Hyperdiluted Calcium Hydroxylapatite for skin laxity and cellulite of the skin above the knee: a pilot study

Running head: Calcium hydroxylapatite for rejuvenation of knees

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Keywords

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Abstract (196)

Background Despite an increasing request for skin rejuvenation above the knee, very few treatment options have been reported in literature.

Objective To evaluate the efficacy and safety of 1:4 hyperdiluted calcium hydroxylapatite (CaHA) in the treatment of skin laxity and dimples of the skin above the knees.

Methods A retrospective evaluation of hyperdiluted CaHA treatment for skin laxity and dimples above the knee was performed. Efficacy was classified as blinded evaluation of pre (T0) and 3-month post-treatment (T1) photographs by 3 investigators according to the validated knee cellulite severity score (KCSS) and patient satisfaction. Safety was evaluated through pain scores and adverse events evaluation.

Results A significant reduction of KCSS at T1, as compared to T0, mainly in subjects with lower KCSS at T0, was observed ($p<0.05$). All blinded assessments resulted in a correct identification of

T0 and T1 pictures and evaluations of all investigators were found to be consistent and reliable. All patients were satisfied. Only minor adverse events (swelling, erythema, bruising and skin irregularities) were reported, lasting 2-3 weeks after treatment.

Conclusion Our preliminary results highlight the efficacy and safety of hyperdiluted CaHA in the treatment of skin laxity and cellulite above the knees.

INTRODUCTION

The request for non-invasive skin rejuvenation techniques is increasing. Skin rejuvenation options should ideally address the issues of both laxity and cellulite (or cellulite-like aspect, or depressions or dimples) which represent two major variations observed with ageing in this area. Previous studies have described the interconnectivity of cellulite and skin laxity; cellulite corresponds to an increase in skin laxity [1,2] and skin laxity contributes to the evidence of cellulite [3]. Therefore, treatments aiming at the dermal remodeling, contributing to new collagen and elastin formation, seem to be suitable for both cellulite and laxity treatment [4,5].

Recently, injections of diluted/hyperdiluted calcium hydroxylapatite (CaHA) (Merz, Frankfurt, Germany) into the skin of the neck and décolletage were shown to stimulate the synthesis of collagen and elastin and to increase neovascularization with histology, leading to an overall increase in dermal thickness, and therefore, the reduction of skin laxity.[6]

Despite the increasing request for the treatment of the area above the knees, only a few treatment options have been described to date.[7] The aim of the current study is to evaluate the efficacy and safety of 1:4 hyperdiluted CaHA for the improvement of skin laxity and dimples, or depressions, above the knee.

PATIENTS AND METHODS

Study Subjects

This retrospective study enrolled women seeking improvement of skin laxity and depressions above the knees treated with the hyperdiluted CaHA 1:4 between September 2017 and June 2019. Patient eligibility for study inclusion was ≥1 point according to the previously described and validated knee cellulite severity score (KCSS).[8] Exclusion criteria for the study included the presence of any illness or use of medications that may affect wound healing, the presence of significant scarring, open wounds or lesions in the area to be treated, previous skin tightening procedure before the proposed treatment area within the past year, immunosuppression or autoimmune disease.

All procedures performed in studies involving human participants were in accordance with the Helsinki declaration and its later amendments or comparable ethical standards. All patients provided informed, written consent.

1:4 hyperdiluted CaHA treatment

All patients were treated by a single investigator (S.G.), with the hyperdiluted CaHA technique, in a private practice scenario. The areas to be treated were marked with a pen with the subject in a standing position. The treatment was performed following the scheme shown in Fig. 1.

The skin was prepared with a disinfecting agent (Germoxid® liquid, Germo Spa, Cormano, MI, Italy). Each 1.5 ml of CaHA syringe was diluted with 0.5 ml of 1% lidocaine and 5.5 ml of 0.9% saline. The total volume was injected on each knee.

An adequate solution was obtained connecting a 10 mL LuerLock syringe, containing the diluent, to the original syringe of the CaHA product, through a transfer adaptor, as previously described. [9] According to guidelines[10], at least 20 passes between the 2 syringes were performed, to ensure product homogeneity and the solution was used immediately after reconstitution to avoid the separation of each component.

Using a 25G, 50-mm-long cannula, the solution of hyperdiluted CaHA was injected, using the fanning technique (Fig. 1) to cover the areas of interest of each area above the knee. After that, a vigorous massage of the treatment area was performed to ensure even dispersion, and subjects were instructed to refrain from exercising the treatment area for 24 hours. The treatment was performed in a single session and did not require any post-treatment medication.

Efficacy

Photographic images of the area above the knee of the left and right legs were taken before (T0) and 3 months after treatment (T1). All photographs were taken in the same room, by the same investigator. The lighting was kept constant; artificial central lighting was supplemented with indirect illumination from a lamp tangential to the zone being photographed.

