

## Response of Immune System and Wound Healing to Laser Puncture in Burned Patients

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### ABSTRACT

**Background:** laser acupuncture due to its anti-inflammatory and immunocorrection action has great role in improving immunity parameters (neutrophils and total lymphocytes) and accelerate wound healing in patients with depressed immune system after burn.

**Aim:** This study aimed to investigate the response of immune system and wound healing of laser puncture in burned patients through comparing the impact of laser puncture and routine medical treatment on treatment of burned patients.

**Subjects and Methods:** After one week of burn injury, sixty patients (31 males and 29 females) suffering from thermal burn injury (second degree) were randomized into two groups: laser puncture group (A) (n = 30) as well as control group (B) (n = 30). The intervention was performed 3 sessions each week for two months. Wound measurement – working out surface area in cm<sup>2</sup> and complete blood cell counts (Neutrophils, total lymphocytes) were evaluated pre- and post-intervention.

**Results:** In groups A and B, there was a significant decrease in wound surface area and a substantial increase in lymphocytes as well as neutrophils post-treatment compared to pre-treatment (p > 0.001).

**Conclusions:** laser puncture is an effective technique to increase the immunity parameters and wound healing in burned patients.

**Keywords:** Immune, Wound Healing, Laser puncture, Burn.

### INTRODUCTION

Burns are the 4th most common type of injury worldwide, following car accidents, falls, and acts of violence <sup>(1)</sup>. The systemic inflammatory reaction syndrome is triggered by burn injuries and can lead to tissue and organ destruction throughout the body <sup>(2, 3)</sup>. Neutrophils as well as macrophages are the initial immune cells that reach at the wound site following burn injury that triggers the acute phase of the immune reaction <sup>(4)</sup>. The bone marrow is responsible for producing and renewing the blood's neutrophils as well as macrophages. These innate immune cells clear the body of infections and necrotic tissue through processes called phagocytosis and the generation of reactive oxygen species <sup>(5)</sup>.

Acupuncture (ACU) is an integral part of the initial response in the multifaceted treatment of burn trauma (BT). The anti-inflammatory, analgesic, and immune-stimulating effects of ACU, as well as its influence on the veins and lymphatic system, all contribute to its ability to reduce the symptoms of post-traumatic stress. When it comes to healing wounds, ACU is crucial, especially for avoiding hypertrophic scarring <sup>(6)</sup>. All burn patients, but especially those in mass disasters and medium and low-income countries, can benefit from ACU's easy and inexpensive application. The use of ACU in BT complex therapy has several benefits, including those in the fields of medicine, economics, and psychology, as well as the avoidance of medical-law conflicts. Laser acupuncture therapy has bio

excitation, tissue regenerative, antimicrobial, anti-inflammatory, and analgesic properties <sup>(6)</sup>. To the author's knowledge, there is no previous study on the response of immune system and wound healing of laser puncture in burned patients.

### SUBJECTS AND METHODS

#### *Randomization and study design*

A cluster randomised-controlled trial with two arms was carried out. When patients were admitted, they were given an identification number. Group 1 was assigned odd numbers, while group 2 was assigned even numbers. Before beginning treatment, participants' consent was obtained after a thorough explanation of the study's details.

#### **Participants:**

From the Burn Department of Hehia Hospital in Sharqia, Egypt, 60 patients who fulfilled the inclusion criteria were chosen for this study. Total of sixty patients (31 males and 29 females), their ages ranged from 25 to 55 years with thermal burn injury (second degree). After one week of burn injury, they were randomized into two groups: laser puncture group (A) (n = 30) and control group (B) (n = 30).

**Inclusion criteria:** Patients with ages ranged from 25 to 55 years old, both genders, patients were diagnosed with 20 to 30% TBSA, and able to follow instructions or complete self-report scales.

**Exclusion criteria:** Patients with age more than 55 years or less than 25 years, patients with infection, other causes of immune deficiency, burns affected location of acupuncture points, concomitant psychiatric disorders (including major depression, anxiety, or personality disorders), pregnancy, inability to follow instructions or complete self-report scales, patients had metallic implants at or near the treatment site, and patients had cardiac pacemakers or other implanted electronic devices.

#### **Interventions:**

**Group A (laser puncture group):** The treatment procedures started after one week from burn injury. The patient was in a comfortable position and was put on protective eyewear. All patients in the study group were submitted to A gallium–aluminum–arsenide (GaAlAs) diode laser (PhysioGo 400/401C, Poland) <sup>(7)</sup> and Gallium arsenide (Ga-As) laser diode. Maximum average power output: 50 mW. Output wavelength: 820 nm. Beam spot size: 0.156 cm<sup>2</sup>. Power density: 0.39 W/cm<sup>2</sup>. Energy density: up to 30J/ cm<sup>2</sup>. Laser probe was placed perpendicular over the body acupoints (GB-20) (KI-27) (ST-36) (LI-4) (LU-7) in both sides, with duration of 90 seconds for each point three times/week for two months along with routine medical treatment.

