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New Non-Invasive Method with Hyaluronic Acid for Skin Rejuvenation - A Pilot Study

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Abstract

Hyaluronic Acid (HA), a product used both in topical cosmetics and in invasive procedures, aims to promote rejuvenation. However, the injectable application of HA can cause several complications in the applied area. New non-invasive method with HA may be an alternative for the treatment of facial rejuvenation, such as Equipment with High-frequency high-intensity ultrasound that can contribute to facial rejuvenation reducing the complications invasive process.

Objective: The aim was to determine clinically the effectiveness of a new non-invasive method for reducing facial wrinkles.

Methods: All patients underwent three treatment sessions. Assessment of skin characteristics and wrinkle reduction was carried out by clinical evaluation, questionnaire, photographic images and moisture assessment.

Results: All treatments were painless and were well accepted by patients. Among the protocols evaluated, the association of HA with equipment provided better results in relation to skin characteristics and the appearance of wrinkles throughout the patient's follow-up.

Conclusion: The results confirm that it was possible to improve skin characteristics and reduce facial wrinkles through a non-invasive and painless methodology.

Keywords: Wrinkles; High-frequency ultrasound; delivery system; non-invasive method; skin rejuvenation

Introduction

Hyaluronic acid (HA) is a linear glycosaminoglycan consisting of N-acetyl-D-glucosamine and D-glucuronic acid and each repetitive disaccharide unite is linked by β-1,4-glycosidic bonds [1,2].

HA is an important component of the extracellular matrix, together with collagen and elastin. These components decrease with age, resulting in the loss of hydration in the skin causing wrinkles, sagging and aging [3,4].

Skin aging is a complex biological process, result of intrinsic and extrinsic factors. Intrinsic factors include, ageing of the skin occurs inevitably a natural consequence influenced by hormonal changes. Extrinsic factors are result of exposure to external factors, such as ultraviolet (UV) irradiation, that is also referred to as photoaging. Both factors result in collagen degradation, dryness skin, loss of elasticity, epidermal atrophy and wrinkling of the skin [5,6].

Among all these changes, wrinkles are observed, marked lines that appear over the years, showing the signs of aging, being classified as dynamic wrinkles and static wrinkles.

Dynamic wrinkles are wrinkles that appear with facial movements. Static wrinkles are wrinkles visible at rest, i.e. wrinkles that appears regardless of relaxed muscle and usually emerge in the early 30s and grow in severity with aging [7].

HA is one of the most used fillers currently, enabling its application in approximately 21 regions of the face [8]. However, the use of HA fillers is contraindicated in pregnancy, breastfeeding women, systemic autoimmune diseases and immunocompromised patients, local inflammation or infection, coagulation disorders or users of anticoagulants [9,10]. Thus, HA, even though it is considered safe, has adverse reactions, such as inflammatory events, small bruises, infection, edema, erythema, nodules, local abscesses, hypertrophic scars, granuloma, blindness, ischemia, tissue necrosis [9,10].

Thus, given all the complications that can occur with the application of HA fillers, the present proposal aims to achieve the benefits of HA, such as skin rejuvenation and hydration, through a safe and non-invasive procedure that combines the topical application of HA with high-frequency high-intensity ultrasound equipment.

Materials and Methods

Equipment

Equipment used in this study was provided by NAPID Pesquisa

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e Desenvolvimento Ltda. (EMBRAPII/NAPID/USP - Project number: PIFS-2212.0058). This equipment has Q-switched Nd:YAG laser with pulse repetition rate of 10 Hz, a second harmonic generator and a diffuser to deliver a laser fluence of 50mJ/cm^2 at 532nm [11,12] All laser pulse energy is converted into ultrasound, schematic of the equipment is depicted in Figure 1.

Formulations

1) Formulation with hyaluronic acid (HA) (called of formulation 1): contained 20% HA (93%, molecular weight 15 kDa, cosmetic quality class, provided by Contipro Biotech, Czech Republic), 20% H_2O, 58% glycerol and 2% propylene glycol mixed with hydrogel (2% ethanol, 86% H2O, 10% glycerol and 2% hydroxypropyl methylcellulose)

2) Formulation without hyaluronic acid (HA) (called of formulation 2): contained 2% ethanol, 86% H2O, 10% glycerol and 2% hydroxypropyl methylcellulose

Participant selection and study design

This was a prospective, randomized, double-blind study.

