

The Role of HE-NE Laser 632.8 nm in the Management of Hypertrophic Scars

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ABSTRACT

Hypertrophic scar formation is a major clinical problem in the developing and industrialized worlds. Scar control is a major concern in burn wound management. When scarring occurs, the outcome may be associated with a loss of function or an undesirable cosmetic result, as once scars have formed; they are known to be difficult to treat because of their tendency to worsen with hypertrophy and contractures. Various experimental, conservative clinical and surgical efforts have been made but the problem has not been solved yet. Therapies such as surgical excision, dermabrasion, compression with silicon, and corticosteroids don't provide optimal results in treatment of burn scars. The benefits of LLLT in wound healing are still controversial. In spite of many discussions about possible effects of low power laser light and widespread clinical application, the effects of LLLT on burn scars have not been the subject of clinical studies in human beings up till now. This present prospective study was designed to objectify the effects of LLLT in the treatment of burn scars.

INTRODUCTION

Hypertrophic scar formation is a major clinical problem for burn patients [1]. Prevention of keloid and hypertrophic scars is the best treatment strategy. There is no universally accepted treatment modality that results in complete and permanent hypertrophic scar or keloid amelioration [2].

Available tools to modify the progression of hypertrophic scar formation are limited in number and effectiveness. These tools include scar massage, compression garments, topical silicone, steroid injections, and surgery. In some contractures over major joints, serial casting may be useful [3].

Surgical excision is usually followed by recurrence unless adjunct therapies are employed since the new surgical wound is subject to the same mechanical and biochemical forces of the original lesion. The recurrence rate has been reported to range from 45-100% when surgical excision is performed as monotherapy [4,5].

Radiation therapy is infrequently used as monotherapy. When combined with surgical excision, the recurrence rate following radiation treatment has been reported between 10 to 20% [6,7]. A dose of at least 1500Gy, delivered in fractions within 10 days of surgery, is recommended by some investigators [8].

Compression therapy exerts its effect by producing tissue ischemia, decreasing tissue metabolism and increasing collagenase activity [9,10]. It is necessary to wear the pressure dressing for at least 6 months for a minimum of 18 hours a day. Scars older than 6-12 months often respond poorly and it may be difficult to achieve the required amount of pressure (24-40mm Hg) in locations over a joint because of excessive skin movement [11]. Moreover, many patients find the pressure dressing uncomfortable and cumbersome, limiting their compliance to the prescribed regimen [12]. Application of topical silicone gel sheeting or cushions for at least 12 hours daily for 2-4 months has been used for treatment and for prevention of hypertrophic scarring [13].

Intralesional corticosteroids have become a cornerstone of both treatment and prophylaxis of hypertrophic scars [14]. The most commonly used drug for intralesional corticosteroid injection is triamcinolone acetonide, which can be diluted with lidocaine to decrease the discomfort of the injection [15].

Based on the premise that interferon can decrease the production of types I and III collagen from fibroblasts, several groups have demonstrated improvement in keloid or hypertrophic scars following intralesional interferon injection [16,17]. Although improvements of up to 50% have been reported, the efficacy of interferon for the treatment of keloids has been questioned [18,19].

Advances in laser technology and refinements in technique have made laser therapy one of the most advantageous modalities for the treatment of hypertrophic scars and keloids. In the 1980s, there was great controversy among laser surgeons regarding the benefits of keloid vaporization with various lasers (carbon dioxide, argon, and neodymium: yttrium-aluminum-garnet [Nd:YAG]). Ultimately, none of the preliminary studies with the aforementioned laser systems demonstrated an advantage over scalpel excision, with unacceptably high rates of scar recurrence and other adverse effects including pain, atrophy, and dyspigmentation [20]. During the past decade; multiple studies using the pulsed-dye laser (PDL) have demonstrated striking improvements in scar erythema, texture, height, and pliability [21]. In 1993, Alster and colleagues [22] were the first to demonstrate improvement in argon laser-induced hypertrophic scars over a 10-month period following five PDL treatments. Initially, the PDL was used to target the vascular component of scars to reduce or eliminate persistent erythema, with the flattening and increased pliability of the treated scars being an incidental finding. Currently, there is no consensus on the mechanism by which the PDL achieves these additional clinical effects. It has been hypothesized that laser-induced tissue hypoxia leads to decreased cellular function, laser-induced heating leads to disulfide bond disruption with subsequent remodeling of the fibers, or collagenolysis occurs following cytokine stimulation [23,24].

