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The Effectiveness of Dry Needling on Dimensions and Elastic Modulus of the Nasolabial Fold due to Aging Process: An Uncontrolled before-after Clinical Trial Study

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Original Article

Abstract

Introduction: Biomechanical properties of the skin change with pathological conditions and aging process. Nasolabial fold (NLF) is a degenerative manifestation of the skin tissue, which facilitates biomechanical properties measurement due to its quantifiable scales. The aim of this study was to evaluate the effectiveness of dry needling on decreasing NLF dimensions and improving NLF skin biomechanical properties.

Materials and Methods: 11 women aged between 35-55 years with NLF class between 2-2.5 were selected for study, and received 7 sessions of dry needling treatment. Another group of young women between 20-30 years considered as control group and received no treatment. NLF depth and area, epidermis and dermis thickness, and young modulus of NLF skin were analyzed with high resolution ultrasonography and a custom-made forceguage. For reliability of sonographic data the interclass correlation coefficient, and for between and within group comparisons, independent and paired t tests were used, respectively.

Results: NLF depth and area decreased significantly ($P < 0.001$). Moreover, epidermis and dermis thickness and young modulus increased significantly ($P < 0.001$).

Conclusion: Dry needling induced decrease in depth and area, and improved biomechanical properties of the NLF, without any side effect.

Keywords: Dry needling, Nasolabial fold, Young modulus, Epidermis, Dermis

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Introduction

Skin tissue is an integrated outer layer that covers the whole body and is paid much attention in medical science due to the various vital functions it performs to maintain the body's internal environment (1). The structural integrity of the skin is created by the complex interaction of the various layers of the skin, which include epidermis (rich in Keratin), dermis (composed of nerves, blood vessels, and collagen and elastin fibers), and subcutaneous layer (including adipose tissue) respectively from the outer to inner layers (2). The biomechanical properties of the skin are of special importance due to their important role in health and disease, structural cohesion, and aging process (3). The connective tissue of the skin is mainly composed of collagen and elastin fibers, with collagen fibers accounting for 70 to 80% of the dry weight of the skin which play a major role in maintaining the structural and mechanical integrity of the skin, the synthesis of which gradually decreases with age (4).

Many skin changes occur with increasing age. One of the most obvious morphological changes due to aging is the emergence and development of facial wrinkles (5). This process takes place as a result of slow tissue degradation due to the loss of collagen fibers, destruction of the elastin fiber network, and loss of water (6). In addition, as the age increases, the

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skin thickness decreases (7), so that the skin thickness of the elderly is 0.7 to 0.8 mm lower than that of the young people (8). Since the dermal collagen fiber network plays a key role in maintaining the Young's Modulus of the skin tissue (9), the loss of order of placement of the fibers in this network can change its mechanical properties (Young's Modulus). Nasolabial fold (NLF) is one of the facial feature and the most prominent deep fold of the dermis (10). With age and loss of biomechanical properties of the skin as a result of repetitive facial movements and facial expression, NLF is created perpendicular to the stretching line of the muscles creating it [Levator labii superioris (LLS) and Zygomaticus major (ZM)] (11).

Different treatments have been presented so far to improve the biomechanical properties of the skin. Increased demand for less invasive techniques and greater effects along with fewer side effects and shorter treatment duration have paved the way for less invasive techniques (12). In general, the mechanism of action of these methods is to create controlled skin lesions with the aim of enhancing collagen biosynthesis and dermal matrix remodeling (13). In the field of physiotherapy, applying different methods with the above mechanisms leads to changes in the features of various connective tissues including muscles, articular cartilage, bone, and skin (14, 15). The dry needling method is one of the physiotherapy techniques that involves the application of the needle to the skin with various therapeutic purposes. Through the creation of microtrauma and the release of vasoactive substances, dry needling leads to the increased blood flow, accelerated tissue repair process, and synthesis and regulation of the collagen fibers (16). Another cause of NLF is the increased facial muscle tone and their approach to the contracture tone, which causes general stiffness of the facial muscles and aggravates the skin folding (17).

By applying dry needles to the muscles, their contractile properties can be changed and their tone can be reduced as well, and thus reducing their effects on the structural and mechanical changes of the skin tissue (18). Diagnostic ultrasound is one of the tissue characterization methods, which is a low-risk and accurate method to obtain descriptive information on the tissues and their biomechanical properties (19). Using ultrasound images, the features of the skin tissue are quantitatively examined in a non-pressurized mode and perfectly in the natural direction of the facial skin texture, with very accurate analysis results. So far, no detailed studies have been accomplished to quantitatively evaluate the biomechanical properties of the NLF skin tissue in the native Iranian population before and after treatment with physiotherapy techniques and modalities. Therefore, the present study is performed aiming to investigate the use of the dry needling technique on structural and mechanical changes of the facial skin tissue such as changes due to aging, and NLF area was used to evaluate this method due to its validity and applicability because of its quantitative criteria for this purpose.

