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Contents

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Aesthetic Medicine is a multidisciplinary Journal with the aim of informing readers about the most important developments in the field of Aesthetic Medicine.

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The title page should include:
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· Materials and Methods
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· Discussion and Conclusions
· Acknowledgments
· Conflict of interest
· Reference list
· Legends (max 10)

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Journal article - online* *if there is no DOI, provide the URL for the specific article	Coppinger T, Jeanes YM, Hardwick J, Reeves S. Body mass, frequency of eating and breakfast consumption in 9-13- year-olds. *J Hum Nutr Diet.* 2012; 25(1): 43-49. **doi: 10.1111/j.1365-277X.2011.01184.x**
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Newspaper article - in print* *if the city name is not part of the newspaper name, it may be added to the official name for clarity * if an article jumps from one page to a later page write the page numbers like D1, D5	Wolf W. State's mail-order drug plan launched. *Minneapolis Star Tribune.* May 14, 2004:1B.
Newspaper article - online	Pollack A. FDA approves new cystic fibrosis drug. *New York Times.* January 31, 2012. http://www.nytimes.com/2012/02/01/business/fda-approves-cystic-fibrosis-drug.html?ref=health Accessed February 1, 2012.
Websites	Outbreak notice: Cholera in Haiti. Centers for Disease Control and Prevention Web site. https://www.cdc.gov Published October 22, 2010. Updated January 9, 2012. Accessed February 1, 2012.
Entire book - in print	Modlin J, Jenkins P. *Decision Analysis in Planning for a Polio Outbreak in the United States.* San Francisco, CA: Pediatric Academic Societies; 2004.
Book chapter - in print	Solensky R. Drug allergy: desensitization and treatment of reactions to antibiotics and aspirin. In: Lockey P, ed. *Allergens and Allergen Immunotherapy.* 3rd ed. New York, NY: Marcel Dekker; 2004:585-606.

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Example Article

1. Zoellner J, Krzeski E, Harden S, Cook E, Allen K, Estabrooks PA. Qualitative application of the theory of planned behavior to understand beverage consumption behaviors among adults. J Acad Nutr Diet. 2012;112(11):1774-1784. doi: 10.1016/j.jand.2012.06.368.

In-Text Citation Example

LARGE INCREASES IN AMERICANS' CONSUMPTION OF sugar-sweetened beverages (SSB) have been a topic of concern. Between 1977 and 2002, the intake of "caloric" beverages doubled in the United States, with most recent data showing that children and adults in the United States consume about 172 and 175 kcal daily, respectively, from SSB,[1] it is estimated that SSB account for about 10% of total energy intake in adults[2,3]. High intake of SSB has....

References Section Example

References

1. Duffey KJ. Popkin BM. Shifts in patterns and consumptions of beverages between 1965 and 2002. *Obesity.* 2007:15(11):2739-2747.
2. Nielsen SJ. Popkin BM. Changes in beverage intake between 1977 and 2001. *Am J Prev Med.* 2004;27(3):205-210.
3. Drewnowski A. Bellisle F. Liquid calories, sugar, and body weight. *Am J Clin Nutr.* 2007;85(3):651-661.

Use commas to separate multiple citation numbers in text, like you see between references 2 and 3. Unpublished works and personal communications should be cited in the text (and not on the reference list).1 Superscript numbers are placed outside periods and commas, and inside colons and semicolons. When citing the same source more than once, give the number of the original reference, then include the page number (in parentheses) where the information was found. See pages 41-44 of the AMA Manual of Style for more information.

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aesthetic medicine

Original Article

Transforming negative emotional expressions into positive using a full face holistic approach with hyaluronic acid fillers and botulinum toxin

Radina Denkova

MD, Founder, CEO and Medical director at Dermatology Clinic Dr-Denkova Dermatology

Abstract

Background: a proper assessment of the changes in one's facial structure, that are associated with negative emotions expressed by the face, and the preparation of a holistic full face therapeutic plan aimed to improve the emotional expression of patients is essential for their satisfaction with the therapy.
Aim: analysis and evaluation of the achieved results in a holistic full-face approach, with the help of hyaluronic acid fillers and botulinum toxin applied during a single patient visit, with a patient who has a treatment plan based on the predisposed emotions shown by their features.
Materials and methods: the study presents the author's clinical experience and the results of 47 patients treated in a single session. Treatment plan formulas were used to change the expressions of negative emotions into positive ones.
Results: the patients were injected with an amount of Juvederm fillers ranging from 8 to 20 ml, and using Vycross technology from Allergan. Each treatment was aimed at eliminating the expression of tiredness and sadness in the periorbital and perioral area, reduce the sagginess in the whole face, and balance out the facial features.
Conclusion: this article shows how MD CodesTM and MD-ASATM patient assessments can be applied in a real medical practice. Using an algorithm to transform the negative emotional expression of the patients into a positive one, is set to lead to a greater psycho-emotional satisfaction caused by the manipulation of their features.

Keywords
Hyaluronic acid fillers, botulinum toxin, tiredness, sagginess, sadness, full face approach

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Corresponding Author

Radina Denkova, MD

Address: Dr-Denkova Dermatology, bul. Simeonovsko shose 62, Sofia, Bulgaria
E-mail: dr.denkova@abv.bg

Introduction

The way a person looks like has become increasingly important in recent years, and as a result there has been an increase in the number of aesthetic procedures. The use of hyaluronic acid fillers in clinical practices was approved in 2003 by the FDA[1]. Since then, it has gained a lot of popularity and is currently one of the most preferred aesthetic procedures. The main reason for this is the predictable effect, efficacy, short recovery period and significantly low complication rate of this method[2]. In 2017 a study by the American Society for Dermatologic Surgery reported that there was more than double the increase in the number of cosmetic procedures performed compared to 2013[3]. Despite the expected increase for this procedure, there was a decline of over 20% in 2020 compared to 2019, due to the pandemic of COVID-19 reported by the American Society of Plastic Surgeons[4].

Patients have different motivations to look for aesthetic procedures. It is established that the main reason is to increase the state of psychological well-being, quality of life, and increase their level of comfort and confidence in both social and professional environments[5]. Traditionally, aesthetic non-invasive procedures with hyaluronic acid fillers and botulinum toxin are mainly associated with aging and have an anti-aging focus. It is also important to notice that progressively more young people are turning to aesthetic medicine[6]. These patients want to improve their facial structures, but very often they focus on a separate area - a fine wrinkle or crease, and even when they disappear after the procedure is done, patients are still not satisfied[7,8].

This used to be a major treatment objective in the past: it often used a small amount of hyaluronic acid filler[9,10], which achieved short-term results in the direction of the refinement. The use of hyaluronic acid fillers and botulinum toxin in the clinical practice has undergone a variety of significant changes. Some of the causes for this are: the increase of performed procedures, more in-depth studies in the field of aesthetic medicine, the accumulated long-term data from the application of hyaluronic acid fillers, the changes of a patient's skin and facial structures related to age, and the patients' requirements related to the final result and their satisfaction with it, resulted in significant changes in the method of application of hyaluronic acid fillers and botulinum toxin in the clinical practice. Approaches involving a comprehensive assessment of a person's needs are more common and have a better and longer-lasting end result, as well as a higher satisfaction on the patients' part[11,12].

The holistic full face approach is a challenge for the clinician. It is important to know the anatomy well, the possible effects of different types of hyaluronic acid fillers and their proper application, as well as having a clear idea of what the patient desires from the outcome. A proper consultation is essential to achieve a good result and the patient's satisfaction. Patients often come to the aesthetic clinic with a clear idea of looking more refreshed, more beautiful or younger. These are their dreams. To achieve them with that procedure, it is important to clearly comment on their goals, their individual characteristics and needs. During the consultation, the patient should be photographed from different angles in both static and dynamic positions. This allows for patients to observe themselves from an exterior perspective, which however does not often correspond to their true feelings. As described by De Maio, the four main emotions that patients tend to communicate to a third party individual are sadness, tiredness, sagginess and anger.

Numerous studies have found that negative messages given by the patients' faces are associated with specific signs of aging. They may be exacerbated by age-related changes, but are also seen in younger people.

Tiredness is mainly associated with volumetric deficits and changes in the periorbital area, most often depression, and a lack of support in the temporal area, changes in the position of the lateral part of the eyebrow and lateral edging, excess skin in the upper eyelid, changes in the lacrimal cavity (tear trough deformity), and fine crow's feet[13].

An angry expression is most observed in patients with hyperkinetic muscles in the glabellar area, lines in the area between the eyebrows, medial brow ptosis, changes in the perioral area - thin lips, fine wrinkles, chin with a position slightly up and straight (mental hyperactivity in the chin area)[14].

Sagginess can be observed in the middle part - saggy cheekbones and cheeks, which respectively lead to changes in the lower third of the face - loss of contour in the chin and lower jaw, a double chin, a lack of volume in the cheeks, the presence of nasolabial folds and smile lines[15], as well as a repositioning of the adipose tissue in the area of the lower third of the face.

Sadness is manifested in the periorbital and perioral area, as well as in the texture and complexion of the skin. Marks of sad a expression in the periorbital area can be downward-facing eyebrow corners, downward-facing lateral edging, sagging cheekbones, a lack of volume in the temporal area, and lack of volume in the area of the lower eyelid.

In the perioral area, sadness is manifested by a downward-facing corner of the lips, pronounced smile lines, a lack of sufficient volume in the lip area, pronounced nasolabial folds, a lack of anterior and lower projection of the chin, most often due to the hyperactivity of the mentalis muscle, or a retruded lower jaw and any loss of volume in the area.

Although some patients find it difficult to acknowledge and discuss the emotional projection they on others due to their physical characteristics, after a thorough and properly conducted consultation, a consensus is reached on the needs and objectives of the procedure. The expectations of the patients in comparison to more realistic outcomes are also discussed.

Methods

Consultation

At the initial consultation, a detailed history of the patient is taken. Attention is paid to the presence of current and past diseases or conditions that are counter-indicative for the procedure. The risks and possible side effects are explained in detail. All patients

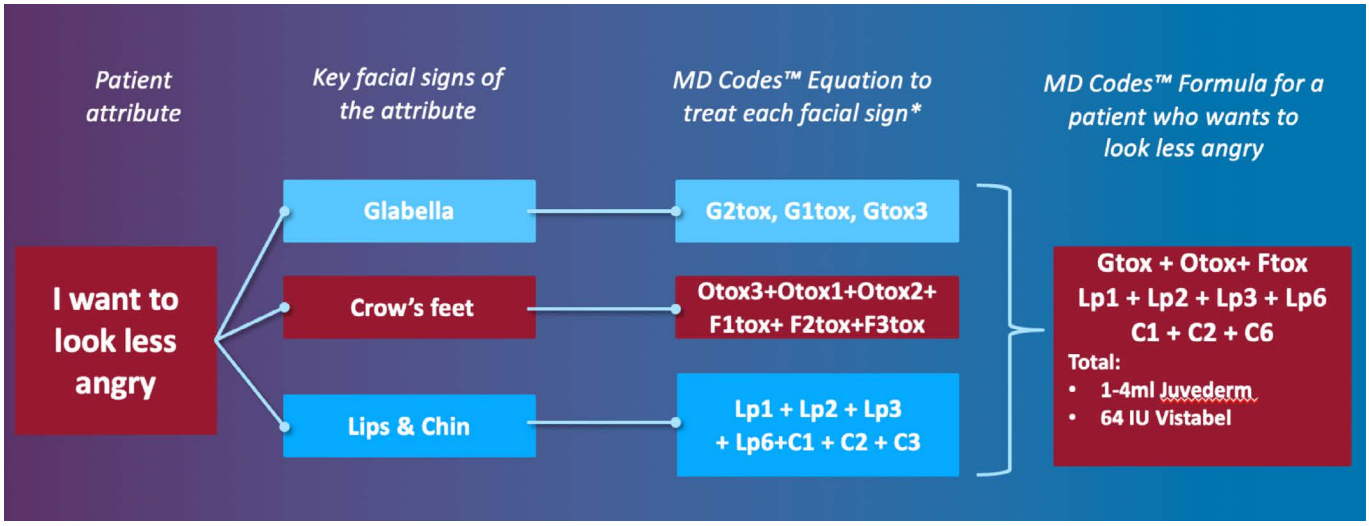
sign an informed consent form before the procedure is performed.
For the purposes of the study, Juvederm Vycross hyaluronic acid fillers and botulinum toxin, which are officially registered and approved for use in the country, were used. The therapeutic plan is based on the author's experience with MD codes, in order to transform the negative face expression of the patients, taking into account the individual characteristics of the whole face of each subject. The whole therapeutic plan, adapted to the specific needs of the patient, is executed in a single session, as the execution of the therapeutic plan begins with transforming the look of tiredness into a positive expression, then continues, if necessary, with transforming the look of sagginess and finally turning the sad expression into a better one.

Assessment

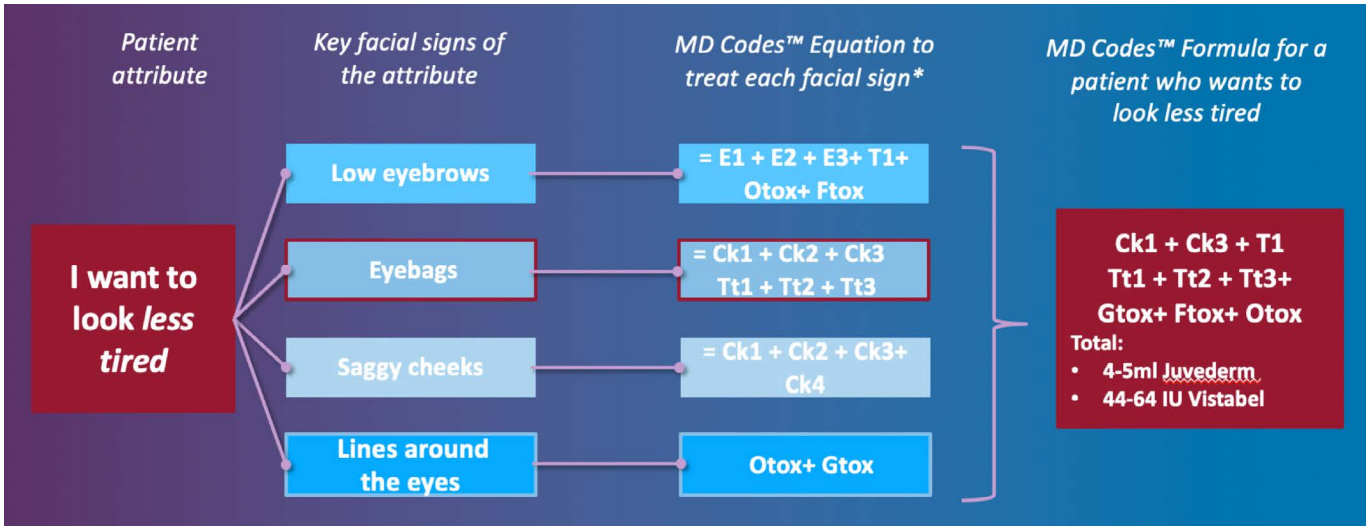
Mauricio de Maio's MDASA system[17] for assessing the patient's needs is used as a method for assessing the patients' faces, using a hierarchical system where 1 (H1), shows that the patient's emotional status is determined. However opposite to that is 5 (H5), with an assessment of crow's feet and wrinkles which characterize the patient. The MDASA system allows for the doctor to first treat the any features that show aging or a negative emotion, and to get a correct patient diagnosis.
Therapeutic plan formulas
Therapeutic formulas for each negative appearance are developed on the basis of a detailed consultation with the patient, "what the patient wants", what are their goals (what the patient feels) and, accordingly, the expertise of the doctor or their idea of "what the patient needs". The author creates a therapeutic plan for each patient, depending on the signs of aging, which she associates with the corresponding external negative emotional manifestations and finally converts them into MD codes equations to identify the patient's needs (as referenced in de Maio's latest study).

