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THE ROLE OF PHOTOBIOMODULATION IN MODULATING CYTOKINE DYNAMICS: INSIGHTS INTO IL-10, TNF-α, AND IL-6 REGULATION

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Abstract *Photobiomodulation (PBM) represents an emerging non-invasive clinical practice for treating inflammatory conditions through therapeutic benefits. PBM achieves its results by controlling cytokine distribution alongside regulating biomarkers that increase or decrease inflammation. Cytokine measurements in human peripheral blood mononuclear cells (PBMCs) under inflammatory stimulation showed that PBM controls interleukin-10 (IL-10) and tumor necrosis factor-alpha (TNF-α) and interleukin-6 (IL-6) production. PBMCs derived from ten healthy donors to establish three separate groups: control PBMCs, inflamed PBMCs injected with 1 g/mL lipopolysaccharide (LPS), and PBMCs submitting to PBM treatment at 810 nm wavelength with 100 mW/cm2 and 10 J/cm2 following LPS stimulation. An enzyme-linked immunosorbent assay (ELISA) measured cytokine quantification and quantitative real-time PCR (qPCR) evaluated gene expression changes. one-way ANOVA statistical analysis was used with a p-value set at (p≤0.05). PBM therapy leads to substantial changes in the expression of cytokines. IL-10 levels in PBM-treated monocytes measured 45.64±3.22 pg/mL and exceeded inflammatory group values at 22.31±2.83 pg/mL shows statistical significance (p=0.014). Similarly, PBM decreases TNF-α and IL-6 to 18.99±2.15 pg/mL (p=0.001) and 23.44±3.03 pg/mL (p=0.023) from the inflammatory phase. The resulting gene expression showed that PBM cells released 2.88 times more IL-10 (p=0.011) than inflammatory cells while producing significantly reduced levels of TNF-α and IL-6 (p=0.035) and IL-6 (p=0.019). PBMs successfully adjust cytokine relationships through elevated IL-10 production while reducing the presence of TNF-α and IL-6 thereby proving their ability to treat inflammatory states. The results validate PBM's capability for restoring immune equilibrium thus establishing its potential value for clinical implementation.*

Keywords*:* *Photobiomodulation; IL-10; TNF-α; IL-6; Cytokine Dynamics; Inflammation; Immune Modulation*

Introduction

The body's tissue repair and defense against illness functions through a complex natural reaction called inflammation. Modern healthcare recognizes chronic inflammation along with autoimmune disorders, cardiovascular diseases, and neurodegenerative conditions as major contributors to diseases (Glass, 2021). Cytokines function as fundamental inflammation mediators which control immune system responses. Studies show that Interleukin-10 functions as an anti-inflammatory cytokine although pro-inflammatory cytokines interleukin-6 and tumor necrosis factor-alpha play vital roles in chronic inflammation development (Gupta et al., 2015, Da Rosa et al., 2023). Low-level light therapy (LLLT) has brought photobiomodulation (PBM) into prominence as a non-invasive treatment option because it activates successful cell operations including inflammation management. Through its activation of cytochrome C oxidase (CCO) in mitochondria PBM generates higher levels of ATP while facilitating reactive oxygen species (ROS) transfers that lead to cellular metabolic benefits. Gene expression regulation moves downstream through the signal network by carrying these molecular changes along and controlling the production of cytokines (Ghasemi et al., 2014, Hamblin, 2017, Ayala et al., 2021). Research findings show that PBM augments IL-10 transcription and decreases TNF-α and IL-6 production. PBMs trigger anti-inflammatory effects for reducing inflammation (Zanotta et al., 2020). The application of PBM results in enhanced levels of angiogenic factors that parallel vascular endothelial development factor (VEGF) to speed up tissue repair and the maintenance of tissues (Zhevago et al., 2006, Catao et al., 2016). Future research is necessary to understand precisely how PBM achieves its cytokine regulatory outcomes and how practitioners can

potentially utilize these findings. The purpose of this research involves examining how PBM affects human PBMCs' expression levels of TNF-α and IL-6 together with IL-10 when exposed to inflammatory conditions (Wang et al., 2018, Sun et al., 2020).

Methods

Study Design and Ethical Approval

The main focus of this experimental work studied how photobiomodulation (PBM) affected the regulation of cytokines throughout inflammatory human peripheral blood mononuclear cells (PBMCs). The Institutional Review Board of the School of Pain and Regenerative Medicine (SPRM), The University of Lahore, approved the research and contestants gave their informed consent before participation.