This study was conducted to evaluate the efficacy of the laser irradiation (HE NE 632.8 nm laser) on hypertrophic scar by using two various

methods of evaluation (grading systems Vancouver Scar Scale, Ultrasonography imaging).

MATERIAL AND METHODS

30 patients (males and females) with acute post burn hypertrophic scar, aged from 20 to 45 years, are selected randomly from the Out Patients Clinic at Faculty of Medicine, Cairo University.

Patients were randomly subdivided into two groups, each group consisted of 15 patients, the first group was the study group who received HE NE (632.8 nm) laser therapy, and the second group was the control group who received routine physical therapy treatment. The treatment was applied every day after day for 8 weeks.

All patients are approximately the same age, they had acute post burn scar. They have no associated disorders, pregnancy, immuno deficiency, HIV, AIDS, diabetes. Patients who had received oral retinoids within the past year, who had skin abnormalities as active skin disease within the treatment areas (i.e., psoriasis, cancer, or autoimmune disease), who had a history of photosensitivity, and had dark skin were excluded from the study.

The Vancouver scale (Fig. 1) was the clinical assessment tool that rated and scored scar according to pigmentation, vascularity, height/elevation, and pliability. From this assessment, a score was obtained; the lower the score, the better the scar. The scores compared over periods of time and across potential treatment modalities [15,25].

Vancouver Scar Scale			
Vascularity	Pigmentation	Pliability	Height
Normal (0)	Normal (0)	Normal (0)	Flat (0)
Pink (1)	Hypopigmentation (1)	Supple (1)	<2 mm (1)
Red (2)	Mixed hyperpigmentation (3)	Yielding (2)	2-5 mm (2)
Purple (3)		Firm (3)	>5 mm (3)
		Ropes (4)	
		Contracture (5)	

Fig. (1)

A total score range from 0 to 14.

Fig. (1): Vancouver scale.

Ultrasonography [26] was used to evaluate the thickness of the skin at the affected area before the treatment (pretest) and after 2 months (post test), for all groups. The Measurement was carried out by the same investigator with the patient in a resting position. The same area was measured every time by determining it in relation to any landmark. Thickness of the ultrasound coupling gel layer was

adjusted to about 1mm to ensure standardization. All values were given as the median of three recordings to avoid measuring inaccuracies.

A laser device “bravo terza serie HE NE laser (ASA s.r.i)” with a Wave length 632.8 nm (head source with aimed beam) was used. The Time of application was 25min duration with a Power density of 119mw/cm² and Energy density of 16J/

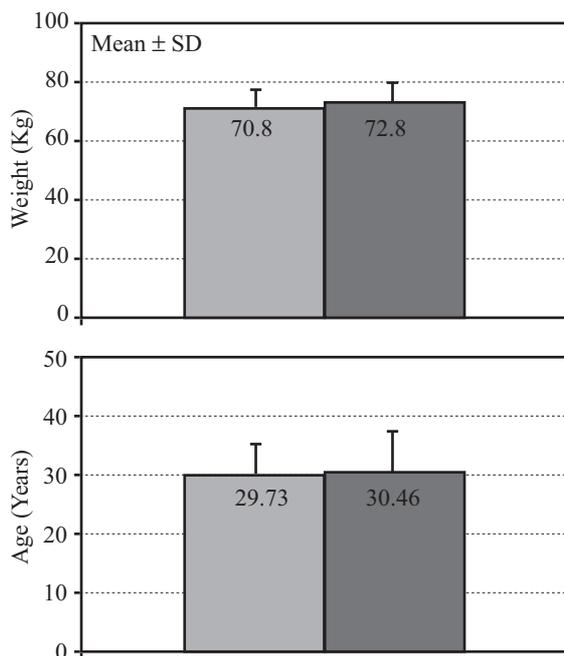
cm². The treatment started 3 months after wound healing. Both patient and therapist wear protective eye glasses, The head of the 632.8 nm laser was stabilized in horizontal alignment opposite to the patient but the beam of laser was in perpendicular direction to the hypertrophic scar. The distance between the laser probe and burn was 70cm length. The patients in 1st group (15 patients) received 632.8 nm laser irradiation at (16J/cm²) for 25min. The treatment was applied day after day for 2 months. Routine physical therapy through the treatment period (Non-invasive methods include individual compressive treatment, splinting, massage therapy, ultrasonic, stretching exercise well as a great variety of additional means such as hydrating creams, antihistamine drugs) was administered respectively with laser therapy.

