Immediate Vascular Photochemical Reactions to Infrared Laser Irradiation in Normal Volunteers

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Abstract: Background: There are evidences that low level laser therapy (LLLT) stimulates wound healing. Objective: The study aimed at investigating the exact vascular mechanisms through which infrared (IR) laser acts to promote wound healing. Participants: Thirty normal female volunteers were selected from the female section, Faculty of Applied Medical Sciences, King Abdul-Aziz University. They were randomly divided into three equal study groups (G1, G2, and G3). Methods: Five ml. of whole blood were collected in a plane tube from all volunteers for the analysis of lipid profile including cholesterol (Chol); triglyceride (TGL); low density lipoprotein (LDL) and high density lipoprotein (HDL). Other five ml of blood were collected for the performance of glycated hemoglobin (HbA1c), hemoglobin (Hgb) and red blood cells (RBCs) count before and immediately after receiving continuous IR laser (810 nm, 100 mW). The irradiation doses were 12 J/cm^2 for 120 sec, 6 J/cm^2 for 60 sec and 1.4 J/cm^2 for 14 sec in groups one, two, and three respectively. Results: There was a significant increase in Chol, TGL, HbA1c%, Hgb concentration and RBCs count after irradiation. On the other hand, there was no significant difference in LDL or HDL concentration in the three groups. Conclusion: Infrared laser was effective in increasing the levels of different blood components that are important for wound healing processes with the best results obtained from laser dosage of 12 J/cm^2.

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1. Introduction:

Large number of neurological disorders may be complicated with the development of ulcers and bed sores such as spinal cord injuries and diabetic polyneuropathy due to autonomic and/or sensory disturbance. Those ulcers need different kinds of treatment including medical, physical therapy, and nursing care. Recently, there has been an increase in the clinical application of LLLT in various therapeutic fields. One of the most important functional aspects of laser therapy is photobiostimulatory effects on various biological systems especially microcirculation [1]. Varicose ulcers and bedsores are appropriate indications for laser therapy combination with conventional therapies [2]. It was found that LLLT was effective in open wounds, which showed better regeneration and faster restoration of structural and functional integrity [3]. In one survey, LLL was ranked as more effective in promoting wound healing than other electrophysiological agents such as particular types of electrical stimulation and ultrasound [4].

Hopkins et al. [5] investigated the effects of LLL on wound healing. They applied laser (8 J/cm^2, 700 Hz) on an induced abrasion on the anterior surface of the forearm in twenty-two healthy subjects. Follow-up photograph testing revealed smaller wounds in the laser group than in the sham one. They also found that other abrasion in the forearm of the study group was contracted more than that of the control group without direct laser application denoting that LLL produced an indirect systemic healing effect on surrounding tissues.

Other study also confirmed the positive efficacy of LLL in enhancing wound healing. Schubert [6] evaluated the effects of IR and red pulsed monochromatic light, with varied pulsations and wavelengths, on the healing of pressure ulcers in a prospective, randomized, controlled study. He concluded that pulsed monochromatic light increased healing rate and shortened healing time.

The use of radiations of all kinds to accelerate wound healing has a long history but radiations in the red part of the visible spectrum have been particularly employed and found to be effective. Despite of those promising effects, little is known about the exact mechanisms through which LLLT promotes healing.

Aim of the study:

The aim of the research was to investigate the possible vascular mechanisms through which IR laser accelerates wound healing. In addition, the study aimed to examine the effects of three different IR laser dosages on different blood components.

2. Materials and Methods

Enrollment of Subjects

Prior to inclusion, a complete medical history was taken. Intended enrollment comprised of 30 healthy female subjects who were selected from faculty of applied medical sciences at King Abdul-Aziz University, their age ranged from 18–24 years (mean=22 ± 0.1). For the initial evaluation, each subject was examined medically by a physician at King Abdul-Aziz University out patient clinics. Subjects were excluded in cases of psychological history or stresses, pregnancy, use of drugs, like corticosteroids, during menstruation period, bleeding or vascular disorders and liver or renal disease. Subjects were randomly divided into three equal groups (G1, G2 and G3). Procedures used in this study were approved by the Ethics Committee of the faculty of applied medical sciences at King Abdul-Aziz University, Saudi Arabia. Written informed consent was obtained from subjects prior to the start of the study.