Therapeutic algorithms by Mauricio de Maio (treatment plan formulas)

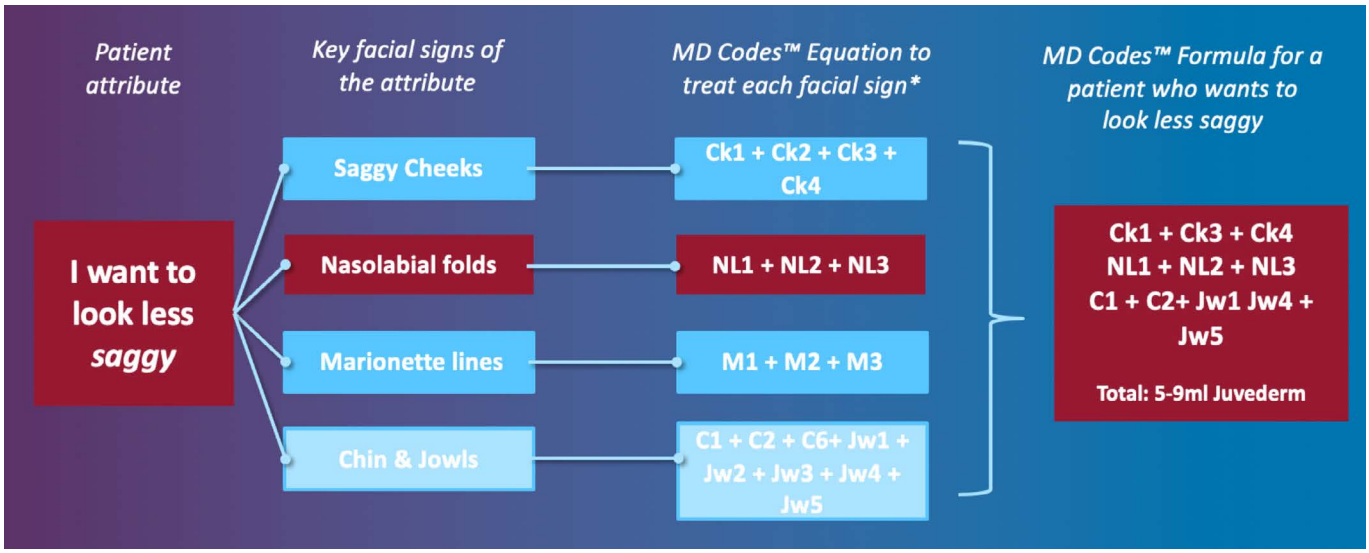
Less tired look



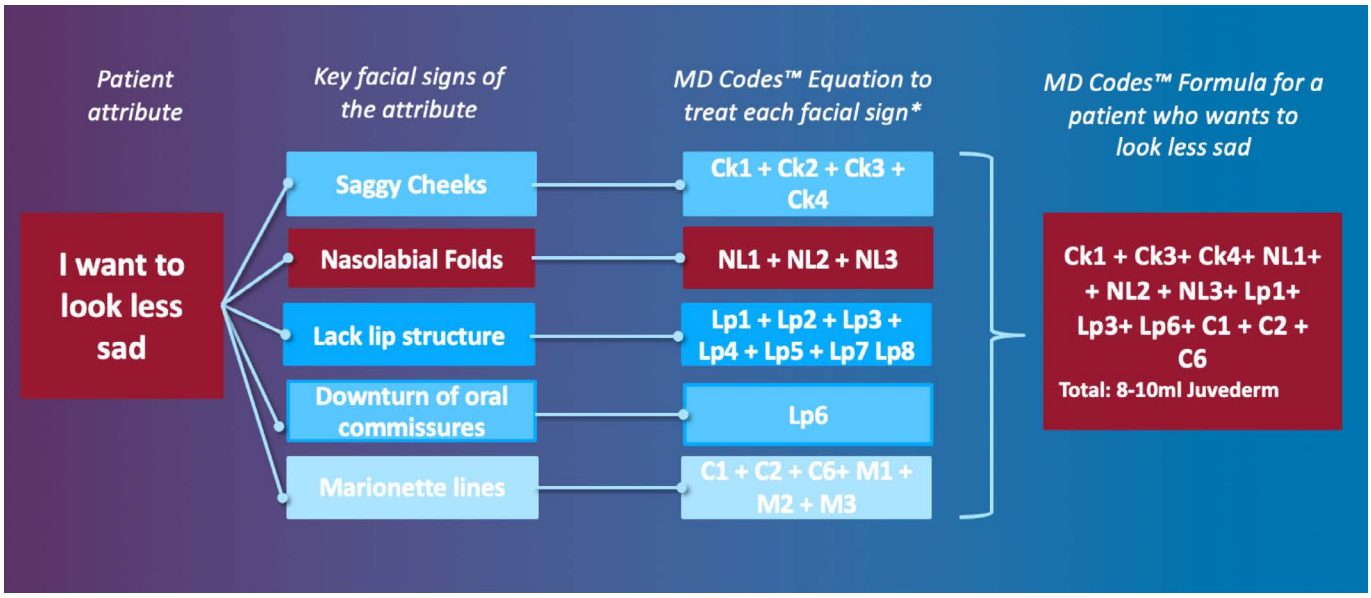
Less angry look



Less saggy look



Less sad look



Patients

47 patients, ages ranging from 30 to 64 years old were enrolled, 8% male patients between 30 and 62 years old, and 92% female patients of ages ranging between 30 and 64 years old. The procedures were performed from Februrary 2021 up until August 2021. All patients have signed an inform consent form before the procedure.

Results

In almost half of the patients, no adverse events were observed after the procedure. The other half reported having at least one of the following adverse events: discomfort/pain, bruising or swelling (*Figure 1*).

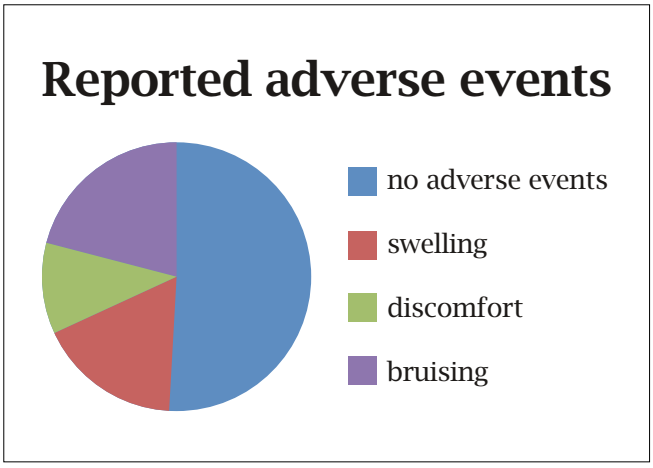


Figure 1 *– Reported adverse events after the procedure.*

All the patients reported that the main goal of the procedure - to transform their negative emotional appearance into a positive one was successful (*Figure 2*). All of them reported that they were either highly satisfied or satisfied with the result, and would repeat or recommend the procedure.

Figure 3 displays information about the amount of hyaluronic acid fillers injected in each patient, units of botulinum toxin, adverse events, such as bruising, swelling and/or discomfort reported one week after the procedure and rate of satisfaction one month after the procedure. The average amount of syringes used in a single session treatment was 12. In almost 40% of the cases, botulinum toxin was also used to achieve the desired goal.

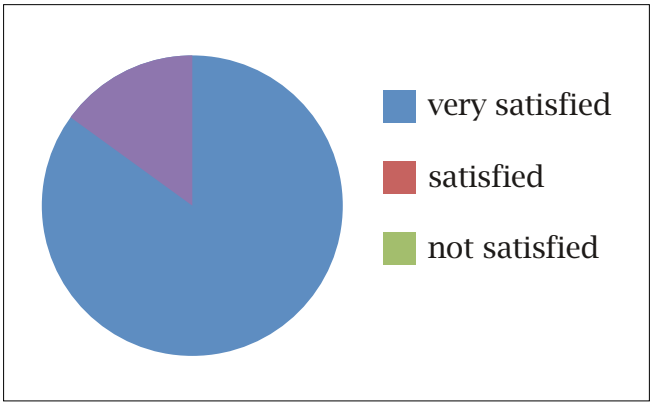


***Figure 2** – Results of the patients' satisfaction after the procedure.*

Patient	Amount hyaluronic acid fillers	Botulinum toxin units	Reported adverse events	Satisfaction rate
1	13	44	Swelling, bruising	satisfied
2	12	44	None	very satisfied
3	16	0	Discomfort	very satisfied
4	10	60	None	very satisfied
5	11	60	None	very satisfied
6	11	0	bruising	very satisfied
7	11	60	None	very satisfied
8	13	0	None	very satisfied
9	13	0	Bruising	satisfied
10	9	60	None	very satisfied
11	8	60	None	very satisfied
12	16	0	Swelling, discomfort	satisfied
13	15	44	Bruising, swelling	satisfied
14	12	60	None	very satisfied
15	11	60	None	very satisfied
16	12	0	None	very satisfied
17	9	60	Swelling	very satisfied
18	10	60	None	very satisfied
19	11	44	None	very satisfied
20	11	0	Swelling	satisfied
21	15	0	Bruising	very satisfied
22	12	0	None	very satisfied
23	14	0	Bruising	very satisfied
24	12	0	None	very satisfied
25	16	0	Swelling	very satisfied
26	8	60	None	very satisfied
27	12	0	None	very satisfied
28	10	60	None	very satisfied
29	11	0	Discomfort	very satisfied
30	13	0	None	very satisfied
31	15	0	None	satisfied
32	14	0	None	very satisfied
33	14	0	Discomfort	very satisfied
34	12	0	Bruising	very satisfied
35	9	60	None	very satisfied
36	10	60	None	very satisfied
37	10	60	None	very satisfied
38	11	0	None	very satisfied
39	10	0	None	very satisfied
40	13	0	Swelling	very satisfied
41	13	0	None	very satisfied
42	13	0	Swelling	satisfied
43	14	0	Swelling	very satisfied
44	12	0	Bruising	very satisfied
45	12	0	Bruising	very satisfied
46	13	60	Discomfort	very satisfied
47	13	0	None	very satisfied

Case 1- 30-year-old patient

30-year-old patient with visible signs of tiredness, sadness and sagginess before the procedure (*Figure 3*).

Figure 3 *- Pictures before a full face treatment.*

To show how the concept of emotional attributes works in the real practice, and using specific therapeutic formulas adapted to the patient's needs, the author presents two patients, women, 30 and 39 years old respectively, and applies the assessment and treatment plan explained above. Patients are photographed in five different positions, and the treatment plan follows the therapeutic formulas developed by Mauricio de Maio, but is adapted to the specific needs of the respective patients. In the following two cases, the author shows an example of an adapted therapeutic plan and "before and after" pictures.

Look of tiredness - regardless of her age, the patient presents a relatively low lateral end of the eyebrow, a primal sagginess in the skin of the upper eyelid with negation of the vector of the lateral edging, sagging cheeks and a well-formed tear trough.

Sagginess - the loss of volume in the middle part and the formed deficit of adipose tissue (deep and superficial) results in sagginess in the area of the lower jaw, an uneven line and diversely shaped smile lines.

Sadness - the patient presents a sad look in the periorbital area with low lateral eyebrow ends, the downwardly oriented lateral canthus, the fully formed tear trough and a loss of volume in the area of the lateral groove.

In the perioral area, the signs of sadness are expressed in the downward-facing lateral end of the lips, the shaped and sunken smile lines, and the nasolabial folds.

Therapeutic plan: the author begins the implementation of the therapeutic plan working with the signs of tiredness. There are two reasons for this: 1. Tiredness as a negative emotion is the first to appear on the faces of patients, as signs of tiredness can be observed even in their early 30s. In addition, to transform tiredness into a positive radiation, the author always starts with the MD codes that are responsible for the foundation of the face, and then works with the codes that are responsible for the effect of the problem.

Personalised treatment plan for less tired look

MD Code	Product used	Right side	Left side
Ck1	Voluma	0.3 ml	0.3 ml
T1	Voluma	0.7 ml	0.7 ml
Ck3	Voluma	0.5 ml	0.5 ml
TT1,2,3	Volbella	0.5 ml	0.5 ml

Personalised treatment plan for less tired look

MD Code	Product used	Right side	Left side
Ck4	Voluma	0.5 ml	0.5 ml
C1	Volux	0.7 ml	0.7 ml
Ck2	Volux	0.3 ml	0.3 ml
Jw3,4	Volux	0.5 ml	0.5 ml
Ck1 (Top model look)	Voluma	0.5 ml	0.5 ml

Personalised treatment plan for less sad look

MD Code	Product used	Right side	Left side
Lp1+6	Volift	0.5 ml	0.5 ml
ML	Voluma	0.5 ml	0.5 ml
Nl1,2,3	Volift	0.5 ml	0.5 ml

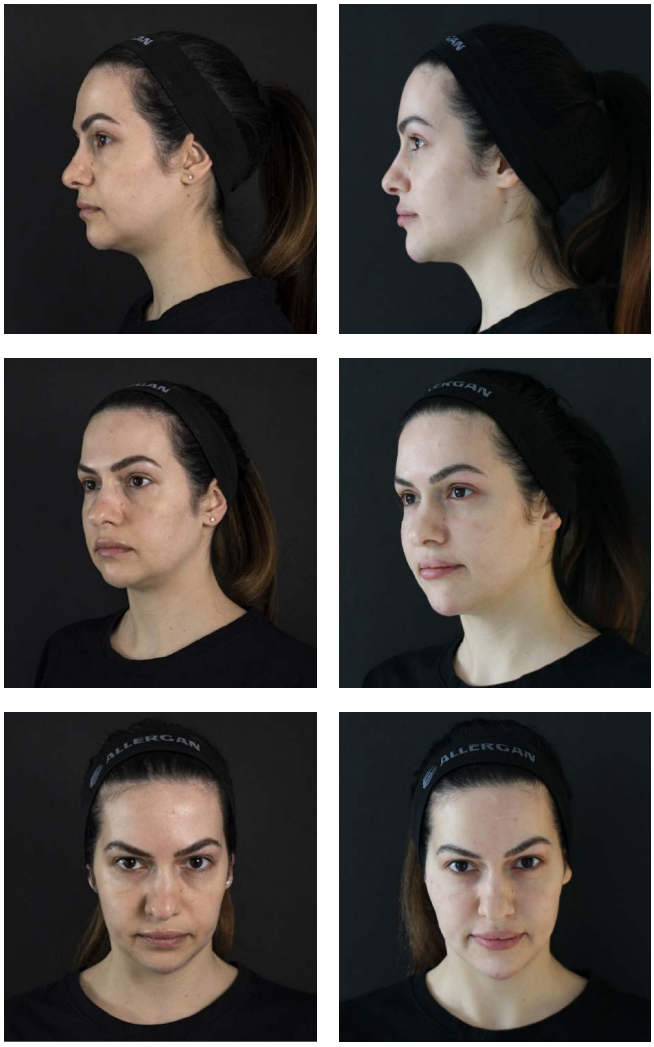


Figure 4 *- Pictures before and after the procedure.*

Case 2 – 39-year-old patient

Figure 5 *- Pictures before the procedure.*

The patient shows signs of tiredness in the periorbital area, signs of sagginess and signs of sadness.
Tiredness: the patient shows a low position of the lateral end of the eyebrow, a negative vector of the lateral canthus, sagging cheeks and a visibly shaped lacrimal cavity and lateral groove.
Sagginess: signs of sagginess are manifested by sagging cheeks, a saggy lower part of the face, an uneven line of the lower jaw.
Sadness: signs of sadness in this patient are seen in the periorbital area as well as in the perioral one.
In the periorbital area we can see a low position of the eyelids with a sunken temporal area, a downwardly oriented lateral canthus, a visible tear trough deformity, as well as a deep lateral groove.
In the perioral area we can observe pronounced nasolabial folds, smile lines, and saggy ends of the lips.

