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Assessment of low intensity laser irradiation on RBC parameters, morphology and free plasma hemoglobin

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Abstract

Background: Red blood cells (RBCs) are an essential component of blood and play a vital role in hemoglobin's ability to carry oxygen to tissues. The propertiesof these cells and the integrity of their membranes can be impacted by exposure to light of different wavelengths and durations, which calls for an assessment of such alterations and the use of suitable laboratory markers. Aims: This study aimed to evaluate the effects of light irradiation on RBC hemolysis, plasma-free hemoglobin (PFHb) levels, and other hematological parameters at wavelengths of 450 nm and 650 nm and during different exposure times. Materials And methods: To assess the effects of various irradiation states in contrast to a non-irradiated control group, ex vivo laboratory study was conducted. Prior to and following irradiation, measurements protected the measurement of plasma-free hemoglobin using spectrophotometry, the computation of the percentage of hemolysis, and an evaluation of RBC indices, (Mean Corpuscular Volume MCV), (MCH Mean Corpuscular Hemoglobin), (MCHC Mean Corpuscular Hemoglobin Concentration). To evaluate the measurements, suitable statistical analyzes have been employed. Results: The mean plasma-free hemoglobin PFHb levels in the control group increased from 3.216 ± 1.017 mg/dL to a few, indicating a slow and steady expansion.601 ± 0.990 mg/dL after 30 minutes of radiation at 650 nm. Additionally, the suggested reported hemolysis percentage increased from 1.345 ± 0.341% to 1.510 ± 0.338%.There were no statistically significant variations in the MCV, MCH, and MCHC indices, although depending on the statistical analysis used, the overall hemoglobin categories displayed different levels of significance. Conclusions: The results support a relationship between changes in plasma-free hemoglobin levels and irradiation duration and wavelength. These findings are still preliminary, though, and further study with bigger pattern sizes, standardization of irradiation conditions, and testing of the hemolysis equation are needed to confirm those results.

Keyword : *Laser Irradiation, RBC Cells, Free Plasma Hemoglobin, 450nm, 650 Nm, Biomodulation.*

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INTRODUCTION

The main protein found in red blood cells (RBCs) is hemoglobin, which is primarily in charge of carrying oxygen. Because there are clear differences in light absorption between hemoglobin's oxygenated and deoxygenated phases, hemoglobin plays a major role in determining the optical properties of whole blood—inside the visible and near-infrared light spectra [1,2]. Through a primary membrane protein called "Band three," which aids in the exchange of chloride and bicarbonate ions and maintains the cytoskeleton of the membrane, red blood cells also contribute to the transfer of carbon dioxide [3]. The integrity of the cell's membrane determines its shape and function; the phospholipid bilayer and the "spectrin" protein community function as an integrated mechanical device, while membrane fluctuations, powered by ATP (power molecules), help to maintain the cell's biconcave shape [4]. The membrane's viscoelastic properties help red blood cells withstand osmotic and mechanical stress [5,6]. Reduced deformability is a characteristic that precedes the development of pathological disorders, whether they are caused by various illnesses or damage related to mechanical change [6,7].The complex structure of the red blood cell membrane is made up of a cytoskeleton based on the protein spectrin and a lipid bilayer. The cell's shape, elasticity, and resistance to shear pressures are maintained by proteins such as spectrin, ankyrin, actin, and Band 3, which are found alongside various connecting proteins [8,9,10]. The membrane's composition also plays a crucial role; the

cytoplasmic membrane includes ldl cholesterol-rich regions, organized lipid domain names, proteins that contribute to the formation of "membrane rafts," and connections between the lipid bilayer and the cytoskeleton—all of which affect the mobile's capacity to undergo deformation [11,12]. Additionally, oxidative stress affects all proteins and lipids, resulting in lipid peroxidation, spectrin and ankyrin aggregation, and hemoglobin structural alterations that ultimately jeopardize membrane integrity [13]. Long-term storage in the presence of EDTA results in the formation of echinocytes and spherocytes, increased osmotic fragility, and a higher charge of hemolysis, whereas oxidative damage linked to hypoxia causes defects inside the proteins "band 3" and "spectrin" [14,15].

The rupture of the red blood cells membrane, known as hemolysis, causes the contents of the mobile to leak into the plasma. Osmotic imbalance, oxidative damage, mechanical strain, or thermal strain might be the cause of this rupture. When the membrane breaks, the hemoglobin inside the cell becomes "plasma-unfastened hemoglobin" (PFH), which is an immediate sign of cell lysis [15,16,17]. Additionally, changes in cellular morphology are common indicators in ex vivo (outside the body) analyzes; pressure can result in the development of spherocytes, echinocytes, stomatocytes, and degenerative paperwork—either before or after detectable hemolysis [16,18]. As a result, measurements of cellular morphology, hemolysis degree, and plasma-free hemoglobin (PFH) provide a thorough set of criteria for evaluating blood integrity, which makes them ideal for ex vivo evaluations.

The term "Low-Level Light Therapy" (LLLT) describes the use of low-intensity light to control biological interest without causing tissue damage. The existence of photoreceptors, including those connected to the cytochrome device, is linked to photobiomodulation—using purple or near-infrared light—in a variety of cellular types. Mature red blood cells, on the other hand, do not have a nucleus or maximum mobile organelles. As a result, their photobiology is different and likely involves interactions between hemoglobin, components of the cellular membrane, and the surrounding medium [5,8]. The optical properties of blood have a significant impact on such interactions: red blood cells are the best sellers of light scattering, whereas hemoglobin dominates light absorption within the 400–1100 nm wavelength range. Additionally, the blood's oxygenation kingdom modifies its scattering and absorption conduct [19,20].