Analytical Methods

Blood Sampling and Laboratory Methods

Blood samples (10 ml) were collected, before and immediately after laser irradiation, from antecubital veins with a 20G butterfly without use of tourniquet; hemolysed and lipemic samples were excluded. Samples were centrifuged at 3500 rpm for 5 minutes, to separate the plasma. All samples stored after centrifugation at -70°C until time of processing. These samples were collected in two different vacutainer tubes. One tube was used for measuring lipid profile (Total Chol, TGL, LDL and HDL). The second tube contains $EDTAK_2$ and $EDTAK_3$ for measuring complete blood count and glycated hemoglobin (HbA1c).

Blood Count

Blood count was measured in Beckman Coulter AC-T (Beckman Coulter, Fullerton, CA, USA) at king Abdul-Aziz University Hospital. Blood count provided red blood cells count (RBC) and Hemoglobin (Hgb) concentration.

Glycated hemoglobin (HbA1c)

Quantitative determination of HbA1c% was measured by using COBAS INTEGRA 400 system, Roche, German. The total Hgb concentration was measured calorimetrically. Glycated hemoglobin was determined immuno-turbidimetrically. The ratio of both concentrations yields the final HbA1c%.

Biochemical analysis

Biochemical parameters included serum for total Chol, TGL, LDL, and HDL. All were measured at the same time when the medical pre-check up was performed. All of the biochemical assessments were performed using a fully automated analyzer using the principle of Electro-chemiluminescence's immunoassay (Modular Analytics E170, Roche, German) at king Abdul-Aziz University Hospital.

Laser

Sys*Stim, ME-540, USA was used, with Aluminium-Galium Arsenide (AlGaAs) diode and wavelength of 810 nm. The beam delivery system is hand held probe, continuous/ pulsed, the output power is 100 mW, and the dose ranges from 0.01 to 99.99 Joules.

Laser Application

Each Subject was asked to sit on a chair with back support and a table of suitable height was beside her to support the forearm in supination position. The area of application was determined, two cm proximal to the palmer crease of the wrist joint in the midline. The area was cleaned well with alcohol swab. The three groups were exposed to IR laser as follow: G1 received 12 J/cm^2 for 120 seconds [7], G2 received 6 J/cm^2 for 60 seconds [8], and G3 received 1.44 J/cm^2 for 14 seconds [9]. For safety measure, both the subject and the researcher wore goggles for eye protection.

Statistical Analysis

Data was presented as the mean and standard deviation. Paired t-test was used to analyze the data within each group and one way Anova test was used to analyze the data between the three groups. The p-value was <0.05.

3. Results

Lipid Profile

Cholesterol

The results revealed no significant difference regarding chol. mean values between groups, neither pre nor post irradiation (5.2±0.9, 4.7±0.9 and 4.7±0.75 mg/dL, respectively) (p= 0.888 and 0.331, respectively). On the contrary, there was a significant increase in chol. mean values after laser application compared to that before in the three groups (p_1 = 0.0001, p_2 = 0.017 and p_3 = 0.001) (Table: 1).

Table (1): Comparison of groups 1, 2 and 3 regarding cholesterol mean values before and after laser irradiation.

Cholesterol (mg/dL)	G 1 (Mean ± SD)	G 2 (Mean ± SD)	G 3 (Mean ± SD)	*p*
Pre	4.3 ± 0.8	4.5 ± 0.9	4.4 ± 0.76	0.888
Post	5.2 ± 0.9	4.7 ± 0.9	4.7 ± 0.75	0.331
p	0.0001	0.017	0.001	

Triglyceride

There was a significant difference in TGL mean values between the three groups after irradiation compared to that before (p= 0.115 and 0.0001, respectively). Also, the results revealed a significant difference after laser application compared to that before in the first two groups (p_1= 0.001, p_2 = 0.048) and non significant difference in the third one (p_3 = 0.875) (Table: 2).

