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Research Paper

Effect of Helium Neon Laser Irradiation on Calcium Levels in Blood "An Experimental Study"

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Abstract:
This study was conducted to find out the physiological effect of low-level Helium-Neon radiation on serum calcium in human blood. The laser was of 5 mW power, wavelength being 632.8 nm, with a spot size of about 0.03 cm² to ensure uniform exposure. Energy density of 1.0 J/cm² was delivered to the samples. Twenty healthy, nonsmoking volunteers of different age groups from 19 to 65 participated. Blood was drawn, serum was separated by centrifuging at 3000 RPM for about 3 to 5 minutes at 25 °C, and then the serum was divided into four portions: one portion being the control and the three other aliquots putting to laser exposure for 1, 3, and 5 minutes. In results, it was seen that after 1 minute of exposure, there were trivial changes in calcium levels (about 0.2-0.3 mg/dl average increase). Increasing exposure to 3 minutes seemed to noticeably raise variabilities in calcium concentrations (about 0.5-1.5 mg/dl average increase), indicating possible effects on the cell membrane permeability and enzymatic activity. With 5-minute exposure, the results diverged; some continued rising (say, sample 18: from 8.7 to 18.4 mg/dl, and sample 12: from 9.3 to 15.3 mg/dl), but others either stabilized or decreased just slightly. gender-dependent variations were observed: males showed more consistent increases in calcium levels with longer exposure times, whereas females exhibited more varied responses, with some samples showing significant elevations. This significant and differential increase in calcium levels is mainly attributed to the photobiomodulatory effect of the low-level helium-neon laser, which stimulates cellular processes without significant thermal effects.

Keywords: *Helium-Neon Laser, Calcium Levels, Laser Irradiation, samples.*

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I. INTRODUCTION:

Nowadays, the number of calcium-deficient people is rising enormously in the world. Calcium acts as one of the fundamental minerals in the process of hardening of bones, transmission of nerve signals, and muscle contraction (Pravina et al., 2013)[1]. In the body, can be affected by outside factors, imbalanced nutrition on top of modern lifestyle. Calcium makes this one of the major soluble soil minerals and the most abundant element in the human body, with over 99% existing in bones and teeth (Olendorf et al., 1999)[2], with the residual probably being in the blood, muscles, and intercellular fluids; calcium also serves in intercellular cement, allowing cohesion of tissues (Pravina et al., 2013)[1]. Calcium homeostasis is maintained by complicated hormone systems involving parathyroid hormone (PTH), vitamin D, and calcitonin (Nazal et al., 2016)[3]. If disturbed, this calcium balancing mechanism can cause severe health disorders, including muscle weakness, neuromuscular disorders, metabolic dysfunctions, and circulatory problems (Pravina et al., 2013)[1]. Though diet andcalcium levels are controlled by hormones and vitamin D (Nazal et al., 2016)[3], yet these levels external influences, such as radiation exposure. Among these, the use of low-intensity Helium-Neon (He-Ne) lasers—which emit coherent light at a fixed and specific wavelength—has been increasing in medical and therapeutic applications (Haimid et al., 2019)[4]. Several studies have highlighted the properties of this laser in promoting wound healing, relieving pain, and activating biological processes, raising questions about its potential impact on the balance of essential minerals in the human body. This study aims to investigate the potential physiological effects of low-intensity He-Ne laser radiation on calcium concentration in human blood serum, as an effort to address a significant knowledge gap in this critical scientific field. In the absence of prior research specifically addressing the effect of Helium-Neon lasers on

magnesium concentrations in blood, this review will examine studies that explore the broader effects of helium-neon lasers on blood components and related biological processes. This approach aims to provide a contextual framework for understanding the potential influence on magnesium levels. Csele (2004) demonstrated the early impacts of low-power laser treatment on in vitro blood samples, noting changes in both absorption and FTIR spectra, indicating the laser's effect on the chemical properties of blood[5]. Ghadage et al. (2015) later corroborated these findings, reporting similar changes in the blood's chemical makeup due to low-power laser therapy [6].In a related study, de Oliveira et al. (2021) observed significant changes in the numbers of red and white blood cells, offering valuable insights into the effects of low-level laser therapy on blood cells in a rat model [7] "Anju et al. (2019) found that low-level laser therapy significantly increased magnesium and vitamin D levels in DPN patients, along with improved nerve function and reduced pain indicators [8]

