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Effect of Helium Neon Laser Irradiation on Magnesium Levels in Blood ”An Experimental Study”

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ABSTRACT

This study investigates the effect of Helium-Neon laser radiation on magnesium levels in blood, a vital element involved in numerous physiological and biochemical processes in the body. The Helium-Neon laser used in this study had a power output of 5 mW and a wavelength of 632.8 nm, known for its stability and low thermal effect. The spot size was approximately 0.03 cm², ensuring uniform exposure of the samples. The energy density delivered to the samples was calculated at 1.0 J/cm², providing an adequate level of irradiation without significant thermal damage. Twenty healthy, non-smoking volunteers aged between 19 and 65 participated in the study. Blood samples were collected in Red Top Tube (RTT) tubes and immediately centrifuged to separate the serum. Centrifugation was performed at 3000 RPM for 3-5 minutes at 25 °C. The separated serum was then divided into four equal portions. The first, the control, had its magnesium level measured using a biochemical analyzer (BS-230), and the mean was 1.84 mg/dL, within the normal range. The remaining three aliquots were exposed to the Helium-Neon laser for 1, 3, and 5 minutes, respectively. Results showed a 60% increase in magnesium in the immediate response, 25% with a decrease, and 15% with no noticeable change. Females showed a greater average magnesium increase (30%) with a more sustained response and greater consistency over time compared to males. Males exhibited more pronounced oscillations, a tendency for initial reductions, and faster returns to baseline. At the 1-minute mark, no significant difference was found between males and females ($p > 0.05$). However, at 3 and 5 minutes, the difference became statistically significant ($p < 0.05$), with females showing a higher increase in magnesium levels than males. The precise mechanism underlying these in vitro effects on serum remains unclear. However, it is hypothesized that Helium-Neon laser irradiation might induce subtle conformational changes in serum proteins (e.g., albumin), potentially altering their magnesium-binding affinity and thereby influencing measured levels within the serum milieu. These findings suggest complex, non-thermal interactions between Helium-Neon laser light and serum components in vitro, warranting further research to elucidate the specific biomolecular interactions

Kay words: Helium-Neon Laser, Magnesium Levels, Laser Irradiation, samples.

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1.INTRODUCTION

A significant portion of the population is estimated to be magnesium deficient, a crucial mineral impacting functions from muscle contraction to mood.[1],[2]. Magnesium is an essential driver of vital processes; its role in muscle contraction and nerve transmission[3] means low levels cause serious health problems. A balanced diet should provide enough Mg2+, but intake is decreasing due to processed food consumption, which is lower in magnesium than unrefined foods.[4,5]. Hypomagnesemia manifests in various ways, affecting the neuromuscular system (fatigue, weakness), cardiovascular system (hypertension), and metabolism (insulin resistance) [6]. While diet and lifestyle are known factors, questions arise about less obvious external influences. Among these, radiation exposure, especially from certain medical and research lasers, is of particular interest.[7,8].

The Helium-Neon (He-Ne) laser is a device that produces a stable beam of light with unique properties, such as monochromaticity (single wavelength), coherence (wavelengths in phase), and directionality (small variations) [9] . These properties distinguish lasers from traditional light sources [10]. A laser consists of an active medium, a pump source, and an optical cavity [11]. The red helium-neon laser, emitting at a wavelength of 632.8 nm, is known for its stability and low power consumption [10]. This laser has been used in medicine and science to study its effects on wound healing, pain relief, and tissue regeneration. Research has also been conducted on its therapeutic potential in areas such as vascular disease [12]. Due to its low thermal efficiency, the helium-neon laser has been used to study blood structure, and studies have shown its potential to affect blood oxygenation and immune function.

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

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blood[13]. Ghadage et al. (2015) later corroborated these findings, reporting similar changes in the blood's chemical makeup due to low-power laser therapy [14].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 [15] "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 [16]

Regarding the physical properties of blood, Nazal (2016) provided evidence of the helium-neon laser's effect on the erythrocyte sedimentation rate (ESR) [17], 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 [18,19]. 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 [20], Mohseen et al. (2020) added a temporal dimension by tracking changes in blood components over various periods following laser exposure [21].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 [22], 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

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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 [23].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)[24] , 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 magnesium 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 magnesium 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 magnesium 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 [25,26,27]. The study adopts a structured methodology, focusing on quantifying magnesium concentrations in human blood serum samples before and after exposure, as well as examining possible gender-dependent variations in responses to laser irradiation

2. MATERIALS AND METHODS

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,

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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 are shown in Figure 2: 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 magnesium 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 magnesium concentration.