**Group B (control group):** This group involved thirty patients who obtained routine medical care such as wound care, burn cream, and narcotic pain medications <sup>(8)</sup>. Burn wound care included the use of cold compression to detach rings and piercings to remove bracelets and watches, as well as the removal of open skin blisters. Silver sulfadiazine burn cream with Tetanus immunisation. Pain medication including narcotics effective for moderate to severe pain not for long-term usage.

#### **Measurements**

The following instruments were used to evaluate all participants pre- and post- the two months' treatment period:

**1. Wound measurement:** - calculating surface area in cm<sup>2</sup>  
A 30% or greater change in wound surface area for four weeks has been shown to be a reliable prognosticator of healing <sup>(9, 10)</sup>.

#### **We used the tracing method in our study.**

Compared to traditional assessments of wound size, tracing the wound's outline is a quick, cheap, and effective alternative. Although it provides more information regarding the wound's shape, it is not without limitations <sup>(11, 12)</sup>. In this treatment, a layer of acetate is placed on top of the wound, accompanied by a layer of transparent film. The wound contact layer is placed in clinical waste after becoming traced with a

fine-tipped permanent marker, while the acetate sheet is dated and also labelled and kept in the patient's record. Indicating the desired direction at the front is mandatory. Overlapping multiple sheets is an option for larger wounds. Most acetate sheets have grid lines that are 1 cm in size.

#### **Determining the percentage reduction in wound surface area <sup>(13)</sup>:**

Re trace the wound as previously explained. Work out the surface area in cm<sup>2</sup>. Work out the reduction in surface area by using the following equation:

$$\text{Percentage reduction in wound surface area} = \frac{\text{New surface area}}{\text{last surface area}} \times 100$$

#### **2- Determination of immune response assessed by laboratory equipment (KX-21N Hematology Analyzer) <sup>(14)</sup>:**

Two blood samples were taken by certified technicians with the participate in comfortable positions before and after two months of the treatment. The lab examined the sample using (KX-21N Hematology Analyzer) it was used to measure complete blood count (neutrophils and total lymphocytes). [Sysmex Corporation KX-21N Automated Hematology Analyzer. Manufacturer: Sysmex Corporation. Shipping weight: 115 pounds].

#### **Ethical approval:**

**All human subjects research was approved by the Faculty of Physical Therapy at Cairo University in Egypt (approval No.: P.T. REC/012/ 003137) and was conducted in accordance with all applicable national regulations and institutional policies, as well as in accordance with the principles outlined in the Declaration of Helsinki. In 2020-11-13, the trial was registered with Clinical Trials (www.clinicaltrials.gov) under the identifier NCT04629456. All individuals included in this study gave their informed consents.**

#### **Statistical analysis**

Unpaired t-test was utilised to evaluate the correlation between the groups' ages and TBSAs. Chi-square test was performed to investigate differences in the ratio of males to females across different groups. To make sure that our data followed a normal distribution, we tested it through the Shapiro-Wilk test. Levene's test for variance homogeneity was utilized to do this assessment. An unpaired t-test was used to compare the two groups' means for wound surface area, neutrophils, and lymphocytes. The pre- and post-treatment outcomes of each group were compared using a paired t-test. All statistical tests were performed at a p value of 0.05 as the threshold for significance. Statistical analysis was performed using SPSS for Windows, version 25. (IBM SPSS, Chicago, IL, USA).

**RESULTS**

Subject characteristics for groups A and B were presented in Table (1). Distributions of age, TBSA, and sex were similar among groups ( $p > 0.05$ ).

**Table (1):** subject characteristics between groups A and B

	Group A		Group B		p-value
	Mean	± SD	Mean	± SD	
Age (years)	32.9	± 6.95	31.53	± 5.23	0.39
TBSA (%)	26	± 3.81	26.16	± 4.48	0.87
Sex					
Females	13	(27%)	16	(40%)	0.43
Males	17	(73%)	14	(60%)	

SD, standard deviation; p value, probability value

**Effect of treatment on wound surface area, neutrophils and lymphocytes:**

**- Within-group compared:**

Within-group compared: In group A, the percent change in wound surface area, neutrophils, and lymphocytes was 53.09, 10.8, and 84.16%, respectively, while in group B, it was 33.57, 6.53, and 60.6%. (Table 2).

**- Comparison of groups:**

Pre-treatment, there were no statistically substantial differences among groups ( $p > 0.05$ ). Post-treatment comparisons showed that wound surface area was substantially lowered in group A compared to group B ( $p > 0.01$ ), and that neutrophils and lymphocytes were substantially increased. (Table 2).

