Approval No. 23, dated 17 February 2025, Babil, Iraq). This was a laboratory-based, ex-vivo experimental investigation utilizing the peripheral blood samples from patients with breast carcinoma. A sample of 100 patients participated in the study and were manually randomized at a 1:1 ratio into control (no irradiation) or experimental (laser irradiation) groups, with 50 patients per group. Randomization was achieved by simple random (hand-drawn) methods.

After blood was taken, the coordinator who was not engaged in the analytical procedure applied a coded, non-informative label to each tube, thereby blinding laboratory personnel who handled the sample and the analysis of data.

Inclusion criteria: Patients were adults (≥18 years) with histologically proven breast carcinoma.

Exclusion criteria: Patients were excluded if they had any of the following: active febrile illness or infection in the last 14 days; confirmed hematologic disorders (for example, myeloproliferative disease, hemoglobinopathies); platelet disorders or dysfunction; autoimmune disease requiring treatment with systemic therapies; use of systemic corticosteroids or immunosuppressive medications in the last 30 days; transfusion of blood within the last 30 days; pregnant or nursing; uncontrolled renal or hepatic dysfunction; used any medications that produced photosensitization that clearly interfered with photobiomodulation treatment (PBMT). The rationale for these exclusions was to limit confounding factors associated with hematologic and inflammatory measures. After blood was taken, the coordinator who was not engaged in the analytical procedure applied a coded, non-informative label to each tube, thereby blinding laboratory personnel who handled the sample and the analysis of data.

Each participant underwent blood collection at the site of involvement, by qualified phlebotomists using standard sterile technique to minimize contamination and hemolysis (5mL of peripheral venous blood into EDTA tubes). To reduce pre-analytical variability, laser irradiation took place within 1 hour of blood collection. All blood samples were stored at room temperature (20–24 °C), shielded from ambient light, and the sample was similarly gently inverted 8-10 times to mix the anticoagulant in the blood sample immediately before laser irradiation.

Splitting of groups

The experimental group was split into four groups based on the exposure time of the laser:

Group A: 1 minutes
Group B: 1.5 minute
Group C: 2 minutes
Group D: 2.5 minutes

This stratification was to identify the dose-response relationship of exposure time to laser with hematological changes. Through investigation of these time-dependent effects, the study aimed to provide information about the optimal therapeutic window and the affected biological process with laser treatment.

Laser Irradiation Procedure

A low-level laser device operating at 810 nm and producing 500 mW (near-infrared spectrum) was used to irradiate ex vivo whole-blood samples. The 810 nm wavelength was chosen due to significant literature supporting documented advantages for photobiomodulation, such as the improvement of mitochondrial respiration and modulatory effects on oxidative stress, inflammation, and the immune system. Venous blood was drawn from each participant and aliquots were placed into sterile clear flat bottom tubes that allowed light to penetrate evenly. The laser was placed perpendicular to the surface of the tube and was restricted to a laser source distance of 1 cm to ensure repeated exposure geometry. The illuminated spot area was restricted to 1 cm² which produced a total power density (irradiance) of 500 mW/cm².

To assess the dose-response relationship, experimental samples were divided further and exposed to laser light for periods of 1.0, 1.5, 2.0, and 2.5 minutes, representing the following energy densities (fluence):

30 J/cm² (1.0 min)
45 J/cm² (1.5 min)
60 J/cm² (2.0 min)
75 J/cm² (2.5 min)

Energy density (fluence) was determined using the following formula:

Energy Density (J/cm²)=Power (W)×Time (s)÷Area (cm²)

Following irradiation, each sample was mustered for hematological analysis to evaluate acute cellular response. All procedures were completed under aseptic conditions and underwent duplicate calibration before starting each session to confirm dose accuracy and reproducibility. The control group samples were treated under similar conditions but did not undergo exposure to the laser light.