Sample Collection and PBMC Isolation

Five milliliters of blood through sterile EDTA vacutainers from a study participant of the age range 20-40 (n=10). Ficoll-Paque™ from GE Medical Care enabled the retrieval of PBMCs by density-gradient centrifugation. Later the cells received a PBS wash before being integrated into Gibco's RPMI-1640 medium with 10% FBS, 2 millimeter L-glutamine, and the supplemental antibiotics of 100 U / mL penicillin and 100 g / mL streptomycin.

PBM Treatment Protocol

A density of 106 cells per well was employed to seed PBMCs onto the surface of the 24-well traditional plate. The research was divided into three major sections through categories.

- **Control Group:** PBMCs cultured without any treatment.
- **Inflammatory Group:** Administered 1 μg/mL LPS to PBMCs to create inflammatory responses.
- **PBM-Treated Group:** Following LPS stimulation at 1 μg/mL PBMC cells received PBM treatment.

PBM was administered using a Class IV laser device with the following parameters:

- Wavelength: 810 nm
- Power Density: 100 mW/cm²
- Energy Density: 10 J/cm²
- Spot Size: 1 cm²

During the 100-second PBM session, the cells received exposure which continued for the subsequent 24 hours.

Cytokine Quantification

The assay kit from Thermo Fisher Analytical determined the levels of cytokines (IL-10, TNF-α, and IL-6) in the collected supernatant by enzyme-linked immunosorbent assay standards at 450-nanometer wavelengths.

Gene Expression Analysis

Cells extraction using Invitrogen's TRIzol™ reagent. The NanoDrop™ spectrophotometer determined both RNA concentration and purity levels. The High-Capacity complementary DNA Reverse Transcription Kit produced by Biosystems enabled complementary DNA synthesis. A SYBR™ Green PCR Master Mix solution from Thermo Fisher Scientific combined with a StepOnePlus™ Real-Time PCR System from Applied Biosystems performed the quantifiable PCR run with specific primers. The evaluation of gene expression utilised GAPDH as a house gene reference while the 2Ct method determined relative expression levels.

Statistical Analysis

All research treatments ran three times alongside their statistical results shown as mean standard deviations. GraphPad Prism version 9.0 performed the statistical analysis. Analytical measurements began with Tukey's post hoc test followed by one-way ANOVA parameter evaluation for categorical comparisons. Results consider statistical significance at p-value (≤ 0.05).

Results

The cytokine levels measured across the Control, Inflammatory, and PBM-treated groups revealed statistically significant changes. Anti-inflammatory cytokine IL-10 was significantly increased in the PBM-treated group (45.64±3.22) pg/mL compared to the Inflammatory group (22.31±2.83 pg/mL) indicating a 2-fold increase (p=0.014). The control group has a baseline level of (10.23±1.57) pg/mL. The TNF-α of PBM resulted in a significant decrease in the proinflammatory cytokine TNF-α to (18.99±2.15) pg/mL from (47.52±4.37) pg/mL in the Inflammatory group (p=0.001). The control group maintain a minimum concentration of (5.77±0.93) pg/mL. PBM support was decreased in IL-6 levels (23.44±3.03 pg/mL) compared to the Inflammatory group (59.74± 5.66 pg/mL, p-value=0.023) versus control group (8.19±1.35) pg/mL. Additional insight into PBM outcomes is provided by the to fold variations in IL-10, TNF-α, and IL-6 gene expression. IL-10 gene expression 2.88-fold increase (p=0.011) in IL-10 utterance was detected in the PBM-treated group compared to the inflammatory group. The present study reflects a strong increase in anti-inflammatory cytokine gene expression in PBM treated group. TNF-α. Gene expression PBM downregulated TNF-α expression by 1.99-fold in comparison with the inflammatory group (p=0.035), which contributes to the suppression of the proinflammatory pathway. IL-6 gene expression correspondingly; PBM reduces IL-6 gene expression by a 2.33-fold relative to the inflammatory group (p=0.019), demonstrating its efficacy in suppressing the inflammatory response. These findings are summarized in Table 1, Figures 1, and 2, showing the PBM influence on cytokine and gene expression.