Data obtained from both groups before initiation of treatment (Pre) and after 2 months (Post) from the initiation of the treatment regarding, ultrasonography and Vancouver Scar Scale were statistically analyzed and compared. Descriptive and analytic statistical data were used: The mean, the standard deviation and range were used as a primary source to measure central tendency. Paired *t*-test was used to compare the variable within each group to detect level of significance in each group. Unpaired *t*-test was used to compare the variable between two groups to detect significance level between two groups (comparison).

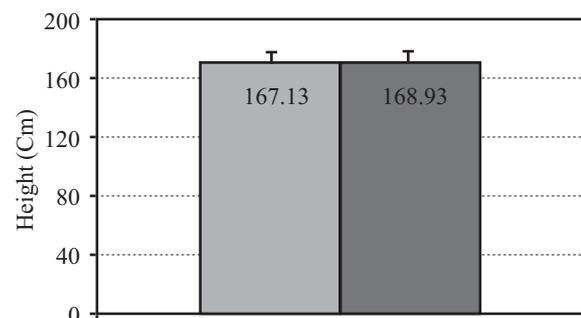
RESULTS

The mean age was (29.73±5.2) and (30.46±6.75) years, mean weight was (70.8±6.61) and (72.8±7.13) kilograms (Kg), and mean height was (167.13±8.27) and (168.93±5.14) centimeters (cm) for group A and B respectively denoting no significant difference between both groups in their ages, weights, and heights where their *t* and *p*-values were (0.33, 0.74), (0.79, 0.43), and (0.71, 0.48) respectively (Fig. 2).

There was a significant difference in the paired *t*-test between pre and post treatment Skin thickness of the hypertrophic scar by ultrasonography in group A and group B as the mean value of pre treatment was (7.4±1.62) (7.32±1.81) and for post treatment was (3.96±0.74) (6.56±1.75) where the *t*-value was (10.86) (7.75) respectively. The *p*-value was (0.0001) for both. However the percentage of improvement was 46.48 % and 10.38 % respectively (Figs. 3,4). The independent *t*-test results for the Skin thickness of the hypertrophic scar by ultrasonography pre and post treatment between groups A and B showed no significant difference in pre treatment values where the *t*-value was (0.12) and *p*-value was (0.9) But there was a significant difference in the post treatment values (*p*<0.05) where the *t*-value was (5.29) and *p*-value was (0.0001) (Fig. 5).



Physical characteristics of patients in both groups (A & B).



Item	Group A		Group B		Comparison		S
	Mean	±SD	Mean	±SD	<i>t</i> value	<i>p</i> value	
Age (yrs)	29.73	±5.2	30.46	±6.75	0.33	0.74	NS
Weight (Kg)	70.8	±6.61	72.8	±7.13	0.79	0.43	NS
Height (Cm)	167.13	±8.27	168.93	±5.14	0.71	0.48	NS

SD: Standard deviation, P: Probability, S: Significance, NS: Non-significant.