Therapeutic plan

Tired look

MD Code	Product used	Right side	Left side
Ck1	Voluma	0.3 ml	0.3 ml
T1	Voluma	0.7 ml	0.7 ml
Ck3	Voluma	0.5 ml	0.5 ml
Ck3 (SOOF)	Volift	0.5 ml	0.5 ml
TT1,2,3	Volbella	0.5 ml	0.5 ml

Saggy look

MD Code	Product used	Right side	Left side
Ck4	Voluma	0.5 ml	0.5 ml
C1	Volux	0.7 ml	0.7 ml
C2	Volux	0.3 ml	0.3 ml
Jw4,5	Volux	0.5 ml	0.5 ml
Ck1 (Top model look)	Voluma	0.5 ml	0.5 ml

Personalised treatment plan for a less sad look

MD Code	Product used	Right side	Left side
Lp1+6	Volift	0.5 ml	0.5 ml
ML	Voluma	0.5 ml	0.5 ml
Nl1,2,3	Volift	0.5 ml	0.5 ml

Results

Figure 6 *- Pictures before and after.*

Disscussion

The growing interest in non-surgical aesthetic procedures over the last two decades has resulted in more in-depth studies on the patients' satisfaction with the end result[18,19], taking into account any improvements in the patients' quality of life and well-being[20,21]. Many patients worry about surgeries because of the high cost, long recovery period, and high risk of complications[22], however injection procedures with hyaluronic acid fillers and botulinum toxin are relatively safe and have a low complication rate[2,23-25]. Young patients want to slow down the aging process or improve their appearance[5,26]. Aesthetic procedures are elective. It means that patients always come to the clinic with some clear purpose and motivation. They often focus on the "defect" that worries them and think that if it improves or disappears, they will feel better and like themselves more. In the past, doctors have often focused on working on the symptom, for example, direct filling of the nasolabial folds with hyaluronic acid filler[27,10]. Currently, more and more clinicians are taking a different approach. By restoring volumes and working in the cheek area, the condition of the nasolabial folds[16,28,29] as well as the periorbital area[30], are indirectly improved. At the same time, volumes must be taken into account, because overfilling can also lead to unnatural results. When we work on the upper and middle part of the face, the lower part is also affected[31,32]. The interconnection of all the different parts of the face, and a good knowledge of the anatomical structures, provoke more and more clinicians to work on complete facial modeling[24,33-36], taking into account efficiency and comparative safety. These authors did not report any serious adverse reactions during or after the procedures. Their occurrence is rare, however, possible, and is described in some studies. A good aesthetic doctor must be well aware of the dangers of injecting hyaluronic acid fillers and botulinum toxin and follow the basic principle in medicine "primum non nocere". Neither beauty, nor medicine, can be labeled as exact sciences. The individual therapeutic plan and approach targeting each patient must be in accordance with the needs of their facial structures, the desire of the patients, and the expertise of the injector. A basic set of directions and the formation of an algorithm to aid specialists in preparing a therapeutic plan aimed at improving the emotional expression of their patients, is key to a satisfying result. Each part of the face is interconnected, meaning that every facial structure contributes, directly or indirectly, to constitute an emotional expression. A holistic full face approach, and the making of a therapeutic plan based on emotional attributes, leads to optimal results. The proposed algorithm used to transform the negative emotional expression into a positive one, cannot cause the patients to look the way we demonstrated in the presented cases. MD codes are based on three main steps - foundation, contouring and refinement[17], and a proposed therapeutic approach split into several sessions. This approach, however, is partial and requires more time, which would result in the patient's dissatisfaction.

Conclusion

The article aims to show that a holistic full face approach, which is based on the expertise of the clinician, a properly performed consultation, and the development of a therapeutic approach based on transforming the negative radiation into a positive one performed in one session, leads to better results and satisfaction. The proposal to work with these algorithms would lead to a higher patient satisfaction, compared to the therapeutic plan including a foundation, contouring and improvement. This is especially true in cases where the patient cannot afford a complete face modeling. Improving even just one emotional attribute, indirectly results in the improvement of the surrounding facial parts. The author has not used the patient's assessment questionnaires, and therefore has no objective value measures to support this claim and the proposal for an algorithm and an approach to work. The article shows a personal experience, and the results based on a full face approach with a therapeutic plan, based on transforming the negative emotions into positive ones in one session instead of the widely used method of foundation, contouring and improvement in several sessions. Further studies need to be carried out to establish the effectiveness of the proposed strategy.

Conflict of interest

Radina Denkova, MD is a trainer and speaker for Allergan.

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aesthetic medicine

Original Article

High molecular weight hyaluronic acid (HMWHA) for the treatment and prevention of skin aging

Enrique Lorente Prieto[1,2], Pérez MLL[3,4]

[1]Aesthetic Medicine and Surgery Unit, Sagrada Familia Clinic, Barcelona, Spain
[2]Aesthetic Medicine and Surgery Unit, Eiviluxury Clinic, Ibiza, Spain
[3]Graduated in Physical Sciences from the U. A. B. Vall d'Hebron, Barcelona, Spain
[4]Scientific Advisor. ToskaniMed. Barcelona, Spain

Short title: TKN HA3® for the skin aging

Abstract

The treatment and/or prevention of skin aging is one of the main reasons for consultation in our offices. We have a new generation of skin biorevitalizers with a high molecular weight (2700-3700) kDa non-cross-linked hyaluronic acid similar to that of the skin. The anti-aging effectiveness of injected hyaluronic acid is well known. Its injection deep into the dermis stimulates collagen production, increases skin hydration, firmness and elasticity, improves the morphology and function of fibroblasts, and reinforces the epidermal barrier. Manufactured with a specific technology that guarantees the final molecular weight, the size of the molecules of these new biorevitalizers offers significant resistance to the action of endogenous hyaluronidase, prolonging their permanence in the dermis and thus their action and effectiveness.
To demonstrate the effectiveness and safety of high molecular weight hyaluronic acid (HMWHA) as a treatment option and prevention of skin aging, it was injected using 30G / 4 mm needles in 6 volunteers of both male and female genders, at an interval of three weeks.
The results show improved firmness, elasticity and hydration, evidenced obtained using the Cutometer® and Corneometer® probes.
The dermal injection of HMWHA with a molecular weight between (2700-3700) kDa has proven to be effective and safe in the treatment and prevention of skin aging.

Keywords
Treatment, prevention, aging, non-cross-linked hyaluronic acid

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Corresponding Author

Enrique Lorente Prieto, MD

Address: ToskaniMed, Pol. Ind. Can Magre. Barcelona (Spain)
Phone: +34 617749098
E-mail: e.lorente@toskani.com

Introduction

The treatment and prevention of skin aging is one of the main reasons for consultations in our offices. A new generation of skin biorevitalizers is now available, based on a high molecular weight non-cross-linked hyaluronic acid (HA) similar to the one that is naturally produced by the skin, and which contributes to both combat and prevent skin aging.

Aging is the result of two biologically independent processes. On one hand, intrinsic or innate aging, which is an unavoidable phenomenon that affects the skin in the same way that it affects all the internal organs of the body. On the other hand, extrinsic aging is the result of the exposure to external factors, mainly ultraviolet (UV) radiation, in the context of a process also known as *photoaging*[1]. Intrinsic skin aging is influenced by hormonal changes that occur with advancing age[2]. It has been well established that an estrogen and androgen deficiency results in the degradation of collagen, dryness, loss of elasticity, epidermal atrophy and skin wrinkles[3]. Hyaluronic acid is the predominant molecule in the extracellular matrix and belongs to a series of molecules involved in the humidification of the skin. It also provides firmness and softness through the lubrication of the collagen fibers, and acts as a defensive barrier, as it complicates the action of certain pathogens. A lack of water in the skin is associated to aging[4].

Hyaluronic acid (molecular formula C28H44N2O23) is a non-sulfated glycosaminoglycan composed of repeated polymeric disaccharides of D-glucuronic acid and N-acetyl-D-glucosamine joined by a β- (1 → 4) glycoside bond and β- (1 → 3) bonds[5]. The structural stability of HA depends on the stereochemistry of the disaccharides. Given the natural abundance of this biopolymer (human and animal tissues) and its biodegradability and biocompatibility, HA is considered in versatile applications as a prognostic marker, and for the treatment of a broad range of human and animal disease conditions.

Hyaluronic acid is characterized by its marked capacity to retain approximately 1000 times its own weight of water and possesses hygroscopic and homeostatic properties[6]. The hydration of the skin is fundamentally dependent upon the water bound to HA in the dermis and in the vital zone of the epidermis, while the maintenance of said hydration is essentially conditioned by the granular layer (stratum granulosum)[7].

Hyaluronic acid is essential for the formation of proteoglycans. The latter are composed of a long hyaluronate chain to which numerous core protein molecules bind non-covalently at intervals of 40 nm, in turn bound covalently to many shorter glycosaminoglycan molecules such as chondroitin sulfate, keratan sulfate, heparan sulfate and dermatan sulfate. Fibrous proteins such as collagen and elastin in turn interweave with these extremely large extracellular proteoglycans, forming a meshwork that contributes resistance and elasticity to the entire extracellular matrix[8].

High molecular weight hyaluronic acid (HMWHA) is HA with a molecular weight of over 1000 kDa, and is found in a normal healthy tissue. Oligosaccharides of HA (OHA) in turn consist of chains composed of ≤ 20 monosaccharides. The rest of the molecular species of HA are referred to as *low molecular weight hyaluronic acid* (LMWHA)[9-10]. The concentration of HA is 500 mg/g in the skin, with a molecular weight of about 3000 kDa[11]. Hyaluronic acid is synthesized in the membrane of different types of cells[12], mediated by a family of membrane glycosyltransferases composed of three isoenzymes called *hyaluronic acid synthases* (HAS) 1, 2 and 3[13-15]. Although the three isoforms are similar and synthesize an identical product, they differ in their half-life, stability, rate of synthesis and substrate affinity. Accordingly, each HAS synthesizes HA chains of variable lengths. Specifically, HAS3 synthesizes small polymers (100-1000 kDa), while HAS1 and HAS2 synthesize larger polymers (200-2000 kDa) - particularly HAS2 (yielding a product of up to 6000-7000 kDa)[16].

Outside the cells, the newly formed HA chains can join together directly or through specific proteins (hyaloadherins), forming large complexes, or may also act as ligands for certain membrane receptors such as CD44 and TLR. The expression of HAS is regulated by different growth factors and cytokines[17].

The degradation of HA comprises two possible pathways: a specific pathway, mediated by hyaluronidases (HYALs), and a non-specific pathway, which is common to many other molecules, mediated by reactive oxygen and nitric oxide species that mainly act upon the large fragments of HA[18-20]. The HYALs are enzymes pertaining to different families, and include HYAL, PH20 (hyaluronoglucosaminidase PH-20) and HYALP 1 (pseudogene of hyaluronidase 1)[21].

The HYALs, and particularly HYAL1 and HYAL2, hydrolyze specific β-1,4 bonds within the HA molecule. HYAL2 acts in the cell membrane, and the resulting HA fragments can be internalized and transported first to endosomes and then to lysosomes. Within the latter, lysosomal HYAL1 degrades the HA fragments into tetrasaccharide units that are eliminated through exocytosis and can be used in the neosynthesis of HA[22].

The receptor-mediated internalization of HA is essential for its full degradation, with HARE (hyaluronic acid receptor endocytosis) or stabilin-2 and CD44 (cluster antigen differentiation 44) being the main implicated receptors. Recently, a complex protein has been identified, called *KIAA1199*, which binds, internalizes and induces the degradation of HA[23].

The chain size and molecular weight of the HA influence the activation of the receptors and their signaling mode. It has been suggested that signal transmission depends on cooperative and multivalent interactions, or on the capacity of HA to group the membrane receptors. The interaction of hyaluronic acid-CD44 is strongly influenced by specific factors, particularly the size of the HA chain: HMWHA chains facilitate grouping, while OHA exerts no effect[24].

In mesotherapy, injection into the dermis and depositing of the biorevitalizer at this level within the skin causes microlesions of the dermal tissues. In the damaged tissue, HMWHA of the extracellular membrane decomposes into LMWHA, which has a molecular weight of between 0.8-800 kDa[25].

Following the tissue damage, the local fibroblasts proliferate to heal the wound[26-27]. In addition to

fibroblasts, hematopoietic progenitor cells infiltrate the lesion site and differentiate into cells similar to fibroblasts, known as fibrocytes[28].
The fibrocytes produce cytokines, collagens, angiogenic and fibrogenic growth factors, and matrix metalloproteinases that contribute to reconstruct the tissue following the damage[28-35].
The peripheral blood monocytes generally transform into macrophages, and much remains to be learned about the factors that determine whether a monocyte will or will not transform into a fibrocyte[36].
One of the main receptors expressed by monocytes and lymphocytes for detecting HA is CD44[37-38].
HA-CD44 interactions facilitate the migratory cell displacement during development and help the immune cells to migrate towards damaged sites[12,39]. Such interactions also promote the adhesion and motility of fibroblasts, which facilitates tissue repair and the remodelling of the damaged sites[40]. Monocytes, dendritic cells and lymphocytes also bind to HA through toll-like receptors (TLRs) such as TLR2 and TLR4[32-33]. LMWHA binds to TLR2 or TLR4 to induce proinflammatory effects, while HMWHA reduces the inflammation by inhibiting TLR2 or TLR4 signaling.
In 2011, Anu et al. showed that HMWHA facilitates the differentiation of human monocytes into fibrocytes, while LMWHA inhibits the differentiation of fibrocytes. The digestion of HMWHA mediated by hyaluronidase produces small fragments of hyaluronic acid, which inhibit the differentiation of fibrocytes. Monocytes internalize HMWHA and LMWHA with equal ease, which suggests that the opposite effects upon fibrocyte differentiation are not attributable to any differential internalization of HMWHA or LMWHA[41].
The addition of CD44 antibodies promotes the differentiation of fibrocytes, which suggests that CD44, at least in part, mediates the effect of HA upon fibrocyte differentiation. The fibrocyte differentiation inhibiting factor serum amyloid P (SAP) inhibits the differentiation of fibrocytes induced by HMWHA and enhances the inhibition induced by LMWHA. In contrast, LMWHA inhibits the capacity of HMWHA, interleukin-4 (IL-4) or interleukin-13 (IL-13) to promote fibrocyte differentiation[41]. Hyaluronic acid is currently the gold standard in aesthetic medicine, since it allows the youthening of different body zones. It is very widely used in products for a topical application, formulations for injection in mesotherapy or as a dermal filler. The present study focuses on the use of non-cross-linked HMWHA in mesotherapy, not only for hydration of the skin, but also to trigger the biological mechanisms secondary to the injection of non-cross-linked HMWHA within the deep dermal zone.

Materials and methods

The present pilot study was carried out in abidance with the principles of the Declaration of Helsinki (2013).

