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Contents

Editorial

Can the pandemic by COVID-19 (SARS-CoV-2 infection) increase the number of adverse effects after the use of dermal fillers?

On January 7th, 2020, the appearance of a new coronavirus (CoV) was officially reported in Wuhan (China). At the time, no one could have suspected the chain of events that, with unusual speed, would lead to the greatest pandemic affecting the world population today.

Medical literature on COVID-19 is currently overwhelming, with new findings being published every day. However, we are far from knowing all the intricate details about its mechanism of action, its physiopathology, the response it elicits on different subjects or even its symptoms. Questions are accumulating and we still have a lot of work ahead of us to learn about and fight this virus.

We do agree that it spreads easily, that it is not just a lung disease, and that it causes significant changes in the immune system.

We also know that there are many asymptomatic carriers and people who have suffered mild, and even moderate, forms of COVID-19, whose diagnosis could not be confirmed by the different tests available.

In Spain, as well as in other countries, the severity of the pandemic called for a mandatory confinement of the general population and the declaration of a state of emergency, which entailed the closure of Aesthetic Medicine clinics from mid-March until mid-May.

In general, those measures intended to ensure the safety of the medical body and patients have been recorded in a protocol.

However, we are solely responsible for the reevaluation of our actions with regard to potential risks that some of our therapies may entail.

In recent days, we have witnessed the increasingly number of warnings issued by several sources about possible complications that we will have to deal with in our daily practice. Despite that an analysis of said sources exceeds the aim of this brief text, a few are mentioned below:

- The *Joint Council of Cosmetic Practitioners* (JCCP) from the United Kingdom has proposed a recommendation guideline that states the following: "There is increasing evidence that dermal fillers given in the presence of any viral infection can increase the risk of delayed hypersensitivity reactions."
- The publication of a review in *J Cosmet Dermatol* (2020; 00:1-4): Aesthetic Dermatology Procedures in Coronavirus Days highlights the following: i) permanent filler materials and some resorbable ones may cause chronic inflammation; ii) in comparison, reactions are minor (in principle) when the material used is hyaluronic acid; iii) there are also late hypersensitivity reactions to hyaluronic acid; iv) viruses may activate cytokines and T cells, and promote a proinflammatory state, therefore they recommend: v) to return to antibiotic empirical treatment (macrolides or tetracyclines); vi) use needles with less caliber; and vii) avoid high-risk areas.

In order for this to be reflected in patients' medical history, their documentation must be duly adjusted and this new information must be included in the informed consent provided to the patient. The professional, for his/her part, must consider this possibility in terms of the care that patients may require after the performed procedure.

Reflections by Paloma Tejero, MD, PhD

In my personal experience, because of my doctoral thesis on adverse effects of filler materials in 2013 and because I was part of the SEME committee of adverse effects, my colleagues usually referred patients to me or consult with me on different issues regarding filling materials. From May to July 2020, I have received several reports of exacerbated inflammatory responses after implant placement. Some have called my attention, particularly: i) non-permanent fillers in the perioral area; and ii) two patients with granulomatous abscess of permanent fillers that had been inactive for 14 and 7 years, respectively. All patients had negative serology results, although they reported having been near patients with COVID. However, there are also studies that support the disappearance of antibodies after two or three months of exposure.

In the nearby future, we will be able to weigh on the usefulness of these events considering several factors, which will prevent biased observations: i) accept that AE reporting has been very low (or non-existent) during the months of confinement, and measure it; ii) assess and compare data against reported AEs between March and July of 2019; and iii) assess the frequency with which permanent fillers have AEs within five years of their implantation.

In a word: we currently have more questions than conclusions, but I believe it is important to be alert, try to minimize risks and conduct prospective studies that allow us to learn about the possible interaction between COVID-19 and our practice.

Reflections by Hernán Pinto, MD, PhD

As it happens with any other topic that becomes fashionable, words fly. However, this is a fashion that has been imposed by the circumstances we are living in. And each one of us must do whatever we can to improve our own lives and safety, as well as those of our families, friends, professional colleagues and patients.

Every day we learn more about this virus and COVID-19. But, as usual, a serious search for knowledge gets us answers that, in turn, raise more questions. And, unfortunately, the relationship is not linear: for each answer we get, several new questions emerge. That is, each day we know more than the day before but, at the same time, what we have left to learn also increases. The more we study, the more we have left to study. It is normal.