Materials and Methods

This study was a pre- and post-clinical trial in which the subjects were chosen using the selective sampling method. The aim in this study was to investigate the effect of 7 sessions of the dry needling treatment (twice weekly for 4 weeks and once in the last week week) on the NLF depth and area, epidermis and dermis thickness, and the skin Young's Modulus in the NLF area among women aged 35-55 years old in the Department of Physical Therapy, School of Medical Sciences, Tarbiat Modares University, Tehran, Iran. Prior to data collection, ethical approval was obtained from the Ethics Committee, Tarbiat Modares University with code IR.TMU.REC.1396.559. Additionally, the study information was recorded on the Iranian Registry of Clinical Trials (IRCT) website with number IRCT20170818035759N2.

Following the publication of the call at Tarbiat Modares University, 11 women aged 35-55 years with clear NLF in all three internal, middle, and external parts were selected based on the classification performed in the study by Pessa and Brown (20) and enrolled in the study considering the inclusion and exclusion criteria. In order to evaluate the baseline data and compare the before-and-after treatment results with normal values, a healthy control group (n = 5) with an age range of 20-30 years was used. Based on the afore-mentioned changes in the biomechanical properties of skin due to aging, the age range of the control group as a healthy control group was significantly lower than that of the intervention group in order to provide the ground for comparing the skin conditions in the normal conditions with the case it has changed due to aging. For instance, it is clear that the thickness of the skin is higher in young individuals. Therefore, if the skin thickness in the intervention group is close to that of the control group at the end of treatment, the treatment will be more effective and desirable. Accordingly, the present study was designed as a non-randomized, non-controlled clinical trial to discuss the clinical value of the proposed treatment.

The study inclusion criteria included women aged

35-55 years old, body mass index (BMI) between 25 and 35 kg/m^2, type III-IV skin (swarthy and wheatish complexions) based on the Fitzpatrick classification scale, and the NLF depth ranged from 2-2.5 based on the Modified Fitzpatrick Wrinkle Scale (MFWS) (21) including average and totally obvious depth (1-3 mm). For further confidence, a stainless hardened digital caliper (Shenzhen, China) with a precision of 0.01 mm was used.

Other ways to treat NLF (such as Botox, needling, and facial surgery) during the six months prior to the study, history of blood coagulopathy, use of blood coagulants, diseases such as acquired immunodeficiency syndrome (AIDS), diabetes mellitus (DM), connective tissue diseases such as lupus, scleroderma or other systemic diseases, surgical or non-surgical scars in the treated area, active inflammation or infection in the treated area, and alcohol and tobacco addiction were considered as the exclusion criteria.

After qualifying in the inclusion criteria, the needle test was performed on the back of the hand of the subjects (due to the high sensitivity of this area and its similar features to those of the face skin). In case of the lack of consent, the volunteers did not enter the study and in case of consent, they completed the informed consent form and entered the study. Then, the participants were informed about the various aspects of the needle therapy. To ensure the accuracy and evaluate the treatment, the subjects washed their faces completely two hours prior to the evaluation and treatment and were kept in a room at constant temperature and humidity for 30 minutes.

The dimensions of the NLF area were measured on the images taken by an ultrasound device (B-mode, Ultrasonix Medical Corporation, Canada) with a linear converter of 8.12-40 L, a resolution of 0.001 mm, a frequency of 40 MHz, and a penetration depth of 2 cm. The applicator of the sonoelastography device was positioned longitudinally on the NLF (Figure 1), and its measurement site was in the middle of a line connecting the corner of the lip to the root of the alar cartilage.

Figure 1. Dimensions of Nasolabial Fold Area (NLF) using an ultrasound device

At the same time of imaging, the NLF depth was calculated in millimeters and used as a criterion for adjusting and matching the ImageJ software scale [United States National Institutes of Health (NIH)].

In order to calculate the NLF depth using the ImageJ software, the mean of the three lines that were at a distance of 0.1 mm from each other from the center of a continuous line connecting the two edges of the NLF was measured (Figure 2). The surface area of this site was also determined using the *Area* part of the software and calculating the average of the three times of measurement of the V-shaped NLF area and the line connecting its two edges. The epidermal and dermal thickness based on the ultrasound studies was as follows: the first hyperechoic band, the epidermis, and its lower band, which included the hyperechoic band mixed with the hypoechoic parts, were considered as the dermis area (22). The area was divided into five equal sections and each area was measured three times and in total, 15 measurements were performed and the mean value was reported as epidermal and dermal thickness.

