HELIUM NEON LASER VERSUS GALLIUM ARSENIDE LASER IN HEALING OF VENOUS ULCERS

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ABSTRACT

Venous leg ulceration is a chronic condition characterized by cycles of healing and recurrence with healing times ranging from weeks to years. Purpose: to evaluate the effect of Helium Neon and Gallium Arsenide in healing of venous ulcer using wound surface area and colony count. Methods: - sixty (53 males and 7 females) patients with leg venous ulcers were randomly divided into three group. Group (A) received Helium Neon laser in addition to standard ulcer care. Group (B) received Gallium Arsenide laser in addition to standard ulcer care. Group(C) (Control group) received only the standard ulcer care. Results: - The result showed that there was a significant decrease in ulcer surface area and number of bacteria using Low Level Laser Therapy (Helium Neon and Gallium Arsenide) better than conservative ulcer care in addition to there were no significant differences between He-Ne 632 and Ga-As 808-904 in decreasing ulcer surface area and colony count in the healing of the venous ulcers. Conclusion: Both types (He-Ne 632 and Ga-As 808-904) are effective in enhancing healing of venous ulcer that reflected by decreasing ulcer surface area and colony count without superiority of one type over the other.

Key words (Low intensity laser, Helium Neon, Gallium Arsenide, venous ulcer, wound surface area and Colony count).

I. INTRODUCTION

Leg ulcers are a chronic and recurring condition that affect between 1.5-3.0 people in every 1000 at any one time. The prevalence of leg ulceration increases with age and can be as many as 20 in every 100 people over the age of 80. There is no single etiology; however, it is thought that approximately 70% of people with a leg ulcer are as result of venous hypertension. Arterial or mixed arterial/venous disease is responsible for a further 20% and the remaining are from other conditions such as diabetes, rheumatoid disease, malignancy and vasculitic conditions [1].

Venous ulcers are wounds that have the character of a full thickness wound involving the subcutaneous tissues and fat are thought to occur due to improper functioning of valves that cause chronic venous insufficiency of the lower limb veins [2],[3].

It represents an enormous percentage of chronic wounds. Venous ulcers occur in soft tissue as a result of continuous, unrelieved microcirculatory changes leading to tissue necrosis. Over time prolonged occlusion of the capillaries limited exchange of nutrients and oxygen leading to ischemia, cell death and tissue ulceration [4].

Many compound factors contribute to venous ulcer formation, but the primary cause is venous insufficiency, which cause pressure in deep veins falls less than normal, so called venous hypertension which leads subsequently to venous ulceration [5]. Immobility, abnormality of the calf muscle pump, or valvular dysfunction of the venous...
The study was performed on 60 patients who had venous leg ulcers just above the medial malleolus in the territory of the long and short saphenous veins. It has been described in the upper calf and they can also occasionally occur on the foot, though these are very rare. It usually presents as shallow, irregular to oval in shape, with macerated borders. Size varies from pinhead to extensively covering the distal-medial aspect of the leg [7],[8].

Venous ulcer characterized by wound bed usually consists of slough (yellow tissue) and granulation tissue (red tissue), tend to be shallow in depth, moderate to high exudate levels, usually positioned around the ankle and medial aspect of the leg, brown staining on the legs, skin is warm to touch, capillary refill usually within normal limits, pain can be relieved by elevation and rest, foot pulses present. [9],[10].

Not all lasers are useful for bio-stimulation. A laser whose wavelength is more than 600 nm can be used for bio-stimulation. LLLT seems to be the best for wound and ulcer treatment. Low level laser treatment is non-invasive method causes no damage to tissue structure or temperature elevation. It stimulates and accelerates the activity of wound healing with an anti-inflammatory effect on chronic non-healed skin wounds, and is an effective tool for treatment of soft tissue injuries and pain. Because of these qualities it is also named "therapeutic laser" [11].

The two most commonly used are the Helium Neon (He-Ne) laser and the Gallium Arsenide (Ga-As) laser.

Helium-neon (He-Ne; 632 nm) is a gas laser with visible red light and shallow depth of penetration. Depending on the type of tissues involved, the maximum depth of penetration achieved is 6–8 mm for a power out of 3.5 mW and 8–10 mm for 7 mW. It is used for wound healing and superficial applications like laser acupuncture [12].

Gallium-Arsenide Laser (Ga-As) is a low levels semi-conductive laser system used in management of pain and wound healing with wave length 904 nm, and radiation power of 5 milli watts. It is the commonest therapeutic laser with the greatest depth of penetration. It is used therapeutically for treatment of pain. It is available as pulsed light or continuous wave lasers and used in lower doses for wound healing as they have deeper tissue penetration than the He-Ne laser, these lasers have the disadvantage that their light is invisible and therefore eye protection is required [13].