MATERIALS AND METHODS

Study design

Twenty blood samples were used in the investigation. A Complete Blood Count (CBC) analyzer was used to collect hematological data both before and after radiotherapy. Drabkin's method was also used for the spectrophotometric measurement of hemoglobin, and blood smears were made to examine morphological changes in red blood cells.

Blood collection and preparation

Depending on the kind of tube used, twenty venous blood samples were collected into tubes that contained either tripotassium or dipotassium EDTA as an anticoagulant. In order to maintain homogeneity and prevent mechanical hemolysis of red blood cells during handling, the samples were gently blended. Maintenance was also made to reduce the amount of time between blood collection and the completion of laboratory procedures. To maintain accuracy and make it easier to compare data collected before and after radiation, each sample is given a unique identifying identifier.

Irradiation with laser

For irradiation times of 15 and 30 minutes per pattern, blood samples were exposed to moderate sources with wavelengths of 450 and 650 nm, with a constant distance of 5 cm between the light source and the pattern. For each wavelength, a consistent power density was maintained: 5.28 mW/cm² at 450 nm and 27.78 mW/cm² at 650 nm. As a result, the computed light doses have been 4.The 15-minute

and 30-minute publicity periods are comparable to 75 J/cm² and 9.5 J/cm² at 450 nm and 25 J/cm² and 50 J/cm² at 650 nm, respectively. Pre-irradiation data were used as a reference point for comparison with post-irradiation observations. Before using these figures, the exact electrical density and the location of the light spot on the sample role should be determined experimentally.

Measuring blood parameters using a complete blood count CBC

Using a CBC analyzer and the appropriate reagents, a complete blood count (CBC) was performed in accordance with the instructions supplied by the equipment manufacturer. Positive samples were measured both before and after irradiation, and each sample's effects were noted individually. Like other measured indices, the recorded parameters included hematocrit (Hct) and total hemoglobin (Hb).

Measurement of hemoglobin using the Drabkin method

This method is based on the "cyanmethemoglobin" technique for estimating the level of free hemoglobin in plasma. Plasma is separated from blood cells (collected in a tube containing EDTA) via centrifugation; then, 20 microliters of the plasma are added to 5 ml of Drabkin's reagent. The mixture is left at room temperature for 5 to 10 minutes to allow for the formation of the colored complex. A "blank" solution, consisting of 5 ml of Drabkin's reagent, is used to zero the spectrophotometer. Subsequently, the absorbance of the samples is measured at a wavelength of 540 nm, and the concentration of free hemoglobin is calculated using the following established equation: Plasma Hemoglobin = (Absorbance of the sample / Absorbance of the standard) × Concentration of the standard.

Blood film preparation

Before and after irradiation, blood smears were made from the samples, stained with Leishman stain in accordance with the hooked up laboratory protocol, and examined under a light microscope to assess the size and shape of the red blood cells as well as any morphological alterations, such as the presence of spherocytes, echinocytes (spiculated cells), and fragments of red blood cells.

Ethical approval

The study was conducted in accordance with the ethical guidelines found in the Helsinki Declaration. The participants verbal and analytical consent was obtained prior to sample collection. In addition the consent and information from an ethics committee at the university of Babylon, Hammurabi college of medicine (issue no. 72, date:28/June/2026) , Iraq, assessed and approved the study permission.

Statistical analysis

The measurements obtained before and after irradiation have been regarded as paired (associated) measures, whilst the twenty samples have been considered as objective organic units. Quantitative variables were provided as the median and interquartile variety (IQR) when the requirements for a normal distribution were not satisfied, or as the implied ± broad deviation (SD) when the data showed a regular distribution. A paired-samples t-test was utilized when its assumptions were met, but the Wilcoxon signed-rank test was employed when they weren't. The demand for a statistical check became largely dependent on the nature of the statistics. Taking into account the sample size and the nature of the experimental design, a statistical significance level of ($p<0.05$) was chosen.

RESULT AND DISCUSSION

Laser effect on FPHG

The results demonstrated a progressive rise in the implied concentration of plasma free hemoglobin (PFHb) as the irradiation duration increased, varying by wavelength. The radiated group's mean increased to 3.216 ± 1.017 mg/dL, 3.280 ± 1.020 mg/dL after 15 minutes of 450 nm radiation, and 3.408 ± 1.006 ±/dL after 30 minutes. Table (1) and Figure (1) demonstrate that at a wavelength of 650

nm, the suggestion increased to (3.427 ± 0.979) mg/dL after 15 minutes and to (3.601 ± 0.990) mg/dL after 30 minutes.

Table 1. The arithmetic mean and standard deviation of PFHb concentration in the control group and the four irradiation groups.