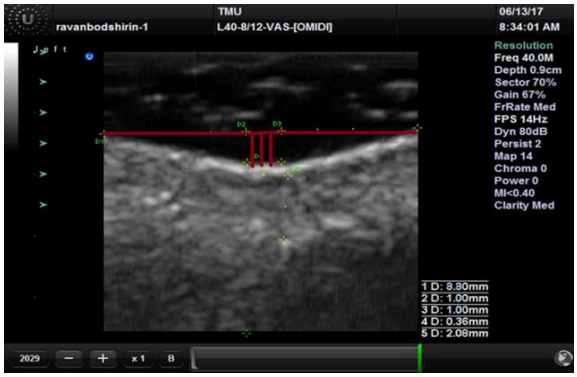

Figure 2. Evaluation of the Nasolabial Fold (NLF) indexes (the depth calculation is shown in the figure.)

Besides, the Young's Modulus was determined based on the Hook's law by applying a force of 0.25 to 0.35 N by a digital force gauge (Lutron Electronic Enterprise, Taiwan) connected to the ultrasound device transducer (Figure 1) and calculation of the force-induced stress. The force gauge was connected to the computer and the force was controlled using Lutron 801 software (Lutron Electronic Enterprise, Taiwan). Accordingly, using the ImageJ software, a point with specific echogenicity was determined and its distance to the skin surface before (L1) and after applying the force (L2) was determined and its difference was recorded as the strain (ΔL). The Young's Modulus was then calculated using Equation 1 where A is the cross-sectional area of the applicator.

$$\text{Young's Modulus} = (F/A)/(\Delta L/L1) \quad (1)$$

After initial evaluation, the treatment was carried out in 7 sessions (twice a week). The needle used (Wuxi Jiajian Medical Instrument Company, China) had dimensions of 0.18 mm × 13 mm and 0.16 mm × 7 mm in the NLF area and in the muscle area, respectively. On average, the length of the NLF in participants was 4.5 to 6 cm. Therefore, 3 to 4 needles were injected in each side of the face in the desired area. The LLS and ZM muscle masses found based on the function and contraction, respectively (23), each was injected a needle. According to the detailed and illustrated guidebook of needling (24), it was applied as inclined (45°) and horizontally (15°) in the muscles and NLF area, respectively. In general, the treatment time was 30 minutes and to accelerate the needle effect, the rotation technique was applied every 5 minutes in the clockwise and counterclockwise directions. The needle was then left in place for 10 minutes and then removed. After completion of the treatment process and 48 hours after the last treatment session, the ultrasound evaluation was performed again.

The sample size was determined based on the studies on acupuncture (25,26) due to the lack of previous studies with dry needle intervention on the dependent variables similar to the present study. Given the mean and standard deviation (SD) of the data on the skin thickness and Young's Modulus in these studies (25,26) and considering $\alpha = 0.05$ and $\beta = 0.8$, the number of samples in each group was obtained.

First, the interclass correlation coefficient (ICC) was utilized to evaluate the reproducibility of the ultrasound data. In addition, the Shapiro-Wilk test was employed to compare the frequency distribution of the quantitative variables with the normal distribution and since $P > 0.05$, parametric tests were used for the comparisons. The independent t-test was used to compare the two independent groups and paired t-test was used to compare the results before and after the treatment. Finally, the data were analyzed in SPSS software (version 20, IBM Corporation, Armonk, NY, USA). $P < 0.05$ was considered as the significance level.

Results

The results of the ICC test for all variables were more than 0.88.

Based on the Shapiro-Wilk test results and the normal distribution of the data on all variables, parametric tests were used to compare the data. All participants were present from the beginning to the end of the study and the percentage of the drop was zero.

There was a significant difference between the two groups in terms of age, but there was no significant difference in other demographic characteristics. Moreover, all subjects in the control group were healthy and non-menopause. The demographic characteristics of the subjects in the two groups are presented in table 1.

The comparison of the data of the intervention and healthy control groups before the start of treatment indicated that a significant difference in all variables measured. There was also a significant difference in the NLF depth, NLF area, epidermal and dermal thickness, and Young Modulus between the intervention group and healthy control group after the treatment (Table 2) ($P \leq 0.001$). The mean NLF depth and area of the intervention and control groups decreased from 0.63 mm and 2.16 mm^2 to 0.34 mm and 1.32 mm^2, respectively. Furthermore, the mean epidermal and dermal thickness decreased from 0.11 and 0.47 mm to 0.06 and 0.28 mm, respectively. Additionally, the difference in the mean Young's Modulus between the two groups decreased from 14.64 kPa to 11.05 kPa.