Table (2): Comparison of groups 1, 2 and 3 regarding concentration of TGL mean values before and after laser irradiation.

TGL (mg/dL)	G 1 (Mean±SD)	G 2 (Mean±SD)	G 3 (Mean±SD)	*p*
Pre	0.87 ± 0.3	0.74 ± 0.27	1.01 ± 0.3	0.115
Post	1.3 ± 0.5	0.91 ± 0.4	0.98 ± 0.5	0.0001
P	0.001	0.048	0.875	

High density lipoprotein

The results revealed no significant difference regarding HDL mean values between groups before and after irradiation (p = 0.834 and 0.558, respectively). Also, the results showed no significant difference after laser irradiation compared to that before in the three groups (p_1= 0.269, p_2= 0.119 and p_3= 0.666) (Table: 3).

Table (3): Comparison of groups 1, 2 and 3 regarding concentration of HDL mean values before and after laser irradiation.

HDL (mmol/L)	G 1 (Mean±SD)	G 2 (Mean±SD)	G 3 (Mean±SD)	*p*
Pre	1.8 ± 0.2	1.8 ± 0.4	1.7 ± 0.5	0.834
Post	1.92 ± 0.5	1.97 ± 0.3	1.8 ± 0.6	0.558
p	0.269	0.199	0.666	

Low density lipoprotein

Also, There is no significant difference regarding LDL mean values between groups before and after irradiation (p=0.907 and 0.754, respectively). The results revealed no significant difference after laser application compared to that before in the three groups (p_1=.0.116, p_2=0.143, p_3=0.914 respectively) (Table: 4).

Table (4): Comparison of groups 1, 2 and 3 regarding concentration of LDL mean values before and after laser irradiation.

LDL (mmol/L)	G 1 (Mean ±SD)	G 2 (Mean±SD)	G 3 (Mean±SD)	*P*
Pre	2.7 ± 1.1	2.9 ± 0.7	2.7 ± 0.9	0.907
Post	2.96 ± 0.7	2.7 ± 0.8	2.7 ± 1.1	0.754
p	0.116	0.143	0.914	

Red Blood Cells

There was no significant difference regarding RBCs mean values between groups before and after irradiation (p=0.454 and 0.153, respectively). But there was a significant increase after laser application compared to that before in the first two groups (p_1=0.0001, p_2=0.001) and non significant difference in the third one (p_3 =0.126) (Fig: 1).

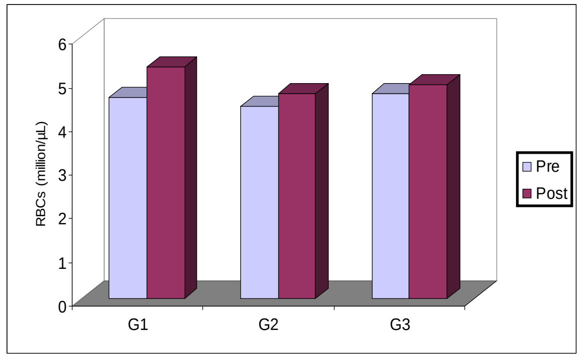

Fig (1): Mean values of RBCs count in the study groups before and after laser irradiation.

Hemoglobin

There was a significant difference regarding the Hgb concentration mean values among the three groups after laser irradiation compared that that before (p= 0.737 and 0.038, respectively). Also, there was a significant increase after laser application compared to that before within G2 and G3 (p_2= 0.0001 and p_3= 0.0001) while non-significant difference was found in G1 (p_1= 0.07) (Fig 2).