Study subjects

An efficacy and safety evaluation was obtained with 6 volunteers / cases of both sexes, with different grades of aging in the facial zone based on the Glogau scale.
The study covered the months from October to December, 2020.
The characteristics of the volunteers and the study inclusion and exclusion criteria are described in *Table 1*.

SUBJECTS	EXCLUSION CRITERIA
6 volunteers 5 females 1 male Age: 44-70 years Study period: October to December 2020	Pregnancy Breastfeeding Hypersensitivity to HA Treatments in the 6 previous months Autoimmune diseases No collagen topical use or ingestion No history of Covid-19
	INCLUSION CRITERIA
	Prior informed consent Agreement to follow the home treatment

Table 1 *- Characteristics of the volunteers and inclusion and exclusion criteria.*

Materials used in the clinic

The materials used in the clinic to carry out the procedure for six volunteers are shown in detail in *Table 2*.

MATERIALS USED	DESCRIPTION	INGREDIENTS
Preloaded syringe for injection of non-cross- linked HMWHA	3 ml Concentration: 9 mg/ml pH: 7.0 Osmolality: 300 mOsmol/kg Dynamic viscosity: 16,000 cP Sterile Bacterial endotoxin: <0.25 EU/device	9 mg/ml of HA 2.7- 3.7·10^6 Da 0.9% sodium chloride solution for injection
Hypodermic needles	30 G / 4 mm	Steel
QuantifiCare Live Viz 3D®	Photographs Camera for skin analysis	Not applicable
Cutometer® (firmness and elasticity) Corneometer® (hydration)	Measurement probes	Not applicable

Table 2 *- Materials used in the clinic to carry out the procedure for 6 volunteers.*

The biorevitalizer used consisted mainly of non-cross-linked HMWHA (2700-3700 kDa), at a concentration of 9 mg/ml, highly purified and manufactured under aseptic conditions in all the stages of the process. This avoided the need for final sterilization and yielded an end product in which the molecular weight remained the same as that of the starting material, and very similar to that found in the skin. Stainless steel mesotherapy needles (30 G / 4 mm) were used.

Photographs were obtained with a high-resolution system (QuantifiCare®, Biot, France), at baseline and at the end of the study. Skin firmness and elasticity in turn were assessed with the Cutometer®, based on the skin suction and relaxation action of the probe of the device, where an optical system measures the level of skin penetration into the probe aperture, as well as its deformation and return to the original state prior to the deformation.

The parameters selected for the study of the mechanical properties of the skin using the Cutometer® were set at a constant pressure of 450 mbar with a deformation mode/time of 3 seconds, followed by a relaxation time of 3 seconds, with a single suction.

The values obtained were: R0, R1, R2, R3, R4, R5, R6, R7 and R8. The variables R3 and R4, for a single suction are equivalent to R0 and R1 respectively.

The variables R0 and R8 refer to the firmness of the skin at different times of deformation/relaxation; According to different authors, the R0 variable is the most used to characterize changes in skin firmness.

The variables R2, R5, R6 and R7 refer to the elastic capacity of the skin at different times of deformation and relaxation. For these 6 volunteers, we selected the variables R0 (to characterize skin firmness) and R5 (to characterize net elasticity), as main variables, since they are the ones that best characterize internal skin changes, without the influence of external factors[42-43].

Lastly, the skin's hydration was evaluated with the Corneometer® (Courage+Khazaka Electronic GmbH, Köln, Germany). This system consists of a probe, which in contact with the skin measures the water content of the latter based on the principle of capacitance of a dielectric medium.

Home treatment materials

The prescribed home treatment consisted of the daily use of a sunscreen with a sun protection factor (SPF) of 50, upon demand.

Treatment protocol

An anesthetic gel was applied two hours before the visit and was replaced three times at half-hour intervals. The gel was formulated by a pharmacy (Barcelona, Spain) and contained the following: 7% lidocaine, 7% prilocaine, 6% tetracaine hydrochloride in gel base. Chlorhexidine digluconate (Cristalmina® 1%, Laboratorios Salvat, Esplugues de Llobregat, Spain) was used as a disinfectant. After cleaning and disinfecting the face of the participant, the point-by-point technique was used to inject the non-cross-linked HMWHA into the dermis at a depth of ≤ 4 mm, with a 5 mm distance between the injection points. A possible treatment overcorrection was avoided in order to prevent the appearance of papules over the subsequent days. The recommended needle specifications were 30 G / 4 mm. In the first step we aimed to reinforce the debilitated anatomical structures, exploring the face of the patient in search of weak zones. The positioning of the zygomatic arch was reinforced, and in the preauricular zone we applied two injection lines parallel to each other and along the mandibular arch, without reaching the jowl. We likewise injected further points at the hairline in order to reinforce this zone, with the above-mentioned depth and distance specifications. Lastly, strategic injections were made over the rest of the face, forming ascending antigravitational vectors to reinforce those areas exposed to overlapping as a result of sagging caused by the weight of the tissues, carefully avoiding an overcorrection.

A gentle massage is advised after the treatment session. It is not necessary for the patient to perform the massage at home, though if he or she prefers to do so, it must be emphasized that massaging should be extremely delicate.

Treatment schedule

Three treatment sessions spaced three weeks apart were performed in the period between October and December 2020. After the completion of the procedure, no immediate post-treatment measures were needed, and the participants were allowed to proceed with their daily activities. A sunscreen (SPF 50) was prescribed throughout the duration of the treatment, and the participants were advised to continue applying it on a daily basis to prevent photoaging.

Time course of the objective parameters. Cutometer® and Corneometer®

The objective variables were recorded at two different timepoints: before the start of treatment (T baseline) and 30 days after the third and last session (T final). The parameters were recorded using the following systems:

Cutometer®:
- Parameter R0 as an indicator of skin firmness (mm)[42].
- Parameter R5 as an indicator of intrinsic skin elasticity (%)[43].

Corneometer®:
- Skin hydration, in arbitrary units (AU) (range of values 0-130), where <40 AU is indicative of dehydration and >40 AU indicates sufficient hydration[44].

QuantifiCare Live Viz 3D®:
- Photographic monitoring of the evolution of treatment over time.

Outcome assessment based on the GAIS scale

The Global Aesthetic Improvement Scale (GAIS) – I was used to assess the aesthetic improvement according to the investigator, while the GAIS – S was used to assess the subjective aesthetic improvement according to the participant.
- Degree of satisfaction with brightness
- Degree of satisfaction with firmness
- Degree of satisfaction with hydration
- Degree of satisfaction with biorevitalizing effect
- Degree of satisfaction with decrease in wrinkles
- Degree of satisfaction with global skin effect
- Degree of post-treatment social disability

Statistical analysis

The objective parameters were reported as the mean ± standard error of the mean (SEM), while the subjective parameters were reported as percentages. The normality of the data distribution was assessed with the Shapiro-Wilk test, and the non-parametric Wilcoxon test was used for the comparison of means between the two study timepoints (baseline and final). The Statistical significance was considered for $p < 0.05$. The SPSS version 20 statistical package (IBM, Madrid, Spain) was used throughout.

Results

The 6 volunteers completed all the phases of the pilot study, despite the complications posed by the rise in cases of the SARS-CoV-2 infection. All the participants came to all the sessions, despite the geographical mobility restrictions, telecommuting and time schedules imposed by the viral pandemic. No adverse effects were recorded either during the treatment or in the post-treatment period. The side effects were those inherent to the technique: ecchymosis, transient erythema (approximately one hour) and an itching sensation in some participants, that disappeared within 24 hours after treatment.
The 6 volunteers/cases of both sexes that met all the inclusion criteria and none of the exclusion criteria, with a mean age of 54.66 ± 3.96 years (range 44-70). Of the 6 participants, 5 (83%) corresponded to grade III and one (17%) to grade IV of the Glogau aging scale.

Objective study parameters:
- Net elasticity of the skin (parameter R5, measured with the Cutometer®)
- Firmness (parameter R0, measured with the Cutometer®)
- Hydration (measured with the Corneometer®)

Subjective study parameters:
- Degree of satisfaction with brightness
- Degree of satisfaction with firmness
- Degree of satisfaction with hydration
- Degree of satisfaction with biorevitalizing effect
- Degree of satisfaction with decrease in wrinkles
- Degree of satisfaction with global skin effect
- Degree of post-treatment social disability

1) Net elasticity
Net elasticity is defined as the capacity of the skin to return to its original position following deformation induced, in this case, by suction with the Cutometer® probe.
Figure 1 shows the results obtained (mean ± SEM). Net elasticity was seen to improve by 66.1% with respect to the baseline condition. The increment between the two study timepoints was 25.1 ± 6.9% on average, and proved to be statistically significant ($p = 0.028$). All the participants showed an increase in elasticity.

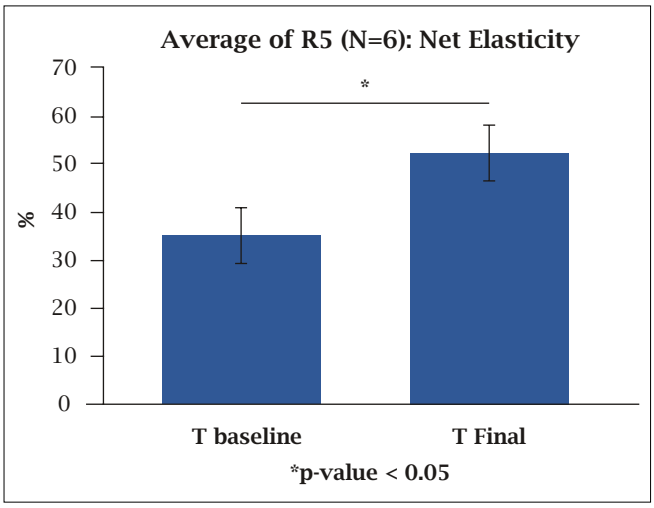

Figure 1 *- Net elasticity (mean ± SEM) at the two study timepoints, measured in the preauricular zone.*

2) Firmness
Firmness is defined as the resistance of the skin when it is sucked with the Cutometer® probe.
Figure 2 shows the results obtained (mean ± SEM). Firmness was seen to improve by 15.2% compared to the baseline condition. The increment between the two study timepoints was 0.032 ± 0.02 mm on average, without reaching a statistical significance ($p = 0.116$). Eighty-three percent of the participants showed an increase in firmness.

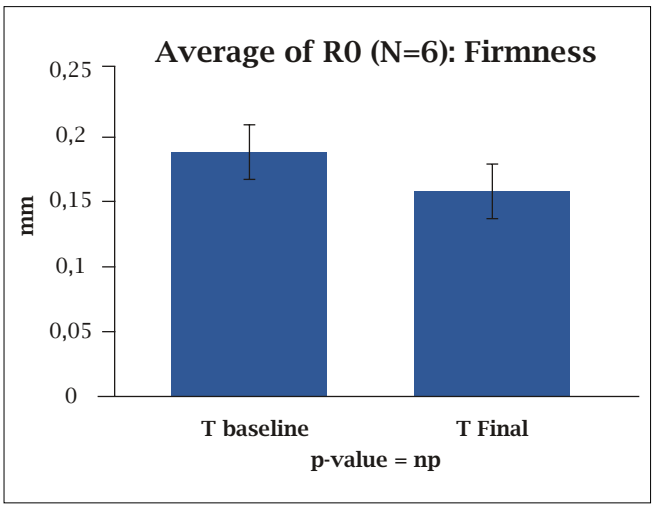

Figure 2 *- Firmness (mean ± SEM) at the two study timepoints, measured in the preauricular zone.*

3) Hydration

Figure 3 shows the results obtained (mean ± SEM). Hydration was seen to improve by 19.8% with respect to the baseline condition. The increment between the two study timepoints was 9.5±6.49AU on average, without reaching statistical significance (p = 0.173). Eighty-three percent of the participants showed an increase in hydration.

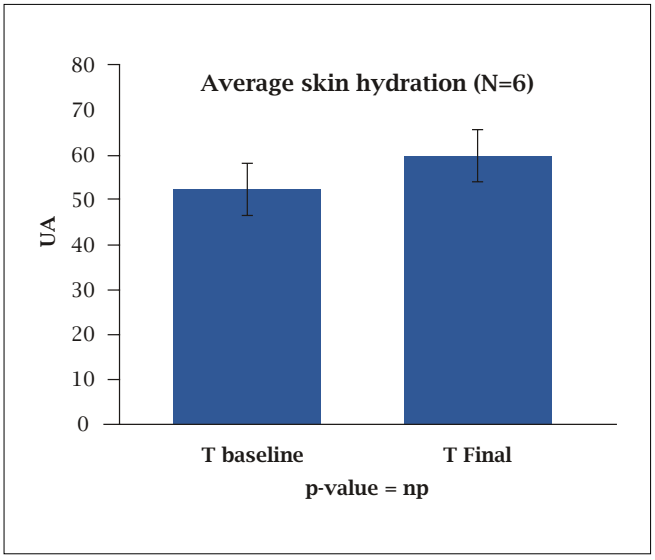

***Figure 3** - Hydration (mean ± SEM) at the two study timepoints, measured in the preauricular zone.*

4) Subjective parameters

Data provided by the participants based on the subjective GAIS-S scale

a) Post-treatment brightness
- 67% of the participants reported great improvement
- 16% of the participants reported considerable improvement
- 17% of the participants reported no changes

b) Post-treatment firmness
- 50% of the participants reported great improvement
- 33% of the participants reported considerable improvement
- 17% of the participants reported no changes

c) Post-treatment hydration
- 50% of the participants reported great improvement
- 17% of the participants reported considerable improvement
- 33% of the participants reported no changes

d) Post-treatment biorevitalizing effect
- 83% of the participants reported great improvement
- 17% of the participants reported considerable improvement

e) Post-treatment decrease in wrinkles
- 67% of the participants reported great improvement
- 17% of the participants reported considerable improvement
- 16% of the participants reported some improvement

f) Post-treatment global effect
- 66% of the participants reported great improvement
- 34% of the participants reported considerable improvement

g) Post-treatment social disability
- 83% of the participants could lead a totally normal life
- 17% reported minor ecchymosis that proved difficult to cover with makeup

Data provided by the investigators based on the GAIS-I scale

a) Post-treatment brightness assessed by the professional
- 66% of the participants showed great improvement
- 34% of the participants showed considerable improvement

b) Post-treatment firmness assessed by the professional
- 83% of the participants showed great improvement
- 17% of the participants showed considerable improvement

c) Post-treatment hydration assessed by the professional
- 83% of the participants showed great improvement
- 17% of the participants showed considerable improvement

d) Post-treatment biorevitalizing effect assessed by the professional
- 83% of the participants showed important revitalization
- 17% of the participants showed average revitalization

e) Post-treatment decrease in wrinkles assessed by the professional
- 67% of the participants showed important improvement of the wrinkles
- 34% of the participants showed average improvement of the wrinkles

f) Post-treatment global effect assessed by the professional
- 83% of the participants showed important global improvement
- 17% of the participants showed considerable global improvement

g) Post-treatment social disability assessed by the professional
- 67% of the participants did not need makeup
- 17% of the participants presented very minor ecchymosis requiring no application of makeup
- 17% required makeup during three days to cover ecchymosis secondary to treatment

Figures 4 and *5* show the clinical condition before treatment (A) and 30 days after the last treatment session (B).