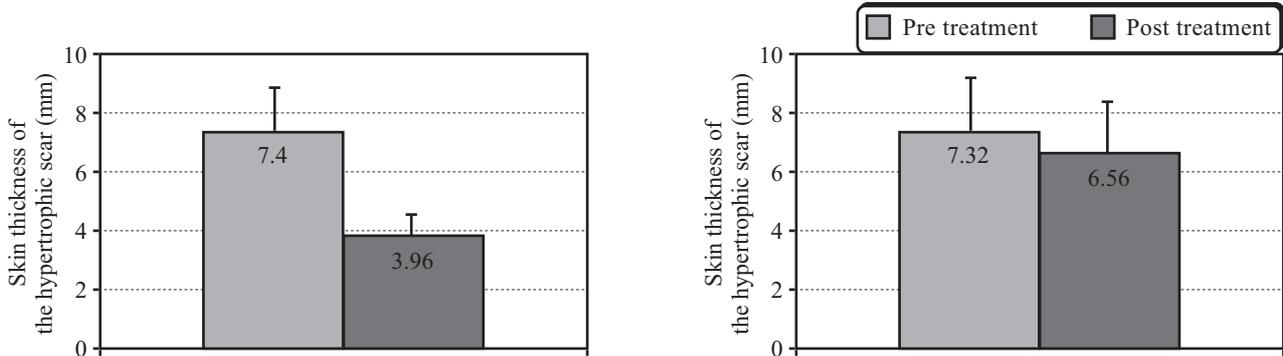


Fig. (2): Physical characteristics of patients in both groups (A & B).

Group A	Skin thickness of the hypertrophic scar by ultrasonography	
	Pre treatment	Post treatment
Mean	7.4	3.96
±SD	±1.61	±0.74
Mean difference	3.44	
Percentage of improvement	46.48%	
DF	14	
t-value	10.86	
p-value	0.0001	
S	S	

Group B	Skin thickness of the hypertrophic scar by ultrasonography	
	Pre treatment	Post treatment
Mean	7.32	6.56
±SD	±1.81	±1.75
Mean difference	0.76	
Percentage of improvement	10.38%	
DF	14	
t-value	7.75	
p-value	0.0001	
S	S	

SD: Standard deviation, P: Probability, S: Significance, DF: Degree of freedom.



Mean and ±SD of skin thickness of the hypertrophic scar by ultrasonography pre and post treatment.

Fig. (3): Thickness of hypertrophic scar by U/S.

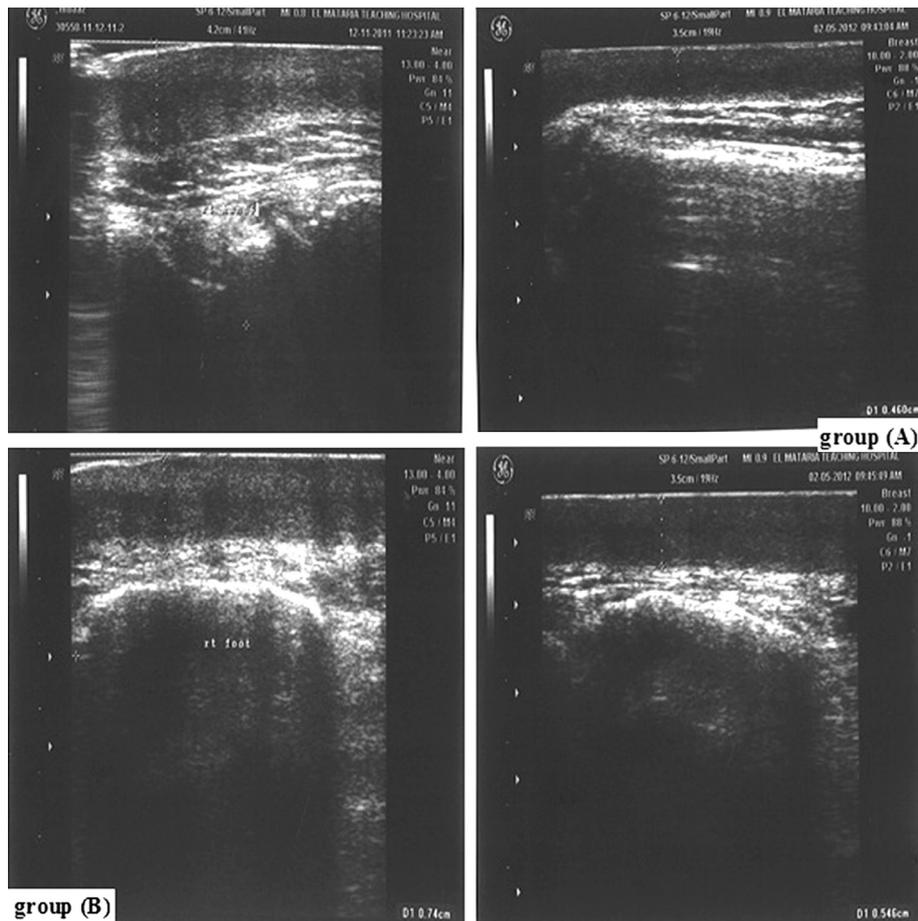
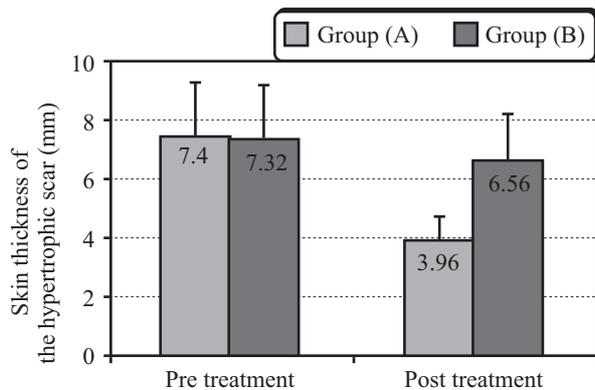