**Table (2):** Mean wound surface area, neutrophils and lymphocytes pre and post treatment of group A and B

	Group A		Group B		MD	t- value	p value
	Mean	± SD	Mean	± SD			
<b>Wound surface area (cm2)</b>							
Pre treatment	82.69	± 16.8	77.47	± 20.09	5.22	1.09	0.27
Post treatment	38.79	± 13.24	51.46	± 16.64	-12.67	-3.26	0.002
MD	43.9		26.01				
% of change	53.09		33.57				
t- value	18.95		13.41				
	<i>p = 0.001</i>		<i>p = 0.001</i>				
<b>Neutrophils (%)</b>							
Pre treatment	84.61	± 4.66	83.83	± 4.45	0.78	0.66	0.51
Post treatment	93.75	± 4.21	89.3	± 5.05	4.45	3.7	0.001
MD	-9.14		-5.47				
% of change	10.8		6.53				
t- value	-14.63		-12.21				
	<i>p = 0.001</i>		<i>p = 0.001</i>				
<b>Lymphocytes (%)</b>							
Pre treatment	10.92	± 2.54	10.71	± 2.26	0.21	0.34	0.72
Post treatment	20.11	± 2.05	17.2	± 2.88	2.91	4.5	0.001
MD	-9.19		-6.49				
% of change	84.16		60.6				
t- value	-24.41		-16.44				
	<i>p = 0.001</i>		<i>p = 0.001</i>				

SD, standard deviation; MD, mean difference; p-value, probability value

## DISCUSSION

The findings of this study revealed that laser puncture can modulate immunity parameters (neutrophils and total lymphocytes) and accelerate wound healing in patients with depressed immune system after burn.

Multiple studies have demonstrated that acupuncture treatment can modulate autonomic nervous system activities including blood pressure regulation, sphincter Oddi relaxation, as well as immune system control. Even though there have only been a small number of randomized-controlled trials to assess the effectiveness of acupuncture, there is increasing clinical evidence that EA treatment is beneficial for a wide range of immunological diseases, such as allergic disorders, infections, autoimmune diseases, as well as immunodifficulty-syndromes<sup>(14)</sup>. Laser acupuncture has been used medically for decades, mainly in the treatment of tissue healing as well as inflammatory conditions. Specifically focusing on the concept of laser acupuncture as a non-invasive and non-thermal treatment. They discovered that laser acupuncture is an effective non-pharmacological intervention that can be used as an adjunctive or alternative treatment for chronic pulmonary conditions<sup>(15)</sup>.

The effect of acupuncture treatment on WBC and neutrophil counts was investigated<sup>(16,17)</sup>. When the two groups were compared, the AcuMoxa group was shown to have significantly higher white blood cell (WBC) and neutrophil count (NC) levels ( $p=0.036$ ). Acupuncture treatment induces immunomodulation of neutrophil function and count, as well as CD3+, CD4+, and CD+8 cell counts<sup>(18)</sup>.

Patients with stomach cancer with post-operative leukopenia benefited from acupuncture's ability to control neutrophil and lymphocyte counts<sup>(19)</sup>. **Lu et al.**<sup>(20)</sup> discovered that laser light influences cellular immunity. It has an immunomodulatory effect on T lymphocytes and an immunostimulatory effect on B lymphocytes, enhancing neutrophil phagocytosis.

A manuscript detailing the results of a randomized, single-blinded, placebo-controlled clinical study investigating the impact of acupuncture on neutrophil respiratory burst is currently available. Eleven volunteers received acupuncture at the LI 11 point over 30 minutes, twice weekly, for 4 weeks (8 sessions in total), and the results showed that the respiratory burst of neutrophils was greatly stimulated following the acupuncture<sup>(21)</sup>. **Tadakuma**,<sup>(22)</sup> have demonstrated that exposed cells to either visible or infrared light causes them to become more active, which is a key part of the immune response. Increased phagocytic and chemotactic activity of human leukocytes after infrared laser therapy. Animal model research provides reasonable grounds to expect laser therapy to improve human immune function via a variety of mechanisms.

Acupuncture can help to balance the innate and acquired immune systems. Acupuncture has been shown to influence the pattern of leukocytes (granulocytes and lymphocytes) in humans<sup>(23)</sup>. **Hawkins et al.**<sup>(24)</sup> showed that laser therapy is useful for a wide range of wound healing, acute and chronic pain syndromes. The laser therapy journal has published 30 papers on the subject over the last 7 years. The GA-As diode, which emits light in the near infrared at 830 nm, is the preferred laser. The majority of the reports specify a continuous wave power output of 60 mm.

The current study and previous studies concluded that using laser acupuncture has a significant role in improving burned patients immunity due to its anti-inflammatory and immunocorrection action, as the current study showed that there was a significant increase in the mean values of neutrophils, lymphocytes, and percentage of wound reduction post-treatment in the study group compared to the control group.

**Limitations:** To generalize the findings, additional research must include a diverse patient population as well as a 3-month following-up to determine whether the improvement is preserved or if outcomes reduction following treatment is discontinued.

## CONCLUSION

Based on the findings of the current study and the subsequent discussion, it was determined that laser acupuncture, in addition to routine medical treatment, had a statistically significant effect on immune system and wound healing in patients with burns compared to the other group that received routine medical treatment.

**Relevant conflicts of interest/financial disclosures:** The authors stated that they had no financial or commercial relationships that could be seen as a conflict of interest with the research.

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