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Effect of laser puncture on liver enzymes post burn

Wpływ laseroterapii punktowej na enzymy wątrobowe po oparzeniu

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Abstract

Objective. The present study was carried out to investigate the impact of laser puncture as an adjunctive therapy on liver enzymes after a burn. Material and Methods: The study included 30 injured patients with burned body surface areas ranging from 30 to 50%. They were aged from 25 to 40 years. Cases from Orabi Hospital were randomized into two groups, equal in number. Group A included 15 cases as they received laser acupuncture (infrared diode laser, wavelength 808 nanometers, with pulse interval: 200 nanoseconds, power density: 0.4 Watt/cm^2, and energy density 4 Joule/cm^2); it was located in direct connection with skin perpendicular to the body acupoints (ST36), (SP6), (LR3), (GB34), (BL18) on each side of the body as well as Zhongwan (RN12) and conducted for 90 seconds for every point three times a week, for 4 weeks after leaving the intensive care unit, conventional physiotherapy program as well as medical treatment. Group B included 15 patients as they received only their conventional physiotherapy program as well as medical treatment. Aspartate transaminase (AST) and liver enzymes, alanine transaminase (ALT) were determined using a spectrophotometer. Results. After four weeks of our intervention, it was found that the laser puncture group had significantly lower ratios of plasma liver enzymes than those in the control group. Thus, it can be concluded that laser puncture serves as an efficient adjunctive therapy for improving liver function after a burn.

Keywords

laser puncture, liver enzymes, burn

Streszczenie

Cel. Celem niniejszego badania było zbadanie wpływu laseroterapii punktowej jako terapii wspomagającej na enzymy wątrobowe po oparzeniu. Materiał i metody: Badanie objęło 30 pacjentów z oparzeniami, których powierzchnia ciała oparzona wynosiła od 30 do 50%. Ich wiek wahał się od 25 do 40 lat. Pacjenci z Szpitala Orabi zostali losowo podzieleni na dwie równe grupy. Grupa A obejmowała 15 osób, które otrzymały laseroterapię punktową (laser diodowy podczerwieni, długość fali 808 nanometrów, z interwałem impulsu 200 nanosekund, gęstością mocy 0,4 Wata/cm^2 i gęstością energii 4 Dżule/cm^2); stosowano ją bezpośrednio na skórę, prostopadle do punktów akupunkturowych ciała (ST36), (SP6), (LR3), (GB34), (BL18) po obu stronach ciała oraz Zhongwan (RN12), przeprowadzając zabieg przez 90 sekund na każdy punkt trzy razy w tygodniu przez 4 tygodnie po opuszczeniu oddziału intensywnej terapii, w połączeniu z konwencjonalnym programem fizjoterapii oraz leczeniem medycznym. Grupa B obejmowała 15 pacjentów, którzy otrzymywali tylko swoje standardowe leczenie fizjoterapeutyczne i medyczne. Stężenie asparaginianowej aminotransferazy (AST) i alaninowej aminotransferazy (ALT) w surowicy krwi mierzono przy użyciu spektrofotometru. Wyniki. Po czterech tygodniach naszej interwencji stwierdzono, że grupa poddana laseroterapii punktowej miała znacząco niższe stężenia enzymów wątrobowych w osoczu niż grupa kontrolna. Można zatem wnioskować, że laseroterapia punktowa jest skuteczną terapią wspomagającą poprawę funkcji wątroby po oparzeniu.

Słowa kluczowe

laseroterapia punktowa, enzymy wątrobowe, oparzenie

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Introduction

Increases in core body temperature, blood sugar, oxygen intake, carbon dioxide generation, proteolysis, glycogenolysis, lipolysis, as well as futile substrate cycling, define the hypermetabolic reaction that follows a severe burn. Lean body mass reduction, muscle impairment, bone density reduction, as well as delayed wound healing, are all symptoms of this response, which begins on day 5 after the injury and lasts for up to 24 months post-burn [1, 2].