Regarding the physical properties of blood, Nazal (2016) provided evidence of the helium-neon laser's effect on the erythrocyte sedimentation rate (ESR) [9], a line of investigation later expanded by Falih and Msayer (2023) and Alnayli et al. (2017) through their analysis of the relationship between laser exposure and various parameters including ESR, packed cell volume (PCV), and blood viscosity [10,11]. Further examining specific blood components, a recent study by Slewa et al. (2022) investigated the effect of low-level red Helium-Neon laser therapy on human blood. They found that this irradiation led to a decrease in white blood cell (WBC) and red blood cell (RBC) counts, while observing increases in neutrophils (NEUT), platelets (PLT), and the erythrocyte sedimentation rate (ESR), suggesting potential therapeutic applications related to managing blood viscosity.Subsequently [12], Mohseen et al. (2020) added a temporal dimension by tracking changes in blood components over various periods following laser exposure [13].More recently, Chuang et al. (2024) presented a comprehensive narrative review on the application of intravenous laser irradiation of blood (ILIB) using helium-neon lasers for treating multiple clinical conditions. The study highlighted anti-inflammatory and antioxidative mechanisms, improved oxygen-carrying capacity, and enhanced mitochondrial activity in white blood cells, supporting the relevance of laser therapy in modulating blood components and overall cellular function.Additionally [14], Zaichkina et al. (2016) demonstrated in a mouse model that exposure to helium-neon laser doses ranging from 0.16 to 50 MJ/cm² activated natural protective reserves, as evidenced by reduced DNA damage in whole blood leukocytes. However, no adaptive response was observed with prior laser exposure. Furthermore, the study found that reactive oxygen species (ROS) generation capacity in neutrophils was diminished in animals pre-exposed to laser followed by X-ray irradiation, indicating laser-induced modulation of immune cell activation dynamics [15].Despite the valuable data presented by these studies, research on the impact of He-Ne laser irradiation on blood magnesium levels remains lacking. Although direct studies on the effect of He-Ne laser irradiation on blood magnesium levels are currently lacking, a strong theoretical rationale supports investigating this relationship. This rationale is predicated on the established ability of laser therapy to modulate fundamental cellular processes critical for ion homeostasis. Specifically, laser exposure is known to influence cell membrane transport mechanisms, enzymatic activities related to ion conductance, and intracellular signaling pathways, all of which could potentially regulate magnesium channels and transport. Furthermore, laser-induced alterations in the cellular metabolic and redox environment, potential modifications in the gene expression of relevant transport proteins (Hawkins & Abrahamse, 2005)[16], and direct biophysical interactions (Schmitz et al., 2004; Margarone et al., 2018; Maruyama et al., 2018) collectively suggest a plausible impact of He-Ne laser on calcium dynamics. Thus, exploring this potential interaction is crucial for understanding the broader physiological effects of laser therapy.

This knowledge gap is especially relevant considering the critical role calcium plays in various physiological functions and the implications its manipulation could have for therapeutic applications involving laser technology. This current research seeks to address this significant research gap by exploring the effects of He-Ne laser exposure on calcium levels in blood. Acquiring such knowledge is essential for determining the broader implications of laser therapy on mineral balance and for exploring potential clinical uses [17,18,19]. The study adopts a structured methodology, focusing on quantifying calcium concentrations in human blood serum samples before and after exposure, as well as examining possible gender-dependent variations in responses to laser irradiation.

II. Materials and Method

2.1 Sample collection

Twenty healthy volunteers, all non-smokers and ranging in age from 19 to 65, took part in our study. Before starting, informed consent was obtained from all participants. Blood samples were collected using Red Top Tubes (RTT). To ensure sample purity, sterile needles were used to draw the blood (two milliliters from each participant), following standard collection procedures. Immediately after collection, the cases and samples were collected inside the Salaya Care Laboratory in the city of Sirt for processing. The samples were then quickly centrifuged to separate the serum. Centrifugation was performed at 3000 RPM for 3 to 5 minutes at 25 degrees Celsius.

