Effects of Low Level Laser Irradiation on Blood Plasma and blood parameters of Breast Cancer patient

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Abstract
This study designed to evaluate the effect of Low level laser in vitro on Some blood parameters such as Erythrocytes Sedimentation Rate, Mean cell volume, hematocrit and MCHC of patients' with Breast Cancer by irradiated a human blood plasma samples by using a green laser and comparing this effects pre- and post- irradiated at fixed power density (100mW/cm2) and wavelength (532nm) of the laser that we used. The 8 ml of fresh blood samples collected in EDTA- tubes and separated in to four equal quantities: one for control (non-irradiated) sample and other three for irradiation by laser. The blood samples separated by bench centrifuge and cells sediment in the bottom of the tube and the plasma are supernatant in the up of the tube. After separated, the plasma irradiated by laser with different exposure time (10, 20, and 30) minutes the blood parameters are measured by using measured Automatic hematology analyzer and then ESR values are measured by using a Westergren method. The RBCs volume (MCV) increased while MCHC are decreased and also the values of ESR decreased significantly at 30min exposure time.
Keywords: Blood plasma, low level laser, Breast Cancer, Erythrocyte sedimentation rate, green laser pointer.

1. Introduction

Laser light has special characteristics which make it important way, more effective, and dangerous than conventional light that has the same power, and There have several important properties that distinguish it from the normal light (1). Low level laser is the most accurate and commonly used term to describe the types of lasers used in rehabilitation. The instrument is described as a "therapeutic laser." LLLT has been characterized as a non-thermal modality in the past. LLLI provides varying degrees of clinical effect in medicine depending on using. LLLI has been used in blood therapy for a range of clinical applications due to its capacity to enhance microcirculation and modify blood rheology at various powers, wavelengths, and exposure times (2, 3). Low-level laser therapy (LLLT) is a non-invasive process, painless, and this method that used photonic energy to supply biological therapeutic advantages, including analgesic effects. It's used to treat indolent or infected wounds, tissue necrosis, nerve injury, osteoarthritis, and other chronic pain syndromes like myofacial pain, fracture healing, tendinous or ligamentous injury, and post-surgical incision care (4, 5).
Laser therapy generally uses red and infrared laser light. Blue, violet, green, and ultraviolet laser therapy devices have recently been introduced (6).
The interaction between tissue and the laser light beam through therapy is determined by the wavelength of laser, power density, and exposure time. The selective effect of laser light on biological tissues is due to the monochromatic nature of laser light. Light can be transmitted, scattered, reflected, or absorbed as it comes into contact with the tissues. This is dependent on the tissue's composition and the wavelength of the light (7, 8).
Water is the main component of tissue that can absorb in infrared light, while hemoglobin absorbs in visible light, especially green light, and melanin tissue can be absorbed in both ultraviolet and visible light (8). The specific absorption characteristics of each tissue are based on its composition and chromophore content. Hemoglobin, Melanin, Water, and Protein are the primary chromophores found in mammalian tissue. Water absorbs the majority of infrared light, while hemoglobin and melanin absorb the majority of visible and ultraviolet light, respectively. As the wavelength reduces toward violet and ultraviolet, scatter or absorption from covalent bands in protein limits penetration depth in the range (9).

2. Materials and Method

Ethical approval

The protocol of this study was approved by local ethics committee in the department of physiology, college of Medicine, Baghdad University, Baghdad, Iraq. Each patient signed a written consent informed form to agree to participate in the study with keeping the patient records private during the entire research.

The collection and preparation the Plasma of blood

About thirty of Blood samples are collected from

doi.org/10.31838/hiv23.01.187 Received: 05.09.22, Revised: 02.10.22, Accepted: 04.11.22.

adult females with breast cancer patients after informing them of the objectives and ensuring that the data is kept confidential with a mean age of (46.9 ± 8.03) years. The protocol of this study was approved by local ethics committee in the department of physiology, college of Medicine, Baghdad, University, Baghdad, Iraq.