Comparison of 20 sample	Control mean	Treatment mean	Mean paired difference	Difference SD	95% CI lower	95% CI upper	Paired t	Paired t p	Wilcoxon W	Wilcoxon p	Cohen dz	Paired t p (Holm)	Wilcoxon p (Holm)
450 nm - 15 min vs Control	3.2165	3.2800	0.0635	0.0701	0.0307	0.0963	4.0492	0.0007	0	0.0010	0.9054	0.0007	0.0010
450 nm - 30 min vs Control	3.2165	3.4080	0.1915	0.1033	0.1432	0.2398	8.2924	0.0000	0	0.0001	1.8542	0.0001	0.0004
650 nm - 15 min vs Control	3.2165	3.4270	0.2105	0.1486	0.1410	0.2800	6.3370	0.0000	0	0.0001	1.4170	0.0001	0.0004
650 nm - 30 min vs Control	3.2165	3.6005	0.3840	0.1931	0.2936	0.4744	8.8945	0.0000	0	0.0001	1.9889	0.0001	0.0004

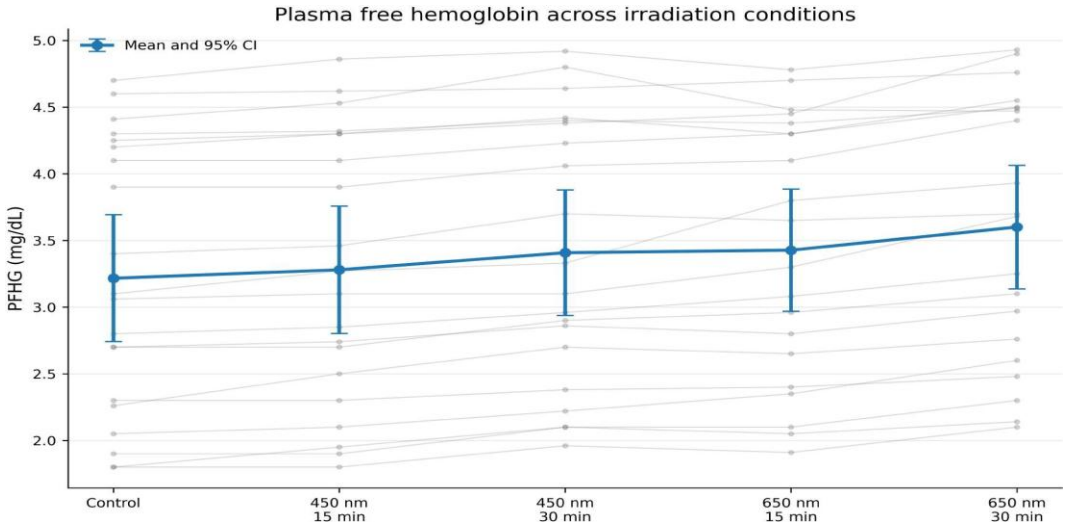


Figure1. Mean PFHb across the five groups, with error bars representing the standard deviation (SD)

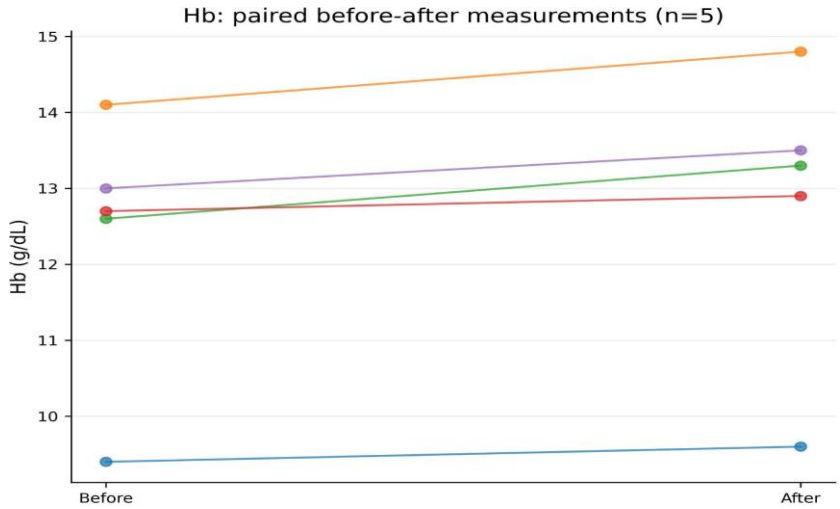


Figure 2. Comparison of hemoglobin (Hb) concentration before and after irradiation in the studied samples.

Based on the analysis of the data, the Friedman check found a statistically significant average difference between the experimental conditions (p= 0.0001, =0.0001, =0,007). The best implied PFHb awareness was measured at 650nm, ,450nm after 15 and 30 minutes, showing a rise of (0.384) mg/dL in comparison to the manipulation, according to descriptive tendencies. However, this difference alone does not prove that the wavelength or duration of irradiation caused an increase in red blood cellular hemolysis; other factors that need to be taken into account include the energy dose added to the sample, irradiation conditions, and the methods used for plasma training and size. The importance of detecting plasma-free hemoglobin (PFHb) is consistent with the study conducted by Chung et al. (2020), which used spectrophotometric data at 380, 415, and 450 nm to automate the Harboe approach for predicting PFHb. Although this approach is useful for estimating free hemoglobin, the validity of the data, the ability to handle spectral interferences, and adherence to appropriate analytical techniques determine how accurate the results are. As a result, the increase seen on this test indicates a different focus on PFHb that requires further investigation; it can no longer be regarded as conclusive evidence of membrane damage or clinically significant hemolysis on its own.

