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The Effects of a 2-W Laser on the Nasolabial Fold of Ageing Individuals Investigated by High-Frequency Ultrasonography: A Nonrandomized Controlled Trial

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Abstract

Introduction: Biomechanical qualities of the skin tissue change following numerous pathological and natural (ageing) conditions. The best skin treatments are those resulting in a positive outcome with the fewest adverse effects. In this study, high-frequency ultrasonography (US), which provides a quantifiable scale, was utilized to evaluate the impact of a 2-W laser on characteristics of nasolabial fold (NLF) including depth and area, epidermis and dermis thicknesses, and Young's modulus (YM).

Methods: Nine female subjects (54.09 ± 0.09 years old) with Fitzpatrick skin types III-IV and five young control participants (26 ± 1.28 years old) for providing the control data were included in this study. Laser therapy was applied twice weekly for seven consecutive sessions. A class IV laser with 810-nm and 980-nm wavelengths, 2-W power, and 25-Hz frequency was applied twice a week for seven consecutive sessions. The energy administered had a density of 8 J/cm^2. An evaluation using high-frequency ultrasound (40 MHz) was accomplished before and 48 hours after the final treatment session. The Face-Q questionnaire was used to assess the patient's perspective on this procedure.

Results: For each US parameter, the intraclass correlation coefficient was high (ICC > 0.9). After the laser treatment, NLF depth, area, epidermis, dermis, YM, and Face-Q significantly improved compared to baseline ($P < 0.05$).

Conclusion: For the assessment of skin tissue, high-frequency US is a reliable technique. The 2-W laser is a safe, effective, and non-invasive procedure for enhancing skin features.

Keywords: Laser; Nasolabial fold; Skin; High-frequency ultrasonography; Epidermis; Dermis.

Introduction

The skin tissue undergoes numerous changes including the development and evolution of wrinkles as it ages. These changes can be observed in females between the ages of 30 and 60[1] and are characterized by a decline in the amount of collagen and elastin fibers and skin cell turnover.[2,3] Collagen fibers make up 70%–80% of the dry skin's weight and are crucial for maintaining the mechanical and structural integrity of the skin.[4,5] Due to the flexibility of elastic fibers and the distortion of the collagen fibers' network-like arrangement in the dermis, the skin can stretch and contract. With repeated movements due to facial expressions, some fibers could be broken. Long collagen fibers are used to repair the broken elastic fibers, and subsequently, short collagen fibers are used to replace the long collagen fibers. The deposition of these improperly repaired structures affects the mechanical and elastic properties of the skin, causing wrinkles to appear.[6]

Nasolabial folds (NLFs) and perioral wrinkles are the most prominent sign of ageing in the lower face and appear early at the age of 30 or before while most people would like them to be less prominent.[7] With ageing, the fat volume in the lower portion of the NLF increases, the nasolabial sulcus loses volume, and the upper cheek area folds,[8] which causes the nasolabial groove to protrude.[9]

Ageing also results in a decrease in skin thickness, which affects the biomechanical properties of the skin such as Young's modulus (YM)[10] Older individuals have epidermis and dermis that are 0.7–0.8 mm thinner than younger ones.[11,12] YM is one of the biomechanical properties of elastic materials that represent the tissue's resistance to deformation caused by the application of force. It is a property of the material that tells us how easily it can stretch and deform and is defined as the ratio of tensile stress (σ) to tensile strain (ε). Stress is the amount of force applied per unit area ($\sigma = F/A$) and strain is extension per unit length ($\varepsilon = dl/L$).[10]

YM of the skin tissue is affected by a variety of parameters, including age, gender, hydration, measurement location,

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and skin thickness, and much research has demonstrated that it decreases with age.[13-15]