After treatment, the NLF depth and area in the intervention group decreased significantly (13.67% and 14%, respectively). The epidermal and dermal thickness and the Young's Modulus were significantly increased in the intervention group after treatment (35%, 15%, and 25%, respectively) (Table 3).

The power test showed that for $\alpha = 0.05$ and $\beta = 0.8$ with the data of the variables examined in the present study, 12 subjects were required in each group.

Table 1. Demographic characteristics of the participants

Group	Variable				
	Age (year)	Height (cm)	Weight (kg)	BMI (kg/m^2)	Menopause (%)
Intervention	50.88 ± 2.42	1.65 ± 0.19	67.44 ± 2.53	25.99 ± 2.67	55
Healthy control	26.00 ± 7.28	1.66 ± 0.58	60.40 ± 4.50	21.90 ± 2.51	0
P value	≤ 0.001	0.760	0.670	0.720	≤ 0.001

Data are reported as mean ± SD.
BMI: Body mass index

Table 2. Comparison of the variables measured in the two groups

Time of recording of information	Group	Variable				
		Depth of NLF (mm)	NLF area (mm^2)	Epidermis thickness (mm)	Dermis thickness (mm)	Young's Modulus (kpa)
Before treatment	Intervention	2.12 ± 0.05	5.96 ± 0.16	0.14 ± 0.03	1.24 ± 0.05	14.06 ± 1.83
	Healthy control	1.49 ± 0.08	3.80 ± 0.21	0.25 ± 0.01	1.71 ± 0.05	28.70 ± 2.28
	P value	≤ 0.001	≤ 0.001	≤ 0.001	≤ 0.001	≤ 0.001
After treatment	Intervention	1.38 ± 0.05	5.12 ± 0.90	0.19 ± 0.03	1.43 ± 0.02	17.65 ± 1.20
	Healthy control	1.49 ± 0.08	3.80 ± 0.21	0.25 ± 0.01	1.71 ± 0.05	28.70 ± 2.28
	P value	≤ 0.001	≤ 0.001	≤ 0.001	≤ 0.001	≤ 0.001

Data are reported as mean ± SD.
NLF: Nasolabial Fold

Discussion

The present study was conducted with the aim to investigate the effect of dry needling on the repair and improvement of biomechanical properties of the degenerated tissue due to aging. In order to determine the effect of dry needling on the biomechanical properties of the skin, part of the skin with quantitatively specific scales (NLF area) was examined.

The NLF depth and area were measured in the present study based on the studies previously performed on the facial folds (27-29), however these variables were measured more times for greater accuracy in the present study.

Applying the needle to the muscular and non-muscular areas, with a micro injury and increasing the blood flow, stimulates the healing process and regular formation and growth of new collagens against the disrupted and irregular collagens (25,26). Since one of the causes of the skin degenerative changes and wrinkles is a decrease in the amount of collagen content in the tissue (6), the increase and accumulation of the collagen fibers and underlying substance through needling can be effective in reducing the depth and area of the NLF region.

In their study, Matsumoto et al. investigated the mechanical aspects of wrinkle formation and found that by removing subcutaneous muscle layers, specific mechanical responses were observed in the skin, and by the decrease in the compressive force due to muscle contraction, the depth of wrinkles decreased significantly (17). Since dry needling to stiff muscles reduces their tone (30,31), part of this decrease in the depth and area seems to be due to the decreased muscle contraction force.

Skin thickness is one of the effective factors in determining the effect of various treatments on the skin (32), affecting the physical and mechanical properties of the skin (33). The use of the B-mode ultrasound device in the present study provided unload and accurate images of the cross-sectional area of the tissue the analysis of which in the ImageJ software yielded reliable and accurate results.

In a study on corpses, Tsukahara et al. found that the dermal thickness in the areas with wrinkles was less than in other areas. They only examined the thickness of the area beneath the wrinkle tip (27). In the present study, the epidermal and the dermal thickness of the entire NLF region was calculated which was significantly lower compared to that of the healthy control group. Tsukahara et al. declared that the decrease in the dermal thickening with age can be as a result of the morphological changes, as well as the decreased levels of collagen and elastin fibers and the underlying substance (27). Additionally, they claimed that this measurement cannot be examined in the healthy subjects and only the decrease or increase in the thickness of the skin can be attributed to the decrease or increase in these structural materials. During dry needling and due to the placement of more of its length in the dermis, by the release of substances such as calcitonin gene-related peptide (CGRP), Substance P, and prostaglandins, the cellular microdamage created increases the blood flow, gene expression of growth factors, and production of more collagen fibers and underlying materials, thereby increasing the skin thickness (16,34).