The study was performed at the Photodynamic Therapy Unit of Hospital Santa Casa in São Carlos, state of São Paulo, Brazil. The Human Research Ethics Committee of Hospital Santa Casa reviewed and approved the study (CAAE number: 65904622.0.0000.8148)

The participants were fully informed about the study and before inclusion in the treatment, all participants signed an informed consent form. Fifty-two women from the city of São Carlo, aged 35 to 82, participated in the research. All volunteers had dynamic wrinkles and static wrinkles, classified as Type II, III, or IV according to the Facial Wrinkle Scale [13].

Exclusion criteria

Patient's smokers, pregnancy or breastfeeding, bariatric patients, with a history of oncology, as well as patients who have used injectable products on the face in the preceding 6 months such as Botox and injectable hyaluronic acid were excluded.

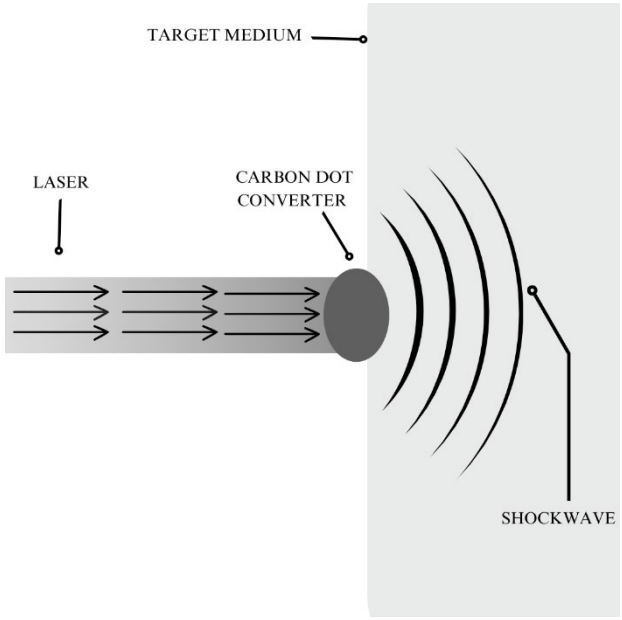

Figure 1: Schematic effect of the generation of laser for induced shock wave.

Groups

Patients were randomized to receive different treatments (Group 1-6) as described below:

1) Group 1: 35+ HA+EQ (n= 10): Patients aged 35 to 60 received formulation 1 associated with equipment

2) Group 2: 35+ EQ (n=11): Patients aged 35 to 60 received formulation 2 associated with equipment

3) Group 3: 35+ HA (n=5): Patients aged 35 to 60 received formulation 1 associated with the equipment turned off.

4) Group 4: 60+HA + EQ (n=11): Patients aged 60 to 82 received formulation 1 associated with equipment

5) Group 5: 60+EQ (n=10): Patients aged 60 to 82 received formulation 2 associated with equipment

6) Group 6: 60+HA (n=5): Patients aged 60 to 82 received formulation 1 associated with the equipment turned off.

All patients carried out 3 treatment sessions with an interval of 7 days between sessions.

All groups were evaluated for both motion and rest wrinkles through clinical assessment, skin moisture, and photographs.

Skin moisture assessment

Skin moisture of the treated face areas was measured with the Skin Moisture Sensor MY-808S Equipment (Scalar Corporation). Measurements were taken before and after treatment and during patient follow-up. The measurement was carried out after cleaning the skin with an alcoholic solution. The equipment was positioned and placed under the patient's face area and the display showed humidity in percentage (%).

Clinical evaluation

Clinical evaluations following 60 days of treatment were realized to through photographic images and evaluation of skin characteristics such as texture/smoothness, brightness, tone and firmness/elasticity.

Standardized photographs were acquired using camera of the IPHONE XR® device, allowing the visual evolution of wrinkles. Static photographs were acquired using LifeViz® Mini 3D Camera (QuantifiCare) allowing quantifying the number of wrinkles in the analyzed region. All images were acquired at different moments of the research: Initial and after 60 days of treatment.

Patient satisfaction survey

At the end of the clinical research, all patients answered the Visual Analogue Scale, a visual scale that allows measuring the pain level.

Furthermore, all patients answered the subjective questions, according to the considerations below:

1. Are you satisfied with the result? Yes or No
2. Did you notice a difference in your skin? Yes or No
3. Would you do a new session of the procedure? Yes or No
4. Would you recommend this procedure? Yes or No

Statistical analysis

Average values of the scores given by the panel and standard error of the mean were obtained the whole population. The significance

of the difference between the populations was analyzed using the Kolmogorov-Smirnov normality test and subsequently the Tukey-Kramer post-Hoc test for multiple analyses, considering $p<0.05$. The software used was Instat 3.0 for Windows.