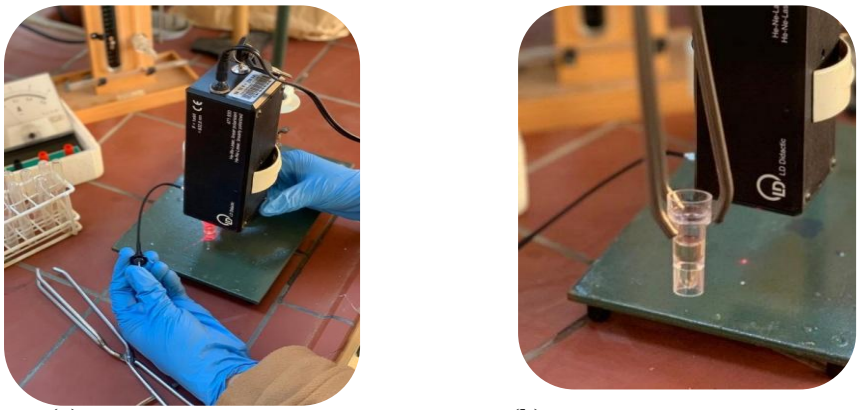
2.2 Sample preparation

Sample Preparation and Laser Exposure: After centrifugation, the separated serum was divided into four equal portions:

• Control Sample: The first aliquot was immediately analyzed using a BS-230 biochemical analyzer to establish baseline calcium levels. The analysis was carried out according to standard protocols.

• Laser Radiation Exposed Samples: The other three aliquots were prepared for laser exposure. The samples were placed at a fixed distance of 5 centimeters away from the helium-neon (He-Ne) laser (wavelength: 632.8 nm, beam diameter: 0.48 mm) to provide uniform exposure.

- Second Aliquot: Exposed to the laser for 1 minute, then analyzed for calcium concentration.
- Third Aliquot: Exposed to the laser for 3 minutes, followed by measurement of its calcium level.
- Fourth Aliquot: Exposed to the laser for 5 minutes, after which its calcium level was measured. Each sample exposed to the laser was compared to the control sample to evaluate the effect of laser exposure duration on serum calcium levels. A BS-230 biochemical analyzer was used to measure calcium levels throughout the study."



(a) (b)

Figure 1 : Illustrates how to prepare the samples and set up the helium-neon laser device. (a) Serum sample placed in a cuvette ready for laser irradiation. (b) Sample being manually prepared and positioned by the operator

III. Results and Discussions

Table 1. Serum calcium concentrations (mg/dL) in 20 human samples measured before and after helium-neon (He-Ne) laser exposure at intervals of 1, 3, and 5 minutes. These data illustrate the potential effects of laser irradiation on calcium levels in human blood**.**

Sample ID	Age	Gender	Laser Power (mW)	Wavelength (nm)	Distance (cm)	Pre-Exposure Ca (mg/dl)	Post-Exposure Ca (mg/dl) 1min	Post-Exposure Ca (mg/dl) 3 min	Post-Exposure Ca(mg/dl) 5 min
1	19	Male	1	632.8	5	9.1	9.4	10.2	13.4
2	65	Male	1	632.8	5	8.6	8.9	10.0	10.6
3	15	Female	1	632.8	5	8.7	8.7	9.1	10.2
4	42	Female	1	632.8	5	8.6	8.1	9.3	10.6
5	18	Male	1	632.8	5	9.3	10.6	10.5	13.0
6	30	Male	1	632.8	5	8.9	9.2	9.4	9.1
7	44	Male	1	632.8	5	8.6	8.9	10.3	10.7
8	20	Female	1	632.8	5	8.9	9.1	10.4	11.5
9	22	Female	1	632.8	5	8.8	9.1	10.3	15.5
10	24	Female	1	632.8	5	8.6	8.7	9.4	10.2
11	25	Female	1	632.8	5	8.9	9.7	9.9	10.2
12	26	Female	1	632.8	5	9.3	9.0	10.4	15.3
13	43	Female	1	632.8	5	8.9	9.2	9.8	10.8
14	29	Male	1	632.8	5	9.0	10.2	10.4	12.8
15	27	Male	1	632.8	5	10.1	10.4	15.0	19.0
16	28	Male	1	632.8	5	9.1	9.4	9.1	9.9
17	21	Female	1	632.8	5	8.6	8.4	10.3	10.7
18	16	Female	1	632.8	5	8.7	8.3	11.8	18.4
19	21	Male	1	632.8	5	8.6	8.7	9.3	10.2
20	23	Male	1	632.8	5	8.6	8.9	10.2	11.2