Tissue catabolism, caused by the body's increased metabolic demands, results in the loss of nitrogen and the potentially fatal depletion of critical protein stores. Proteins, as well as amino acids, are broken down to release energy. This serious critical illness is characterized by elevated protein turnover, breakdown, as well as negative nitrogen balance [3].

Consequently, this disrupts the structure along with the function of vital organs like the liver, heart, skeletal muscle, skin, the body's immune system, as well as cellular membrane transport processes [4].

Lipid peroxidation, an auto-catalyzing mechanism that causes oxidative damage to cellular membranes and eventual cell death, is closely linked to liver disease. Hepatic Malondialdehyde (MDA) is increased by 48%, plasma C-reactive protein (CRP) levels are increased by 30%, and plasma alanine transaminase (ALT) is elevated by 3.5-fold, but aspartate transaminase (AST) is elevated by 2-fold after thermal skin damage [5, 6].

Following the burn injury, both ALT and AST levels increased in the blood and remained high for almost three years. AST and ALT levels are highly indicative of hepatocellular damage. Throughout gluconeogenesis, they catalyze the conversion of amino groups from aspartic acid or alanine to ketoglutaric acid, resulting in the generation of oxaloacetic acid or pyruvic acid, respectively [7, 8].

Traditional Chinese Medicine (TCM), the basis on which acupuncture is founded, is a medical practice with a long and extensive history in China. By stimulating certain areas along the pathways (Meridians), acupuncture is thought to control and restore energy balance, thereby treating disease [9].

Laser puncture, also known as laser acupuncture or photopuncture, is the use of a therapeutic laser on specific acupuncture points. It has been demonstrated to be a reliable treatment tool; it is a simple, efficient, and non-invasive approach. Mechanisms involved in enhancing liver regeneration were activated by low-power laser irradiation. LPLI increases hepatic mitotic index and mitochondrial activity and promotes liver angiogenesis as well as hepatocyte proliferation [10, 11].

Thus, the goals of this work were to validate the curative impacts of laser puncture as an adjunctive therapy on liver enzymes following burn injuries and to help design the most effective physical treatment strategy for restoring normal liver function shortly after major burn injuries.

Materials and methods

Study design

Between February 2023 and May 2023, participants were enrolled, and the study was conducted at the Physical Therapy's Department for Burns at Orabi Hospital for Burns in Al Obour City, Qalyubia, Egypt. The study was permitted by the Physical Therapy Ethics Committee at Cairo University in Giza, Egypt (No: P.T.REC/012/004203), as well as registered in the Clinical Trials Registry retrospectively with the identifier (NCT05728450).

After leaving the ICU, 30 burned cases with burned body surface areas ranging from 30% to 50% were engaged in this investigation. They were all between the ages of 25 and 40. They were recruited from Orabi Hospital and randomized into two categories of equal numbers. Exclusion criteria included burn at areas of acupuncture points, lower extremity amputation, prior history of liver disorders, as well as body mass index (BMI = kg/cm²) of more than 30%. Before enrolling in our study, all cases provided written informed consent in which they confirmed that they had been notified of the work's objectives and methods.

G*POWER (version 3.1.9.2) statistical software was utilized for calculating the sample size, and the results showed that 15 individuals were required in each group to prevent type II errors. Values of $\alpha = 0.05$, Power ($1-\beta$ err prob.) = 0.90, as well as allocation proportion N2/N1 = 1, were used in the calculations.

Randomization

A consent form was obtained from all participants after an explanation of the study procedures. Sequentially numbered index cards were secured in opaque envelopes. A blinded researcher opened the sealed envelope and allocated the patients according to their groups. After randomization, five participants dropped out of the study (Figure 1).

Intervention

Group A (Study group)

This group included 15 burned patients who received laser puncture for one month (3 times/week) in addition to their physical therapy program (splinting, stretching exercises, and ROM exercises) and medical treatment.

Group B (Control group)

This group included 15 burned patients who received only their physical therapy program (splinting, stretching exercises, and ROM exercises) and medical treatment.