Photo bio-modulation is a process by which the light produced by low-level laser either stimulates or inhibits biological processes in tissues by interacting with chromophores within the human tissue. The power, wavelength and duration of application of laser affect the photobiological effects of LLLT [14]. The cytochromes in the mitochondria absorb the laser radiation and convert it into ATPs which are used for stimulation of cell proliferation and synthesis of proteins resulting in photobiological activation of the cell. LLLT has analgesic, anti-inflammatory actions along with stimulatory effects on wound healing, tissue repair and regeneration [15].

II. SUBJECTS AND METHODS

Study Design

A prospective randomized controlled clinical trial was done to compare between helium neon laser versus gallium arsenide laser in healing of venous ulcer using wound surface area and colony count. The study was performed between February 2019 and May 2021.

Subjects:

This study was carried out by 60 patients who had venous leg ulcers just above the medial malleolus due to venous insufficiency, their age was ranged from 50 to 60 years, they were free from any diseases that can affect healing process and influence the results (no diabetes or blood problems). They were recruited from the outpatient clinic of department of vascular surgery unit at Teaching Hospitals in Cairo and Beni-Suef university after signing consent form. The consent form has been recorded from all subjects involved in this study.
**Randomization**

A total of eighty venous ulcer patients were examined for eligibility. Twenty patients were excluded (fifteen patients didn’t meet the inclusion criteria and five patients declined to participate in the study). Sixty patients were divided randomly into three equal groups. Group (1) received Helium Neon laser in addition to standard ulcer care. Group (2) received Gallium Arsenide laser in addition to standard ulcer care. Group (3) (Control group) received only the standard ulcer care. (fig. 1). The patients were divided randomly by a blinded, independent research assistant who used a random card generated automatically by a computer.

**Intervention**

Patients were randomly divided into 3 equal groups in number: 2 study group and control group.

**Group 1:** (first study group), this group was composed of 20 patients who received low intensity laser (He-Ne) with wavelength 632 and the regular ulcer care for two months.
**Group 2:** (second study group) that was composed of 20 patients who received the low intensity laser (Ga-As) with 808-904 wavelength and the regular ulcer care for two months.

**Group 3:** (control group) that was composed of 20 patients who received the same regular ulcer care only through the treatment period.

The treatment frequency was three sessions per week for two months.

**Outcome measures**

All measurements were done pre and post treatment by another assessor who was blinded about subject group allocation.

- **Wound surface area:** Smart phone application (imito wound) calculates automatically the wound area after taking a photograph as following: determine type of wound (venous leg ulcer). Taking a photograph of wound and marking the border of the wound and finally encircle the area for measurement then select measure to calculate (length, width, area and circumference). It was measured before the beginning of the experiment as a first record and at the end of the second month of therapy as a second final record [16].

- **Colony counting and quantitative culture:** Sterile swaps were used for taking samples for culture before the beginning of the experiment and at the end of the second months [17].

### III. DATA ANALYSIS

ANOVA-test was conducted for comparison of age between groups. Chi squared test was conducted for comparison of sex distribution between groups. As the data was not normally distributed, Kruskal-Wallis test was conducted for comparison of the median values of ulcer surface area and number of bacteria between groups and was followed by Mann–Whitney U test to identify the significance difference between each two groups. Wilcoxon Signed Ranks Test was conducted for comparison of pre and post treatment in each group. The level of significance for all statistical tests was set at $p < 0.05$. All statistical analysis was conducted through the statistical package for social studies (SPSS) version 25 for windows (IBM SPSS, Chicago, IL, USA).

### IV. RESULTS

**Subject characteristics:**

Table (1) showed the subject characteristics of the group A, B and C. There was no significant difference between groups in age and sex distribution between groups ($p > 0.05$).

Table 1. Basic characteristics of participants.

<table>
<thead>
<tr>
<th></th>
<th>Group A</th>
<th>Group B</th>
<th>Group C</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age, mean ± (SD), years</td>
<td>55.7 ± 2.36</td>
<td>56.15 ± 3.01</td>
<td>56 ± 3.27</td>
<td>0.88</td>
</tr>
<tr>
<td>Sex, n (%)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Females</td>
<td>2 (10%)</td>
<td>2 (10%)</td>
<td>3 (15%)</td>
<td></td>
</tr>
<tr>
<td>Males</td>
<td>18 (90%)</td>
<td>18 (90%)</td>
<td>17 (85%)</td>
<td>0.85</td>
</tr>
</tbody>
</table>

SD, standard deviation; p-value, level of significance

**Effect of treatment on ulcer surface area and number of bacteria:**

Within-group comparison revealed a significant decrease in ulcer surface area post treatment compared with that pre-treatment in group A ($p = 0.001$), B ($p = 0.001$) and C ($p = 0.002$). Also, there was a significant decrease in number of bacteria post treatment compared with that pre-treatment in group A ($p = 0.007$), B ($p = 0.004$) and C ($p = 0.01$). (table 2).
Between group comparison pre-treatment revealed a nonsignificant difference (p > 0.05). There was a significant decrease in ulcer surface area (p = 0.001) and number of bacteria (p < 0.01) of group A and group B compared with that of group C post treatment. There was no significant difference in ulcer surface area and number of bacteria between group A and B (p > 0.05). (table 2).