The mechanical characteristics of the skin have been assessed using a wide range of techniques, including stretching, torsion, vibration, indentation, elevation, and suction.[16] These investigations produced contradictory findings; some claimed that the YM increased with age, while others claimed that it decreased. Using the suction approach, Diridollou et al[13] found that YM increased for both men and women beyond the age of 30. They also observed that skin thickness increased between the ages of 10 and 20 and decreased beyond the age of 50. Using the indentation approach, Dulińska-Molak et al[17] observed that YM increases with age. According to Boyer et al, the YM values decrease from 10.7 kPa to 7.2 kPa as people get older.[18] Despite the numerous methods available for improving skin biomechanical properties, such as various invasive lasers, many individuals may prefer a laser treatment that offers reasonable results, has fewer side effects, is less invasive, and requires no recovery.[19]

Low-level laser therapy (LLLT) has been utilized in previous studies for a range of dermatological conditions, including wrinkles, scar tissues, and other skin diseases. The mechanism of action of LLLT includes promoting collagen synthesis, dermal remodeling, and skin tightness.[16]

In Sator's study, 90 participants received eight LLLT therapy sessions, and at least one score decreased on the Fitzpatrick wrinkle severity scale.[20]

It must be mentioned that high-power laser (HPL) therapy has recently been developed, with a maximum FDA-approved power of 7 W.[21] However, there is no information on how this type of laser affects the biomechanical properties of skin tissue, which may be helpful for both pathologic (scars) and cosmetic purposes.

Ultrasonography (US) has been used in dermatology as an evaluation technique since the 1970s. The development of equipment with a frequency greater than 15 MHz has made it capable to identify the various structures and layers of the skin. For the observation of the skin's surface properties, the high-frequency US with minimal penetration depth provides excellent resolution.[22] The NLF is a facial landmark suitable for the identification and quantification of different treatment effects. The NLF also has a patient-reported outcome measure (Face-Q) for satisfaction and quality of life following different treatments of the face for research purposes (Face-Q).[23,24]

Therefore, this study aimed to investigate the impact of 2-W laser therapy on NLF characteristics related to ageing through high-frequency US and Face-Q questionnaires.

Materials and Methods

This was a pretest-posttest experimental study.

Patients

Nine female subjects (35-55 years, 25-30 body mass index) with Fitzpatrick skin types III-IV and 5 young control females (20-30 years) for providing normal control data (received no treatment) were enrolled. The exclusion criteria included any treatment for NLF six months before the procedure, systematic diseases like HIV, diabetes, connective tissue diseases like lupus, scleroderma, and sarcoidosis, surgical and non-surgical scars in the treatment area, infectious diseases in the treatment area, cyst, skin rashes, herpes, pregnancy, and psychological disorders. The flow chart of the study is depicted in Figure 1.

Outcome measures included the Face-Q a validated questionnaire composed of several scales for measuring a patient's perception of a facial procedure,[23,24] and NLF properties measured by the US. The Face-Q questionnaire scales included an appraisal of NLF, satisfaction with facial appearance, appraisal of age, satisfaction with the decision, satisfaction with outcomes, and social function. The Memorial Sloan-Kettering Cancer Center has acquired authorization to utilize the Face-Q questionnaire.

Ultrasonography Measurements

All outcomes were collected at the medical physics department of Tarbiat Modares university. The US evaluated wrinkle depth and area, epidermis and dermis thicknesses, and YM were evaluated by the US. A linear transducer (L40-8/12), 40 MHz, US (Ultrasonix Medical Corporation, Richmond, Canada), with a resolution of 0.001 mm and 2-cm penetration depth was used. The transducer was positioned perpendicular to the NLF in the middle of the line connecting the mouth corner and the alar cartilage while the patients were seated upright. For additional analysis, two images were taken and uploaded to the ImageJ program (National Institutes of Health in Wisconsin, USA). To assess NLF depth, we connected two edges of the NLF with a continuous line. Another line was drawn perpendicular to the fold's center starting from the midpoint of this line.[25] Two lines 0.1 cm apart, were added beside the center line for improving fold depth accuracy. These lines were each measured three times, and the NLF depth was determined as the mean of the nine measurements (Figure 2A). The NLF area was measured three times with Image J's area selection tool, and the mean value was reported (Figure 2B). Additionally, the final measurement was assigned as the mean value of the right and left sides of the face. The visual echogenicity features were used to separate the epidermis and dermis.[26,22] The collected images were separated into five sections, which were all measured three times (a total of 15 measurements), and the average thickness value was calculated (Figure 3). The mean value of the right and left sides of the face was recorded, along with NLF depth and area.