Hemolysis percentage

The results in Table 4 show that the proportion of hemolysis in the irradiated organizations increased gradually in comparison to the control group. after 450 nm for 15 minutes, the suggested difference from the control group increased from 0.0270 percentage points to 0.0830 percent factors after 30 minutes. The mean difference in the 650 nm businesses increased to zero after changing to 0.0915 percent points for 15 minutes.30 minutes at 1655 percentage points. These results show that using a wavelength of 650 nm for 30 minutes produced the optimum stage of hemolysis, which is consistent with the PFHb effects shown in Table (3).

The following formula is used to determine the proportion of hemolysis based only on the attention of plasma-free hemoglobin (PFHb) in relation to the attention of total hemoglobin (Total Hb): Hemolysis (%)= PFHb / Total Hb *100 .

In this case, Total Hb indicates the total hemoglobin attention in the blood pattern, whereas PFHb indicates the concentration of unfastened hemoglobin in the plasma. The resultant percentage, which is a key indicator for hemolysis estimation, represents the percentage of total hemoglobin found free within the plasma. The correlation between the hemolysis price results and the PFHb results supports the interpretation that modest publicity—beyond the circumstances of the state-of-the-art test—turned into a protracted release of hemoglobin from red blood cells. Hemoglobin and other cell contents leak into the surrounding media when the integrity of the membrane is compromised physiologically. As a result, the simultaneous increase in each indication provides mutual assistance for a change in membrane integrity; nevertheless, it must be noted that each sign relates to the same underlying phenomena and does not now constitute entirely objective threads of evidence. The current study's findings differ from those of prior studies that have suggested that light irradiation has a protective effect on purple blood cells membranes. Iijima et al. (1991) found that red and green helium-neon laser irradiation decreased hemolysis induced by a hypotonic response, indicating a stabilizing action at the red blood mobile membrane. Similarly, a study by Mohammed et al. (2024) found that irradiating stored blood with wavelengths of 589 nm and 650 nm was linked to a decreased charge of hemolysis for the duration of the garage length, with the 589 nm wavelength exhibiting an additional beneficial impact. The experimental settings, including wavelengths, light dosages, exposure duration, sample type, and the physiological nation of the red blood cells, differ significantly between different research, therefore this disparity does not necessarily indicate a contradiction between the findings. While other research have looked at preserved blood or hemolysis brought on by hypotonic solutions, the current study studied human blood samples ex vivo under specific irradiation conditions. Since chemical reactions to light might be dose-established, an increase in exposure duration or intensity does not necessarily translate into a protective effect. This is consistent with the findings of Al-Yasiri (2018), who found that prolonging the irradiation time at 650 nm altered red blood cell membrane proteins.

This finding may help explain the slow increase in hemolysis markers seen in the modern study. Additionally, study by Zhu et al. (2022) emphasizes the significance of moderate dose in determining the cell response, highlighting the necessity of interpreting results based entirely on a mix of wavelength, dosage, and exposure duration rather than wavelength alone. Even though there were statistically significant differences between the irradiation and control groups, the rise in hemolysis rate was severely limited, reaching nil.For thirty minutes, 1655 percent of the group was exposed to 650 nm. Therefore, it is critical to distinguish between statistical significance and physiological relevance. Although the results indicate a quantifiable trade within the marker, they are insufficient on their own to establish the prevalence of significant practical damage to red blood cells or to infer any clinical impact in humans.

Table 2. Mean hemolysis percentages and standard deviation for each group

Comparison of 20 sample	Control mean	Treatment mean	Mean paired difference	Difference SD	95% CI lower	95% CI upper	Paired t	Paired t p	Wilcoxon W	Wilcoxon p	Cohen dz	Paired t p (Holm)	Wilcoxon p (Holm)
450 nm - 15 min vs Control	1.3445	1.3715	0.0270	0.0296	0.0132	0.0408	4.0826	0.0006	0	0.0009	0.9129	0.0006	0.0009
450 nm - 30 min vs Control	1.3445	1.4275	0.0830	0.0426	0.0631	0.1029	8.7210	0.0001	0	0.0001	1.9501	0.0001	0.0003
650 nm - 15 min vs Control	1.3445	1.4360	0.0915	0.0686	0.0594	0.1236	5.9636	0.0001	0	0.0001	1.3335	0.0001	0.0003
650 nm - 30 min vs Control	1.3445	1.5100	0.1655	0.0863	0.1251	0.2059	8.5767	0.0001	0	0.0001	1.9178	0.0001	0.0003