Table 2. Median values of ulcer surface area and number of bacteria pre and post treatment of group A, B and C:

<table>
<thead>
<tr>
<th></th>
<th>Group A</th>
<th>Group B</th>
<th>Group C</th>
<th>p-value</th>
<th>A vs B</th>
<th>A vs C</th>
<th>B vs C</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ulcer surface area (cm²)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Pre treatment</td>
<td>6.58 (12.45-3.12)</td>
<td>5.85 (9.09-2.78)</td>
<td>5.05 (10.52-2.82)</td>
<td>0.22</td>
<td>0.55</td>
<td>0.81</td>
<td></td>
</tr>
<tr>
<td>Post treatment</td>
<td>0.65 (2.77-0)</td>
<td>1.34 (3.37-0)</td>
<td>4.06 (10.15-2.52)</td>
<td>0.4</td>
<td>0.001</td>
<td>0.001</td>
<td>0.002</td>
</tr>
<tr>
<td>Number of bacteria</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>0.007</td>
<td>0.004</td>
<td>0.01</td>
</tr>
<tr>
<td>Pre treatment</td>
<td>2450 (4225-1925)</td>
<td>2650 (6225-1125)</td>
<td>2750 (3600-2000)</td>
<td>1</td>
<td>0.78</td>
<td>0.86</td>
<td></td>
</tr>
<tr>
<td>Post treatment</td>
<td>0 (285-0)</td>
<td>250 (1400-0)</td>
<td>1000 (1175-825)</td>
<td>0.07</td>
<td>0.001</td>
<td>0.01</td>
<td></td>
</tr>
</tbody>
</table>

IQR, Interquartile range; p-value, Level of significance

V. DISCUSSION

The aim of this study was to compare the effect of helium neon laser versus the gallium arsenide laser on healing of venous ulcers. The WSA had been measured before starting laser application and after two months from the beginning of treatment for all groups of the study.

The results of the current study showed that the median (IQR) value of ulcer surface area post treatment of group A, B and C were 0.65 (2.77-0), 1.34 (3.37-0) and 4.06 (10.15-2.52) cm² respectively. There was a significant difference in the ulcer surface area between the three groups post treatment (p = 0.0001). There was no significant increase in median value of ulcer surface area between group A and B (p = 0.4). There was a significant decrease in median value of ulcer surface area of group A compared with group C (p = 0.0001). There was a significant decrease in median value of ulcer surface area of group B compared with group C (p = 0.001).

Once laser absorbed a photochemical effect can be induced by several mechanisms which include neural mechanisms in which Laser causes changes in nerve action potentials, conduction velocities and distal latencies. Studies demonstrated significant pain relief following low power He-Ne and Ga-Al-As laser stimulation due to increase levels of serotonin in chronic pain patients. Another mechanism may include Photoactivation of enzymes in which one photon can activate one enzyme molecule which in turn can process thousands of substrate molecules. This mechanism provides a theoretical framework in which a very small amount of energy can cause a very significant biological effect [18].

Primary photo acceptors, which are activated by laser, are thought to be flavins, cytochromes (pigments in the respiratory chain of cells) and porphyrins. They are located in mitochondria. They can convert laser energy to electro-chemical energy. Low doses of laser stimulation ATP in mitochondria, activation of the Ca/ATPase pump, Ca in the cytoplasm, (via ion channels) cell mitosis cell proliferation. Higher doses of laser stimulation hyperactivity of the Ca/ATPase pump and exhaust the ATP reserves of the cell failure to maintain osmotic pressure cell explodes [19].

Vibrational and rotational changes in cell membrane molecules: Infra-red radiation results in rotation and vibration of molecules in the cell membrane leading to activation of the Ca++ pump as in the cascade above. Different wavelengths may stimulate different tissue responses which may be synergistic and thus produce better clinical effects [20].
According to Kopera et al., 2005, low-energy photoemissions given at a wavelength range of 600nm to 900nm accelerates cell proliferation and wound healing processes. Its action is thought to stimulate respiratory chain components such as flavin and cytochromes which increase adenosine triphosphate (ATP) synthesis, thus enhancing the rate of mitoses and increasing fibroblast numbers through stimulation of collagen and elastin production, leading to better re epithelialization, stimulation of microcirculation and dilatation of the capillaries and neo-vascularization to increase tissue oxygenation, liberate mediator substances such as histamine, serotonin and bradykinin to influence macrophages and regenerate lymphatic vessels [21].