Fig. (4): Pre and post imaging.



Mean and \pm SD

Skin thickness of the hypertrophic scar by ultrasonography pre and post treatment of group (A,B)

Independent <i>t</i> -test	Skin thickness of the hypertrophic scar by ultrasonography	
	Pre	Post
Mean difference	0.08	2.6
<i>t</i> -value	0.12	5.29
<i>p</i> -value	0.9	0.0001
S	NS	S

*SD: Standard deviation, P: Probability, S: Significance, NS: Non-significant, S: Significant.

Independent *t*-test between groups A and B

Fig. (5): Hypertrophic scar by U/S independent *t*-test.

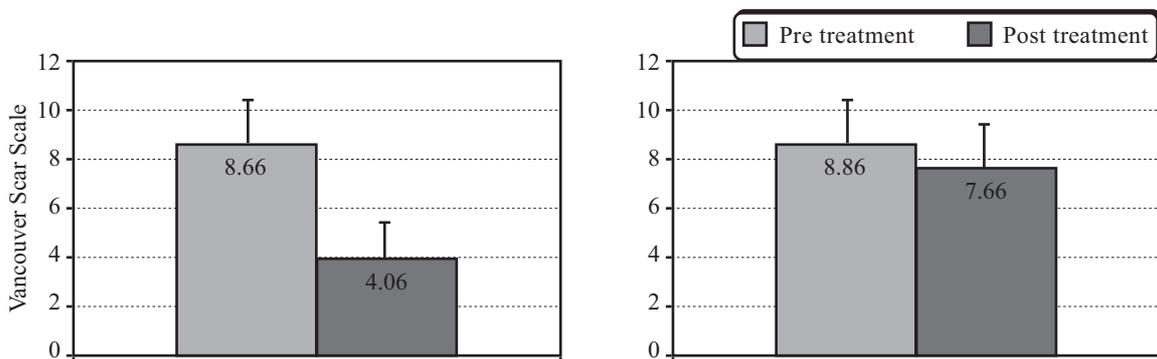
There was a significant difference in the paired *t*-test between pre and post treatment Vancouver Scar Scale in both group (A and B) as the mean value of pre treatment was (8.66 \pm 1.67) (8.86 \pm 1.84) and for post treatment was (4.06 \pm 1.38) (7.66 \pm 1.95) where the *t*-value was (21.51) (6.87) respectively. *p*-value was (0.0001). However, the percentage of improvement was 53.11%, 13.54%

respectively (Fig. 6). The independent *t*-test results for the Vancouver Scar Scale pre and post treatment between groups A and B. Showed no significant difference in pre treatment values where the *t*-value was (0.31) and *p*-value was (0.75) But there was a significant difference in the post treatment values (*p*<0.05) where the *t*-value was (5.82) and *p*-value was (0.0001) (Fig. 7).