Results

A total of 55 female were enrolled in the study. The mean age of the patients of Groups 1, 2 and 3 was 35-59 years old and Groups 4, 5 and 6 was 60-82 years old. All 55 patients completed all the sessions and follow-up for 60days.

Skin moisture measurements were taken on all patients before treatment (without intervention), immediately after each session (1st, 2nd and 3rd) and throughout the patients' follow-up (15 days, 30 days, 45 days and 60 days). Skin moisture data are demonstrated in Figure 2 and Figure 3.

According to the humidity data, we can observe that the groups that were treated with the intervention containing hyaluronic acid (Groups 1, 3, 4 and 6) showed a reduction of approximately 10% in humidity in relation to the initial measurement, indicating a possible effect of hyaluronic acid in this variable. The results demonstrated a statistically significant difference ($p<0.05$) in relation to the % moisture before treatment and immediately after each session, except for Group 5 (60+EQ), suggesting that the presence of hyaluronic acid may be a variable that will influence more on the moisture parameter humidity during treatment.

In relation to each treatment protocol, the groups treated with the association of HA and equipment (Group 1 and 3) showed a statistically significant difference ($p<0.05$) in relation to the group without the presence of HA (Group 2 and 4), corroborating that the presence of HA influences the humidity parameter during treatment. After the 3 treatment sessions, all patients were monitored for a period of 60 days and humidity measurements were evaluated on 15, 30, 45 and 60 days, aiming to verify a possible change in moisture values without carrying out any type of clinical intervention Patient follow-up data are presented in Figure 3.

According to the humidity data, we can observe that there was an increase in humidity values over the 60 days, presenting a statistically

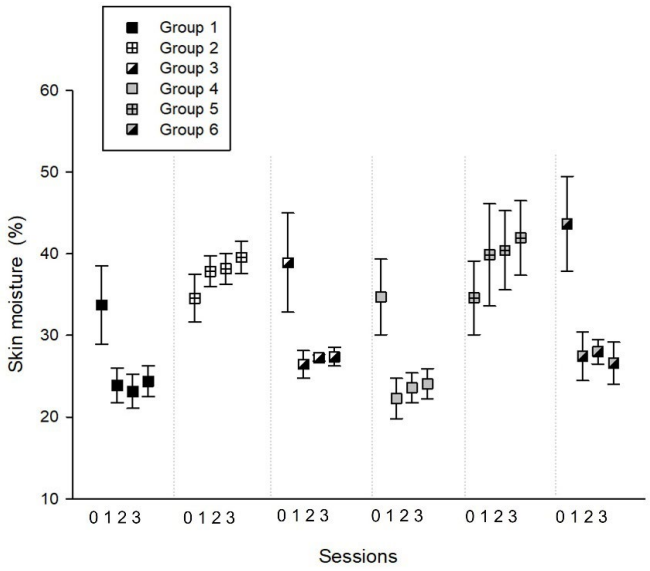

Figure 2: Skin moisture evaluation during treatment.

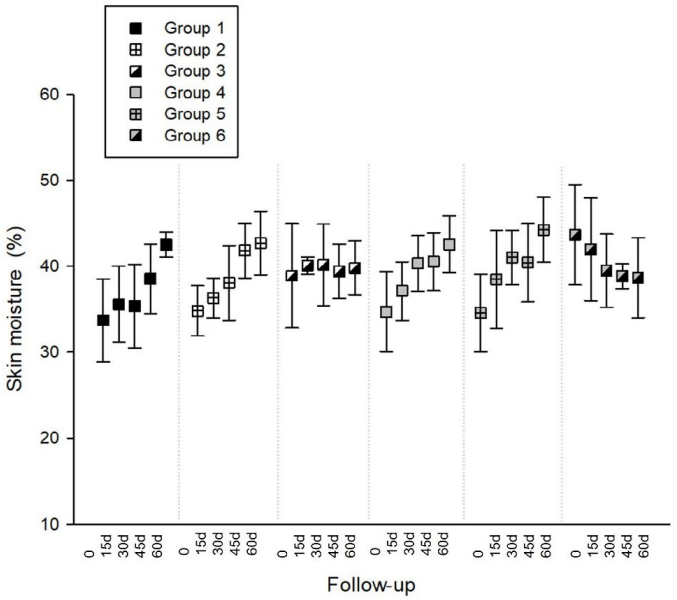

Figure 3: Skin moisture evaluation during patient follow-up for 60 days.

significant difference ($p<0.05\%$) except for the groups that did not use the equipment (Groups 3 and 6), indicating that the presence of the equipment can contribute to an increase in the permeation of hyaluronic acid or water molecules present in the hydrogel to deeper layers of the skin, not limited to just the stratum corneum, providing an increase in skin moisture in the long term (period of 60 days).