In agreement with the current study, Kipshidze et al., 2001 demonstrate that, low-power laser irradiation increases production of vascular endothelial growth factor by smooth muscle cells, fibroblasts, and cardiac myocytes and stimulates EC growth in culture demonstrate that LPLI increases production smooth muscle cells [22].

Also, Yasukawa et al., 2007 concluded that LLLT with He-Ne laser can promote the healing of operative wounds by which the optimal condition was at least the 17.0 mw, 4.2J/cm a day with a frequency of every other day [23].

In a retrospective study of 42 patients with venous leg ulcers, Kleinman et al., 1996 reported full granulation and closure of ulcers in a total of 36 patients (87.5%) who were treated with a laser scanner with 632 nm [24].

Results of this study concerning the effect of Ga-As for promoting healing of venous ulcer in human are supported by Rai et al., 2017 reported that after the end of 4th week wound size and pain reduction was seen in irrespective of treatment along the three groups, and all are helping in treatment of venous ulcers. They didn’t find any significance in wound size and pain reduction on each group statistically but clinically there is significant changes observed (since mean difference in wound size and pain pre and post intervention is more) in patients treated with Low Level Laser Therapy (Ga-As). From the result of the study we observed that the Low Levels Laser Therapy is found to be relatively better than the Therapeutic Ultrasound and the Conservative management of venous ulcer [25].

In a similar study Tantawy et al.,2018 finds that HNLT (632 nm) and ILLT (904 nm) have positive effects on diabetic foot ulcer. Both enhance cell metabolism and production of adenosine triphosphate through enhancing the action of cytochrome oxidase, an active ingredient of the electron transport chain and it can be concluded that diabetic foot ulcer can be improved in the short term (4 weeks) by two types of laser therapy (HNLT and ILLT) and also after 8 weeks of follow-up (from the beginning of the study). They observed that both HNLT and ILLT are valuable in the treatment of diabetic foot ulcer and are equally efficacious in accelerating the healing of diabetic foot ulcer [26].

In contrast, the results of this study were not in agreement with the results of the study conducted by Kopera et al., 2005 which reported that, the least impressive ulcer reduction was seen in the low-level laser group. The difference in the results with the previous study may be attributed to the fact that, randomization produced a difference in ulcer size within the three study groups: at baseline the ulcers in the placebo and control groups were larger than those in the low-level laser group. It may be hypothesized that larger ulcers heal relatively faster than smaller ones. On the other hand, smaller ulcers may have a better prognosis for complete healing than larger ones. However, this did not occur in the laser group, in which there was no statistically significant reduction in ulcer size from baseline to days 28 and 90. This might indicate that low-level laser treatment impaired ulcer healing, which is contrary to our expectations [21].

Also, Malm and Lundeborg 1991 reported that there was no significant difference between the effects of GaAs laser and placebo in the treatment of venous ulcers of the leg. This may be the result of the mode of laser used, the dosage, the duration of irradiation, the treatment intervals, or the state of the wounds. The present results do not support the use of low energy GaAs laser in the treatment of venous leg ulcers [27].

Regarding to colony count, in the present study the result show that the median (IQR) value of number of bacteria post treatment of group A, B and C were 0 (285-0), 250 (1400-0) and 1000 (1175-825) respectively. There was a significant difference in the number of bacteria between the three groups post treatment (p = 0.0001). There was no significant increase in median value of number of bacteria between group A and B (p = 0.07). There was a significant decrease in median value of number of bacteria of group A compared with group C (p = 0.0001). There was a significant decrease in median value of number of bacteria of group B compared with group C (p = 0.01).
The results were supported by Nesrien et al., 2017b who reported that, a significant bacterial reduction on the group treated with laser therapy compared to colony count pre-treatment due to its ability to recruit some important cytokines and growth factors such as interleukin-1 and interleukin-8 and its ability to stimulate phagocytosis of macrophages, eliminating bacteria which in turn accelerate the end of the inflammatory process and so accelerate the healing process through its bactericidal effect [28].

Also, Bayat et al., 2006, concluded that, LL He–Ne LT induced the destruction of S. aureus and P. aeruginosa in third-degree burns of rats, yet at the same time our histological findings showed that LL He–Ne LT caused a significant increase in the mean of blood vessel sections on day 7 after third degree burns and a decrease in the mean of the depth of new epidermis on day 16 after the same burns in rats[29].

Conclusions
After the discussion of the results and according to reports of the previous investigators in fields related to this study, it can be claimed that the application of both types laser (He-Ne 632 and Ga-As 808-904 ) have a similar valuable healing effects on the leg venous ulcers as evidenced by the significant decrease in USA and CC in patients with leg venous ulcers.

REFERENCES