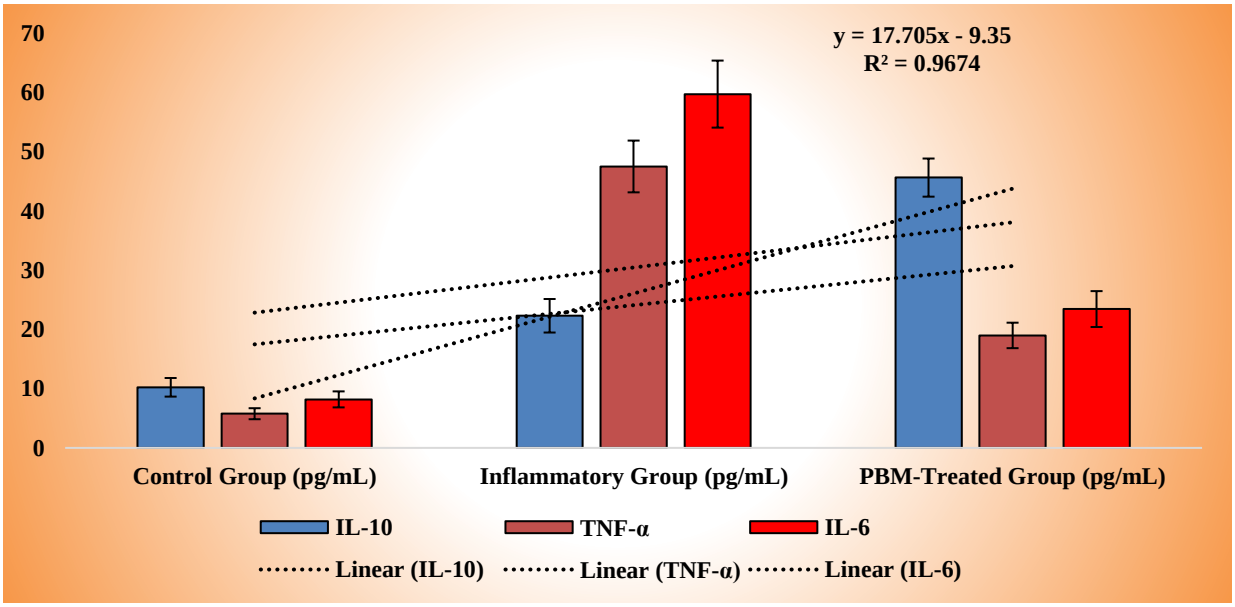

Figure 1: Regulation of interleukin-10 (IL-10), tumor necrosis factor-alpha (TNF-α), and interleukin-6 (IL-6) in human peripheral blood mononuclear cells (PBMCs) by photobiomodulation

Table 1: Cytokine Levels

Cytokine	Control	Inflammatory Group	PBM-Treated Group	p-Value
IL-10 (pg/mL)	10.23±1.57	22.31±2.83	45.64±3.22	0.014
TNF-α pg/mL)	5.77±0.93	47.52±4.37	18.99±2.15	0.001
IL-6 (pg/mL)	8.19±1.35	59.74±5.66	23.44±3.03	0.023

Table 2: Gene Expression

Gene	Fold Change (Inflammatory vs. Control)	Fold Change (PBM vs. Inflammatory)	p-Value
IL-10	2.33±0.02	2.88±0.008	0.011
TNF-α	3.91±0.95	1.99±0.006	0.035
IL-6	4.55±1.22	2.33±0.83	0.019

Discussion

Photobiomodulation (PBM) stands as a promising curative option to control immune system reactions mainly in inflammatory diseases. An assessment of photobiomodulation ability to control cytokine flow in peripheral blood mononuclear cells (PBMCs) focuses on IL-10, TNF-α and IL-6. PBM exposure generated significant improvement of anti-inflammatory cytokine IL-10 while simultaneously reducing proinflammatory cytokines TNF-α and IL-6 according to in-vitro studies (Kozin et al., 2018, Ebrahiminaseri et al., 2021, Cayrol et al., 2022). An elevated level of the cytokine IL-10 in this study matches existing literature about PBM's capability to activate the anti-inflammatory process through trackable sign-marked pathways. Previous studies validate IL-10 as an important mechanism that controls the overreactions of immune cells while protecting homeostasis (Li et al, 2021, Kim et al, 2023). Laboratory work reveals PBM affects transcriptional elements like STAT3 which play a key role in regulating IL-10 production (Hamblin, 2018). The research backs previous studies that demonstrate PBM controls inflammation by releasing IL-10 as a crucial initial process (Cayrol et al., 2022). After PBM therapy, the essential inflammatory mediators TNF-α and IL-6 showed significant reductions. Evidence suggests that these cytokines trigger persistent inflammation which stains tissue integrity within auto-inflammatory disease conditions (Muili et al., 2013, Kwilasz et al., 2016, Wang et al., 2020). By treating the cytokine, PBM utilizes the suppression of the nuclear factor-kappa B (NF_kB) pathway because this pathway functions as a proinflammatory cytokine regulator (Li et al., 2021). The treatment with PBM appears to mediate suppression through both reactive oxygen species (ROS) transition and mitochondrial signaling pathway modification (Song et al., 2012, Ferroni et al., 2020). Research studies based on PBM outcomes in clinical settings alongside preclinical research validate our current findings. Yamada et al., (2020) demonstrated that arthritic patient inflammation decreased due to PBM treatment which improved patient clinical outcomes (Li et al., 2021). Animal models of colitis show reduced inflammation according to (Muili et al., 2012, and Gomes et al.,