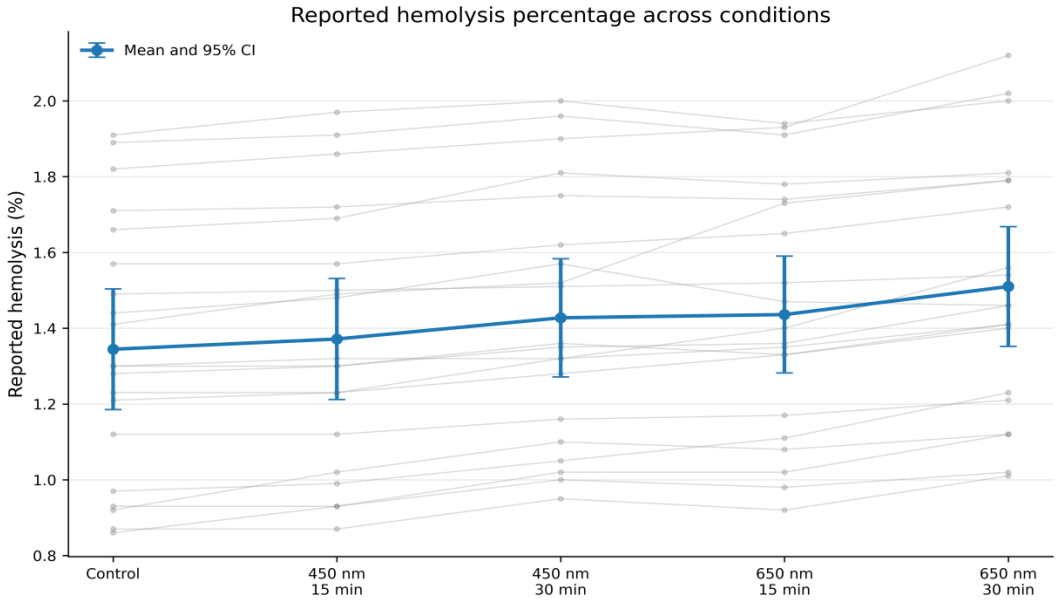


Figure 3. Comparison of reported percentages of hemolysis between control and irradiation groups

Wavelength and irradiation duration effect

All irradiation firms showed a rise when comparing the suggested PFHb degrees with the manipulation group. Based on the numbers shown after rounding, the differences were 0.064 mg/dL (at 450 nm for 15 minutes), 0.192 mg/dL (at 450 nm for 30 minutes), 0.210 mg/dL (at 650 nm for 15 minutes), and 0.385 mg/dL (at 650 nm for 30 minutes).

The comparison reveals that during a 30-minute period, the 650 nm organization was linked to the most significant descriptive increase. On my own, however, I am unable to ascertain if the influence of the

irradiation length is statistically superior to the effect of the wavelength. This necessitates a statistical analysis suitable for the experimental design, including post hoc comparisons where necessary and adjustments to the significance level to take multiple comparisons into account. It should be noted that pre-analytical factors, such as blood series, centrifugation, and plasma separation, as well as the requirement to avoid hemolysis caused by laboratory techniques, have an impact on the accuracy of plasma-free hemoglobin (PFHb) measurement. The need of fine-tuning spectrophotometric data while calculating free hemoglobin was covered by Chung et al. (2020). As a result, while the current findings suggest a descriptive approach to better PFHb tiers under the investigated irradiation conditions, this also needs to be validated by standardizing laboratory procedures and confirming the statistical significance of particular comparisons.

Irradiation effect on RBC parameters

In comparison to pre-irradiation data, the effects shown in Table 5 indicate numerical changes in positive purple blood cellular (RBC) indices after irradiation. The mean corpuscular hemoglobin (MCH) increased from 27.26 to 27, while the mean corpuscular quantity (MCV) increased from 82.86 to 84.00 femtoliters (fL).Eighty picograms (pg) and the suggested corpuscular hemoglobin concentration (MCHC) increased from 32.60 to 33.00 g/dL. Furthermore, the suggested concentration of hemoglobin (Hb) increased from 12.36 to 12.82 grams per deciliter. Despite the fact that numerical increases were found across different types of indices, statistical testing after the Holm correction did not show any statistically significant differences on the zero.Level of importance: 05. For MCV, MCH, MCHC, and hemoglobin attention, the adjusted p-values were 0.1704, 0.1704, 0.2229, and 0.0595, respectively. Because the sample size for this portion of the analysis was limited to the handiest five samples, which lowers the statistical energy to find small adjustments, it is therefore impossible to conclude with certainty that irradiation caused a significant trade in those indices. From a physiological standpoint, MCV, MCH, and MCHC all mimic the amazing properties of red blood cells. While MCH denotes the quantity of hemoglobin present in a purple blood cell, MCV relates to the size of a purple blood cell; MCHC, on the other hand, denotes the awareness of hemoglobin within a specific amount of purple blood cellular mass. As a result, little numerical improvements in those indices are no longer sufficient to confirm a structural or useful trade inside the purple blood cells; they can also duplicate light changes in cellular features or herbal variability among measurements. These results are somewhat consistent with a study conducted by Siposan and Lukacs (2000), which examined the effects of helium-neon (He-Ne) laser irradiation on human blood parameters *in vitro*. Although differences have been noted in many hematological parameters, their analysis indicated no statistically significant changes in MCV, MCH, or MCHC. Although it was unclear if the irradiation was the direct cause, the researchers also observed hemolysis in a few samples. Without necessarily causing noticeable changes in the volumetric or quantitative indices of the red blood cells, these effects support the possibility that membrane integrity or specific blood components can be impacted.