Output power at the sample plane was measured before the start of irradiation and at the end of treatment using a thermopile power meter to confirm both stability and accuracy. Effective spot area (1 cm²) was confirmed for each setup using the mobile camera cross check method to confirm beam spot alignment and uniformity.

This standardized protocol resulted in precise laser dose, consistent energy delivery, and high reproducibility for experimental quality required to evaluate the biological effects of photobiomodulation on hematological and inflammatory indices in breast cancer patients.

Statistical Analysis

Statistical analysis of data was performed with SPSS version 25.0 (IBM Corp., Armonk, NY, USA) and GraphPad Prism 9.0 (GraphPad Software, USA). The Shapiro-Wilk test was applied to assess data distribution normality. Between-groups comparisons of Control and laser-treated were made with independent-sample t-tests for parametric data and with Mann–Whitney U tests for non-parametric data. Within-group comparisons of pre- and post-LLLT used paired t-tests or Wilcoxon signed-rank tests, depending on the distribution. One-way ANOVA with post hoc Tukey's test was applied to compare the effect of varying laser exposure durations, whereas Kruskal–Wallis tests and the following post hoc Dunn's test were applied to non-parametric variables. A p-value of <0.05 was utilized in determining statistical significance.

Results and Discussion

White Blood Cell (WBC) Count (×10³/µL)

White blood cell (WBC) levels were significantly changed after low-level laser therapy (LLLT). The control group had a greater mean WBC level (5.25 ± 1.95 ×10³/µL) compared with all four groups irradiated with lasers, each having a mean of 4.29 ± 1.93 ×10³/µL (Table 1). One-way ANOVA detected a statistically significant difference among the groups ($p = 0.049$), whereas the Kruskal–Wallis test detected a borderline non-significant difference ($p = 0.068$).

As noted in Figure 1, there were significantly lower

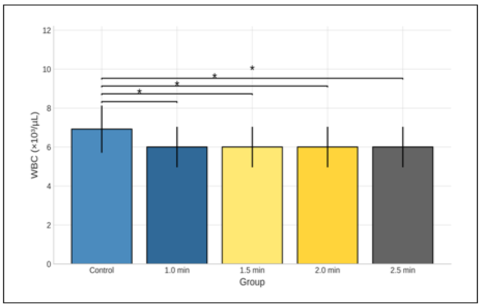

Figure 1. Total White Blood Cell (WBC) Count with Photobiomodulation in *ex vivo* Blood Samples from Breast Cancer Patients. Bars represent mean ± standard deviation (SD) in each group. The control group (no irradiation) is compared with irradiated samples for 1.0, 1.5, 2.0, 2.5 minutes, respectively, at 810 nm, 500 mW . Asterisks (*) denote statistically significant comparisons against the control ($p < 0.05$)

Table 1. White Blood Cell (WBC) Count (×10³/µL) in Experimental Groups and Statistical Comparison

Group	Mean ± SD (×10³/µL)	ANOVA vs Control (p-value)	Kruskal–Wallis vs Control (p-value)	Pairwise vs Control (p-value)
Control	6.92 ± 1.21	0.049	0.068	–
1.0 min	6.00 ± 1.04			0.017
1.5 min	6.00 ± 1.04			0.017
2.0 min	6.00 ± 1.04			0.017
2.5 min	6.00 ± 1.04			0.017

Table 2. Lymphocyte Count (×10³/µL) Among Experimental Groups and Statistical Comparisons

Group	Mean ± SD (×10³/µL)	ANOVA vs Control (p-value)	Kruskal–Wallis vs Control (p-value)	Pairwise vs Control (p-value)
Control	2.46 ± 0.65	0.016	0.063	–
1.0 min	2.00 ± 0.40			0.009
1.5 min	2.00 ± 0.40			0.009
2.0 min	2.00 ± 0.40			0.009
2.5 min	2.00 ± 0.40			0.009