The technique of indentation is used to assess stiffness

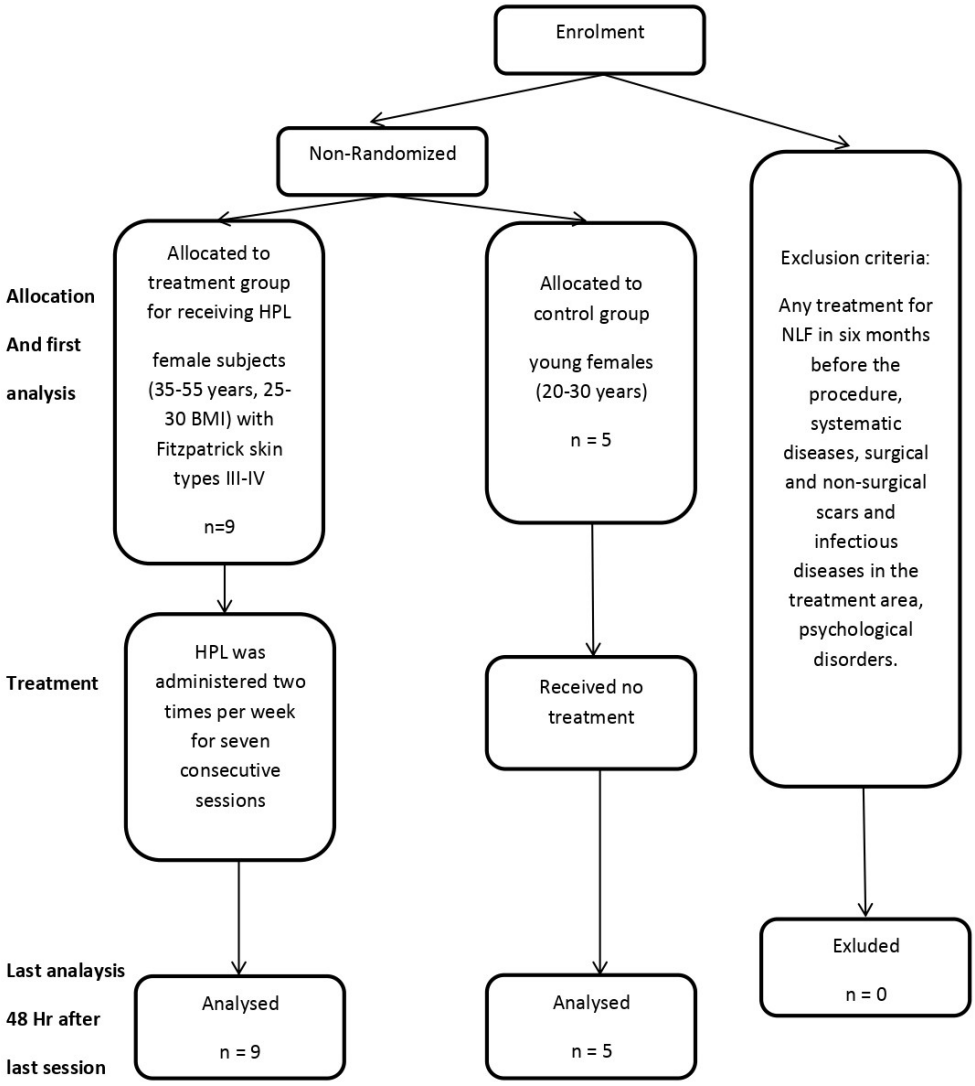

Figure 1. Flow chart of the Study Design

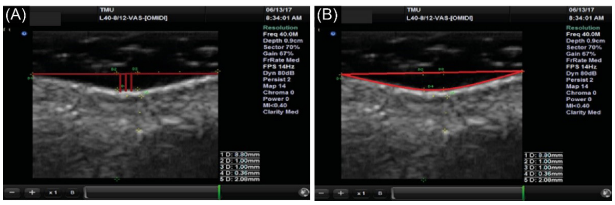

Figure 2. Nasolabial Fold Depth (A), Area (B), and Measurements With 40 Hz Ultrasound Transducer and ImageJ Software