Procedures

Measurement procedures

- Certified technicians took serum blood samples from participants while they were seated in a chair.
- Spectrophotometers were developed to quantitatively analyze the electromagnetic spectrum. They covered regions of the electromagnetic spectrum, including the near-ultraviolet, the near-infrared, and the visible spectrum. To determine the level of absorbance or transmission through a liquid sample, a spectrophotometer was utilized. Since the spectrophotometer's double beam passed one beam through a blank reference sample and the other through the sample under examination, any wavelength within the spectrophotometer's range could be selected for measurement [12].
- The ALT and AST plasma levels were measured using a spectrophotometer, with a 20W halogen lamp and a deuterium lamp as the light sources. A silicon photodiode served as the detector. A CPS controller was linked to the UV/VIS to regulate cell location and temperature. There were five sam-

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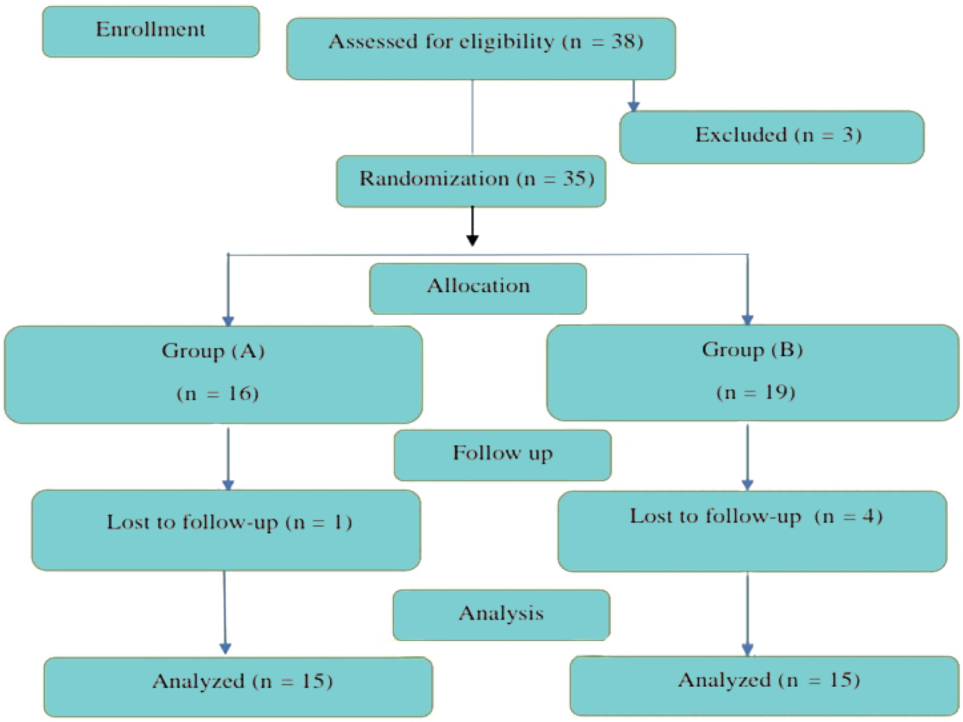

Figure 1. CONSORT flow chart

ple-holding cells, and motors moved the sample holder around in the CPS controller. The thermoelectric temperature controller was fastened to the sample holder for precise temperature management. Cube-shaped cuvettes, which are test tubes used to collect liquid samples, were employed [12].

- Normal values used for patients were 0–45 IU/L for Alanine transaminase (ALT) and 0–35 IU/L for Aspartate transaminase (AST) [8].
- Measurements were obtained prior to the treatment (pre-treatment) and one month later (post-treatment).

Treatment Procedures for Laser Puncture

- The treatment procedures started after the patient's release from the intensive care unit.
- The patient was positioned in a comfortable position.
- The patient wore protective eyeglasses.
- The points for the application of laser acupuncture were cleaned before application. Treatment with an infrared diode laser (808 nm, 200 nS pulse duration) was administered to all study participants. The energy density was 4 J/cm², and the power density was 0.4 W/cm² [13].
- The laser unit's plug was connected to the main power supply, and the power switch was turned on. The therapy form was selected from the main menu, and the parameters were set.