-Third Aliquot: Exposed to the laser for 3 minutes, followed by measurement of its magnesium level.

-Fourth Aliquot: Exposed to the laser for 5 minutes, after which its magnesium 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 magnesium levels. A BS-230 biochemical analyzer was used to measure magnesium levels throughout the study.”

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(a) (b) (c)

Figure (1) : Samples preparation 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. (c) Fine adjustment of the He-Ne laser device to ensure accurate and uniform exposure.

3.RESULTS AND DISCUSSIONS

Table 1 shows Serum magnesium 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 magnesium levels in human blood.

Table (1). Serum magnesium concentrations before and after helium-neon (He-Ne) laser.

Sample ID	Age	Gender	Laser Power (mW)	Wavelength (nm)	Distance (cm)	Pre-Exposure Mg (mg/dl)	Post-Exposure Mg (mg/dl) 1min	Post-Exposure Mg (mg/dl) 3 min	Post- Exposure Mg (mg/dl) 5 min
1	19	Male	1	632.8	5	1.7	2.3	2.0	1.8
2	65	Male	1	632.8	5	1.7	2.2	2.03	1.9
3	15	Female	1	632.8	5	1.8	1.0	2.1	1.8
4	42	Female	1	632.8	5	1.8	1.8	2.4	1.7
5	18	Male	1	632.8	5	2.1	1.8	1.06	1.09
6	30	Male	1	632.8	5	1.8	2.8	1.9	2.3
7	44	Male	1	632.8	5	2.0	2.5	1.7	2.5
8	20	Female	1	632.8	5	2.5	2.3	2.7	2.5
9	22	Female	1	632.8	5	1.7	1.8	1.9	2.5
10	24	Female	1	632.8	5	1.7	1.9	2.3	2.8
11	25	Female	1	632.8	5	1.8	1.4	1.9	2.4
12	26	Female	1	632.8	5	2.4	2.5	2.8	2.8

13	43	Female	1	632.8	5	1.9	1.8	2.0	2.7
14	29	Male	1	632.8	5	1.9	1.02	1.6	1.1
15	27	Male	1	632.8	5	1.7	2.1	1.8	2.2
16	28	Male	1	632.8	5	1.7	1.2	1.8	2.1
17	21	Female	1	632.8	5	1.7	1.8	1.9	2.6
18	16	Female	1	632.8	5	1.7	1.13	1.7	2.0
19	21	Male	1	632.8	5	1.8	1.9	1.7	1.7
20	23	Male	1	632.8	5	1.7	1.8	1.7	1.7

A comprehensive analysis of the alterations in magnesium levels indicates a diverse range of responses among the serum samples following laser exposure. Some samples exhibited increased magnesium levels, while others showed a decrease, and a few cases indicated no notable change. Specifically, 60% of the samples reflected an immediate rise (like the sixth and tenth sample), 25% demonstrated a decline (especially the third and fourth sample), and 15% revealed no significant variation. This variability points to complex interactions occurring within the serum milieu itself upon laser irradiation. While the photobiomodulatory effects of lasers in vivo often involve mechanisms like the modulation of ion transport across cellular membranes – concepts detailed in research on cellular responses to low-power lasers, such as the work by Karu (2003) exploring effects on cellular ion transport [28] – such direct effects on cellular membranes are not the primary drivers in our acellular serum preparation. Therefore, we propose that the observed variability in magnesium levels in this in vitro context stems more directly from the laser's interaction with the inherent biochemical composition of individual serum samples. It is hypothesized that the laser light interacts differently with specific serum components, potentially including subtle effects on the conformation or magnesium-binding characteristics of serum proteins like albumin, leading to the varied responses observed. The mean magnesium level prior to exposure was approximately 1.84 mg/dL, which is within the acceptable range. In terms of the impact of exposure duration, a one-minute exposure resulted in a prompt and substantial effect in the majority of cases, reflecting an immediate and rapid response within the serum samples, as shown in figure