2016) via PBM regulation of TNF-α and IL-6 expression. The clinical value of photobiomodulation emerges clearly from these research results demonstrating its utility in managing inflammatory diseases. The cytokine modulation effects of PBM stem from mitochondrial photo acceptors such as cytochrome C oxidase (CCO) which enhances adenosine triphosphate (ATP) production and cell transformation (Hamblin, 2018). Studies suggest that mitochondrial enhancement impacts the MAPK and PI3K/AKT signaling pathways which function as essential control points for cytokines (Wu et al., 2022). Through oxidative stress biomodulation, PBM demonstrates anti-inflammatory outcomes (Tolentino et al., 2022). Our study has produced laudable findings but a restriction in its results cannot be ignored. The investigative approach based on a test tube environment hinders our ability to directly apply findings to structures found within the human body. Research analysis needs to proceed through animal-based studies together with human medical trials to authenticate its previous findings. Our comprehension of PBM restorative abilities can improve with studies about the sensitivity of cytokine response to different dose levels along with long-term behavioral patterns triggered by PBM treatment mechanics. PBM demonstrates its potential therapeutic ability as a cytokine regulatory method by buoying IL-10 and diminishing TNF-α and IL-6 (Hamblin, 2017). Research data supports current PBM analytical methods that demonstrate the system's capacity to regulate immune response patterns while establishing normal immune equilibrium. Future investigations into PBM treatment as an additional therapeutic approach for inflammatory and autoimmune disease management gain their basis from these recent findings.

Conclusion

PBM photobiomodulation demonstrates value as an effective treatment method to control inflammatory conditions which this research study confirms alongside valuable insights about its clinical applications in cytokine activity regulation. IL-10 cytokine production increased in parallel with the experimental group's PBM treatment where inflammation levels. PBM showed successful pro-inflammatory marker suppression through reducing TNF-α and low levels of IL-6. Gene expression validated PBM effects which demonstrated significant IL-10 increase while showing decreases in TNF-α levels. The findings demonstrate PBM improves immune equilibrium through the rapid transfer of anti-inflammatory signaling mechanisms despite its uncommon pro-inflammatory reaction. The present study demonstrates PBM as a safe procedure that non-invasively controls immune responses for the effective treatment of inflammatory illnesses. Further clinical evaluation may be helpful if these findings progress toward developing their healing potential for future applications.

Key contents

- Photobiomodulation and Cytokine Interactions show meaningful transitions between cytokine IL-10, TNF-α, and IL-6 in inflammatory conditions, highlighting the anti-inflammatory potential of PBM.
- Improved IL-10 expression detects a significant increase in IL-10 and gene expression in PBM-treated categories, demonstrating a role in supporting anti-inflammatory responses.
- The suppression of Pro-Inflammatory Cytokines indicates a significant decrease in TNF-α and IL-6 stages and their gene expression later on in the PBM medication, highlighting its ability to reduce inflammation.
- Curative effects PBM has emerged as a non-invasive therapy for controlling cytokine imbalances in inflammatory diseases.
- The statistical significance of key discoveries, including cytokine transitions and gene expression changes, was confirmed by statistically significant p-values, confirming the credibility of the study.

Learning objectives

- Perceive cytokine Modulation by PBM to find out how photobiomodulation regulates cytokine, in particular, IL-10, TNF-α, and IL-6, in inflammatory conditions.
- Identify the Anti-Inflammatory Mechanisms using the mechanism in which PBM increases IL-10 expression while suppressing proinflammatory cytokine TNF-α and IL-6.
- Identify the therapeutic intent of PBM to assess the capability of photobiomodulation as a non-invasive curative intervention to control cytokine-mediated inflammation.
- Identify the effects of PBM on the gene utterance profile of a critical cytokine and its implications on the transition of an inflammatory disease.
- Obtain information on the relevance of statistically valid consequences to ensure a reliable decision on cytokine governance.

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Declaration

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Authors contribution

AM**:** Conceptualization of the study, experimental design, and manuscript writing. SA**:** Data acquisition, statistical analysis, and result interpretation. HS**:** Supervision of laboratory procedures and validation of experimental protocols. SA**:** Writing and revision of the manuscript. QA**:** Technical support, resource management, and administrative oversight. All authors have read and approved the final manuscript.

Conflict of Interest

The authors declare that there is no conflict of interests with regard to this study. There is no conflict of interest in any financial concern.

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Data Availability statement

All authenticated data have been included in the manuscript.

Ethics approval and consent to participate

These aspects are not applicable in this paper.

Consent for publication

Not applicable