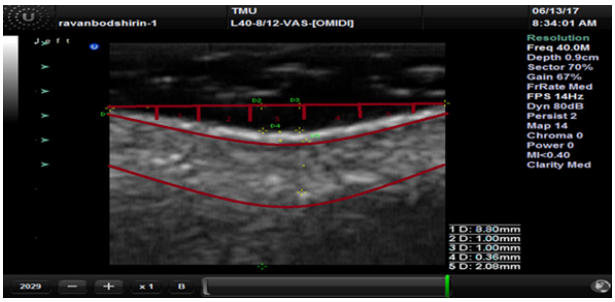

Figure 3. The Method of the Epidermis and Dermis Thickness Measurements in Five Areas of the Captured Image of the Nasolabial Fold

and elasticity (YM) of the tissues. It is a secure, non-invasive procedure that relies on tissue deformation in reaction to external pressure. The method used in this investigation was manually compressing the skin using an ultrasonic transducer. A digital dynamometer (Lutron Electronic Enterprise Co, Taipei, Taiwan) with 0.1N accuracy that was connected to the abovementioned ultrasonic probe was used to precisely quantify the applied stress. Before and following compression, images were collected. A site with special echogenicity was chosen, and the distance from this point up to the skin surface was calculated as L1 to quantify the skin strain using Image J software. Following the application of the compression force (F), which was maintained between 0.25 to 0.35 N through the digital dynamometer,[27] the new distance of this particular point was measured and identified as L2. The Hook law was used to compute YM using the formula (Young's modulus = (F/A)/(L/L1)),[28,29] where A represented the cross-sectional area of the ultrasonic probe.

Blinding

The sonographer was blinded to the patient's group allocation.

Intervention Protocol

Laser treatment was administered two times per week for seven consecutive sessions. It was a class IV laser (OptonPro, Zimmer, MedizinSysteme GmbH, Neu-ULM, Germany) with continuous emission at wavelengths of 810 and 980 nm with a power of 2 W. With a treatment distance of 9mm and a spot size of 10 mm, the applicator was in touch with the skin. Each of the five equally sized portions of the NLF received an energy density of 8 J/cm^2.[30] The energy density in the upper portion of the NLF next to the alar cartilage was doubled to 16 J/cm^2.[31] Since it has been demonstrated that the Levator labii superior and zygomaticus muscles are involved in the formation of NLF prominence with ageing, these muscles also received 8 J of energy.[32,33] Therefore, the total applied energy density for each side of the face was 64 J/cm^2. No adverse effect was seen during the whole procedure. Laser treatment was carried out twice a week for seven sessions that lasted four weeks. All evaluations were repeated 48 hours following the final laser treatment session.

Sample Size

The number of subjects was determined using the data of wrinkle severity score from the study performed by Hong et al.[34] A total of 7 was shown to be necessary based on the alpha level of 0.05 and power (1-beta) of 0.80.

Statistical Analysis

The Statistical Package for Social Sciences (SPSS) software, version 24, was used for every statistical analysis. The normality of data was assessed using the Kolmogorov-Smirnov test. The results before and after laser treatment were analyzed using paired *t* tests. To compare the data between the laser and control groups, we performed an independent-sample *t* test. Statistical significance was determined by the *P* value of 5%.

Results

According to the inclusion criteria, 5 young control females and 9 female volunteers were enrolled in this study.