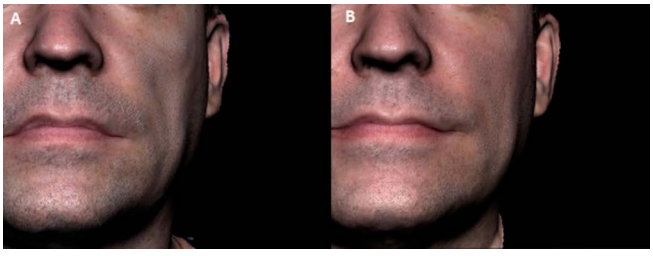

***Figure 4** - Frontal view of a 45-year-old participant. A: before the start of treatment. B: 30 days after the last treatment session.*

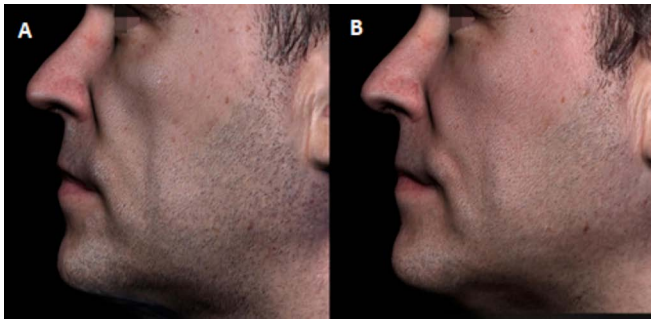

Figure 5 *- Lateral view of a 45-year-old participant. A: before the start of treatment. B: 30 days after the last treatment session.*

Discussion

The present pilot study in 6 volunteers was carried out to validate the mesotherapeutic treatment with non-cross-linked high molecular weight hyaluronic acid (HMWHA), and demonstrates its safety and efficacy in both preventing and treating skin aging.
In the dermis, the main age-related change is the growing affinity of HA for the tissue structures - this in turn being associated to a decrease in the formation of HA. This situation is accompanied by a progressive interweaving and rigidity of the collagen fibers and a loss of collagen production capacity[7]. All these age-related phenomena contribute to the dehydration, atrophy and loss of elasticity that characterizes aged skin. A treatment using HMWHA with a molecular weight between 2700-3700 kDa was found to be effective, with an improvement in the elasticity of the skin, firmness and hydration in 82% of the cases.
The synthesis of epidermal HA is known to be influenced by the underlying dermis, and is under the control of mechanisms different from those involved in the synthesis of dermal HA[7-45]. Injecting HMWHA into the deep dermis may give the impression that the epidermis is not benefiting from the treatment. For this reason, the application technique and posterior massage of the injection site are important. Massaging favors the expansion of HMWHA, not only within the dermis but also to the epidermis. The observed results in terms of skin firmness and hydration confirm this idea. On the other hand, the high molecular weight of the product is similar to that of the molecule produced by the skin. This ensures a longer permanence in time of the injected HA, compared to when other biorevitalizers already available on the market are used. This phenomenon is due to the fact that the degradation of HMWHA is mediated by the HYAL-2 enzyme, which has a very low activity compared with plasma HYAL-1. Consequently, while HYAL-2 specifically hydrolyzes HMWHA, HYAL-1 is more specifically composed of smaller HA chains. As we have commented, HYAL-2 specifically hydrolyzes HMWHA, producing HA fragments of about 20 kDa, which are more extensively degraded to small oligosaccharides by PH[20,46]. In contrast, the role of HYAL-3 in the catabolism of HA is uncertain, though it has been suggested that it can contribute to the degradation of HA, improving the activity of HYAL-1[47].
The mesotherapy technique produces tissue damage, and the local fibroblasts proliferate to regenerate the damaged tissue[26-27]. The fibrocytes produce cytokines, collagens, angiogenic and fibrogenic growth factors, and matrix metalloproteinases that contribute to reconstruct the tissue following any damage[28-35]. Thus, the application technique in the form of deep dermal injections of HMWHA intrinsically stimulates the formation of collagen and elastin. This stimulation in turn is favored and incremented by the biological mechanisms triggered by HMWHA; in this respect, the interaction of HA-CD[44] is known to promote the adhesion and motility of fibroblasts, which in turn facilitates the repair and remodelling of the damaged tissues[40].
Although the study sample size was small, we consider that the findings are scientifically relevant and confirm the efficacy and safety of HMWHA injection based on the principles of mesotherapy.
The present pilot study has not been able to analyse the duration of the results. Further research involving longer periods of follow-ups is therefore required to determine the durability of the outcomes and the need for and frequency of the repetition of the treatment in a larger sample of volunteers.
Although the current evidence does not allow us to confirm the superiority of non-cross-linked HMWHA over other intradermal fillers based on cross-linked HA or other facial youthening techniques, the present study confirms that the proposed treatment, and the technique used, are effective in treating and preventing skin aging.

Conclusions

The administration of non-crosslinked HMWHA in the deep dermis is a promising technique, with evidence of action on the aging process, improving the elasticity, firmness and hydration of the skin. More studies are needed to establish if the results obtained are attributable to the combination of HMWHA and the application technique used. Side effects were those inherent to the technique: ecchymosis, transient erythema and an itching sensation in some participants, the latter two effects disappearing 24 hours after treatment. For all 6 volunteers the treatment was safe and effective. Additional research involving larger samples and longer follow-up periods is required.

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Conflict of interest

The Authors declare that they have no conflict of interest.

Funding source

None.

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aesthetic medicine

Original Article

Using Botulinum toxin injections into depressor anguli oris to adjust drooping mouth corners using a double point injection technique: a new approach

Rubin S John[1], Charanya Suresh[2]

[1]Senior Lecturer, Department of Oral and Maxillofacial Surgery, Saveetha Dental College, Saveetha Institute of Medical and Technical Sciences, 162, Poonamallee high road,
Chennai 600077 Tamil Nadu, India - Email: rubinjohn90@gmail.com (phone 6306258601)
[2]Final year BDS student. Saveetha Dental College, Saveetha Institute of Medical and Technical Sciences, Saveetha University. Chennai 600077, Tamil Nadu, India - Email: 151801072.sdc@saveetha.com (phone 9655590226)

Running title: BTX to correct drooping using a double point technique

Abstract

Aim and objectives: this study aims to propose a new technique using botulinum toxin injections to elevate the corners of the mouth, by administering it at two points on the depressor anguli oris muscle, instead of a conventional single point technique.
Materials and methods: five patients underwent bilateral double-point botulinum toxin injections at the depressor anguli oris. A total of 2U of BTX-A was used for each injection site, with a 8U maximum dose for the entire treatment. Each patient's drooping mouth corners were measured before and after the injection. The effectiveness of the treatment was studied by calculating the perpendicular distance from the angle of the mouth to the chin before and after the BTX application.
Results and discussion: a follow-up visit was carried out after 2 weeks, observing significant elevation in the corners of the mouth. The patient was asked to report to the clinic for a further evaluation six months after the botulinum toxin was injected. The difference from the initial consultation to the 14th day after the injection of botulinum toxin was significantly noticeable.
Conclusion: the use of the botulinum toxin, which is an efficient, safe, quick, and minimally invasive therapeutic option for patients, was found to minimize the patients' drooping mouth corners. This technique is a very helpful addition to the "aesthetic smile improvement" and can raise the patients' self-esteem.

Keywords
Botulinum toxin, drooping, depressor anguli oris, elevation, facial aesthetics

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Corresponding Author

Charanya Suresh

Address: 162, Poonamallee high road, Chennai 600077 Tamil Nadu, India
Phone: 9655590226
E-mail: 151801072.sdc@saveetha.com

Introduction

Lips are a crucial component of the face's anatomy and play an important role in how the face looks and displays expressions[1]. A person with a drooping mouth corner typically exhibits depressive tendencies as well as a stern, old appearance, and their social interactions are frequently negatively impacted[2].
The depressor anguli oris muscle rises up the oblique line of the mandible. It is placed within the mouth's inferior end and interfaces the adjacent muscles. It reduces the mouth's commissure, which leads to a melancholic or annoyed expression. It contributes to the development of the mentolabial fold along with the mandibular ligament[1].
The protein known as the botulinum toxin (BTX) is taken from the gram-positive, anaerobic bacterium Clostridium botulinum, which is the source of botulism. There are seven different toxin serotypes (A, B, C1, D, E, F, and G), however type A toxin is the one that is most frequently employed because it is more effective and less expensive[2]. These are the strongest known toxins, and when they are connected to particular action mechanisms, they are extremely harmful but nevertheless very beneficial for medical research.
By inhibiting the neurotransmitter acetylcholine, which sends signals from the brain to the muscle fibers and causes a contraction, the botulinum toxin causes a muscular relaxation. Although the BTX is generally utilized for cosmetic reasons, it has also been successfully used to treat a wide range of medical ailments, including bruxism, headaches, orofacial pain, sialorrhea, facial spasms, and gummy and asymmetrical smile reductions. For therapeutic purposes, its injection application is done through a muscular route, and in aesthetics, subcutaneously. It is also known that it has a temporary effect, lasting 4-6 months, varying according to the person's metabolism and the hyperactivity of the muscle involved in the process[1]. The highest point of result effectiveness obtained with the use of the BTX is seen after 15 days.
Moreover, its use is very diverse, making it possible to use it for patients with facial changes and changes related to oral health, providing good end results[3]. In dentistry, the use of the BTX has become relevant as it is a non-invasive and safe treatment option, successfully replacing surgical procedures[3,4].
This research study aims to propose a new technique of injecting botulinum toxin to elevate drooping mouth corners, by administering it at two points on the depressor anguli oris muscle in place of a conventional single point technique.

Materials and methods

A total of 5 patients who were observed to have downturned oral commissures, both at rest and while smiling, creating an unaesthetic appearance, were chosen for the study. An ethical approval for the study was obtained from IHEC – the Institutional Human Ethics Committee, following the ICMR guidelines.
The treatment routine included a pretreatment photographic documentation and an informed consent, as seen in *figure A* and *B*. The depressor anguli oris muscle was identified through a palpation, and was divided into upper, middle and lower third, as seen in *figure A*.
Prior to the botulinum toxin injection, the skin surface was cleaned with a 70% ethyl alcohol for oil removal. ONAbotulinum toxin type A was used, at a standard dilution ratio of 2.5 ml of normal saline in 100U of botox. Using a 1.0 ml calibrated insulin syringe, 2 BTX units were injected in the middle third, and 2BTX units were injected in the lower third on either sides of the mouth on the depressor anguli oris muscle as seen in *figure B*. The depth of injection was 3-4 mm. The perpendicular distance from the angle of the mouth to the chin was measured before and after the BTX application.
Following this procedure, the patient was instructed not to interfere with the treated area, not to lay their head or perform physical activities during the first 4 hours after undergoing the procedure. Follow-up visits were carried out every week for 8 months, to closely monitor the elevation of the corners of the mouth and the overall improvement.

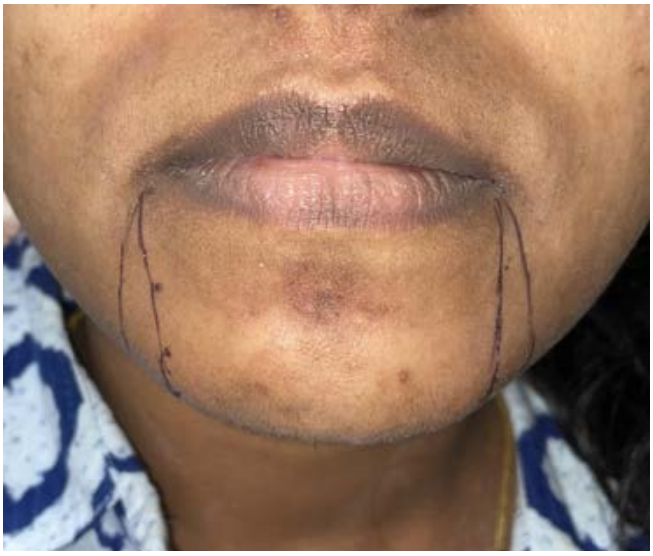

***Figure A** - Pretreatment.*

***Figure B** - 2 BTX units were injected in the middle third, and 2BTX units were injected in the lower third on either sides of the mouth on the depressor anguli oris muscle.*

Results

The results obtained as seen in *figure C*, *D*, *E* and *F*, highlighting the elevation and redefinition of the angle of the mouth, became visible within 2.4 weeks on an average for all 5 patients following the injection, and remained stable for an average period of 6 months as seen in *Table A*. The perpendicular distance from the angle of the mouth to the chin before the BTX application were measured, and an average increase of 0.26cm was observed in all patients as seen in *Table B*, suggesting an elevation of 0.26cm.

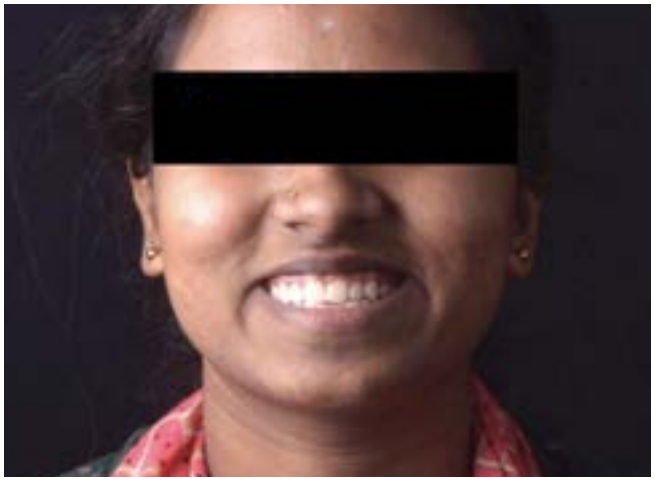

***Figure C** - Patient A before BTX injection.*

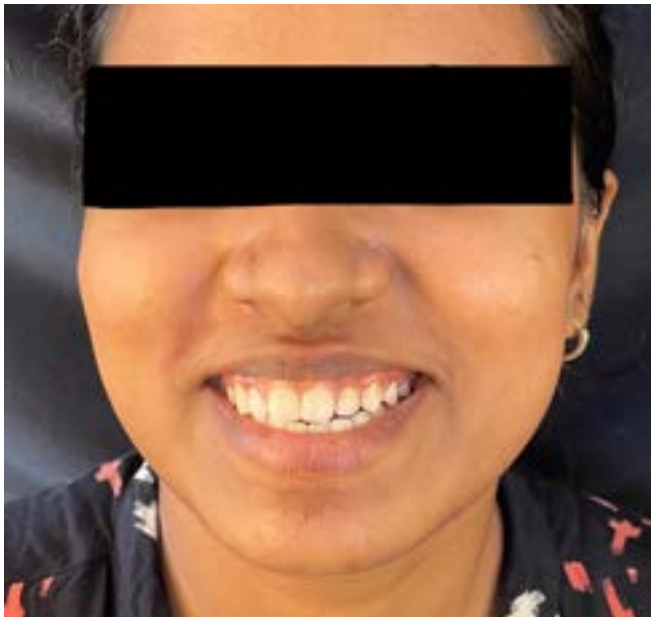

***Figure D** - Patient A after BTX injection.*

***Figure E** - Patient B before BTX injection.*

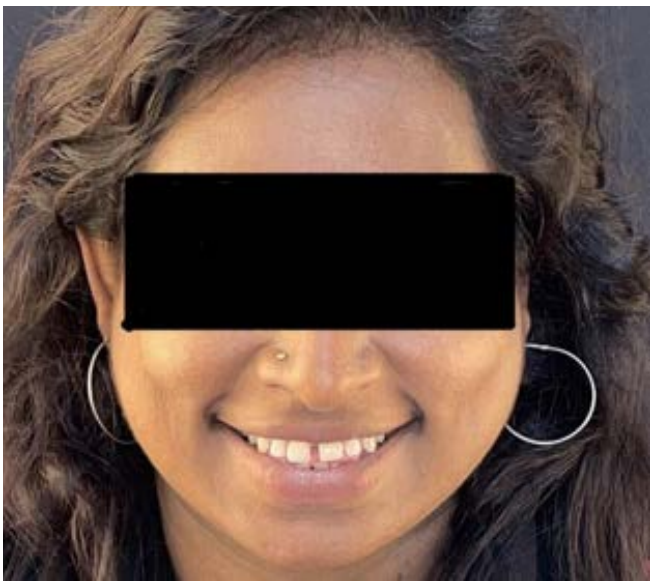

***Figure F** - Patient B after BTX injection.*

	Visible elevation	Elevation lasted
Patient 1	2 weeks later	6 Months
Patient 2	3 weeks later	5 Months
Patient 3	3 weeks later	6 Months
Patient 4	2 weeks later	6 Months
Patient 5	2 weeks later	7 Months

***Table A** - Elevation and redefinition of the angle of the mouth.*

	Perpendicular distance from the angle oh the mouth to the chin BEFORE BTX application	Perpendicular distance from the angle oh the mouth to the chin AFTER BTX application	Evaluation
Patient 1	4 cm	4,2 cm	0,2 cm
Patient 2	4,1 cm	4,4 cm	0,3 cm
Patient 3	4,3 cm	4,5 cm	0,2 cm
Patient 4	4 cm	4,3 cm	0,3 cm
Patient 5	3,8 cm	4,1 cm	0,3 cm

***Table B** - Perpendicular distance from the angle of the mouth to the chin before and after the BTX application.*

Discussion

The use of newer indications for BoNT/A have been increasing over the past years, and it has become the most frequent nonsurgical procedure performed currently[5]. The demand for less invasive treatments that provide a quick recovery and more predictable and safer outcomes is ever more frequent.