An Acupuncture detector (Pointer Excel II) was used for the definite location of acupuncture points.

For 30 minutes, three times weekly for a month, a laser probe was positioned perpendicularly over the skin at the acupoints Zusanli (ST36), Sanyinjiao (SP6), Taichong (LR3), Yanglingquan (GB34), Ganshu (BL18) on each side of the body, as well as Zhongwan (RN12) [14].

The Anatomical Locations of Acupoints Used [15]:

- Zusanli (ST36) acupoint is located on the lateral side of the lower leg, one hand's breadth below the knee crease, and one finger's breadth lateral to the shin bone.
- Sanyinjiao (SP6) acupoint is located on the inside of the lower leg, one hand's breadth above the prominence of the medial malleolus.
- Taichong (LR3) acupoint is located on the dorsum of the foot in the distal hollow at the junction of the first and second metatarsal bones.
- Yanglingquan (GB34) acupoint is located below the outside of the knee, in a tender depression 1 body inch anterior and inferior to the head of the fibula.
- Ganshu (BL18) acupoint is located two fingers' width lateral to the lower border of the spinous process of the 9th thoracic vertebra.
- Zhongwan (RN12) acupoint is located on the anterior median line of the upper abdomen, approximately 5 fingers' breadth above the belly button.

Physical therapy program for both groups

Splinting

Custom splints were made to help keep injured limbs in a functional or anti-contracture position [16]. Based on the

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splints used and the patient's skin condition, monitoring intervals ranged from once an hour to once every four to six hours. The schedule entailed 10 hours of wear followed by 2 hours of rest. Active and/or passive ROM (range of motion) exercises were to be performed once the splint was removed [17].

Therapeutic exercises

Patients performed active range of motion exercises (AROM), such as flexion and extension of shoulders, elbows, hips, and knees from different positions like supine lying, sitting on the bed, and standing against the wall. Patients did active-assistive ROM (AAROM) for shoulder and knee flexion, in which assistance was provided by the therapist. The movements were carried out rhythmically, evenly, and smoothly, with 5–10 repetitions. Treatment frequency was based on the patient's condition and improvement over time [18].

Stretching lasting 30 seconds at a time is the most secure form of long-duration stretch and results in the greatest elastic deformation and longest-lasting plastic modifications to soft tissues [19].

Statistical analysis

An unpaired t-test was employed to compare each group's mean age and body mass index (BMI). The Shapiro-Wilk test was used to check that the data were normally distributed. Levene's test for homogeneity of variances was employed to evaluate the groups' homogeneity. Within- and between-group differences in ALT and AST were analyzed using a Mixed Design MANOVA. For further multiple comparisons, post-hoc tests with the Bonferroni adjustment were conducted. All statistical analyses were done with a p-value < 0.05, which were considered significant. IBM SPSS (Statistical Package for the Social Sciences) Statistics Version 25 for Windows (Chicago, Illinois, USA) was employed for all statistical testing.

Results

The flow diagram for our study's subjects and the randomization procedure is shown in Figure 1. Thirty-eight people were screened to see if they qualified for the study; 3 were eliminated because they did not meet the inclusion criteria, and 35 participants were enrolled in group A or group B. Five participants withdrew in the third week before data collection because they did not complete the entire course of treatment.

Subject characteristics

Group A and B's subject characteristics are compared in Table 1. Distributions of body mass index, age, and sex showed no statistically significant differences between groups ($p > 0.05$).

Table 1. Subject characteristics

	Group A Mean ± SD	Group B Mean ± SD	MD	t- value	p-value
Age (years)	33.66 ± 4.98	31.07 ± 5.22	2.59	1.39	0.17
BMI (%)	21.47 ± 3.55	21.72 ± 4.05	-0.25	-0.17	0.86
Sex, n (%)					
Females	8 (53.3%)	7 (46.7%)		$\chi^2 = 0.13$	0.71
Males	7 (46.7%)	8 (53.3%)			

SD, standard deviation; MD, mean difference; χ^2, Chi-squared value; p-value, level of significance

Impact of Treatment on ALT and AST

There was a significant interaction of therapy and time ($F = 253.82$, $p = 0.001$, $\eta^2 = 0.94$). There was a significant main effect of time ($F = 251.05$, $p = 0.001$, $\eta^2 = 0.94$). There was a significant main effect of therapy ($F = 74.88$, $p = 0.001$, $\eta^2 = 0.84$).