Upon exposing the samples to the He-Ne laser for one-minute, minimal changes in calcium levels were observed compared to the control samples, as shown in Figure 4 (average increase of about **0.2-0.3 mg/dl**). Some samples showed no significant change (e.g., sample 3 and sample 4). This limited effect may be attributed to the brief exposure time, which might be insufficient to induce significant biological alterations in cell membranes or ion transport processes. These findings suggest that short-term exposure does not substantially affect calcium homeostasis in the serum. These findings align with a previous study by Kovács et al. (1996), which investigated the impact of He-Ne laser radiation on human erythrocyte membranes. The study reported an increase in membrane fluidity following irradiation, without significant changes in membrane permeability to hemoglobin. These studies indicate that the effects of He-Ne laser radiation on blood components may depend on exposure duration and radiation dose. Therefore, a one-minute exposure might be insufficient to produce significant changes in serum calcium levels[20] as shown in figure 2

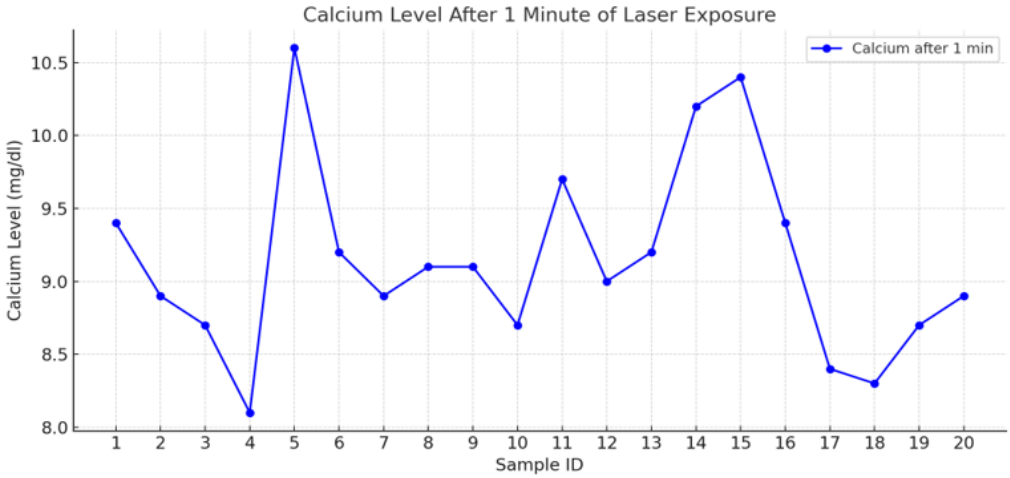


Figure 2 calcium **level at 1 minute**

Increasing the exposure duration to three minutes resulted in a notable rise in calcium concentrations in most samples. (average increase of about **0.5-1.5 mg/dl**). Some samples showed a higher response (e.g., sample 15 increased from 10.1 to 15 mg/dl). As shown in Figure 3, this elevation could be due to the laser's effect on cell membrane permeability, leading to the release of intracellular calcium into the serum. Additionally, the laser may stimulate certain enzymes or metabolic pathways that contribute to increased serum calcium levels. These results indicate that a moderate exposure duration is sufficient to elicit significant biological changes. These findings are in line with the study by Amaroli et al. (2016), which investigated the effect of infrared diode lasers (808 nm and 980 nm) on intracellular calcium concentration and nitric oxide production in Paramecium. The study found that irradiation with the 980 nm laser at a fluence of 64 J cm^{-2} significantly increased intracellular calcium and nitric oxide production[21]. Similarly, in our study, a moderate exposure duration of three minutes significantly influenced calcium dynamics. Both studies emphasize that laser exposure duration and fluence play a critical role in modulating calcium release and cellular activity, highlighting the potential of photobiomodulation in inducing biological changes. Additionally, the study by Pasternak-Mnich et al. (2024) on mesenchymal stem cells (MSCs) also observed that as the energy dose of the laser irradiation increased (3 J, 10 J, and 20 J), there was a significant increase in intracellular calcium concentrations[22]. This supports the notion that higher energy doses and longer exposure durations lead to an elevation in calcium levels, similar to the increase observed in our study. The findings highlight that laser irradiation can have a dose-dependent effect on calcium dynamics, which is consistent with our results showing that increased exposure duration leads to higher calcium concentrations in the serum