The novel double point injection technique proposed herein differs from the conventional single point technique proposed by Goldman et al. where the depressor anguli oris muscles are treated bilaterally only with a single injection of approximately 2-5 U in each muscle[6]. Levy et al. proposed a technique called "Nefertiti lift," where a total dose of 15U BTX is injected along and under each mandible and to the upper part of the depressor anguli oris, unlike our study where we limited the dosage to a maximum of 8 units per patient[7]. Wollina U et al. also used an additional point of injection like the one in our study, but they chose the second point on the nasolabial fold instead. Carruthers JD et al. combined the use of toxins with other traditional techniques in cases involving patients with significant cervicofacial laxity, advanced age, photoaging of the region, highly marked wrinkles, large amounts of submental fat, or significant muscular impairments[4]. Our double point injection technique was carried out to facilitate the understanding of the uniform distribution of the toxin when injected at two points.

There are many studies in literature that have reported mild to severe side effects due to the injection of botulinum toxin into the depressor anguli oris muscle. The study conducted by Gee Young Bae et al., reported slight discomfort in patients, while speaking along with herpes labialis[8]. A study by Wei Qian et al., that investigated the effects of botox in patients with congenital drooping mouth corners, reported discrete asymmetry and ecchymosis in some patients[9]. Nanouk van der Sluis et al., reported haematoma and swelling in 14.3% patients[10]. However, our strict adherence to limited depth of injection of only 3-4mm into the depressor anguli oris muscle which has a soft tissue thickness of 1.8mm[11,12] implemented in this study, decreased the possibility of encountering any major adverse effects. The 5 patients in our study did not report any adverse effects such as muscle paralysis or an asymmetric smile. New fillers, lipotransfer, and the potential uses of stem cells, the clinical application of the tetanus toxin, the evolution of videosurgery and laser technologies, and new indications in the use of BTX represent a new perspective in the treatment of the aged neck and face[13]. The development of new serotypes of this toxin, new commercial preparations with different dosing and advances in the modification of the protein structure of the drug all point toward a broader and safer use of BTX in medicine in the near future[14].

Conclusion

It could hence be concluded that the double point technique of the BTX injection proposed here for patients with drooping mouth corners has proven to produce its visible elevation. However, a comparison with other injection techniques is required to fully understand its significance and adverse effects, if any.

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Conflict of interest

Nil.

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aesthetic medicine

Review

A line of different hyaluronans in skin chrono and photoaging: a review of the literature and usage protocols

Marina Romagnoli[1], Patrizia Piersini[2], Domenico Romano[3], Gilberto Bellia[4], Gabriel Siquier-Dameto[5]

[1]Private Practice, Genoa, Italy
[2]Private Practice, Turin, Italy
[3]Private Practice, Como and Lugano, Italy and Switzerland
[4]IBSA Farmaceutici Italia Srl, Lodi, Italy
[5]Private Practice, Amsterdam and Mallorca, The Nederlands and Spain. Research Group of Clinical Anatomy, Embryology and Neuroscience (NEOMA), Department of Medical Sciences, Universitat de Girona (UdG), 17003 Girona (Spain)

Abstract
Intrinsic and extrinsic factors play a role in the skin aging process, including chronological aging and photoaging respectively. The physical changes associated to skin chrono and photoaging have a significant psychological impact, prompting the search for preventive and maintenance measures. This article presents a range of branded hyaluronic acid-based products with different concentration and molecular weight alone or in combination with antiaging/antioxidant complex. It also presents specific administration protocols for deep skin hydration and for the correction of skin discolouration and signs of chrono and photoaging, based on a literature review and on the clinical experience of a group of experts. Hyaluronic acid-based products with different molecular weights and rheological characteristics are available and may represent useful tools to help reduce, delay, and partially repair age-related skin changes. The selection of the most appropriate products and the application of the most suitable usage protocol may improve skin results.

Keywords
Hyaluronic acid, deep hydration, protocols, chronoaging, photoaging

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Corresponding Author

Marina Romagnoli, MD

E-mail: inforomagnolimarina@gmail.com

Introduction

Aging causes important bodily changes, which are easily visible in the body's most superficial tissue, the skin. Wrinkles, dryness, any loss of elasticity and hydration are some of the most evident changes in the skin with aging, even easily observable by non-expert eyes[1,2]. This review will discuss the structure and function of the skin, with special emphasis on the components of the extracellular matrix of the skin, as well as the changes that chrono and photoaging cause to this tissue, especially on the changes that influence the components of the extracellular matrix of the skin, and the cellular and molecular mechanisms implicated in its chrono and photoaging. In addition, a literature review has been carried out on the line of a hyaluronic acid (HA) based compound illustrated in this article.

Anatomy and Physiology of the Skin

The skin is the largest organ of the body, accounting for about one sixth of the body weight and is a dynamic and regenerating organ. It is composed of three layers: the epidermis, the dermis, and the subcutaneous tissue[3] (*Figure 1*).

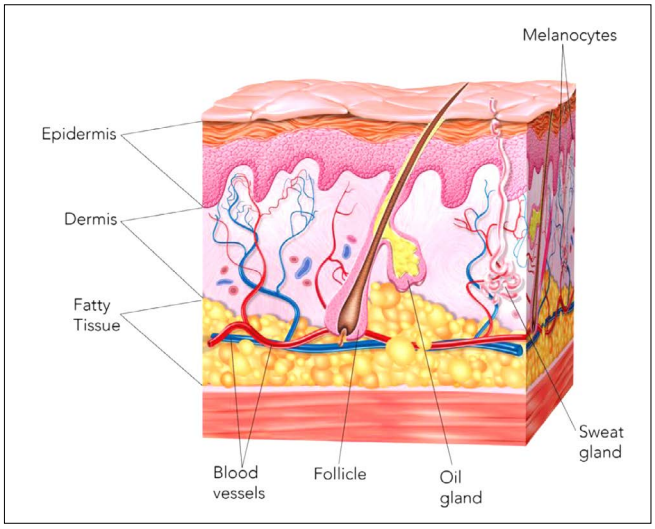

Figure 1 *- Diagram of human skin structure.*

The outermost layer, the epidermis, is a squamous epithelium stratified in five layers, from top to bottom: (I) the *stratum corneum*, (II) the *stratum lucidum*, (III) the *stratum granulosum*, (IV) the *stratum spinosum*, and (V) the *stratum basale* or *germinativum*. The epidermis also contains Langerhans cells, namely dendritic cells acting as antigen presenting cells, melanocytes, which contain melanin for UV protection, Merkel cells, that communicate with cutaneous neurons contributing to our touch, and memory T cells, acting as detectors and controllers of previously encountered pathogens[3]. The middle layer, the dermis, is mainly made up of collagen, a fibrillar structural protein, with a small amount of elastin[4]. The different dermis cells are fibroblasts, mast cells and histocytes. This layer also contains sweat glands and follicles, nerves, and lymphatic vessels. Under the dermis lies the deepest layer, the subcutaneous tissue, or panniculus, which contains adipocytes and has a cushioning effect (*Figure 1*).

The thickness of the different layers varies considerably: for the epidermis it ranges from less than 0.1 mm in the eyelids to 1.5 mm at the level of the palms of the hands and the soles of the feet, whereas the dermis reaches its maximum thickness at the dorsal level, where it is up to 40 times thicker than the overlying epidermis[5].
Functionally, the skin has the biggest challenge of any organ of the body, being exposed and having to first respond to environmental challenges. Beyond protecting the body from external attacks of physical, chemical, and biologic origin, the skin performs several other vital functions, such as preventing excess water loss from the body, regulating body temperature, eliminating waste, acting as a sensory organ for pain, temperature, and touch, synthesizing Vitamin D, and playing a crucial role in social interaction.
The components of the skin that make up the proteins of the extracellular matrix not only form part of the so-called connective tissue of the skin, acting as filling proteins for the extracellular space of the skin, but also play important roles in its physiology. Specifically, HA plays an important role in the proliferation and differentiation of keratinocytes after a skin lesion, regulates the entry of formed blood elements (leukocytes) in the area of skin injury, as well as regulating the secretion of collagen by fibroblasts during the skin healing process, avoiding an excess secretion of this component of the extracellular matrix by these dermal cells[5].

Skin aging: chronoaging and photoaging

Several factors may impair the skin's integrity, including the environment, nutrition, dryness/hydration, and above all the aging process[6]. The factors that play a role in the skin aging process are intrinsic and extrinsic.

Chronoaging

The intrinsic aging of the skin is a natural consequence of the physiological changes that occur over time at rates that vary depending on genetic factors, which are inalterable and is known as chronological aging or chronoaging[7,8]. With aging, skin tends to become more susceptible to pervasive dryness and itching, vascular complications, and an increased risk of skin cancer[6,9].
Age-related skin changes occur in part due to the continuous formation of a reactive oxygen species (ROS) generated by the oxidative cellular metabolism that induces cumulative endogenous damage[8,10]. Skin aging recognizes a genetic background, but the decrease in sex hormone levels also plays a role: sex steroids above all - estrogens, testosterone, dehydroepiandrosterone (DHEA)[11-13] - but also melatonin, insulin, cortisol, thyroxine, and the growth hormone. Furthermore, some signalling molecules, such as cytokines and chemokines, decline whereas others increase with age, overall leading to the deterioration of several skin functions[14]. From an anatomical standpoint, skin thickness decreases with aging, especially due to a decline in the thickness of the stratum spinosum.
The skin turnover time increases from 30% to 50% by age 80, since the mitotic activity in the basal layer is reduced, whereas the transit time to the stratum corneum is increased. The amino acid content of the corneocytes is reduced with a decrease of the natural

moisturizing factors. The overall water content of the skin decreases. The epidermis flattens, reducing its contact with the dermis, as well as the perfusion and nourishment exchanges which occur between the layers. Elastic fibres undergo irreversible structural and compositional progressive changes that eventually lead to a substitution with amorphous elastin[5-9,15]. The overall anatomical changes of the aging skin result in laxity, wrinkles, atrophy, dryness, and the development of pigment and dark spots[16]. Chronoaging causes a decrease in the proportion of large chondroitin sulfate proteoglycans (versican) and a concomitant increase in the proportion of small dermatan sulfate proteoglycans (decorin)[17]. In addition, versican and decorin of the human skin show age-related differences: versican primarily in the size and the sulfation pattern of its glycosaminoglycans (GAGs) and decorin in the size of its GAGs. These age-related differences in proteoglycan patterns may play a role in the age-related changes in the physical properties and injury response of human skin[18,19].

Photoaging

Extrinsic factors are controllable and associated to the exposure to the external environment, including smoking, pollution, lifestyles, and ultraviolet light[15]. In particular, the sunlight exerts deep effects on the skin, which accounts for up to 90% of visible skin aging[15,16,20,21], particularly in subjects with lower levels of melanocytes in the skin[22].

UV radiation causes a molecular chain reaction which eventually upregulates, in dermis and epidermis, the production of collagenase, gelatinase and stromelysin-1 in fibroblasts and keratinocytes.

This results in the deterioration of collagen, elastin, and other components of the extracellular matrix of the dermis.

Chronic exposure to sunlight induces the repeated attempts to repair the dermal matrix, with a cumulative effect on the structure and the organization of collagen itself, that ultimately become visible on the skin surface as thick, dry and rough skin, and deep wrinkles with possible actinic keratoses[23-24]. At the cellular level, UV radiation triggers the production of cytokine (e.g. IL1, IL6, TNF-alpha) by keratinocytes and prostaglandins, and histamine by mast cells in the skin. ROS by UV radiation also activates several transcription factors including NF-kappa-B (NF-k-B) and an activator protein-1 (AP-1), which are two transcription factors involved in the expression of pro-inflammatory factors. UV exposure also induces the expression of inducible cyclooxygenase (COX-2) and lipooxygenase (LOX) in human skin, leading to an increased production of pro-inflammatory mediators such as prostaglandins and thromboxanes[25]. There is enough experimental evidence proving that most of these inflammatory messengers influence skin cells, especially keratinocytes and fibroblasts, in the production of proteins and glycoproteins from the extracellular matrix, and in the generation of proteases that modify the content of the extracellular matrix of the skin[26].

All the above described physical changes associated to skin chrono and photoaging also have a significant psychological impact, since they affect a person's overall image and his/her social interactions[7,27]. This psychosocial impact of skin aging together with the dramatic increase in the aging population, has stimulated the research for an effective prevention and maintenance, where prevention means to hinder age-related skin changes to arise, while maintenance is the process to preserve as long as possible the obtained results. The great advances made in the past two decades in understanding the clinical, biochemical, and molecular changes associated with skin aging, have led to the development of many different approaches to reduce, delay, and in some cases, repair the effects of intrinsic chronological aging and extrinsic environmental damage. One of these approaches is the use of a hyaluronic acid compound, such as the line of medical devices included in this article, as its cutaneous effects can be explained, in part, by the HA induced cellular and molecular mechanisms that were previously described.

Specifically, we are focusing on the decreased production of pro-inflammatory cytokines, that inhibit the secretion of collagens by skin fibroblasts[14], and the induction of the secretion of collagen and other extracellular matrix proteins through cutaneous fibroblasts, minimizing and or counteracting the chrono and photoaging effects in the skin[28-30].

Review of the existing literature

As described above, the dermis becomes thinner and less turgid with aging, mainly due to a reduction in glycosaminoglycan content, especially hyaluronic acid (HA), that progressively decrease with aging and may even disappear after 60 years of age[28]. HA is an essential component of the extracellular matrix, influencing the skin's water content and thus the skin's hydration, flexibility, and firmness. Chemically, HA is a linear polysaccharide, derived from the polymerization of d-glucuronic acid, combined with N-acetyl-glucosamine[29]. HA promotes the proliferation of fibroblasts and its migration, as well as the collagen synthesis. More than 50% of HA in the human body is localized in the skin, where it is synthesized by fibroblasts, keratinocytes, and endothelial cells of the dermal microcirculation in many variants of different molecular weight. In the past few years, there has been an increased use of HA in aesthetic procedures, especially when filling the dermis and reducing wrinkles in facial rejuvenation procedures[30].