Clinical evaluation

Clinical evaluation of improvements in the degree of wrinkles was performed before (without treatment) and after follow-up of 60 days. Photographs representing patients from each group are shown in Figure 4.

According to the photographs shown in Figure 4, we can observe the improvement of wrinkles in patients in the groups treated with the use of HA associated with the equipment (Group 1 and 4) and only with the use of the equipment (Group 2 and Group 5). Patients treated with HA alone (Group 3 and Group 6) did not show improvement in wrinkles, as shown in Figure 4.

In the clinical evaluation, it was observed that the group treated with HA and equipment (Group 1 and Group 4) after the 3 sessions, the patients' skin showed greater smoothness, brightness, firmness and tone uniformity as well as attenuation of wrinkles and expression lines and these characteristics were maintained for 60 days of follow-up.

On the other hand, treatment with equipment (Group 2 and Group 5) resulted in an improvement in smoothness, brightness, firmness and tone uniformity as well as attenuation of wrinkles and expression lines during treatment, but after follow-up these characteristics were not observed, demonstrating that using the equipment alone, the effect of improving skin characteristics are not as prolonged in relation to the combination of equipment and HA.

In relation to the groups treated only with HA, the effects of smoothness and brightness were observed immediately after each session and were not maintained, indicating that the application of HA to provide a topical effect. Furthermore, the parameters of firmness and improvement of expression lines and wrinkles (static and dynamic) were not changed with this protocol.