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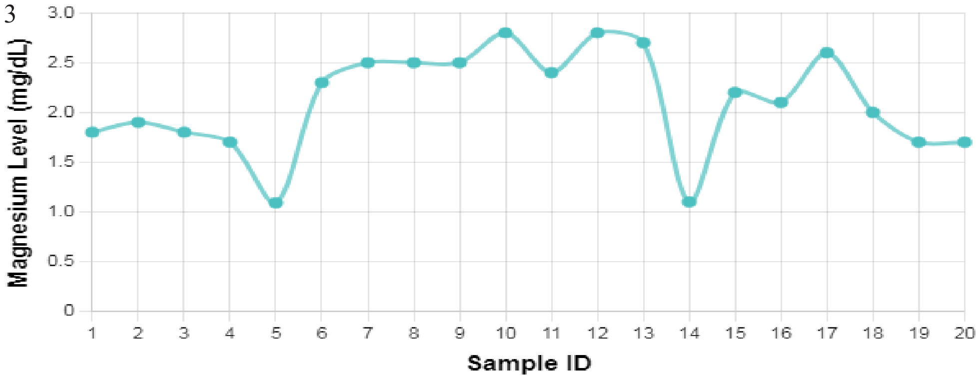

Figure (2) : magnesium level at 1 minute.

On the contrary, the duration of exposure for a period lasting three minutes revealed a moderate physiological response characterized by a discernible level of relative stability in the measured values that are shown in figure (3).

In parallel, the condition of exposure extending over a duration of five minutes, in certain instances, indicated a reversion to baseline levels, thereby implying the existence of an intrinsic regulatory mechanism within the human body that effectively restores homeostatic balance. This is shown in figure (4).

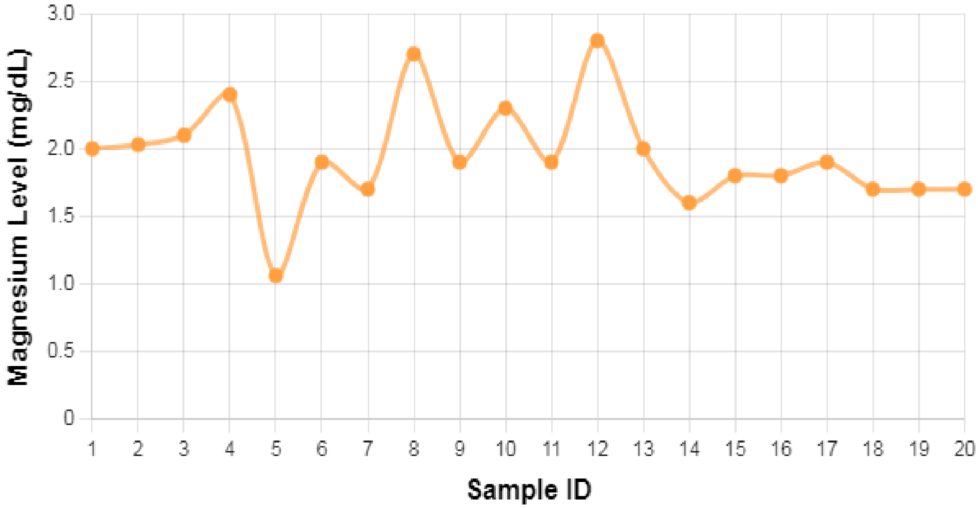

Figure (3) : magnesium level at 3 minutes.

Exposure for five minutes, in some cases, showed a return to baseline levels, suggesting the presence of a regulatory mechanism in the body that restores balance.

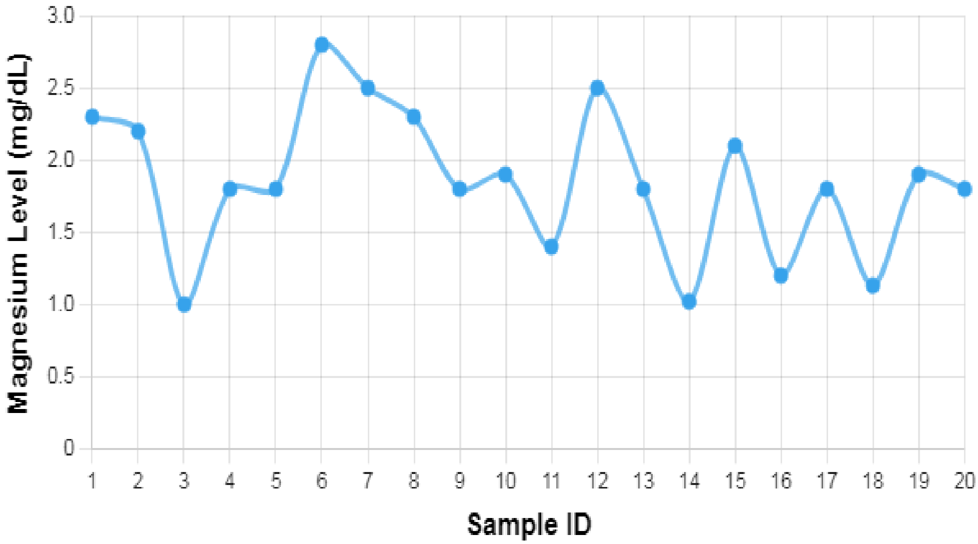

Figure (4) : magnesium level at 5 minutes.