An 8 ml of fresh blood samples collected from venipuncture into anticoagulant tubes that contain about 1.3mg/ml from (EDTA-K3 tubes). Each blood samples was divided into four equal amounts, these aliquot about 2ml per tube, one of these is used as a non-irradiated (control), and the other three aliquots were irradiated samples by exposed to green laser with different exposure times.

The plasma samples Suspension

A bench- top centrifuge was used (HUMAX 5K, SN. 01-0906, 2008) to separate the plasma from the blood cells via Centrifuged the tubes at 3000rpm for 5min {13}. The cells sediment in the bottom of the tube and the plasma is supernatant in the up of the tube.

After 2ml of blood separate they formed 1ml of blood and 1ml of plasma. The Plasma lifted carefully by using a micropipette and placed in the Khan Glass Tubes, a blood sample (RBCs) was collected and placed in an ice box at temperature 4∘ C for 1 hour.

Plasma samples irradiation

We used a green laser pointer (GLP) has a wavelength (532nm) and the irradiation source has output power 33mW, the model of this device (Diode laser pointer JD- 303) ,and fixed power density about 100 mW/cm^2,

The Plasma Samples contain about 1ml of plasma per tube, and then irradiated by a laser beam with a spot diameter beam of about 0.65cm, and a laser placed at fixed distance of 2cm from the tube. Each irradiated set received (60, 120, and 180) J/cm^2 radiation doses at varied exposure times (10, 20, and 30) minutes respectively (Without the control samples). The laser beam was focused on the center of the Plasma sample in the test tube on a frequent basis depending on the exposure time. The irradiation procedure was achieved at room temperature (18-23) °C.

Irradiation plasma re-suspending with Red blood cells

After the plasma irradiated then mixed with non-irradiated erythrocytes to measure the changes of blood parameter after plasma irradiation such as (RBCs Count, MCV, HCT, and MCHC) by using Automatic hematology analyzer (humaCount 5D, Human Gesellschaft fur Biochemica und Diagnostica mbH, Made in Wiesbaden Germany) after various irradiation time and doses and before irradiation to laser. And measured the Erythrocyte sedimentation rate ((ESR)) by the Westergren method [10].

3. Statistical Analyzer

Statistical analysis SPSS software (version 22.0, IBM Corp., Armonk, NY) was used to analyze the data. Using a paired t-test, the differences between the control and radioactive sample sets were assessed. The difference analysis's significance is used to determine the P-value, and when the P-value is less than 0.05, it is considered significant.

4. Results and Discussion

The Results of irradiated blood plasma samples after then mixed with non-irradiated RBCs test for females with breast cancer, this result explain the various in blood parameter before and after irradiation with different irradiation time (10, 20,30) min and different doses (60,120,180) J/cm^2 as shown in table 1

Table1. The changes in blood parameter (RBC count and RBC volume, hematocrit, MCHC, and ESR values) for blood Plasma samples of breast cancer patients (before irradiated and after irradiated by laser).

Blood parameter	Before Irradiation mean ± SD	10 min dose 60J/cm^2 mean ± SD	20 mi dose 120J/cm^2 mean ± SD	30 mi dose 180J/cm^2 mean ± SD	*p*-value
RBCs *10^6 µl	4.415 ± 0.249	4.401 ± 0.395	4.391 ± 0.354	4.362 ± 0.382	0.9848
HCT %	36.803 ± 3.74	36.23 ± 4.213	36.16 ± 4.851	36.023 ± 4.852	0.9099
MCV fL	82.73 ± 5.099	79.59 ± 8.716	79.373 ± 7.383	77.663 ± 8.261	0.0375*
MCHC g/dL	33.75 ± 0.621	35.07 ± 5.090	35.69 ± 4.683	35.996 ± 5.531	0.0121*
ESR mm/h	33.6 ± 1.489	29.1 ± 1.421	28.833 ± 1.389	23.4 ± 1.490	0.0059*
* Significant Difference at level ≤0.05					

In plasma sample irradiated for BC patients when compared before irradiation and after irradiation time 10, 20, and 30min with dose 60,120 and 180 J/cm^2 the blood parameters "RBCs and HCT" decreased slightly with no-significant difference as shown in Tables1. When the values of RBCs count and HCT (as shown in figure1) in plasma irradiated did not significantly different. As a result, HCT was not expected to change, and the decrease in ESR could not have been caused on by a change in HCT or, subsequently, the blood's viscosity [12, 13].