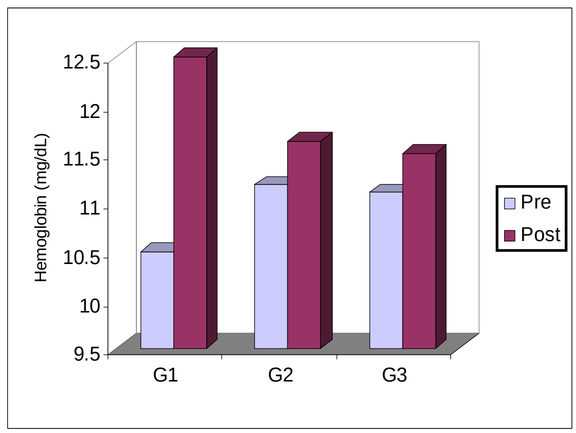

Fig (2): Mean values of Hgb concentration in the study groups before and after laser irradiation.

Glycated Hemoglobin

Significant difference in HbA1c% mean values was shown in the three groups after irradiation compared to that before irradiation (p=0.234 and 0.004, respectively). Also, high significant increase was revealed after laser application compared to that before in the three groups (p_1=0.001, p_2=0.0001 and p_3=.0001) (Fig: 3).

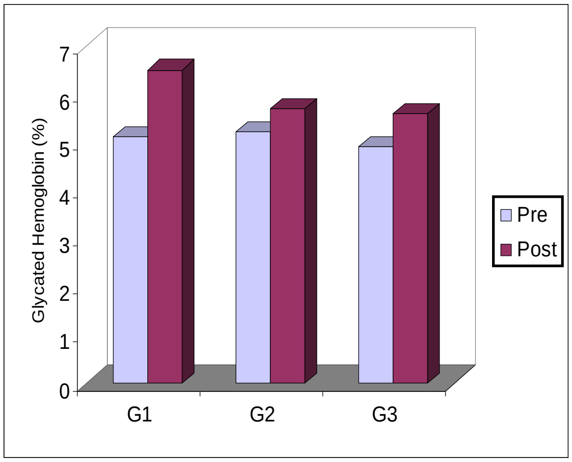

Fig (3): Mean values of HbA1c% in the study groups before and after laser irradiation.

4. Discussion

The current study aimed at investigating the vascular changes that may occur in different blood components in response to IR laser in normal subjects. It was also concerned with comparing the changes that result from exposure to three different laser doses.

The results revealed variable findings in the different measured blood components in the three groups. Some essential components for healing process showed significant increase after laser irradiation, especially in the first two groups, while others did not.

In accordance with the findings of the present work, Adltya *et al*. [10] studied the efficacy of low energy photon therapy (LEPT) in the treatment of venous leg ulcers in nine patients with 12 venous ulcers. Treatment was given three times a week for ten weeks using two different sources. One source provided a wavelength of 660 nm (red), while the second source delivered a wavelength of 880 nm (infrared). They concluded that LEPT of both sources was an effective modality for the treatment of venous leg ulcers.

Moreover, the findings of the current research are supported by those of kujawa *et al*. [7]. Their study aimed at investigating the effect of laser radiation on the structure of protein and lipid components of RBCs membranes and its functional properties. Human RBCs were irradiated with LLLT at different radiant exposures (3.75 - 25 J/cm^2) and laser powers of 10 mW. An increase of the ATPase activity was found with maximal effect at 12-15 J/cm^2 of light dose. On the contrary, the same research procedures were carried out with laser power of 400 mW and inhibition of ATPases activities occurred.

In addition, the positive findings of the current work came in accordance with those of Verdote *et al*. [11]. They studied the LLLT effect for the treatment of open wounds in psycho-geriatric patients. In total, 84 psychiatric patients with 188 open wounds were referred for the treatment of open wounds of varying severity and etiology. Traditional wound care management was used in addition to laser therapy. About 84% of these wounds completely healed.

On the other hand, Lucas *et al*. [12] found no evidence that justifies using LLLT as an adjuvant to the decubitus ulcer treatment. Their negative findings may be attributed to laser parameters they applied. Parameters were infrared laser, 904 nm; total peak power was 12 x 70 W in an 830 Hz pulse frequency mode of 150 nsec pulses with an average beam power of 12 x 8 mW and a radiant exposuree of 1 J/cm, which required an exposure time of 125 sec.