Houssien et al. (2011) investigated the impact of a 632.8 nm helium-neon laser on several human blood parameters. They found changes in hemoglobin awareness and in several indices related to hemoglobin concentration and red blood cell volume. The contrast between these results and our own indicates that the hematological response may also differ depending on the characteristics of the light source, the exposure dose, the kind of samples, and variations in sample size and experimental design. UKM Electronic Journal. In terms of hemoglobin, the current analysis found a numerical increase of 0.46 g/dL after radiation exposure. The Wilcoxon check produced a p-cost of 0.0625, whereas the uncorrected p-price was 0.0149, which changed to zero.0595 following the Holm adjustment. As a result, this boom can no longer be described as a statistically significant difference. Furthermore, since the analysis was done *ex vivo* and did not include erythropoiesis (the production of red blood cells), the increase in hemoglobin concentration in those samples cannot be interpreted as an increase in hemoglobin production or an improvement in red blood cellular function. Regardless of some limited numerical increases, the data shown in this table indicate that, in the pattern size utilized, the CBC parameters investigated did not demonstrate statistically significant modifications following irradiation. When compared to the 1/3 and fourth tables, the relevance of this location becomes clear: an increase

in hemolysis rate and plasma-unfastened hemoglobin (PFHb) does not always result in discernible changes in MCV, MCH, or MCHC during the course of the experiment. This shows that compared to the traditional volumetric and quantitative indices used in a whole blood count (CBC), measures of hemoglobin launch and hemolysis might be more sensitive to sudden changes in membrane integrity.

Table3. Mean values for MCV, MCH, and MCHC before and after irradiation, along with standard deviations and p-values

Parameter for 5 sample	Before Mean	Before SD	Before Mean ± SD	After Mean	After SD	After Mean ± SD	Mean difference (After-Before)	Difference SD	95% CI lower	95% CI upper	Paired t	Paired t p	Wilcoxon W	Wilcoxon p	Cohen dz	(Holm across CBC outcomes)	(Holm across CBC outcomes)
MCV	82.8600	7.5834	82.86 ±7.58	84.00	7.4542	84.00 ±7.45	1.1400	0.9607	-0.0529	2.3329	2.6533	0.0568	1	0.1250	1.1866	0.1704	0.3750
MCH	27.2600	3.2362	27.26 ±3.24	27.80	3.1875	27.80 ±3.19	0.5400	0.4615	-0.0331	1.1131	2.6163	0.0590	0	0.1250	1.1700	0.1704	0.3750
MCHC	32.6000	1.1336	32.60 ±1.13	33.00	0.9618	33.00 ±0.96	0.4000	0.6205	-0.3704	1.1704	1.4415	0.2229	3	0.3125	0.6447	0.2229	0.3750
Hb	12.3600	1.7587	12.36 ±1.76	12.82	1.9357	12.82 ±1.94	0.4600	0.2510	0.1483	0.7717	4.0980	0.0149	0	0.0625	1.8327	0.0595	0.2500