Patients were assessed according to the KCSS[8] at T0 and T1. Each knee was evaluated by three dermatologists (S.G., S.L. and V.D.M.) independently. Skills according to the KCSS scale varied; the first evaluator (S.G.) created the adapted scale, the second (S.L.) was familiar with it and the third (V.D.M.) was unfamiliar with it and received specific training. Each of the three items (number and depth of depressions and skin laxity) was graded from 0 (absent) to 3 (severe); 0 corresponds to

absence and 3 to the most severe condition. The overall scores (0 – 9) were classified as absent (0), mild (1-3), moderate (4-6) and severe (7-9), as previously described.[8]

Further, efficacy was evaluated by a blinded, qualitative identification of T0 and T1 images by 2 investigators (initials). For each subject, the reviewers were asked to identify which was the post-treatment image. Additionally, a 3-point satisfaction questionnaire (unsatisfied, satisfied, very satisfied) was also administered to patients at T1.

Safety

Subject pain assessment was made during treatment using a validated 10-point (1–10) Numeric Rating Scale, where 1 indicates no pain and 10 the worst possible pain. The treated area was examined approximately 30 minutes after injection for evidence of erythema or edema or ecchymosis or skin irregularities. At each subsequent visit, subjects were asked about possible adverse events, which were classified according to minor events, not requiring additional interventions or medications, or major events, including events requiring additional interventions or medications, such as scarring, hyperpigmentation, or granuloma.

Statistical analysis

Results were presented as mean ± standard deviation (SD) for continuous variables while categorical variables were presented as frequency for each group. The Cohen's kappa (κ) statistic was used to measure the agreement between investigators 2 and 3 and investigator 1 (that created the scale, considered the gold standard). We evaluated inter-observer agreement for all items and total score. We selected κ statistic as the measure of agreement because our variable of interest was binary[11,12].

Kappa is a measure of agreement level, indicating a low (κ <0), slight (κ = 0.01 to 0.20), fair (κ = 0.21 to 0.40), moderate (κ = 0.41 to 0.60), substantial (κ = 0.61 to 0.80), almost perfect (κ = 0.81 to 0.99) and perfect (κ = 1) agreement. The p-value indicates the level of agreement between the two methodologies. Margin statistics was used to estimate adjusted predictions and marginal effects after a linear regression based on a previously fitted model, in which values of total score were fixed among all three investigators. Predictive margins of total score for each investigator was estimated. Additionally, the variation between T0 and T1 was assessed according to (T0 score-T1 score)/T0 score*100. χ^2 (or Fisher's exact test) or Student's T-test were used to assess potential correlations for categorical and continuous variables, respectively.

Statistical analyses were performed with STATA 14.0 (Stata Corp LP). A p-value ≤0.05 was considered significant.

RESULTS

A total of 20 women (40 knees) with a mean age of 50±7 (range 38-61). Table 1 reports patient classification according to the KCSS; age and KCSS severity scores were correlated (p=0.01) (data not shown).

Efficacy

The hyperdiluted CaHA 1:4 induced an improvement of the total mean KCSS at T1, as compared to T0 (Table 1). In detail, the mean overall total score was 4.9 ±1.4 (range 2-9) at T0, which was significantly reduced to 1.8 ±1.5 (range 0-6) at T1, p<0.05. Interestingly, a different total score improvement was observed based on KCSS severity at T0, with a reduction ≤50% in women with severe

KCSS, between 50 and 100% in patients with moderate KCSS and 100% in patients with mild KCSS (Supplementary Table 1; Fig. 2).

When considering each item of the KCSS (number, depth of depressions and skin laxity) separately, the improvement at T1, as compared to T0 ($p<0.001$), was confirmed. Interestingly, at T0 each item showed a score ≥1, while at T1 about 40% of patients showed a score of 0 for each item.

There was a strong correlation between the evaluation of investigator 1 and investigators 2 and 3. (Supplementary Table 2, Supplementary Fig. 1).

According to clinical images at T0 and T1, blinded dermatologists correctly identified the T1 images in 100% of patients. Furthermore, patients were found to be very satisfied in 66.7% of cases and satisfied in 33.3% of cases.

Safety

Mean pain score was 2.3±0.9 (range 1-5). Only minor adverse events were observed, including erythema and oedema (observed in almost all patients, lasting 1-5 days with spontaneous resolution), ecchymosis (observed in 9 patients, lasting 7 - 14 days with spontaneous resolution) and skin irregularities (observed in 3 patients, lasting some minutes – few days after a vigorous massage).