Group A	Vancouver Scar Scale	
	Pre treatment	Post treatment
Mean	8.66	4.06
\pm SD	\pm 1.67	\pm 1.38
Mean difference	4.6	
Percentage of improvement	53.11%	
DF	14	
<i>t</i> -value	21.51	
<i>p</i> -value	0.0001	
S	S	

Group B	Vancouver Scar Scale	
	Pre treatment	Post treatment
Mean	8.86	7.66
\pm SD	\pm 1.84	\pm 1.95
Mean difference	1.2	
Percentage of improvement	13.54%	
DF	14	
<i>t</i> -value	6.87	
<i>p</i> -value	0.0001	
S	S	

SD: Standard deviation, P: Probability, S: Significance, DF: Degree of freedom.



Mean and \pm SD of Vancouver Scar Scale pre and post treatment

Fig. (6): Vancouver scale pre and post.

Independent <i>t</i> -test	Vancouver scar scale	
	Pre	Post
Mean difference	0.8	3.6
<i>t</i> -value	0.31	5.82
<i>p</i> -value	0.75	0.0001
S	NS	S

*SD: Standard deviation, P: Probability, S: Significance, NS: Non-significant, S: Significant.

Independent *t*-test between groups A and B

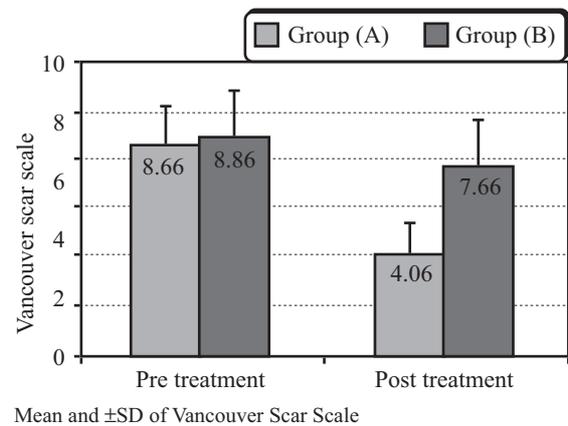


Fig. (7): Vancouver scale independent *t*-test.

DISCUSSION

Low level laser therapy (LLLT) is considered part of light therapy. It is used to heal diverse ulcerations, to treat edema, burns and dermatitis, to relieve pain and treat chronic inflammation and autoimmune disease. The principle of using LLLT is to supply direct biostimulation light energy to the body's cells. Cellular photoreceptors (e.g. cytochromophores and antenna pigments) can absorb low-level laser light and pass it on to mitochondria, which produce the cells fuel, ATP [27].

Simultaneous collagen synthesis and degradation during normal scar maturation result in decreased nodularity and flattening of the scar [2]. Hypertrophic scars occur when the body overproduces collagen, which causes the scar to be raised above the surrounding skin. An imbalance of matrix degradation and collagen biosynthesis resulting in excess accumulation of collagen in the wound has been postulated to be the primary biochemical features of this skin lesion [28,29]. However, it has been stated that hypertrophic scar fibroblasts respond normally to growth factors and demonstrate only a modest increase in collagen production [30]. Also, Some investigators have demonstrated an abnormal balance between proliferative and apoptotic cell death in fibroblasts derived from keloids and hypertrophic scars [31,32].

The experimental findings by Webb and colleagues in 1998 [33] revealed that 660nm LLL of energy density 2.4J/cm² and 4J/cm² had stimulatory effect on the cell counts of both hypertrophic scar fibroblasts and normal dermal fibroblasts cell lines derived from biopsies of donors, who were matched by race, gender, anatomical region and age. The stimulatory effect appeared to be slightly greater in hypertrophic scar fibroblasts than normal dermal fibroblasts and that is related to enhancing the cells' release of basic fibroblast growth factor.