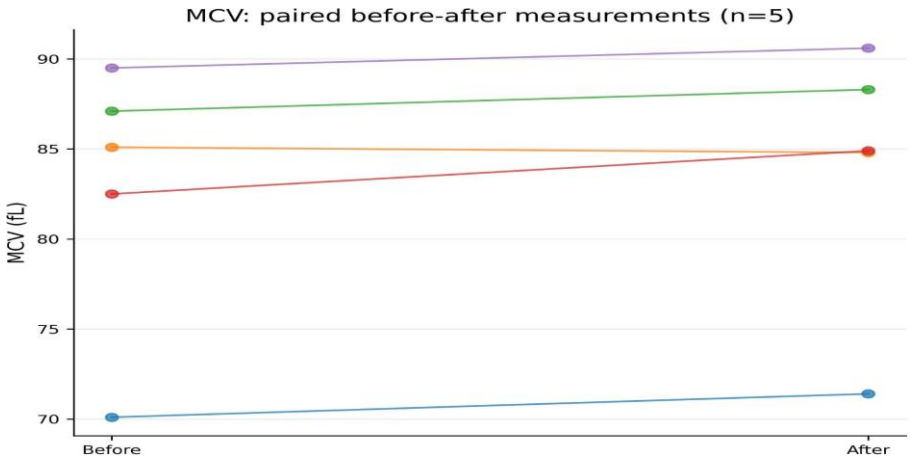


Figure 4. Comparison chart of MCV before and after irradiation

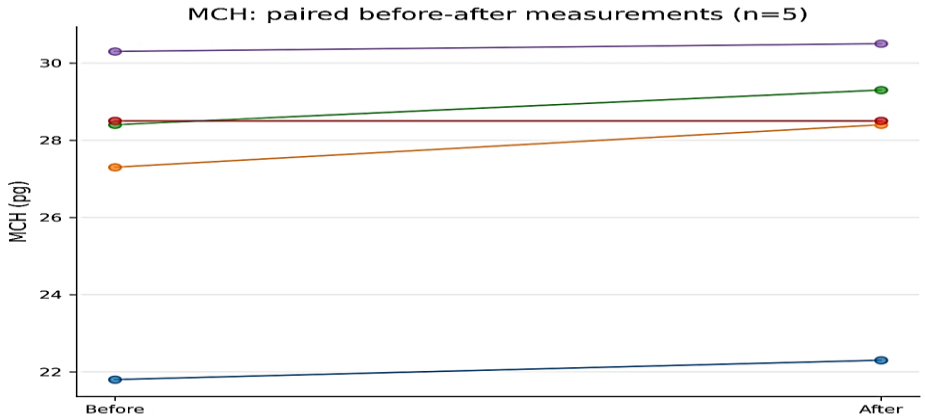


Figure 5. Comparison chart of MCH before and after irradiation

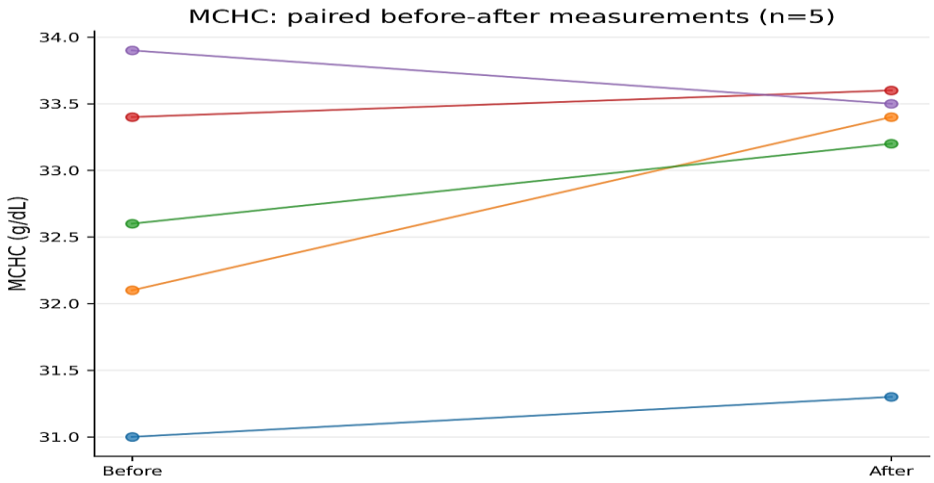


Figure6. Comparison chart of MCHC before and after irradiation

Given the current sample size and experimental settings, these results show that there is insufficient statistical evidence to support a trade within the three indexes. A lack of statistical significance, however, does not always imply that there is no organic influence; the sample size may be too small to detect minute variations. In a lab setting, MCV indicates the approximate volume of red blood cells, MCH indicates the approximate quantity of hemoglobin per cell, and MCHC indicates the approximate hemoglobin attention in the red blood cells. Although such parameters are frequently employed to indicate purple blood mobile homes, hemolysis is not directly measured by them. Therefore, rather than relying just on one index to assess the integrity of the red blood cells membrane, those indices should be evaluated in conjunction with PFHb readings and microscopic inspection.

Changes in red blood cells and their physiological significance

Even though the hemolysis fee and plasma-free hemoglobin (PFHb) showed a gradual increase below different irradiation conditions, I am unable to determine the physiological significance of these adjustments based solely on statistical significance; the increases were extremely limited, and their interpretation depends on the accuracy of the equation used to calculate the hemolysis charge as well as the particular experimental conditions. Additionally, after accounting for multiple comparisons, the impacts for whole blood matter (CBC) parameters did not consistently show statistical significance since they were entirely dependent on a narrow range of samples. Conversely, microscopic analysis of red blood cells (RBCs) is an essential technique for identifying morphological and structural alterations, such as membrane deformation, cellular shape changes, and fragmentation, which may provide supplementary evidence regarding the integrity of the cells. A study conducted by Al-Yasiri (2018) confirmed that the denaturation of RBC membrane proteins and the creation of pass-hyperlinks are linked to extended exposure to a 650 nm laser. According to Zhu et al. (2022), an increased production of echinocytes (spiculated purple blood cells) may also result from exposure to high light doses. Consequently, the results of the current study show a quantifiable change in hemolysis markers after radiation. To gain a more specific understanding of the potential organic consequences of light exposure, however, future studies with larger pattern sizes—including examinations of membrane integrity and microscopic examination of morphological changes—will be necessary to determine the physiological significance of those findings.