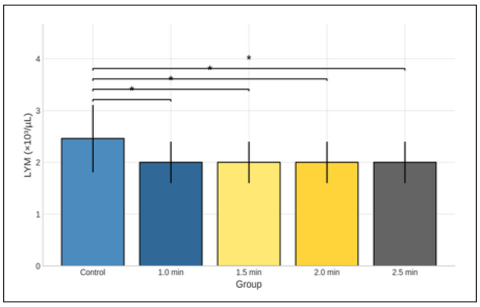

Figure 2. Effect of Photobiomodulation on Lymphocyte (LYM) Counts in *ex vivo* Blood Samples Derived from Breast Cancer Patients. Bars denote mean ± standard deviation (SD) for each group. The controls (no irradiation) were compared against irradiance at 810 nm, 500 mW for 1.0, 1.5, 2.0, and 2.5 minutes. Asterisks (*) denote statistically significant comparisons against the control ($p < 0.05$)

WBC counts for all laser-treated groups compared to the control group (p = 0.016 for all group comparisons), and none of the durations of laser exposure (1.0, 1.5, 2.0, and 2.5 minutes) differed from one another. This uniform suppression indicates that LLLT's impact on leukocyte count may be optimal with very short exposure times.

In this study, the research revealed a consistent reduction of the white blood cell (WBC) count in ex vivo blood samples from breast carcinoma patients following LLLT. Though the WBC count of the control group was measured at 5.25 ×10³/µL, all treated groups (1.0 to 2.5 minutes) revealed a significantly reduced and consistent mean value of 4.29 ×10³/µL.

These findings are in agreement with those in the literature reporting anti-inflammatory actions of LLLT. Near-infrared photobiomodulation was found to influence immune cell function via modulation of mitochondrial activity namely, cytochrome c oxidase activation leading to downstream changes in ATP synthesis and redox-sensitive transcription factors [6]. Inhibition of pro-inflammatory cytokines TNF-α and IL-1β, and interference with leukocyte migration, have been consistently reported in laser-irradiated models [5, 16].

3.2 Lymphocyte (LYM) Count (×10³/µL)

Lymphocyte (LYM) count showed a statistically significant reduction in all irradiated samples compared to the control group. As presented in Table 2, the control group had a mean value of 2.46 ± 0.65 ×10³/µL, while all laser groups (1.0–2.5 min) consistently reported a lower mean of 2.00 ± 0.40 ×10³/µL. One-way ANOVA revealed a statistically significant group difference (p = 0.016), while the Kruskal–Wallis test approached but did not reach significance (p = 0.063). Post hoc pairwise t-tests confirmed significant reductions in all irradiated groups compared to the control (p = 0.009), with no significant differences between the irradiated groups themselves (p = 1.0). This consistent suppression is visualized in Figure 2.

The results indicate that LLLT has a statistically significant inhibitory influence on blood lymphocyte count in breast carcinoma patients. The findings suggest that a very brief duration of exposure to laser (1 minute) is sufficient to cause detectable immunomodulation.

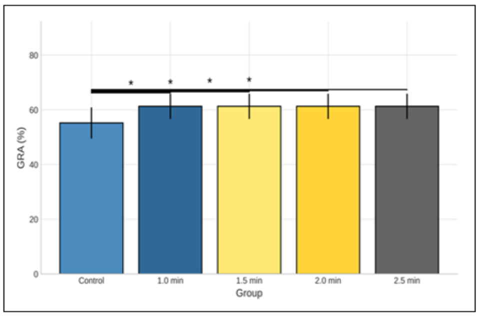

Figure 3. Effect of Photobiomodulation in *ex vivo* Blood Samples from Breast Cancer Patients on Granulocyte Percentage (GRA %). Bars represent mean ± SD in each group. The control group (no irradiation) is compared with irradiated samples for 1.0, 1.5, 2.0, 2.5 minutes, respectively, at 810 nm, 500 mW . Asterisks (*) denote statistically significant comparisons against the control ($p < 0.05$).