Table 1 displays the characteristics of the participants. There was no statistically significant difference between the two groups' starting points. Each parameter was assessed three times using three different images, and the intraclass correlation coefficient (ICC) was used to examine the data to determine the reliability of ultrasound. The NLF depth, area, epidermis thickness, dermis thickness, and YM all had intra-rater, test-retest reliability, $ICC_{3,1}$ values[35] of 0.971, 0.913, 0.967, 0.994, 0.989, and 0.995, respectively.

After the treatment, there were significant decreases in NLF depth and area in the laser group ($P<0.02$). Epidermis and dermis thicknesses also displayed statistically significant changes ($P<0.001$). YM also showed a significant increase following laser treatment ($P<0.001$; Table 2).

Before and following treatment sessions, subjective evaluation of the laser treatment revealed considerable changes ($P<0.001$) in the appraisal of NLF, satisfaction with facial appearance, and appraisal of age ($P<0.001$; Table 3).

Other scales of the Face-Q questionnaire, such as satisfaction with the decision, satisfaction with the outcome, and social function were collected after the laser therapy ceased (Table 4).

Clinical photographs of the patients were also obtained before and after the procedure, and a comparison of the images confirmed that the NLF area had enhanced after 7 sessions of laser treatment (Figure 4).

Table 1. Demographic Characteristics of Study Subjects

	Age (y)	Height (m)	Weight (Kg)	BMI	Menopause (+)
Laser	54.09 ± 0.09	1.64 ± 0.12	70.27 ± 2.64	24.68 ± 3.04	55%
Control	26.00 ± 1.28	1.66 ± 0.58	60.40 ± 4.50	21.90 ± 2.51	
P value	0.000	0.96	0.25	0.67	

BMI, body mass index.

Table 2. Ultrasonography Outcomes in the Young Control and the Laser Group Before and After Laser Treatment, and the Mean Reduction After Laser Treatment

	Laser (Before)	Laser (After)	Control	Mean Reduction, %
NLF depth (mm)	2.08 ± 0.07[a]	1.87 ± 0.06[b,a]	1.49 ± 0.08	10
NLF area (mm^2)	5.98 ± 0.07 [a]	5.51 ± 0.13 [b,a]	3.80 ± 0.21	7.8
Epidermis thickness (mm)	0.15 ± 0.02 [a]	0.18 ± 0.01 [b,a]	0.25 ± 0.01	9.8
Dermis thickness (mm)	1.20 ± 0.10 [a]	1.31 ± 0.09 [b,a]	1.71 ± 0.05	9.1
Young modulus (kPa)	14.94 ± 1.16 [a]	17.07 ± 1.30 [b,a]	28.70 ± 2.28	14.2

[a] Significant difference with the control group.
[b] Significant difference in the laser group before and after treatment.

Table 3. Face-Q Questionnaire Scales Before and After Laser Treatment

	Appraisal of NLF	Satisfaction With Facial Appearance	Appraisal of Age
Before	48.28 ± 8.80	50.06 ± 14.53	43.66 ± 22.09
After	54.11 ± 9.03	55.11 ± 13.75	48.55 ± 22.01
P value	0.000	0.000	0.011

Table 4. Face-Q Questionnaire Scales Related to Patient Satisfaction After Laser Treatment

	Satisfaction With Decision	Satisfaction With Outcome	Social Function
After	60.00 ± 14.77	59.44 ± 12.94	60.88 ± 9.31

Figure 4. Before (A) and after (B) Clinical Photographs of the of One of the Subjects

Discussion

This study evaluated the effects of a 2-W laser on NLF parameters, including epidermis and dermis thicknesses, depth and area, YM, and patient's perception of ageing skin. According to earlier studies, LLLT has two main effects: increasing microcirculation by having a photochemical effect, and stimulating the production of collagen and cellular matrix.[36-38] HPLs have all the qualities listed above in addition to greater power, deeper penetration, and higher thermal effects on tissues in comparison to low-level lasers.[39]