Within-Group Comparison

Group A's ALT and AST levels decreased significantly post-therapy compared to prior to therapy ($p < 0.001$), while group B's AST levels increased significantly after treatment compared to prior to therapy ($p < 0.01$) but there was no significant alteration in ALT ($p > 0.05$), (Table 2).

Between-Groups Comparison

Pretreatment data showed no statistically significant differences between groups. After therapy, ALT and AST levels in Group A were found to be lower than those in Group B by a statistically significant difference ($p < 0.001$), (Table 2).

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Table 2. Mean ALT and AST pre and post treatment of group A and B

	Group A Mean ± SD	Group B Mean ± SD	MD (95% CI)	P value
ALT (IU/l)				
Pre treatment	59.80 ± 5.10	58.86 ± 6.65	0.94 (−3.50: 5.36)	0.67
Post treatment	25.66 ± 4.12	57.80 ± 5.72	−32.14 (−35.86: -28.41)	0.001
MD (95% CI)	34.14 (31.84: 36.42)	1.06 (−1.22: 3.36)		
% of change	57.09 *p = 0.001*	1.80 *p = 0.34*		
AST (IU/l)				
Pre treatment	45.20 ± 6.20	46.80 ± 5.96	−1.6 (−6.15: 2.95)	0.47
Post treatment	24.13 ± 3.04	51.07 ± 4.44	−26.94 (−29.78: -24.08)	0.001
MD (95% CI)	21.07 (18.07: 24.06)	−4.27 (−7.26: −1.27)		
% of change	46.62 *p = 0.001*	9.12 *p = 0.007*		

SD, Standard deviation; MD, Mean difference; CI, Confidence interval; p-value, Level of significance

Discussion

Multiple organ failures, as well as infections, are the biggest reasons for the high mortality rate in cases with burns. It is essential for physicians to recognize the biology of burn injuries and how they affect drug pharmacokinetics. The local as well as systemic inflammatory reactions caused by thermal injury are extremely complicated, leading to local burn tissue destruction and negative systemic consequences on all organ systems other than the burn location itself [20]. Increased edema development in burn patients may result in cell damage with the release of liver enzymes. The most sensitive indicators of damage to hepatocytes are liver enzymes including AST and ALT. Cellular damage or changes in the permeability of cell membranes allow these enzymes to enter the bloodstream. Low Na and high K+ levels occur as a result of a rise in vascular permeability as well as cell edema. These enzymes could be used to predict prognosis in burn patients [21].

The liver is a vital organ whose main functions in the body include the detoxification of drugs, disposal of excess byproducts of red blood cell destruction and regeneration in the form of bile, production of blood coagulant agents, storing glucose in the form of glycogen as well as regulating fat and sugar metabolism. Indeed, the liver's function in the absorption of fat and defense against microbes and toxins from foods should not be overlooked. First noted glycogen accumulation in the nucleus of cells and suggested episodes of diabetes, tuberculosis, septicemia, hepatitis, and autoimmune diseases whose excessive accumulation will leave irreversible damage on liver tissue [22].

Laser-puncture treatment applies laser photon energy to an acupuncture point that holds functioning cells which are highly sensitive to environmental alterations generated via energy pressure [23]. Irradiation is applied to the acupuncture point to allow for cell repair on inflammation and necrotic cells [24]. Recent studies using the infrared diode have shown that it can increase the total number of nucleated cells in the bone marrow, reducing the detrimental effects of cyclophosphamide on the cell cycle, induce the cell cycle for proliferation, decrease apoptosis, improve the intramedullary hematopoietic system, and resolve mechanical or ischemic disorders of organs involving the heart, skeletal muscle, the brain, and the liver [25]. In addition, there is evidence that low-power laser irradiation increases the protein or gene expressions of a number of growth factors, including vascular endothelial growth factor (VEGF), insulin-like growth factor I (IGF-I), transforming growth factor-β (TGF-β), as well as platelet-derived growth factor (PDGF) [26]. Therefore, it is evident that LPLI has the capability to promote differentiation as well as the proliferation of cells via the elevated levels of these growth factors [25].