Table 3. Granulocyte Count (GRA%) Throughout Experimental Groups and Statistical Comparisons

Group	Mean ± SD (%)	ANOVA vs Control (p-value)	Kruskal–Wallis vs Control (p-value)	Pairwise vs Control (p-value)
Control	55.2 ± 5.7	0.003	0.012	–
1.0 min	61.3 ± 4.6			0.004
1.5 min	61.3 ± 4.6			0.004
2.0 min	61.3 ± 4.6			0.004
2.5 min	61.3 ± 4.6			0.004

Table 4. Hemoglobin (HGB, g/dL) by Experimental Groups and Statistical Comparison

Group	Mean ± SD (g/dL)	ANOVA vs Control (p-value)	Kruskal–Wallis vs Control (p-value)	Pairwise vs Control (p-value)
Control	12.91 ± 0.41	0.002	0.006	–
1.0 min	12.41 ± 0.38			0.002
1.5 min	12.41 ± 0.38			0.002
2.0 min	12.41 ± 0.38			0.002
2.5 min	12.41 ± 0.38			0.002

Mechanistically, near-infrared LLLT has been associated with mitochondrial upregulation and modulation of the redox pathways, possibly suppressing lymphocyte proliferation and migration by ROS-sensitive transcription cascades [6]. IL-2 and interferon-γ downregulation have been reported in previous research following LLLT, supporting its immune cell deactivation effect [5].

Granulocyte (GRA %)

As indicated in Table 3, the control group had a mean GRA% of 55.2 ± 5.7, while all irradiated groups had significantly higher and uniform values of 61.3 ± 4.6. One-way ANOVA showed a statistically significant difference between groups ($p = 0.003$), supported by the Kruskal–Wallis test ($p = 0.012$). Pairwise comparisons revealed that all laser-treated groups were significantly different from the control ($p = 0.004$), while no differences were observed between the laser durations ($p = 1.0$). This marked increase in granulocyte ratio is illustrated in Figure 3, likely reflecting a photobiomodulation-induced activation or redistribution of innate immune cells. The figure highlights a clear shift toward higher GRA% in all irradiated groups, with statistically significant differences from control, shown by uniform elevation and asterisks above bars.

Our findings reveal that LLLT induces a large elevation in the percentage of granulocytes (GRA %) in ex vivo blood from patients with breast carcinoma. While the control group had a mean GRA% of 55.2 ± 5.7, all of the irradiated groups displayed a consistent and significantly higher mean of 61.3 ± 4.6, irrespective of exposure duration. LLLT has been shown to influence leukocyte migration through increased NO production and chemokine gradient modulation [17-19]. Alteration in mitochondrial redox status also may trigger granulocyte release from bone marrow niches or marginal zones in the periphery [20].

Hemoglobin (HGB, g/dL)

Hemoglobin (HGB) concentration exhibited a small but consistent decline in all irradiated samples compared to the control. As reported in Table 4, the control group had a mean HGB level of 12.91 ± 0.41 g/dL, while all laser-treated groups showed a uniform reduction to 12.41 ± 0.38 g/dL, regardless of exposure time. This change was statistically significant (ANOVA $p = 0.002$; Kruskal–Wallis $p = 0.006$), and post hoc tests confirmed significant differences between control and each laser-treated group ($p = 0.002$). This trend is clearly illustrated in Figure 4,

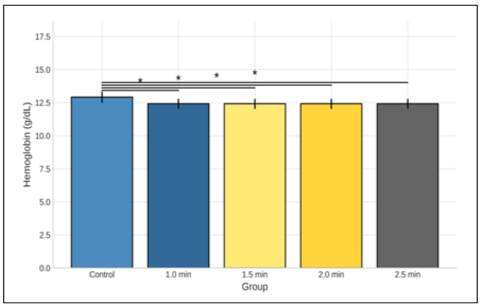

Figure 4. Effect of Photobiomodulation in *ex vivo* Blood Samples from Breast Cancer Patients on Hemoglobin (HGB, g/dL). Bars represent mean ± SD in each group. The control group (no irradiation) is compared with irradiated samples for 1.0, 1.5, 2.0, 2.5 minutes, respectively, at 810 nm, 500 mW . Asterisks (*) denote statistically significant comparisons against the control ($p < 0.05$).

suggesting a potential effect of LLLT on red blood cell dynamics or hemoglobin integrity. Figure 4 reveals a modest yet uniform decline in HGB values across laser exposures, with tight SD ranges and visual confirmation of the statistically significant difference.