On the other hand, when exposed to the helium-neon laser, females showed a bigger increase in magnesium (30%) and a longer and more consistent response than males shown in (Figure 5) and table (2). At the 1-minute mark, there is no significant difference between males and females ($p > 0.05$). However, at 3 and 5 minutes, the difference becomes statistically significant ($p < 0.05$), with females showing a greater increase in magnesium levels than males. Males had more pronounced oscillations and an initial decrease before going back to baseline. These sex differences are consistent with known physiological variations influencing magnesium homeostasis in vivo (Avinash et al., 2013; Lowenstein & Stanton, 1986), such as the role of estrogen in systemic magnesium handling (Avinash et al., 2013). However, given the in vitro nature of this study using serum, these observed differences likely arise from baseline variations in serum composition itself. It is plausible that the laser interacts differently with serum components, perhaps influencing the conformation or binding affinity of magnesium-binding

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proteins like albumin [29,30] , which may vary between sexes. Age also played a big role in the magnesium response. The 15-45 age group responded faster and more consistently while the over 45 age group responded slower and less and with longer recovery time. These age differences may reflect underlying physiological changes associated with aging (Dominguez et al., 2024), which can impact cellular function in vivo [31] . Similarly, in our in vitro context, the diminished response in the older group could be linked to age-related alterations in serum proteins or other components, potentially affecting their interaction with the laser light and subsequent influence on measurable magnesium levels.

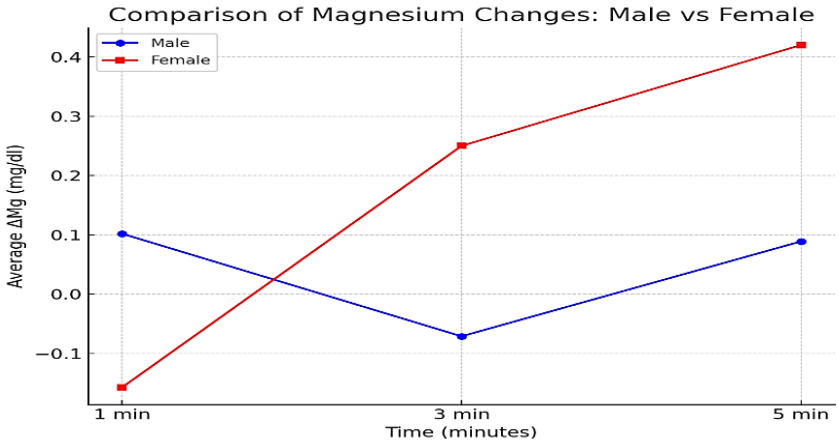

Figure (5) : Comparison of Magnesium Changes Between Males and Females

Table (2) shows the statistical summary of changes in serum magnesium 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.

Table (2). Statistical changes in serum magnesium levels after helium-neon (He-Ne) laser exposure

Standard Error (SE)	Standard Deviation (SD)	Mean ΔMg (mg/dl)	Group	Time (Minutes)
0.18	0.56	0.018	Male	1 Minute
0.11	0.35	-0.218	Female	
0.15	0.48	-0.019	Male	3 Minutes
0.12	0.36	0.230	Female	
0.22	0.71	-0.098	Male	5 Minutes
0.16	0.51	0.430	Female	

4.CONCLUSION

He-Ne laser irradiation affects human serum magnesium levels through exposure dependence on duration as well as through sex and age variations according to this research. Magnesium levels stayed elevated for longer durations in females and subjects under 40 years old increased their magnesium levels faster. Additional research needs to be conducted to determine how low-energy laser therapy affects mineral equilibrium according to the current findings. Additional research must examine what causes these changes in serum magnesium levels while also studying different laser parameters and assessing long-term effects in living tissue. Such complete understanding between these interactions holds potential for developing new non-invasive therapeutic techniques.

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