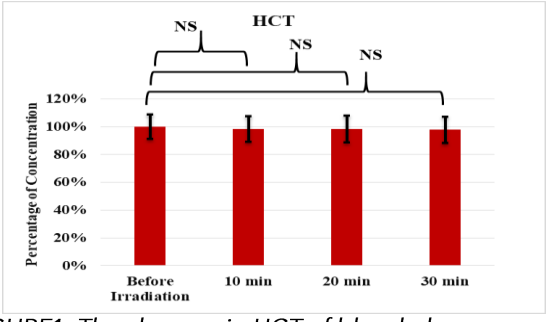

FIGURE1. The changes in HCT of blood plasma samples for the breast cancer patients pre- and post-irradiation with different irradiation time

The other blood parameters (MCV, MCHC, and ESR) of plasma irradiation samples for Breast Cancer patients in table1, there has a significant difference observed at p-value <0.05, the mean ± SD values decreased in MCV and ESR and increased in MCHC when compared between pre- and post-irradiation time 10min, 20min and 30min and doses 60J/cm2, 120J/cm2 and 180 J/cm2, as shown in Figure 2 and 3 respectively.

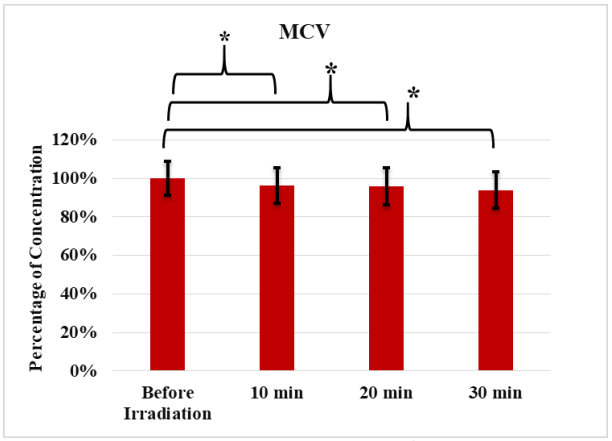

FIGURE2. The changes in MCV of blood plasma samples for the breast cancer patients pre- and post-irradiation with various irradiation times

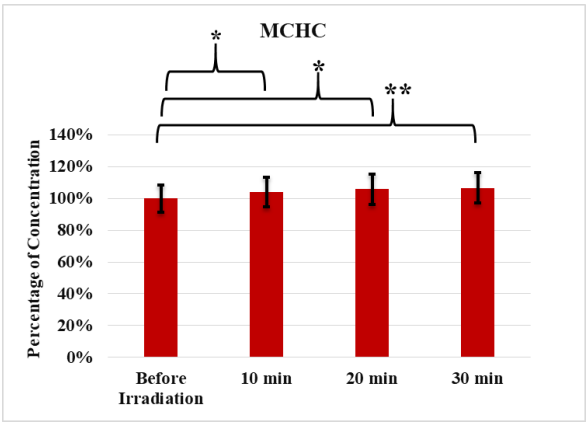

Figure.3 The changes in MCHC of blood plasma samples for the breast cancer patients before- and after -irradiation by laser

The change in MCV in Figure2 when the plasma sample was irradiated after different exposure times this change in MCV after irradiation by (LLL) is in fact a mirror to change in ESR values and reduction when exposed to LLL (low level laser). This reduction in ESR not only affects change in MCV its possible effects on a small change in HCT.

This study shows definitely that irradiation changes the plasma composition, which directly affects cell connections and significantly affects the ESR , the reduction in ESR value indicating a change in ESR of human blood by inducing by laser, Then the maximum decreased of ESR value at exposure time 30min with dose 180J/cm^2 as shown in **Figure 4.** the reduction in the ESR values is independent of the RBC counts (on the number of RBC). In this study, the RBC counts were non-significant different pre- and post- irradiation (in table1). Under present experimental conditions, this study suggests that erythrocytes did not hemolysis after irradiation with the laser.