DISCUSSION

Non-surgical treatment options for skin rejuvenation of the face and the body are increasingly requested by patients, due to reduced downtime and complications as compared to more invasive surgery.[13-17] Specifically, the treatment of the area above the knees, that may show both skin laxity and depressions, is challenging and a valid surgical approach is still lacking. To date, only a few non-

invasive therapeutic options have been reported, and their effectiveness has been proved on skin laxity only.[7]

CaHA treatment has shown efficacy on improving skin laxity.[6,18] After initial skepticism about potential adverse events, CaHA has been safely employed for over a decade.[19,20] It can be used both as a volumizing filler and in its diluted/hyperdiluted form as a biostimulatory material.[6,9,21,22] Several CaHA dilutions/hyperdilutions with 0.9% saline have been described in the literature, such as 1:1 (dilution) to 1:2,1:4,1:8 (hyperdilutions), depending on the location and on specific characteristics of the skin.[18] Injection of diluted/hyperdiluted CaHA into the skin has been histologically proven to stimulate the synthesis of collagen and elastin and to increase neovascularization, leading to an increased dermal thickness.[6] Recently, diluted/hyperdiluted CaHA has been applied to the treatment of skin laxity of regions of the lower face, neck, décolletage, upper arms and abdomen and to the treatment of cellulite on the buttocks and posterior thighs.[5,6,9,16] However, its role in the treatment of the skin above the knees has not been described so far, although its use in this area has been suggested in CaHA consensus guidelines.[10]

This study proposes the 1:4 hyperdiluted CaHA to treat laxity and depressions of the skin above the knees and outlines results in a cohort of 20 female patients. This treatment provided an improvement of the target area in terms of skin laxity and depressions in all patients, according to assessments with the recently described and validated KCSS[8], blinded evaluations performed by investigators, and patient satisfaction. In particular, a significant improvement was observed for both the total KCSS and for each item of the scale (number and depth of depressions and skin laxity). Additionally, evaluation of all three investigators were consistent and reliable.

Interestingly, KCSS at T0 was proportionally related to age, suggesting a progressive increase of KCSS with the ageing process. Furthermore, a low KCSS at T0 was associated with an increased improvement at T1, as compared to T0. This means that early treatment of laxity and depressions of the knees with 1:4 hyperdiluted CaHA may be more effective, as compared to the treatment of women with high KCSS scores.

This study confirmed the safety profile of hyperdiluted CaHA 1:4 since only a few and minor adverse events (erythema, oedema, ecchymosis, skin irregularities) and variable degrees of pain, that did not impair the execution of the procedure, were reported.

Alternative techniques, such as micro-focused ultrasounds with visualization (MFU-V), have been applied to skin laxity treatment of the skin above the knees[7], but have not provided specific effects on depressions in the same area and lack an objective measure of the observed improvement. Other treatments applied in daily routine, such as threads in the area above the knees, have not been reported in the current literature, therefore no comparisons can be made.

Limitations of this study are represented by the low number of subjects enrolled, the retrospective design of the study, and the enrollment of women only. Further, the duration of the effect has not been evaluated in this study. However, the presence of laxity and depressions in the area above the knees is mainly represented in women. Further studies will be performed in order to confirm these results and study the effect of different CaHA dilutions/hyperdilutions, according to different severities of basal KCSS assessments or skin quality.

Our results suggest that hyperdiluted CaHA 1:4 improves the aesthetic appearance of laxity and depressions of the skin above the knees, with high patient satisfaction rates.

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Figure legends

Figure 1. Schematic representation of the technique

Figure 2. Clinical pictures of patients treated with diluted CaHA: a 50-year-old woman at A) T0 and B) T1 and a 61-year-old woman at C) T0 and D) T1

Supplementary Figure 1. Linear prediction of total score for investigator 1 (triangle), investigator 2 (circles) and investigator 3 (square). Means (dots), 95% confidence interval (wriskers), samples at T0 and T1.

Tables

Table 1. Descriptive table of KCSS items at T0 and T1 for all three investigators.

		Investigator 1				Investigator 2				Investigator 3			
KCSS		T0		T1		T0		T1		T0		T1	
		n	%	n	%	n	%	n	%	n	%	n	%
Overall KCSS °. mean ±SD (range)		4.9 ±1.4 (2-9)		1.8 ±1.5 (0-6)		5.1 ±1.5 (2-9)		2.0 ±1.5 (0-6)		4.8 ±1.3 (2-9)		2.1 ±1.5 (0-6)	
Number of depressions*	0	0	0	18	45	0	0	17	42.5	0	0	18	45
	1	10	25	17	42.5	11	27.5	15	37.5	12	30	16	40
	2	27	67.5	5	12.5	23	57.5	8	20	22	55	6	15
	3	3	7.5	0	0	6	15	0	0	6	15	0	0
Depth of depressions*	0	0	0	18	45	0	0	17	42.5	0	0	18	45
	1	19	47.5	21	52.5	19	47.5	21	52.5	24	60	21	52.5
	2	19	47.5	1	2.5	19	47.5	2	5	14	35	1	2.5
	3	2	5	0	0	2	5	0	0	2	5	0	0
Skin laxity*	0	2	5	15	37.5	2	5	16	40	1	2.5	11	27.5
	1	18	45	23	57.5	14	35	22	55	16	40	27	67.5
	2	18	45	2	5	22	55	2	5	21	52.5	2	5
	3	2	5	0	0	2	5	0	0	2	5	0	0

* p-value <0.001
° p-value<0.05