Several HA-based dermal fillers are available that differ in physical and chemical characteristics, affecting their overall performance.

Viscoderm® (IBSA Farmaceutici Italia Srl) is the brand of a range of HA-based products including:

- Viscoderm® (0,8-1,6-2,0%) (V08, V16, V20): containing high-molecular weight linear HA (1400KDa-2000KDa);
- Viscoderm® Skinkò and Skinkò E (VS, VSE): containg medium molecolar weight linear HA (400KDa-700KDa) combined with an anti-aging/anti-oxidant complex composed by vitamines, mineral salts and aminoacids;
- Viscoderm® Hydrobooster (VH): containing two cross linked molecular weight HA (1000KDa and 2000KDa); with peculiar rheological properties[35] conferring the product a high deformability and low stiffness and viscosity, that allow it to exert a dual hydrating and

stretching function and is particularly indicated for the dynamic areas of the face.

Review of the existing literature V08, V16, V20

V16 was studied in 30 subjects (2 males and 28 females) between 29 and 67 years of age (mean 51.30, SD 10.35) to test its efficacy and tolerability in cutaneous biostimulation[31]. The study product was administered through an intradermal infiltration according to a "picotage" micropomphous technique. Ten infiltrations of 0.1 mL each were performed in each hemiface using a 30 G needle, in each of four sessions conducted about 20 days apart from each other. The biorevitalizing effect of the treatment was assessed at each visit, immediately before and after the injection, by a skin testing equipment (Dermalab® USB-Skin testing, Cortex Technology, Denmark) measuring (I) skin hydration (in µS), (II) trans-epidermal water loss[32] (TEWL, in g/m2/h), and (III) skin elasticity expressed as the retraction of skin exposed to forced extension (in MPa). The results showed a progressive increase in hydration, reaching a statistical significance at the 4th visit, as well as in skin elasticity - significant at visit 3 and 4 - whereas TEWL did not substantially change during the treatment period. The physician judged the overall results very good in 60% of cases, good in 36.7%, and fairly good in 3.3%. The patients' mean level of satisfaction with the overall procedure, expressed on a 1-10 visual analogue scale (VAS), was 9.07 (SD 1.01). The tolerability of the procedure was excellent.

A second study was aimed at evaluating a new protocol, consisting of an injection of a cross-linked HA, (CLHA, Aliaxin® GP) followed by the injection of a non-cross-linked HA (NCLHA, V20) one month later, in comparison with the injection of CHLA alone[32]. Thirty subjects (4 men and 26 women) with moderate to severe wrinkles, were randomized to receive either one or the other procedure for the improvement of their wrinkles in different facial areas, including the glabella, upper lips, chin, cheeks and nasogenien and periorbital regions. The treatment with the combination of CHLA and NCHLA, resulted in a significantly greater improvement in the Wrinkle Severity Rating Scale, (WSRS) with a follow-up done up to 24 weeks later compared to the control group. Skin hydration and elasticity were also significantly better in the combination protocol group, and the TEWL was significantly lower. The patients' satisfaction expressed on a ten-point rating scale was 8.36 (SD 0.11).

The third trial was based on a high-frequency (20–100 MHz) ultrasound (US) assessment, a non-invasive technique used to evaluate age-related skin changes[33]. The aim of this study was to assess the long-term effects of microinjections of V20 on the echogenicity of the subepidermal low-echogenic band (SLEB), which is known to be photoaging-related (the lower the SLEB echogenicity, the higher the photoaging).

Twenty-two women with clinical and US signs of moderate photoaging were enrolled. The treatment consisted of multiple microinjections of the study product (1 mL) in the dorsum of one hand, once a week for 4 weeks, and then following a monthly routine. Patients were randomly assigned to receive monthly injections for 4 months (group A) or 9 months (group B). The dorsum of the other hand of each subject was injected with a saline solution and served as control. All subjects underwent a high-frequency US in order to measure the changes in SLEB echogenicity during the treatment. Only 18 of the 22 enrolled patients completed the study. At the end of the weekly injection period, a statistically significant increase of dermal echogenicity compared to the control hand was observed in 13 subjects, classifying them as "responders". In these patients, the difference was still significant after 4 (group A) and 9 (group B) additional months of monthly injections.

The authors conclude that mesotherapy with the studied medical device may effectively improve skin aging and photoaging, as objectively measured by the US, showing significant changes in SLEB density.

Review of the existing literature VS, VSE

VSE was studied in an in vitro model to assess its effect on the human skin fibroblast (HFb) biorevitalization (BR), a process of direct supplementation with HA alone, or in combination with other molecules such as vitamins[34]. For the study of BR, HFb were seeded on a layer of three medical devices with different concentrations of HA: a solution of HA 6.4 mg/mL with amino acids, mineral salts, and vitamins (VSE); a HA gel 10 mg/mL and polynucleotides (Newest, Mastelli); HA gel 20 mg/mL in saline solution (Restylane® Vital, Galderma). BR with the 3 products resulted in the activation of the hyaluronan synthase 1 (HAS1), which was highest using VSE. The stimulation of hyaluronidase 1 (HYAL1) was observed with all the tested products. BR was also used to activate metalloproteinases (MMPs), especially MMP3. The authors concluded that a better comprehension of the biology of fibroblasts fibroblast might allow a more proper clinical application of BR protocols.

An animal study evaluated the safety and histological biocompatibility of VSE and of another crosslinked HA products, Aliaxin® GP, a cross-linked HA filler, both injected into the skin of guinea pigs[36]. The study showed that the two compounds induced no significant inflammatory reactions, and increased collagen and elastic fibres in the skin. The same authors then also performed a test in the clinical setting, administering the same crosslinked gel followed by VSE to patients with moderate-to-severe wrinkles affecting the nasolabial folds[36]. The sequence of the two products resulted in a greater improvement in the hydration of the nasolabial fold, TEWL, and wrinkles compared with the crosslinked gel alone.

The efficacy and safety of VSE were clinically evaluated in a mesotherapy "biorevitalization" (BR) protocol for the treatment of skin aging and chronoaging[37]. A total of 64 female volunteers from 37 to 60 years of age (with a mean age of 52) were enrolled. The BR protocol consisted of multiple microinjections at a distance of 1–2 cm from each other, with either a 27 G or a 23 G needle, in the face (external corner of the eye and cheek), neck, low neckline, and back of the hands. Three mL of the product were injected in the face and 5 mL were injected overall in the other areas.

Four sessions of BR were performed for each subject at 3-week intervals, and the aesthetic result was assessed at baseline and after 6, 9, and 12 weeks. Hydration of the deep skin layers (assessed at 1.5 mm and 0.5 mm

of depth) was obtained by a MoistureMeterD (Delfin Technologies, Kuopio, Finland). Other instrumental evaluations included skin spectrophotometry and optical colorimetry. Pictures of the wrinkles were taken and a profilometry was performed to calculate the average roughness of the analysed profile, the total wrinkle height, and the maximum wrinkle depth. Clinical evaluation scales included the Glogau scale and the Facial Volume Loss Scale plus internal reference clinical scales for the neck, low neckline, and hands. Furthermore, a phototest was performed, by exposing the subjects' dorsal skin to six incremental doses of UV radiation, in order to assess the effect of the BR protocol on the UVB-induced erythema.
The results showed an antiaging effect of the BR protocol, with the studied product as early as from the second treatment session, based on all the instrumental evaluations. A clinical and statistically significant improvement was observed in terms of profilometric parameters, skin brightness, pigmentation, and deep skin hydration. The visual score of the UVB-induced erythema significantly decreased compared to the baseline, indicating a photoprotective effect of the product. The main results at 12 weeks after the start of the treatment are summarized in *Table 1*. The author concluded that the studied product induced clinical and biophysical changes in the skin, showing a multifunctional activity. Thanks to the antioxidant activity of its ingredients, including lipoic acid, VSE also exerted a protective effect against damage caused by UVB exposure.

Review of the existing literature VH

The efficacy and tolerability, and the duration of the effect of VH were assessed in human volunteers with moderate aging/photoaging, both clinically and by non-invasive instrumental evaluations[35]. The clinical evaluation was based on the visual score of deep and fine wrinkles according to the Glogau's reference scale (0 = no wrinkles to 4 = only wrinkles with severe photoaging), and, for the surface micro relief, on the Beagley Gibson reference scale (from 1 to 4). Eighteen volunteers (35-55 years) were enrolled and underwent two injections of VH two months apart from each other. The subjects were evaluated at baseline, 48 hours after each injection and finally 5 months after the first injection. A significantly progressive improvement of the periocular wrinkles' grade, vertical lip lines and the depth of nasolabial folds was recorded at all evaluation timepoints. The Aging/photoaging grade and surface microrelief improved from 2 months after the first injection. These clinical improvements were supported by instrumental improvements for the skin profilometry and optical colorimetry. The injections were very well tolerated as assessed by a self-grading VAS score. The authors conclude that the studied product showed a good aesthetic performance and a long duration of the effect, up to 3 months of follow-up after the 2nd injection, on dynamic facial wrinkles and/or static facial lines.
VH was administered to 100 consecutive women with a Glogau grade of 2-3 wrinkles, requiring deep hydration, according to the judgment of a medical expert. Three Italian dermatologists administered 2 injections of VH 2 months apart[38]. The subjects were assessed at baseline, two months after the first injection and three months after the second injection. The aesthetic results were judged as highly satisfactory by both patients and dermatologists and the tolerability profile was widely reassuring.

Suggested protocols

With the aim of defining specific procedures for the different patient's needs – ie, hydration, dyschromia, chronoaging, and photoaging, – based on the treatment approaches adopted in the clinical studies described above, integrated with the clinical experience, we developed different standardized treatment protocols with homogeneous timescales, consisting in three sessions taking place every three weeks, as explained below. For each protocol, 10 patients were evaluated in a real-life setting, with the collection of photographs by VISIA before and after the treatment.

Hydration protocol

V08, V16, V20 are the products indicated for the superficial hydration of the skin, and densification of the dermis. The clinical experience published by Coacci showed a significant increase in face hydration with multiple infiltrations of V16 in repeated sessions[31], and a significant improvement in wrinkle severity through a combined protocol[32]. Based on these results, the protocol proposed for skin hydration consists of 3 injections of V16, 21 days apart from each other (*Figure 2*). In specific cases, based on the individual skin hydration level, V08 or V20 could alternatively be used. The injection technique used for this procedure involved micro-boli (0,02 mL) in the superficial dermis spaced about 1 cm apart, as they are recommended for a more superficial hydration (*Figure 3 A* and *B*).

Parameter	Instrumental evaluation	% variation vs baseline at 12 weeks
Deep skin hydration 1.5 mm	Tissue electric constant of deep skin layers	+2.3%*
L* parameter skin brightness	Optical colorimetry	+2.2%*
B* parameter skin pigmentation	Optical colorimetry	-7%*
Skin erythema visual score	Phototest	-36.6%*
Crow's feet roughness	Clinical evaluation	+58%*
Hand surface microreliefs	Clinical evaluation	+60%*
*Dunnett test p<0.05		
(Modified by) Sparavigna A et al. 37 Clinical, Cosmetic and Investigational Dermatology 2015		

***Table 1** - VSE Biorevitalization protocol: summary of main results at 12 weeks.*

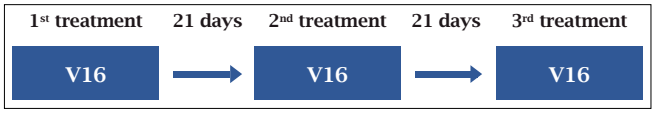

Figure 2 - Hydration protocol scheme.

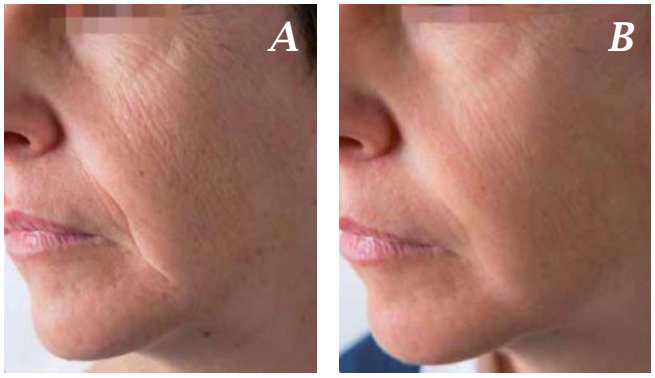

Figure 3 - A) Before hydration protocol; B) 1 month after hydration protocol.

Skin discoloration protocol

VS and VSE are specifically formulated for the protection and prevention of damage from UV radiation, and the attenuation of skin discoloration. Based on the favorable results, measured by instrumental assessments, obtained by Sparavigna et al.[35] in a mesotherapy BR protocol, the proposed protocol for skin discoloration and prevention of UV damage consists of 3 sessions, 21 days apart, (*Figure 4*) of multiple VSE micro-boli in the superficial dermis, 1–2 cm from each other (*Figure 5 A* and *B*).

Figure 4 - Skin discoloration protocol.

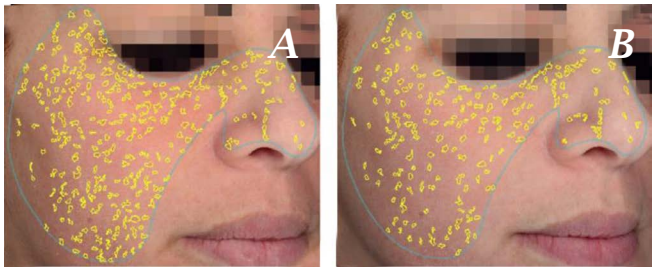

Figure 5 - Dyschromia improvement before (A) and 1 month after discoloration protocol (B) (11% improvement in dyschromia).

Chronoaging protocols

VH is the product of the line with dual hydrating and stretching properties that make it particularly suitable for attenuating the effects of chronoaging.
For the dynamic areas of the face, the suggested protocol consists of a first session of VH injections for a deep skin hydration, followed, after 3 weeks, by a session of V16 injections for a superficial hydration, and concluded after another 3 weeks, by another session of VH injections (*Figure 6*).
For a more deep hydration, the recommended injection technique is a retrograde micro-linear technique, consisting of microinjections of <0,05 mL of product in the mid-deep dermis of the target area (malar/submalar); a subsequent gentle massage will allow for more uniform results. For Glogau grade 2 wrinkles (dynamic wrinkles), stretching is best obtained by <0.01 mL close micro-boli along the path of the wrinkle, followed by gentle massage. In case of wrinkles of Glogau grade 3 (wrinkles at rest) a combined injection technique is suggested: a retrograde micro-linear technique with <0.01 mL immediately below the wrinkle path as support, combined with a <0.01 mL close micro-boli along the wrinkle path (for stretching). A gentle massage is always recommended to even out the results (*Figure 7 A* and *B*).

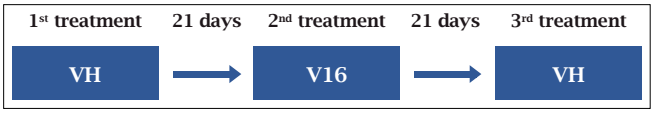

Figure 6 - Chronoaging protocol for the face.