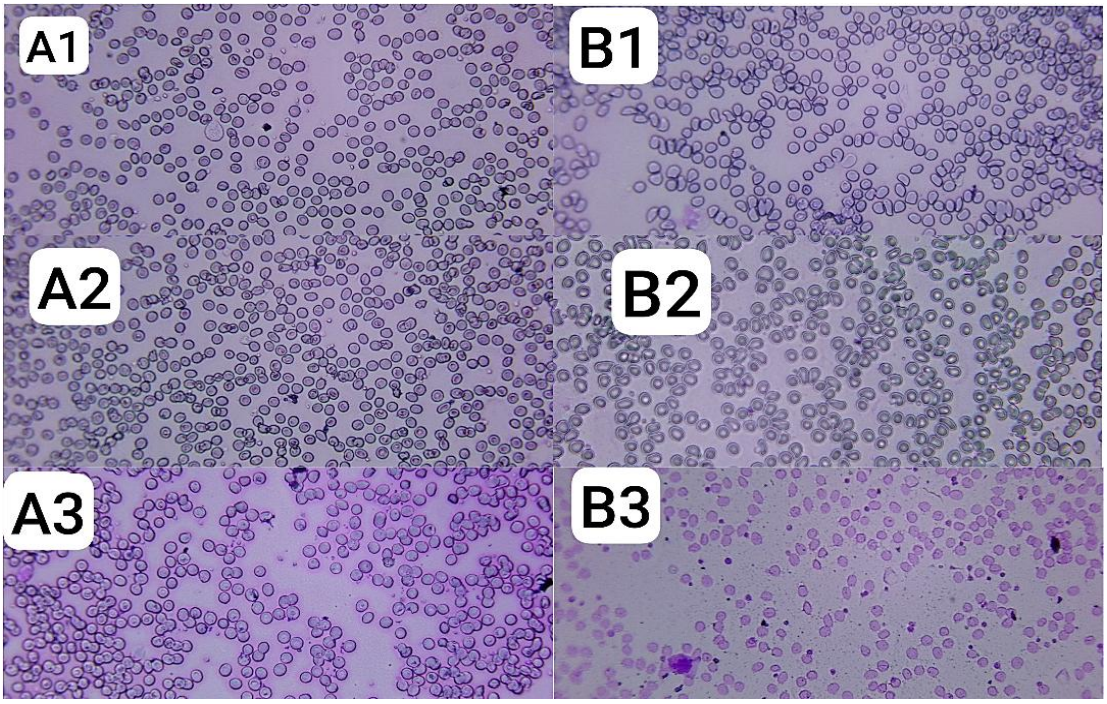


Figure 7. RBC cells shape, size change, (A) represent before and (B) Characterize after treatment

CONCLUSION

The results of the analysis indicate that exposing blood samples to moderate radiation at wavelengths of 450 nm and 650 nm was associated with a slow rise in the concentration of plasma-free hemoglobin (PFHb) and the charge of hemolysis, with the greatest increase found after 30 minutes of exposure at 650 nm. When interpreting their physiological significance, care should be used because these increases were severely constrained in absolute language. Furthermore, after controlling for multiple comparisons, whole blood matter (CBC) values ceased to exhibit consistent, statistically significant changes. Therefore, even though irradiation conditions may also impact the integrity of the red blood cellular membrane, further research involving larger sample sizes, biochemical testing, and direct microscopic analysis of morphological changes within the purple blood cells will be necessary to confirm those effects.

Limitation

The observation's limitations include the limited sample size and the absence of a distinct assessment of the power dosage applied to the samples during irradiation. Additionally, the results are still preliminary and need comparable research with larger sample sizes.

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تقييم تأثير التشعيع بالليزر منخفض الشدة على مؤشرات كريات الدم الحمراء، وشكلها، ومستوى الهيموغلوبين الحر في البلازما

الملخص

خلفية: تُعد خلايا الدم الحمراء (RBCs) مكوناً أساسياً من مكونات الدم، وتؤدي دوراً حيوياً في قدرة الهيموغلوبين على نقل الأكسجين إلى الأنسجة. يمكن أن تتأثر خصائص هذه الخلايا وسلامة أغشيتها بالتعرض للضوء بأطوال موجية وفترات زمنية مختلفة، مما يستدعي تقييم هذه التغيرات باستخدام مؤشرات مخبرية مناسبة. الأهداف: هدفت هذه الدراسة إلى تقييم تأثيرات التعرض للضوء على انحلال خلايا الدم الحمراء، ومستويات الهيموغلوبين الحر في البلازما(PFHb) ، ومؤشرات دموية أخرى، وذلك عند أطوال موجية تبلغ 450 نانومتر و650 نانومتر ولفترات تعرض متفاوتة. المواد والطرق: أُجريت دراسة مخبرية خارج الجسم (ex vivo) لتقييم تأثيرات ظروف التعرض الضوئي المختلفة مقارنة بمجموعة ضابطة لم تتعرض للضوء. تضمنت القياسات -قبل وبعد التعرض للضوء- تحديد مستوى الهيموغلوبين الحر في البلازما باستخدام تقنية القياس الطيفي الضوئي(spectrophotometry) ، وحساب نسبة انحلال خلايا الدم الحمراء، وتقييم مؤشرات خلايا الدم الحمراء، وهي: متوسط حجم الكرية(MCV) ، ومتوسط كمية الهيموغلوبين في الكرية(MCH) ، ومتوسط تركيز الهيموغلوبين في الكرية .(MCHC) كما استُخدمت تحليلات إحصائية مناسبة لتقييم النتائج. النتائج: ارتفع متوسط مستويات الهيموغلوبين الحر في البلازما (PFHb) من 3.216 ± 1.017 ملجم/ديسيلتر إلى 4.601 ± 0.990 ملجم/ديسيلتر بعد 30 دقيقة من التعرض لضوء بطول موجي 650 نانومتر. بالإضافة إلى ذلك، ارتفعت نسبة انحلال خلايا الدم الحمراء من 1.345 ± 0.341% إلى 1.510 ± 0.338%. لم تُسجل فروق ذات دلالة إحصائية في مؤشرات (MCV)و (MCH)و(MCHC)، في حين أظهرت فئات الهيموغلوبين ككل مستويات متفاوتة من الدلالة الإحصائية اعتماداً على نوع التحليل المستخدم. الاستنتاجات: تشير النتائج إلى وجود علاقة بين التغيرات في مستويات الهيموغلوبين الحر في البلازما وبين مدة التعرض للضوء وطوله الموجي. ومع ذلك، تظل هذه النتائج أولية؛ لذا يلزم إجراء دراسات إضافية تعتمد على أحجام عينات أكبر، وتوحيد ظروف التعرض للضوء، واختبار دقة معادلة حساب الانحلال، وذلك لتأكيد هذه النتائج.

الكلمات المفتاحية: التشعيع بالليزر، خلايا الدم الحمراء، الهيموغلوبين الحر في البلازما، 450 نانومتر، 650 نانومتر، التعديل الحيوي (Biomodulation).