Therefore, the present work's aim was to examine whether or not laser puncture could help in reducing plasma liver enzymes levels (ALT and AST) in patients with 30%-50% BBSA. The results showed that the plasma liver enzymes levels of ALT and AST among those in the laser puncture group (group A) decreased significantly while AST increased significantly in the control group (group B) with no significant change in ALT.

The results of this research are in line with earlier investigations that discovered a common photobiological mechanism of light in the respiratory chain's terminal enzymes in the mitochondria of eukaryotic cells [27]. In this area, some research teams have looked into how lasers affect mitochondrial activity. According to the published research, lasers can increase hepatocyte ATP (adenosine triphosphate) generation by inducing conformational changes in the cytochromes' arrangement [28].

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In addition, our results are in agreement with Astuti et al. [29], who studied laser diode puncture to restore normal function in mouse liver cells. Since its capacity to penetrate is greater than that of other visible light laser diodes, the red laser diode was selected. The researchers in this study employed a red laser diode with a wavelength of 650.01 ± 6.11 nm. This laser was capable of transmitting 17.01 mW of power at a distance of 0 mm from mice, allowing it to be utilized as a laser-puncture device. To get the desired dosage of energy, irradiation periods of 30, 60, 90, and 120 seconds at a laser beam temperature of 28°C-30°C were utilized. To avoid damaging liver tissues and cells, the laser will alternatively trigger photochemical reactions, as this is the typical physiological effect on tissues and cells at 37°C.

In contrast, Li et al. [30] found that acupuncture's impact is to restore homeostasis following pathogenic damage by activating several reaction cascades. The laser diode is a tool used in acupuncture treatment. A small collection of acupuncture sites (the Acupoint Network) will be stimulated by the laser energy, and this stimulation will spread along the meridians (the Meridian Network). The cascade serves to reinforce acupuncture-related data while simultaneously activating the NEI via the body's extensive meridian network. The NEI then works on the disease's network to generate acupuncture effects, releasing information impacts to target organs via multilevel and multisystem pathways.

Our outcomes are also in line with Saleh et al. [31], who showed that the energy of photons absorbed in tissues exposed to the same frequency of photon radiation is transferred to the liver tissues via depolarization of the meridian system. The nucleus will interpret this data as chemical instructions and use it to enhance liver cell activity in the regeneration processes; this, in turn, could accelerate the repair of liver histopathology. The meridian system, which consists of tissues linking the internal organs to the skin, is an important focus for restoring equilibrium to the body. Traditional Chinese medicine relies on the connection between acupuncture points and the body's meridian system for diagnosis and therapy.

Whereas Youn et al. [32] validated the impact of low-level laser therapy on the level of serum lipid and weight gain. In LLLT (Low-Level Laser Therapy) (30 mW-10 min), food effectiveness was lower. The level of serum triglyceride, ALT, AST, free fatty acid, ALP were lowered in LLLT30-10. LLLT (5 mW-10 min) and LLLT30-10 raised serum HDL-cholesterol levels. Serum ALT was reduced in LLLT (5 mW-5 min) patients as well. Weight, food efficiency ratio, serum lipid, and liver function were all positively affected by LLLT 30-10, while weight, food intake, and ALT were all negatively affected by LLLT5-5.

Conclusion

This work's findings demonstrate that laser puncture is a promising novel therapy for lowering plasma levels of the liver enzymes ALT and AST, thus enhancing liver function after a major burn in a short time. Thus, this study's results outline the value of including laser puncture in burn rehabilitation plans as an adjunctive therapy for improving liver function.

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