He-Ne laser with 180J/cm² irradiation can inhibit the growth of cultured fibroblasts in HS [34]. The cause is due to the stagnation and apoptosis of G0/G1 cells induced by He-Ne laser probably [35]. Later on, Hawkins and Abrahamse [36,37] stated that cultured skin fibroblasts irradiated with 632.8nm, 830nm, 1064nm with a fluence 16J/cm² had showed increased apoptosis and percentage of cellular viability. Then Houeild and Abrahamse [38] confirmed this finding by stating that human skin fibroblasts viability was determined by the Trypan blue exclusion, showed significant decrease in percentage of cellular viability and increased apoptosis when irradiated with 632.8nm HE NE laser in comparison with normal and un-irradiated cells.

SHU Bin and colleagues [35] concluded that repeated 632.8nm He Ne laser irradiation at the power density of 100mW/cm² or 150mW/cm² can suppress collagen synthesis of cultured fibroblasts in hypertrophic scar. The cause of suppression may be associated with down regulation of type I procollagen mRNA expression. They used confocal laser scanning microscopy and flow cytometry to explore the mechanism of He-Ne laser inducing apoptosis of fibroblasts of cultured hypertrophic scars (HS) at protein level. The cultured fibroblasts in HS were irradiated with He-Ne laser (Wavelength 632.8nm, power density 100mW/cm²) daily for 30 minutes. Findings showed several proteins such as Bcl-2, Fas, ICE, p53 and c-myc proteins were present in scar fibroblasts in culture. The amount of Fas and ICE proteins increased, and Bcl-2 protein reduced, while the amount of Bax, p53 and c-myc proteins remained constant, after repeated He-Ne laser irradiation. The conclusion of the study is that He-Ne laser inducing apoptosis of scar fibroblasts were associated with the changes of Fas, ICE and Bcl-2. They also stated that repetitive irradiation of He Ne for the culture of scar derived

fibroblasts at power densities of 100 or 150mW/cm² can induce cell apoptosis. Nuclear DNA of apoptotic cells displayed ladder bands characteristic of internucleosomal DNA fragmentation after laser irradiation at 100 and 150mW/cm² for 30 minutes. So that, Power density is more important than energy density so far as laser inducing apoptosis of scar fibroblasts concerned.

The increase in the ALP activity may indicate that a high dose of 16J/cm² results in the up-regulation of ALP expression, which requires the cessation of proliferation [39]. Hawkins and Abrahamse [36] in their study, the Wounded fibroblasts showed an increase in the ALP activity after two exposures of 16J/cm² and three exposures of 16J/cm², indicating that multiple exposures of a high dose (16J/cm²) may cause additional stress or damage to the cell membrane of the wounded fibroblasts to cause an increase in the release of the ectoenzyme ALP anchored in the plasma membrane.

They [37] also compared between the effect of different doses of HE NE laser on skin fibroblasts, and he stated that; From the cell morphology results, wounded cells responded to a dose of 2.5 and 5J/cm² with an increase in chemotaxis-chemokinesis and haptotaxis indicating a stimulatory effect. The results indicate that a fluence of 5 J/cm² on 2 days stimulates mitochondrial activity (ATP activity) and cell proliferation without adversely affecting the cell viability or damaging membrane integrity or causing DNA damage. Higher doses (10 and 16J/cm²) result in a decrease in cell viability and mitochondrial activity with an increase in percentage cytotoxicity and DNA damage.

Conclusion:

The finding of this study indicated a considerable difference in measuring Vancouver Scar Scale and Scar thickness (by ultrasonography) between pre-treatment and post treatment within the two groups (control & experimental). Moreover, the Results of this study concerning the effect of (632.8nm-15J/cm²) for promoting hypertrophic scar in humans shows that LLLT has a beneficial effect on burn scars in human beings. In general, the scars became softer and more pliable. The irradiation gave them relief from pruritus and pain and sometimes improved the pattern of scars. The present study suggests that a planned regime of treatment with LLLT can have significant benefit for a considerable proportion of patients during the rehabilitation stage.

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