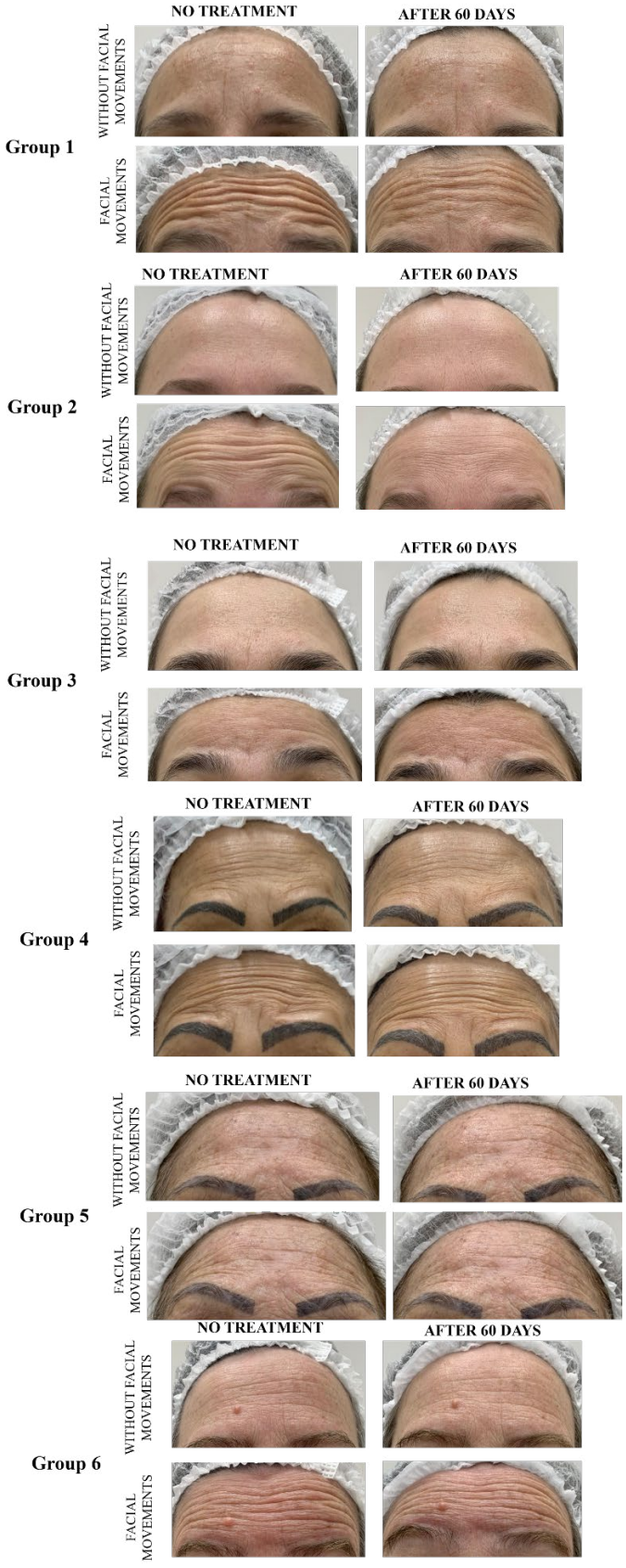

Figure 4: Clinical standardized photography (example of participants in the groups studied).

The number of static wrinkles (wrinkles without movement on the face) were evaluated in patients before starting treatment and 60 days after the last session and photographs representing patients from each group are shown in Figure 5.

Wrinkles number is a parameter that varies according to each patient, that way this study the number of wrinkles in the patients' frontal region was individually assessed and verified whether there was a reduction in this number after applying the different treatments.

According to the results, there was a reduction in the number of wrinkles in Groups 1, 2, 4 and 5, with the most significant reduction ($p<0.05$) presented by the groups that associated HA with equipment (Group 1 and Group 4) in relation to patients treated only with the equipment (Group 2 and Group 5).

Patient Satisfaction Survey

The clinician tolerability assessment showed that all different treatment protocols this study were well tolerated without any adverse events evaluated and all treatments were painless as reported by patients.

All patients in this study reported *"no pain"* according to the VAS assessment in all protocols applied in this study.

Patients were questioned about treatment and responses indicated that almost all participants treated with different protocols, 90%-100%

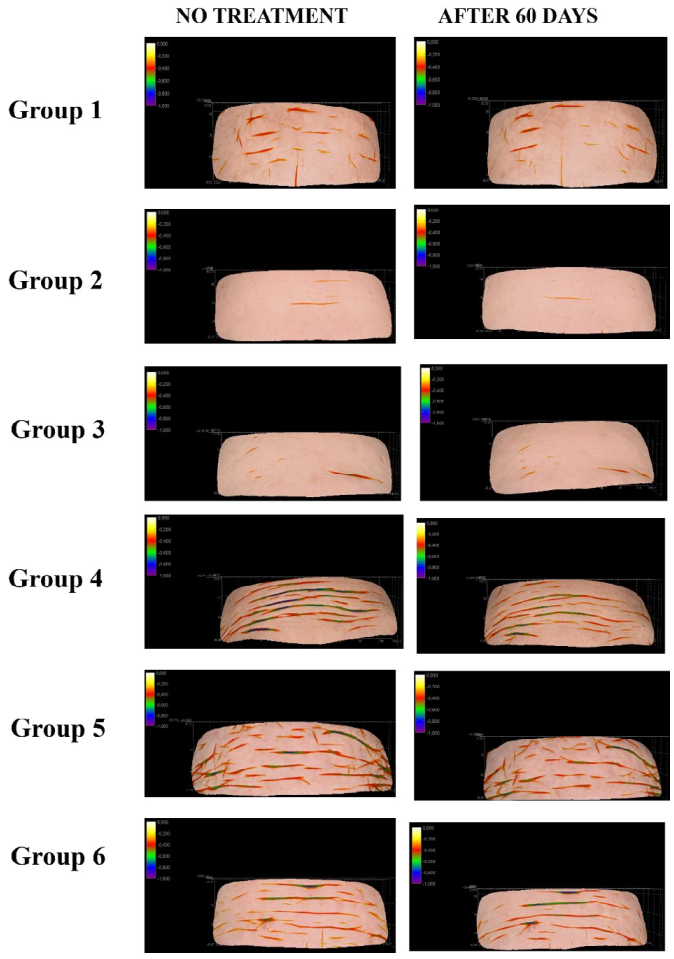

Figure 5: LifeViz® Mini 3D Camera photography (example of participants in the groups studied).

responded positively Subject Satisfaction Survey after 60 days of the treatment. On subject questionnaire, 100%t of patients were satisfied with the results, would undergo a new session of the procedure and recommend the treatment.

Discussion

Studies conducted by Freire dos Santos et al. (2018) [11] demonstrated the effectiveness of using high-frequency high-intensity ultrasound pulses in the permeability and distribution of HA in the skin in a safely for the treatment of facial wrinkles, corroborating the results presented in Figure 4 and Figure 5, which is notable for the improvement of wrinkles and skin characteristics.