Hemoglobin is a redox-sensitive protein, and laser-induced modification may serve as a marker of acute modulation of erythrocyte membrane stability or oxidative balance. Near-infrared laser therapy has been shown to increase intracellular ROS and nitric oxide (NO) and temporarily affect hemoglobin oxygen affinity and cell deformability [21, 22].

Platelet (PLT ×10³/µL) Count

Platelet (PLT) count was significantly reduced in all irradiated samples following low-level laser therapy. As presented in Table 5, the control group had a mean PLT count of 238.48 ± 7.57 $\times 10^3/\mu L$, while all laser-treated groups displayed identical reduced values of 215.14 ± 2.00 $\times 10^3/\mu L$, regardless of exposure duration. One-way ANOVA showed an extremely significant difference ($p = 1.02 \times 10^{-102}$), with confirmation by the Kruskal–Wallis test ($p = 3.20 \times 10^{-25}$). Pairwise t-tests also revealed that all irradiated groups differed significantly from the control ($p = 3.01 \times 10^{-28}$), but not from each other ($p = 1.0$). This uniform reduction suggests a threshold response in platelet count to LLLT, potentially reflecting photonic influence on platelet reactivity or survival. The trend is illustrated in Figure 5. Figure 5 illustrates a marked and consistent drop in PLT values across all laser exposure times, supporting the statistically robust findings.

Table 5. Platelet Count (PLT ×10³/µL) Across Experimental Groups and Statistical Comparisons

Group	Mean ± SD (×10³/µL)	ANOVA vs Control (p-value)	Kruskal–Wallis vs Control (p-value)	Pairwise vs Control (p-value)
Control	238.48 ± 7.57	1.02×10^{-102}	3.20×10^{-25}	–
1.0 min	215.14 ± 2.00			3.01×10^{-28}
1.5 min	215.14 ± 2.00			3.01×10^{-28}
2.0 min	215.14 ± 2.00			3.01×10^{-28}
2.5 min	215.14 ± 2.00			3.01×10^{-28}

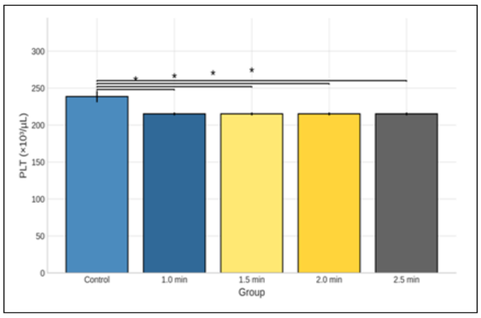

Figure 5. Effect of Photobiomodulation in *ex vivo* Blood Samples from Breast Cancer Patients on Platelet Count (×10³/µL). Bars represent mean ± SD in each group. The control group (no irradiation) is compared with irradiated samples for 1.0, 1.5, 2.0, 2.5 minutes, respectively, at 810 nm, 500 mW . Asterisks (*) denote statistically significant comparisons against the control ($p < 0.05$).

Photobiomodulation impacts platelet aggregation, membrane potential, and granule release via nitric oxide (NO) and mitochondrial ATP synthesis [23, 24]. Reducing circulating platelet count might be proof of photonic modulation of the surface receptors of the membrane that is detrimental to platelet reactivity or adhesiveness capacity [25-27].