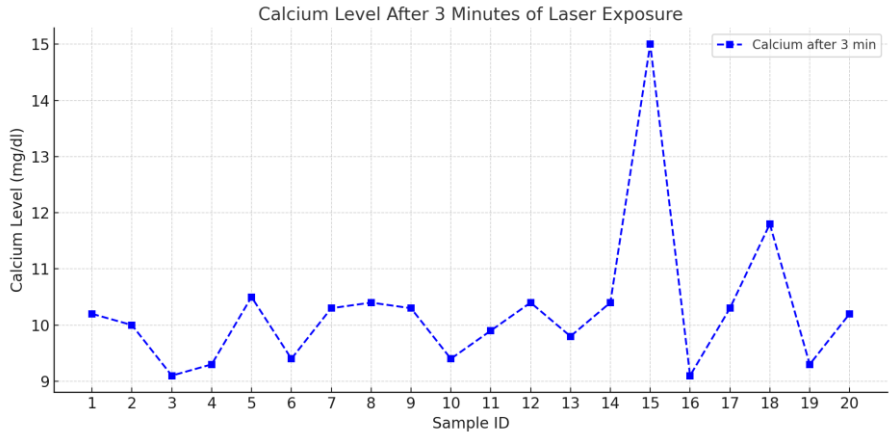


Figure 3 : calcium **level at 3 minute**

Extending the exposure time to five minutes led to varied outcomes: some samples continued to show increased calcium levels (e.g., sample 18 increased from 8.7 to 18.4 mg/dl, and sample 12 from 9.3 to 15.3 mg/dl). while others exhibited stabilization or even a slight decrease in concentration. As shown in Figure 4 , This variability might be due to reaching a saturation point in the laser's effect, where prolonged exposure does not further elevate calcium levels. Moreover, regulatory mechanisms within the body could be activated to restore calcium balance after an initial increase. These observations suggest the existence of an optimal exposure duration, beyond which no additional benefits are observed, and potential adverse effects may arise. This biphasic response in calcium mobilization is consistent with the phenomenon well documented in low-level laser (light) therapy (LLLT). Huang, Y. Y et al. (2011) demonstrated that LLLT exhibits a biphasic dose response—where lower doses effectively stimulate cellular processes (including intracellular calcium elevation), while higher doses lead to a plateau or even inhibitory effects[23]. Similarly, Huang et al. (2011) provided an update on this concept, showing that increasing the dose of light beyond an optimal point can result in diminished stimulatory effects on cellular responses, including calcium signaling. These studies reinforce our observation that extending the exposure time to five minutes can induce a non-linear response in calcium dynamics[23], with some cells showing increased calcium levels and others reaching saturation or displaying a slight decrease.