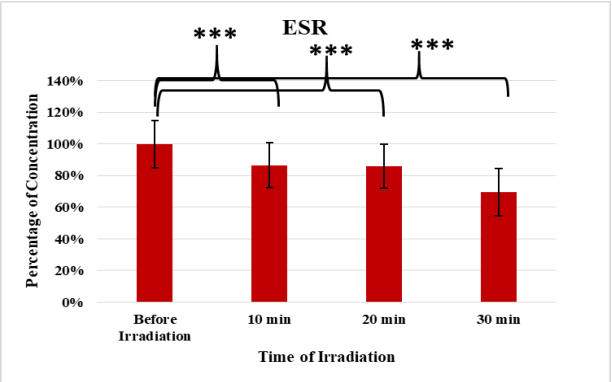

Figure4. The changes in ESR values for blood plasma samples of Brest Cancer patients after different exposure time

Other studies (14-16) which used LLL with different wavelengths resulted in the maximum decrease of ESR values in doses (72 J/cm2) for different wavelengths. while other author (17) proved the relationship between the ESR and wavelengths, when at different wavelengths (405, 589, 780) nm of LLLI the maximum reduction in the ESR values was the most effective at wavelength (405 nm).

The primary variables that influence the ESR are plasma fibrinogen and globulins. The strongest aggregator is the ESR fibrinogen, and it is directly correlated to the ESR and correlates with the concentration in the blood (18, 19). Albumin has the lowest aggregation ability in RBC sediment, and alpha and gamma globulin provide 50% of fibrinogen's aggregation ability in RBC. According to the study's findings, the ESR is changed when blood plasma is exposed to LLLI. The microcirculation and rheological characteristics of diseased blood samples are improved by laser irradiation (20, 21).

These results explain According to one theory, RBCs in blood samples from healthy human donors tend to congregate into what looks like a stack of coins or rouleaux. ESR readings rise as RBC aggregation and rouleaux generation increase. RBCs aggregate when the disaggregating forces caused by electrostatic repulsion outweigh the attractive bridging forces caused by the adsorption of macromolecules, such as fibrinogen and globulin plasma proteins, on adjacent cell surfaces. Aggregation is also impacts affected by the inherent characteristics of RBCs', such as the membrane's elastic nature, which contributes to the RBC cells' resistance to aggregation [22]

The attractive bridging forces are stronger than the electrostatic repulsive forces under typical experimental conditions used to determine ESR. According to researcher [14], an increase in the negative surface charge of RBCs leads to an increase in the electrophoretic mobility of erythrocytes suspended in a phosphate-buffered solution when they are exposed to laser irradiation (with wavelengths of 632.8 and 532nm, laser power of 30mW, irradiation spot of 5mm in diameter, and a

power density rate of 1.5mW/mm^2). As a result, the electric repulsive force rises, which leads to a decrease in the formation of rouleaux and consequently the ESR. This might explain the decrease in ESR observed in the irradiated plasma separated of blood when compared to non-irradiated samples. The light beams scatter as they come into contact with tissues during the laser interaction with it, and some of the scattered light is also simultaneously absorbed by the tissue [23].

5. Conclusion

The blood paramters changes when effected by low level laser irradiation at different exposure time and the LLL using in this study with wavelengh ,doses 532nm and fixed power density.

The results present the ESR, MCV, and MCHC variations at 30min exposure time to laser and dose 180J/cm2. In the blood plasma irradiated mixing with non-irradiated RBCs; it resulted in the ESR maximum decreased in 30min (180J/cm2) more than before irradiation for patients with breast cancer. This decreasing in ESR Values is very useful to use in laser therapy and its should consider in laser therapy procedure (the laser energies, wavelength, doses, and exposure time)

Conflict of interest: The authors declare no conflict of interest.

Source of Funding: This research received no external funding and didn't receive any specific grants from governmental, private, or nonprofit funding organizations.

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