The current investigation shows that low-level laser therapy (LLLT) applied ex vivo to peripheral blood from patients with breast cancer causes observable changes in multiple important hematological parameters. LLLT was associated with decreased total white blood cell (WBC) and lymphocyte (LYM) counts, increased granulocyte percentage (GRA%), a small decrease in hemoglobin (HGB), and a large decrease in platelets (PLT). The responses to LLLT were consistent across exposure duration suggesting a biological response that is threshold dependent rather than simply linear dose–response.

The decrease in lymphocytes and increase in granulocytes, considered from a functional immunological perspective, could suggest a temporary transition from adaptive immunity to innate immunity dominance [28, 29]. The changes observed may also just represent a mobilization response related to acute inflammation or stress. Of course we would be concerned about transiently immunosuppressive effects, especially in patients already at risk for immune suppression due to chemotherapy or radiation therapy. The decline in platelet count, although modest, could suggest a potential alteration in hemostatic balance or megakaryocyte function, and should be thoughtfully examined in the context of cancer-related thrombocytopenia [30, 31].

Hematological perturbations could have functional immune implications and lead to effects such as diminished lymphocyte-mediated immune surveillance or temporary neutrophilia, which would compromise the tumor microenvironment or risk infection [32, 33] . While the present study was conducted ex vivo, it does not necessarily reflect hematologic toxicity in vivo, but rather the intrinsic cellular sensitivity of circulating immune cells to the photobiomodulation dosage used herein. Future translational studies, including controlled in vivo and clinical studies, will need to establish if different impacts are observed in the clinic or in patients when LLLT is used as an adjunct therapy, whether delivered foe systemically or locally.

In considering this data, particularly for immunocompromised or cytopenic patients, it is important to consider the potential risks of exposure to LLLT, especially when one considers that transient changes in leukocyte or platelet counts, could lead to increased sensitivity for these patient populations. LLLT therapy is considered safe and non-thermal, but inappropriate energy densitites or exposure time could produce tissue energy burdens that are above the biostimulatory dose window, and could produce bioinhibitory or cytoxicity responses. The clinical implication of this scenario warrants dosages being time and energy calibrated, particularly related to clinical treatment plan builds or dosing guidelines [34, 35].

With regard to safety implications, the data suggest that LLLT at a wavelength of 810 nm and a power of 500 mW can elicit robust biological effects on blood cell populations even in the context of moderate fluence (30-75 J/cm²). Therefore, it is important to establish a therapeutic safety margin to avoid exacerbated hematologic modulation. The concept of a biphasic dose–response (Arndt-Schulz curve) is particularly relevant here, as sub-threshold or excessive laser dosing may lead to opposite biological mechanisms [36, 37].

From these findings, it is clear that hematological cells have a therapeutically beneficial effect and, furthermore, biological sensitivity to photobiomodulation. Additional studies utilizing functional immune assays (e.g., cytokine profiles, phagocytic activity, T-cell activation) and clinical outcomes (e.g., infection rate, wound healing, tolerance to treatment) are needed to demonstrate whether the observed interesting shifts in hematology cells equate to clinically significant immune modulation. Future investigations will be important for informing LLLT safety parameters, determining dose windows, and safely bringing photobiomodulation into breast cancer supportive care.

In conclusion, the present experiment demonstrates that LLLT, if administered ex vivo to peripheral blood from breast carcinoma patients, induces dramatic alterations in several significant hematological parameters. Specifically, laser therapy induced:

- Decrease in white blood cell (WBC) and lymphocyte (LYM) number
- Dramatic rise in the percentage of granulocytes (GRA%)
- Small decline in hemoglobin (HGB) levels
- Prominent inhibition of platelet (PLT) count

These effects were present invariably irrespective of laser exposure durations, suggestive of a threshold-dependent biological response and not a progressive dose-related pattern.

Acknowledgments

Statement of Transparency and Principles

• The authors declare no conflict of interest.

• The study was approved by the Research Ethics Committee of the authors' affiliated institution.

• The study data are available upon reasonable request.

• All authors contributed to the implementation of this research.

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