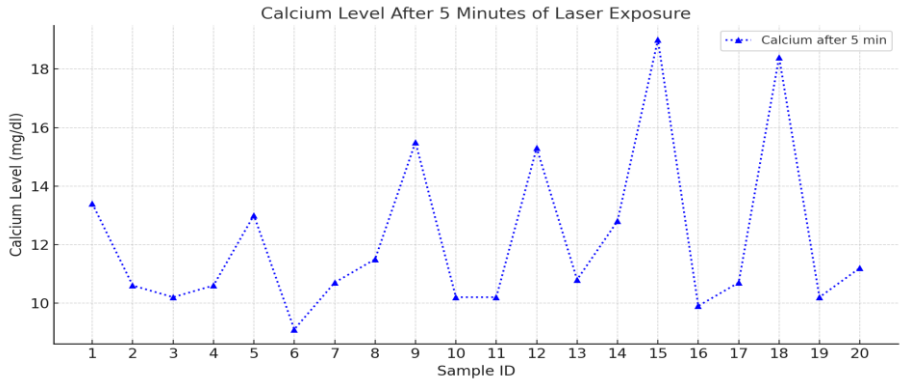


Figure 4 : calcium **level at 5 minute**

Males: Showed more consistent increases and relatively stable responses with longer exposure times. Females**:** Responses were more varied, with some samples showing significant increases (e.g., sample 18). As shown in figure 5. showing significant elevations. These findings align with those of Heaney (2000), who highlighted biological differences in calcium metabolism between males and females, indicating that females tend to exhibit greater sensitivity to calcium fluctuations[24]. Additionally, these outcomes are supported by the findings of Naganathan and Sambrook (2003), who reported sex-based differences in bone and calcium metabolism, even among genetically matched individuals, underscoring the role of gender in calcium-related physiological responses[25] membrane fluidity following irradiation, without significant changes in membrane permeability to hemoglobin. These studies indicate that the effects of He-Ne laser radiation on blood components may depend on exposure duration and radiation dose. Therefore, a one-minute exposure might be insufficient to produce significant changes in serum calcium levels[20] .

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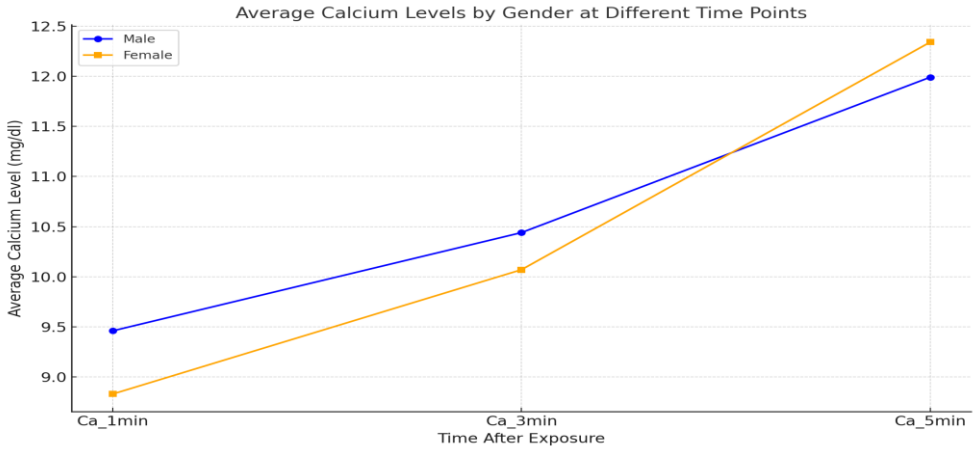

Figure 5 : Comparison of calcium Changes Between Males and Females

Table 2. Statistical summary of changes in serum calcium levels (ΔMg) among male and female subjects following helium-neon (He-Ne) laser exposure at time intervals of 1, 3, and 5 minutes. The table presents the mean change, standard deviation (SD), and standard error (SE) for each group.

Standard Error (SE)	Standard Deviation (SD)	Mean ΔCa (mg/dl)	Group	Time (Minutes)
0.13	0.42	0.470	Male	**1 Minute**
0.12	0.39	0.030	Female	**1 Minute**
0.42	1.32	1.450	Male	**3 Minutes**
0.24	0.76	1.270	Female	**3 Minutes**
0.77	2.45	3.000	Male	**5 Minutes**
0.91	2.89	3.540	Female	**5 Minutes**

IV. Conclusion:

It was found in this study that the time for which the He-Ne laser is used has a bearing on the serum calcium levels. Whereas a brief exposure of about one minute has hardly any effect, a longer exposure for about three minutes can bring about significant increases. However, five minutes of exposure was not capable of furthering this increase and this may speak in favor of an optimal exposure time beyond which the desired effect does not manifest anymore. These results emphasize the need for proper dosage determination in applications of He-Ne lasers for medical and biological purposes in order to obtain the benefits intended and to guard against any negative ramifications.

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