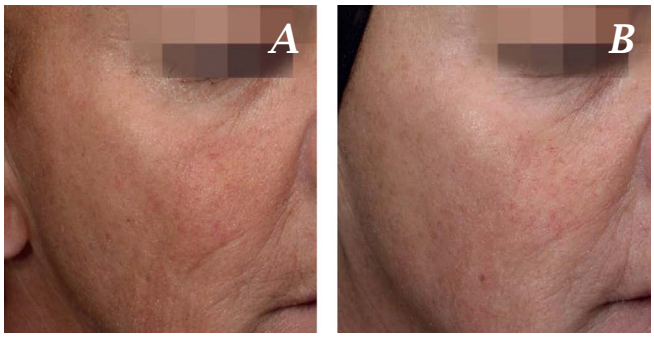

Figure 7 - A) Before chronoaging protocol; B) 1 month after chronoaging protocol.

Photoaging protocol

In the literature, positive results in improving clinical and instrumental signs of photoaging have been obtained by V20 33, VSE 37 and VH 35, used individually. To reduce discoloration and improve skin uniformity in the face, we propose a combination protocol, starting with hydrostretching by VH, followed by the reduction of UV damage by VSE after 21 days, and concluding after another 21 days with another session of VH (*Figure 8*).
The techniques suggested are the same of chronoaging and skin discoloration. A gentle massage is always recommended to even out the results (*Figures 9 A* and *B*).

Figure 8 - Photoaging protocol.

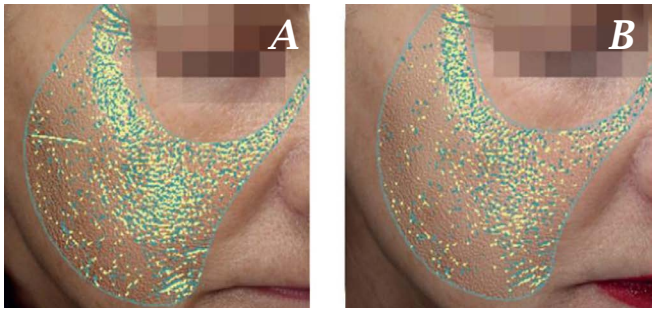

Figure 9 - Images of improvement in uniformity. Before (A) and after 1 month (B) photoaging protocol.

Conclusions

HA-based products with different molecular weights and rheological characteristics may represent useful tools to help reduce, delay, and partially repair age-related skin changes. The selection of the most appropriate products and the application of the most suitable usage protocol may improve skin results.

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Conflict of interest

Medical writing has been sponsored by IBSA Farmaceutici Italia Srl. GB is an employee of IBSA Farmaceutici Italia Srl. The authors report no other conflicts of interest in this work.

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aesthetic medicine

Mini review

Dermatological presentations in COVID-19 patients: perspectives on the present and the future

Suresh Kanna S[1], Shradha L[1], Mathisha Ebby Perin[1], Srinivasa Rao Gopisetty[1], Goutham Kumar AP[1]

[1]Department of General Medicine, Sree Balaji Medical College and Hospital, Chennai, India

Abstract

The Coronavirus 19 (COVID19) disease is a global pandemic caused by the Severe Acute Respiratory Syndrome Coronavirus 2 (SARSCoV2). Extrapulmonary symptoms related with COVID-19 have grown more prominent in recent months, particularly within dermatological manifestations. As a result, dermatologists should be familiar with the different ways in which the COVID-19 disease can present itself, and what to look out for if a COVID-19 patient appears to have skin lesions. When coming in touch with a suspected or confirmed case of COVID-19, personal protective equipment must be used. However, its use has been linked to dermatological adverse effects, which dermatologists practicing during the COVID-19 era should be aware of. Tele dermatology can help to avoid these problems, and should be made more widely available, especially in rural locations. By examining PubMed and a few review articles on dermatological presentations in current and future views for covid19, a systematic review was done. As a result of the variable nature of COVID-19-related cutaneous symptoms, our group identified six basic clinical patterns: Papulovesicular exanthem, a chilblain-like acral pattern, a livedo-reticularis-racemose- like pattern, purpuric “vasculitic” papulovesicular exanthem, and a confluent erythematous/maculopapular/morbilliform rash. With an emphasis on the clinical characteristics and therapeutic treatment options for each subcategory, this review presents an overview of the COVID-19-associated cutaneous symptoms.

Keywords

Chilblain-like acral pattern, confluent erythematou, COVID 19, maculopapular, morbilliform rash, papulovesicular exanthem

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Corresponding Author

Suresh Kanna S, MD

Associate Professor, Department of General Medicine, Sree Balaji Medical College and Hospital, Chennai-600044, India

Email: sureshkannatmc@gmail.com

Introduction

The "severely respiratory clutter coronavirus 2" (SARS-CoV-2) was found in December 2019 in patients with pneumonia in Wuhan, China. "Coronavirus disease 19" (COVID 19) has spread over the world at an alarming rate since then, becoming a global pandemic emergency[1]. Aside from causing a fever and respiratory symptoms, COVID 19 has been related to a number of extrapulmonary symptoms, along with dermatological ones[2]. The increase in Covid 19 cutaneous symptoms is occurring at a rather vague frequency, and the role of SARS-CoV-2 in their pathogenesis is still debated. The following six key clinical types of COVID 19-associated cutaneous symptoms were hypothesised in a recently published review study by our group: Urticarial rash, confluent erythematous/ / morbilliform /maculopapular rash, chilblain-like acral pattern, papulovesicular exanthem, livedo reticularis/ racemose pattern, purpuric "vasculitic" pattern (*Figure 1*). The cutaneous symptoms of COVID 19 have been summarized in this article, which is divided into sections. Clinicians should be familiar with COVID-19's dermatological presentations, as they can sometimes be mistaken for only one symptom of the disease. A greater understanding of these characteristics could aid in the early detection of COVID 19 as well as the prognosis and management of the disease. Skin lesions in people with the SARS-COV-2 infection were a common occurrence during the COVID 19 pandemic.

According to several case reports, urticarial injuries are the most common symptom of COVID-19, with antihistamines being the most common treatment[3,4]. Broad urticaria has been reported in a few patients who have experienced more severe side effects. The first symptom of the infection could be an urticarial exanthem on both sides of the thighs and buttocks[5,6]. Pruritic erythematous plaques over the face and acral regions are another early symptom. Nonpruritic urticaria in pediatric patients is described in detail[7]. Urticaria has also been found to be late dermatologic evidence of a viral infection, according to research. Undefined, pruritic eruptions appeared on the patient's torso, back, and extremities after a month of battling the persistent viral infection. It was revealed that the dermis had perivascular lymphocytic infiltrations and dilated blood vessels. Fortunately, the patient's urticaria subsided after one week, and the patient's swab of the nasopharynx was negative as well[8]. Low-dose systemic corticosteroids have been advocated by Shanshal[9] as a restorative option for the COVID-19-associated urticarial syndrome.

As a matter of fact, the developer hypothesised that low dose systemic corticosteroids combined with nonsedating antihistamines could assist a moderate COVID-19's hyperactivity.

Morbilliform Rash

When it comes to viral exanthems, a morbilliform rash is a prevalent morphology. An erythematous/ morbilliform eruption was seen in 14 of 18 COVID-19 individuals in an Italian cohort who had cutaneous abnormalities outside the norm. COVID-19 patients with generic maculopapular/ morbilliform emissions have also been documented by a few Spanish groups, with at least one case of COVID-19. Therapeutic Options: Intersecting erythematous/ maculopapular/morbilliform hasty shifts in accordance with the severity of the clinical presentation. In most cases, topical corticosteroids are sufficient. In more extreme and broad introductions, systemic corticosteroids should be more controlled[10-12].

Vesicular Eruptions

COVID-19 patients have been shown to experience varicella-like vesicular eruptions[13,14]. Researchers in Italy identified 22 individuals with varicella-like vesiculopapular emissions that were geographically spread, representing the biggest cohort of COVID 19 patients with similar cutaneous eruptions[6].

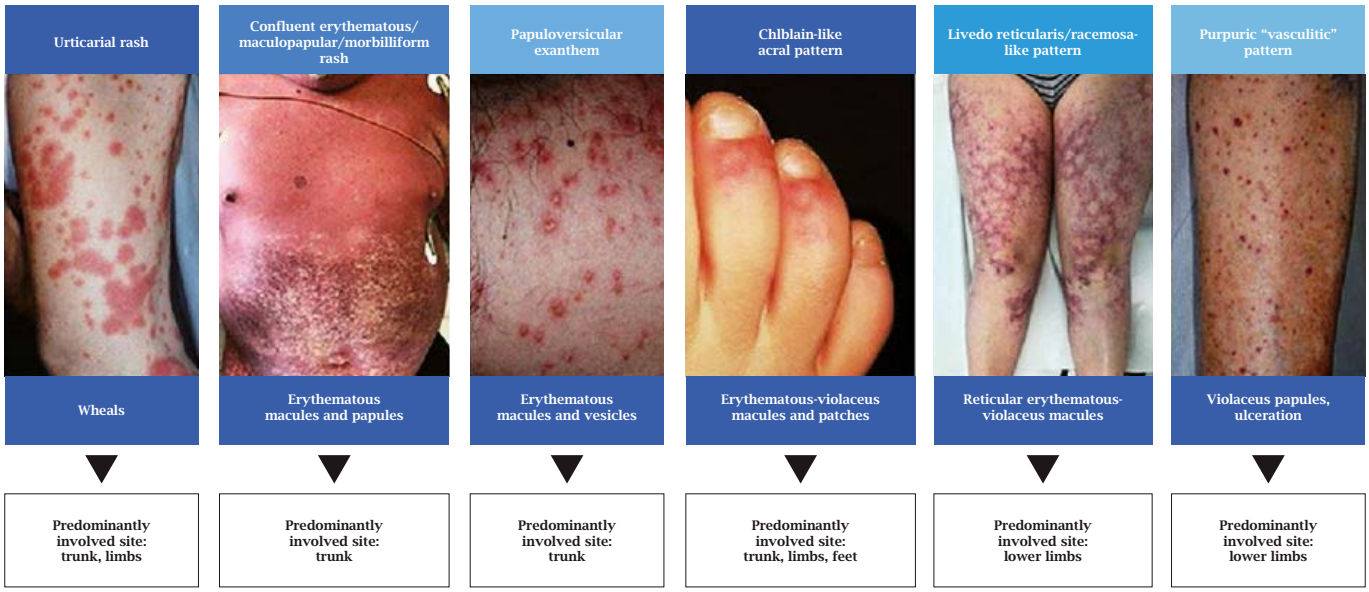

Figure 1 *- Urticaria.*

These vesiculopapular eruptions were found to be unique to COVID-19 patients, according to the creators. The researchers concluded that these vesiculopapular eruptions were unique to COVID 19, citing the fact that no one in their cohort had been exposed to current medicines in the 15 days leading up to the onset of the eruptions. COVID 19 systemic adverse effects resulted in an average of 3 days of inactivity, whereas skin symptoms lasted an average of 8 days. There was no discernible link between the severity of the COVID-19 disease and cutaneous emissions. The main Histopathological highlights were apoptotic keratinocytes on the surface of the exanthem, which is comparable to what has been discovered in prior viral exanthems. Therapeutic Options: There are no conventional treatments for a COVID-19-related papulovesicular exanthem, which is similarly self-healing in a short period of time. As a result, a "wait and see" approach may be advocated.

Chilblain Like Lesions

An Oedematous swelling of the fingers and toes is categorized within chilblain-like injuries. They are often erythematous to violaceous papules and macules with potential bullae on the fingers, and do not leave a scar. Skin biopsies show necrotic keratinocytes and a thicker storm cellar film, indicating a histopathologic pattern of vacuolar interface dermatitis. Lymphocytic penetration occurs in a perieccrine dispersion, and plasma cells are occasionally encountered. The dermatopathologic findings are similar to chilblain lupus, but there is no skin edoema[3]. There have been occurrences of COVID 19, which affects children and young people more frequently, where these eruptions have been observed. When the virus has been infected the patient for around 14 days, the symptoms usually subside. Pruritus or pains are frequently accompanied with chilblain-like outbreaks. They're usually asymmetrical, and they show up on toes more often than on fingers[4]. COVID-19 cases without systemic symptoms have been found to appear with chilblain-like lesions on the dorsal fingers[5]. Milder infections are typically associated with acral erythema and chilblain-like lesions. The presence of these indicators in younger people is another favourable prognostic factor[3].
Alternatively, the "wait and watch" system may be the best way to approach the situation to see if COVID-19-associated chilblain-like acral lesions are able to heal on their own.

Livedoid Eruptions

Several cases of livedo reticularis-like eruptions have been documented in COVID 19 patients in the United States[15,16]. It is possible that the livedoid alterations are unilateral. These lesions are thought to be secondary to COVID19-induced thrombotic vasculopathy, which could be significant. If COVID-19 individuals with systemic thrombotic vasculopathy develop livedoid eruptions, a clinical recognition of these eruptions will be critical, and there may be a predictive value in these patients. At least one of the patients with a livedoid eruption was admitted to the hospital and required supplemental oxygen, but the result is however unknown. It is divided into two types:
(1) Livedo racemose, which appears as bigger, irregular, and asymmetrical rings like livedo reticularis.
(2) Livedo reticularis, which appears as tight, symmetrical, lace-like shadowy patches forming complete rings around a light center and is commonly associated with cold-induced cutaneous vasoconstriction or vascular flow disturbances such as polycythaemia.
(3) Alternatives to the Traditional Treatment COVID-19-associated chilblain-like acral lesions have no effective treatment choices, and they tend to heal spontaneously, therefore a "wait and see" strategy can be recommended.

Purpuric Vasculitic Lesions

Age-related COVID-19-related mortality is associated with purpuric lesions in elderly patients with severe COVID-19[17]. They are probably the most common cutaneous signs of a possible COVID-19-related death. This view is supported by the poor prognosis reported in some cases which is documented in literature[18,19]. The purpuric pattern indicates the existence of vasculitic alterations, which are most likely caused by the virus's direct impact on the endothelial cells, or by COVID-19's dysregulated host inflammatory responses.
Purpuric lesions might be widespread[20], confined in the intertriginous areas[21], or distributed acrally[22]. Hemorrhagic blisters can develop from vascular lesions. In the most severe cases, extensive acute necrosis and significant coagulopathy might be found. Pupils with a yellow globule center, and an incomplete violet border, were seen through a dermoscopy of the purpuric lesions[23].
Therapeutic alternatives such as topical corticosteroids have shown to be effective in the treatment of minor purpuric lesions. Systemic corticosteroids may be used to treat cases with extensive necrotic-ulcerative lesions.

Discussion

COVID-19-related cutaneous symptoms have become more common in recent months, acquiring more importance in the eyes of the international scientific community, as well as the media. Several months following the outbreak of COVID-19, a number of narrative and systematic analyses of the different virus-related skin symptoms were published. A summary of the clinical aspects, histology results, the severity of the different COVID-19 systemic symptoms, and the therapeutic options for the multiple COVID-19 related cutaneous manifestations are shown in the table above. Even though there are various ideas on the pathophysiological mechanisms behind these skin results in literature[26,27,28], none of them are supported by substantial data, and this topic remains mainly unexplored.
Additionally, cutaneous eruptions caused by viruses other than SARS- CoV[22-24,25]or medicines used to treat this infection[29,30] must always be checked out.
To shed a light on this unique, understudied, and fascinating topic, a further experimental pathophysiology research and clinical data collected from extensive case series are needed.

Conclusion

The most important message is that although COVID-19-related cutaneous symptoms are becoming more common, their pathophysiological pathways must be thoroughly investigated. The diseases can be classified into six clinical phenotypes, each with distinct histological features.

Conflict of Interest

None declared.

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President: J. A. Lopez
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Mexican Scientific Society of Aesthetic Medicine
Pepsi Center, WTC Mexico City
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Email 2: coordinadora@groupdmc.com
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