The positive outcomes of the present research suggest that, in case of open wound, healing occurs due to photochemical changes in blood components as a result of IR laser irradiation. Photochemical reactions included increase in hemoglobin, RBCs and lipid levels in blood leading to an increase in blood flow and membrane stability. Hence, there will be increase in the ATP activity, oxygen and nutrition delivery to body tissues that enhances healing process. Such changes were also reported by Siposan and Lukacs [13] who found significant differences between control and irradiated blood samples for RBCs; Hgb; and viscosity. In addition, they found increase in plasma proteins including albumin; alpha one globulin and gamma globulin and fibrinogen.

Other study was carried out by Schindl *et al*. [14] who selected thirty patients with diabetic ulcers or gangrenes. They received either a single LLL irradiation with an energy density of 30 J/cm² or a sham irradiation over both forefoot regions. Skin blood circulation, as indicated by temperature recordings over the forefoot region, was detected by IR thermograph. Data from this first clinical trial demonstrated an increase in skin microcirculation due to athermic laser irradiation in patients with diabetic microangiopathy.

Furthermore, LLLT improves blood microcirculation by soothering blood vessels, which is biologically beneficial. It has been found that LLLT increases the delivery of oxygen and nutrients in the blood cell to the body's soft tissue areas [15]. Photons are picked up by the cell membrane and result in improved membrane stability and increased activity of the ATP dependent Na/K pump. Moreover, light therapy increases blood flow much better, resulting in improved systemic blood circulation thus increasing RBCs which stimulate the release of ATP [16].

Additionally, Wounds require adequate oxygen delivery to heal [17] and hemoglobin, in the blood, is what transports oxygen from the lungs to the rest of the body tissues where it releases the oxygen for cell use. Also, Hemoglobin in the RBCs carries some of the waste product carbon dioxide back from the tissues to the pulmonary capillaries of the lungs [18]. Therefore, the significant increase in hemoglobin level and RBCs, whose cytoplasm is rich in hemoglobin, shown in the current study may be other possible mechanisms that contribute to healing process. In accordance, Vladimirov *et al*. [19] found that nitrosyl complexes of heme proteins, such as hemoglobin and cytochrome c, are the primary chromophores of laser radiation. Upon irradiation, they can easily dissociate to produce free nitric oxide which may be responsible for blood vessel relaxation and activation of mitochondrial respiration. Glycated hemoglobin has a nutritional role as well; it is a form of hemoglobin used primarily to identify the average plasma glucose concentration over prolonged periods of time.

The findings also showed significant increase in TGL, which is a type of lipid that circulates in the blood stream, in the first two groups. Certain vitamins such as vitamin A, D, E, and K are fat-soluble and depend totally on the presence of TGL for absorption. Therefore, TGL helps in ensuring adequate nutrition that is highly useful for wound healing [11].

Other mechanism of accelerating healing was also suggested by P"ontinen [15] which is the removal of accumulated toxins by LLLT through improving lymph circulation. The buildup of toxins in a health body could block the normal blood circulation and impair cellular energy. When IR waves are applied, water molecules that encapsulate the toxins get heat up, and start to vibrate. This vibration reduces the ion bonds of the atoms that are holding together the molecules of water. As the breakdown of the water molecules occurs, encapsulated gases and other toxic materials are released and the body gets rejuvenated.

The findings also revealed significant increase in blood cholesterol level upon irradiation in the three study groups. So, IR laser should be used cautiously with patients who are at risk of hypercholesterolemia. Finally, further studies are recommended with wounds or pressure ulcers monitoring both the immediate and long term effects of IR laser on different blood components and its correlation with the rate of wound closure.

5. Conclusion:

Infrared laser was effective in increasing the levels of different blood components that are important for wound healing processes with the best results obtained from laser dosage of 12 J/cm^2.

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