One of the major variables influencing the ageing process of the skin is cutaneous thickness. In assessing the effects of various treatments on the ageing process, it is crucial as well.[40,41] Compared to invasive skin sampling, radiographic, or skinfold caliper methods, noninvasive ultrasound measurement of skin thickness is a more practical and accurate procedure. Images of the skin folds were taken using ultrasound while they were unloaded, standing upright, and as close as possible to their natural integrity.[42] Furthermore, the dimension A-mode scanner utilized in earlier investigations was less accurate than the B-mode ultrasonic scanner, which captures the image of the tissue cross-section.[43]

In accordance with Tsukahara and colleagues' study, we determined the wrinkle area and depth.[25] To determine the greatest depth of the wrinkle, they measured the vertical distance between the reference level and the lowest valley (highest peak) of the wrinkle. However, they used a silicone replica to measure the depth and area of face wrinkles, which took time and rendered different results on cadaver facial skin than living individuals. To achieve more precise data, we measured the epidermis and dermis thicknesses in this study by separating the NLF into 5 regions as opposed to simply one area.

More practically than silicone replicas, profilometric analysis using imaging and related software was used by Kohl et al[44] to study the effects of the fractional laser. The 2-W laser irradiation of the current study, however, had no adverse effects, unlike the fractional laser, which can cause side effects like crusting, swelling, and erythema after each treatment session. In contrast to the current study, older adults were shown to have thicker skin on their faces compared to younger subjects, except in the infra-orbital regions.[40] However, the patients whose ages ranged from 60 to 90 were older than those included in the current study.

There is evidence that the biomechanical properties of the skin may be affected by the age-dependent loss of collagen and elastin fibers.[16,45] This phenomenon was supported by findings of the current study that older participants had lower dermal thickness (which contains these fibers) and YM than young controls.[25] The firmness of a material and its degree of deformation under high stress are directly related to its YM. So treatments that improve skin tightness are necessary for treating skin conditions, wrinkles, and rejuvenation. As previously mentioned, there are several techniques for determining the YM of the skin, and the results are controversial. Diridollou et al[13] and Alexander and Miller[46] reported that YM increased with age and evaluated YM using the suction method, but Boyer et al[18] reported that YM reduced with age and evaluated YM with the indentation method, the latter of which was consistent with the current investigation. As a result, different investigational methods may have produced different results. The findings of the current study on YM following laser therapy were consistent with those of Hong et al.[34] They claimed that the skin elasticity was greatly improved using a non-ablative long-pulsed 1064 nm Nd:YAG laser with an energy density of 20–24 J/cm^2, which was greater than that of the present study.

All scales of the Face-Q questionnaire showed a significant improvement in the subjects' perceptions of the impact and effectiveness of laser treatment. The subjects' satisfaction with this non-invasive laser treatment was the more noticeable outcome.

It should be noted that the high-power laser used in this study induced controlled tissue injury rather than side effects of the invasive methods such as severe pain, edema, acne, severe scarring, blisters, and pigmentation. As a result, it may be an effective treatment for scar tissue and rejuvenation.

After seven non-invasive laser treatment sessions, all parameters, although they were still different, inclined towards the young control. Presumably, increasing the number of treatment sessions might yield better outcomes. Moreover, further investigations with a larger sample size and follow-up consideration are recommended.

Conclusion

High-frequency ultrasound is a simple, effective approach for examining the properties of skin folds. NLF depth and area considerably decreased compared to baseline after seven treatment sessions using a 2-W laser with an energy density of 8 J/cm^2 ($P<0.05$). YM increased by 14.2%, and epidermis and dermis thicknesses also significantly improved ($P<0.05$).

Conflict of Interests

None declared.

Ethical Considerations

Under the designation IR.TMU.REC.1396.559, the Tarbiat Modares University Ethics Committee has authorized this study. After thoroughly explaining the study protocol to the patients and obtaining their written agreement, the study was conducted. Besides, this study was registered in the *Iranian Registry of Clinical Trials website* (identifier: IRCT20170818035759N2; https://www.irct.ir/trial/26931)

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