This effect, related to that obtained with HA and shock waves, was also described by Sá et al. (2017) [12] in an experiment with a mini pig, in which the epidermis showed an increase in the structure of the stratum corneum with an application of total contact time for 30 minutes.

In this study, the application of HA by non-invasive method with Brazilian women of all phototypes, allowed clinical evaluation of the skin, before, during and after treatment and the results revealed an improvement in skin characteristics after treatments without any complications or complications. and without pain.

The skin moisture results (Figure 2) demonstrated that there was an increase in water loss after the treatment sessions, which could be directed to the immediate effect of the treatment caused by high-frequency high-intensity ultrasound pulses generated when short laser pulses are absorbed, resulting in a conversion of the laser energy into a thermoelastic expansion, which can result an increased trans epidermal water loss healthy skin [11].

All six groups cleaned their skin with 70% alcohol, with the intention of removing dirt and other elements present on the skin, before and after the treatment session. In patients who received HA application associated with the equipment (Group 1 and Group 4), humidity data decreased, different from those observed by patients who did not use HA (Group 2 and Group 5). This effect of reducing humidity also occurred with patients who applied only HA without the use of equipment (Group 3 and Group 6).

Gupta et al. (2020) [14] explains this balanced removal of lipids through intra- and intermolecular bonds with the solvent. Alcohol presented a physiological action without damage, allowing the removal the lipids present in the layer of the stratum corneum, enabling new specific channels for the permeation of molecules.

According to Sá et al. (2013) [12] an important characteristic regarding the conversion of light into pressure and generation of photoacoustic waves with the duration of the laser pulses is that these resources combine can displace the corneocyte layers, which facilitates the passage of the product without harming the barrier function.

This characteristic of the equipment may justify the effect of increased hydration during the 60-day follow-up (Figure 3) of patients who used the equipment (Groups 1, 2, 4 and 5) and consequently this more hydrated skin will have greater benefits in delaying aging

The skin moisture results (Figure 3) corroborate scientific data, due to the characteristics of hyaluronic acid in being able to attract and retain water molecules [15].

The water retention capacity due to only topical use of the cosmetic (groups 3 and 6) was not observed during the 60-day follow-up, indicating that more sessions will be carried out for this application to obtain a significant improvement in the skin's characteristics.

Injectable HA are related to a painful sensation understood as pain at the time of application and attributed to the risk of mild to severe complications and complications. At the injection site, reactions such as erythema, edema and bruising may occur, in addition to delayed reactions such as hypersensitivity, Tyndall effect, vascular occlusions and pathogen infections [11].

To give the patient a feeling of comfort during the procedure, a local anesthetic is normally applied, for example, lidocaine in conjunction with HA [11]

In this study, the methodology applied was completely painless without the need for the application of local anesthetic, according to VAS data.

The clinical assessments in this study are based on photographic according to Figure 4 and facial aspects present at the time of service, such as texture/smoothness, brightness, tone and firmness/elasticity. Patients treated with the association of the equipment with HA (Groups 1 and 4) were those who obtained the best clinical results until the end of the 60-day follow-up, suggesting that this protocol is the most suitable for improving skin characteristics and that contribute to delaying aging.

Skin brightness is essential for skin condition information. In clinical practice, brightness indicates quality of health and well-being, on the other hand, opaque or matte skin can be indicative of nutritional deficiency, hormonal imbalance, pollution and other factors [16-18]. And according to the brightness of the skin, this characteristic was visible in all patients after the first session until the third, with no difference between the groups, however during follow-up the brightness of the skin was only noticed in the group with association of the equipment and HA (Group 1 and 4) this result obtained through a non-invasive method is considered a very favorable event that can be granted for a long period in the field of aesthetics.

It is suggested that because hyaluronic acid has a high layer of skin water, it is suitable for hydration, favoring the tissue in different aesthetic patterns [11,15].

However, in our study the effectiveness of these properties is enhanced with shock waves.

Conclusion

This study evaluated skin characteristics and rejuvenating action through non-invasive methods using equipment with high-frequency and high-intensity ultrasound pulses associated or not with HA. From the results obtained, we can conclude that this methodology can contribute to facial rejuvenation treatment without causing pain to the patient. Therefore, this methodology becomes very promising in the field of facial aesthetics, especially in patients who are sensitive to other treatment methods.

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