Table 3. Comparison of variables measured before and after treatment in the intervention group

Group	Variable				
	Depth of NLF (mm)	NLF area (mm^2)	Epidermis thickness (mm)	Dermis thickness (mm)	Young's Modulus (kpa)
Before treatment	2.12 ± 0.05	5.96 ± 0.16	0.14 ± 0.03	1.24 ±0.05	14.06 ± 1.83
After treatment	1.83 ± 0.05	5.12 ± 0.90	0.19 ± 0.03	1.43 ± 0.02	17.65 ± 1.20
Percentage of changes	13.67	14	35	15	25
P value	≤ 0.001	0.013	≤ 0.001	≤ 0.001	≤ 0.001

Data are reported as mean ± SD.
NLF: Nasolabial Fold

One of the important biomechanical features of the skin tissue is its Young's Modulus, which is related to intra- and extra-molecular cross-links, collagen and elastin fibers, underlying substance, and dermis thickness (35). With aging, the bands and content of collagen decrease (6). In the present study, the significant decrease in the dermal thickness in the elderly compared to the healthy control subjects confirmed this finding. Studies have shown that the central role of collagen in the dermis is to maintain the elastic properties of the skin tissue (9). Thus, the Young's Modulus of the skin changes due to the decrease in the basic material of its composition. Numerous studies have investigated the Young's Modulus of the skin due to the aging process, however there is no agreement among their findings. Some have reported the decreased and some others have reported the increased Young's Modulus with age (2,36). In the present study, the Young's Modulus was calculated using the ultrasound images under normal conditions and with the preservation of stresses and interactions within the skin layers in a non-invasive manner. In this method, the Young's Modulus of the intervention group was significantly lower than that of the healthy control group, which increased significantly after the treatment with dry needling, and the mean difference between the two groups decreased, however it was still significant between the two groups.

It should be noted that the healthy group in this study was chosen only to specify the difference between the elderly and young individuals, and considering the age difference between the two groups, it is quite logical that the two groups had a significant difference in biomechanical and ultrasound characteristics at the beginning of the study. Meanwhile, the objective of the study was to recognize the ability of the proposed intervention to return the characteristics of the tissue under treatment to the healthy and young conditions. Applying dry needling through the afore-mentioned mechanisms, by increasing the main constituent of the skin, i.e. collagen fibers, seems to improve skin firmness and increase its strength to permanent deformations under the forces due to the muscle contraction.

The needling was accompanied by positive effects on some of the biomechanical properties measured in the present study as well as basic and prospective effect on other skin pathologies such as scars.

Limitations

Due to the lack of time and facilities, it was not possible to evaluate the control group in the age range of the individuals under treatment. In addition, since the electromyography (EMG) information of the muscles was not measured, it was not clear how much of the changes were due to changes in muscle tone and how much due to the local changes in the skin tissue.

Recommendations

In future studies, it is recommended that the evaluation and follow-up of the treatment outcomes continue for up to six months after treatment and a control group be used in the age range of the treated individuals. Moreover, the changes in the activity of the treated muscles is suggested to be examined using accurate techniques such as EMG.

Conclusion

The findings in this study indicated that the application of the dry needling technique can improve the biomechanical properties of the skin affected by degenerative changes due to aging and may affect other skin pathologies.

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Authors' Contribution

Parinaz Omidi: Study design and ideation, providing study equipment and samples, data collection, analysis and interpretation of results, manuscript preparation, final manuscript approval for submission to the journal office, responsibility for maintaining the integrity of the study process from beginning to publication, and responding to reviewers' opinions; Roya Ravanbod: Study design and ideation, attracting funding for the study, supporting, executional, and scientific services of the study, providing study equipment and samples, analysis and interpretation of results, specialized statistics services, manuscript expert assessment in scientific terms, final manuscript approval for submission to the journal office, responsibility for maintaining the integrity of the study process from beginning to publication, and responding to reviewers' opinions; Manizheh

Mokhtari-Dizaji: Data collection, final manuscript approval for submission to the journal office; Ghazi Sarhan: Supporting, executional, and scientific services of the study.

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Conflict of Interests

The authors declare no conflict of interest. Dr. Roya Ravanbod received funding for basic studies related to this article from Tarbiat Modares University and has been working as a physiotherapy associate at the university since 2013. Parinaz Omidi has been a Postgraduate Student, Department of Physical Therapy, School of Medical Sciences, Tarbiat Modares University since 2015.

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