The protocolization of Aesthetic Medicine practices surrounding all the implications that this virus may have is now a necessity. However, the evidence we have is dissimilar and contradictory, both in terms of quality and conclusions. In a word, we don't know what it is going on. The creation of evidence from and for our collective has become fundamental because dermal fillers represent a high percentage of aesthetic-medical practice. That is why the Spanish Society of Aesthetic Medicine (SEME) will create a commission to study the relationship between Aesthetic Medicine

and the coronavirus (COVID-19), which will allow us to combine our efforts and, among other things, sponsor scientific evidence-based studies nationwide in order to ensure patients' safety.

Hernán Pinto
Main Handling Editor

Paloma Tejero
Spanish Society of Aesthetic Medicine

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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.

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Original Article

Liplush Analysis. Dimensional analysis of the lips in relation to the lower third of the face during lip augmentation with hyaluronic acid

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Short title: Liplush Analysis

Abstract

Background: Historically, full lips have been considered a symbol of beauty and youth. This is the reason why lip enhancement has become an extremely popular and requested procedure. Hyaluronic acid is the filler of choice for the majority of practitioners due to its undoubtable advantages. Many papers have been written concerning the filling materials and injection techniques but so far no proper guide was created to perform a preoperative assessment of lips in order to reach a pleasant result.
Aim: Our work suggests some useful points and measures that could help the clinician to evaluate the lips themselves and in relation with the face. We called this evaluation method “liplush analysis“.
Methods: All patients enrolled in our study underwent lip augmentation with HA. The amount of filler and the lip areas needing augmentation were carefully assessed applying the “liplush analysis“ before the procedure.
Results: The percentage of satisfaction among the patients was stunningly high.
Conclusion: We suggest “liplush analysis“when approaching lip augmentation in cosmetic practice.

Keywords

Liplush, liplush analysis, lip, lower third, filler, hyaluronic acid

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Introduction

Lips play a major role among facial aesthetic features and for this reason they represent one of the most frequent targets in cosmetic procedures. Photomorphometric analysis and 3D stereophotogrammetry studies show that wider and fuller lips are a mark of attractiveness[1-2].
Anatomically lips create a transition zone between the facial skin and the oral mucosa. Their morphology tends to change with the ageing process, and is further worsened by extrinsic factors such as excessive sun exposure and smoking. Lips tend to increase until puberty, due to muscular and glandular hypertrophy, afterwards they progressively decrease as consequence of skin and supporting tissue changes as well as bony resorption.
Ageing also leads to pallor of the vermilion that results in the loss of sharp vermilion-cutaneous junction demarcation[3-4].
Both Caucasian men and women have similar hard and soft tissue age related volume loss resulting in a thinning of the vermilion and of the cutaneous portions of the lips.
Generally, women have a higher tendency to develop rhytids of the upper and lower lip, especially if smokers. Furthermore, lips play a major role in phonation, mimic expression and light touch sensation. The lips are perhaps the most movable expressive aesthetic unit of the face; they are believed to be a cathartic point by movie stars and fashion managers. Based on a study published in 2004, it was found that models, in general, appear to have fuller and better-defined lips than non-models[5]. Because of this, lip enhancement and redefinition are one of the most often requested procedures in cosmetic practice. Data provided by the American Society of Plastic Surgeons (ASPS) related to 2019 confirm that lips redefinition and augmentation procedures showed an increasing trend. Compared with the previous year (2018), the augmentation with soft tissue fillers increased by 2% and lip surgery augmentation procedures increased by 4% [6].

The aim of upper lip augmentation is a natural three-dimensional (3-D) enhancement of lip volume with good definition of the vermilion border; while for the lower lip it is advisable to magnify the prominence and the projection of the vermilion in proportion with the upper lip[7]. In both cases, it is important to achieve harmonic facial traits, and at the same time maintain the patient's unique features, while avoiding overcorrection.
Ideal face proportions are of common knowledge.
The face may be vertically divided into thirds (*Figure 1*); and it is possible to identify:
- Upper facial third: from trichion (hairline) to glabella
- Middle facial third: from glabella to subnasale
- Lower facial third: from subnasale to soft tissue menton

The vertical facial thirds should be approximately equal, although the lower facial third may be slightly greater than the middle third.
The lower facial third may be further subdivided (*Figure 2*), with the upper lip forming the upper third and the lower lip and chin forming the lower two-thirds; the ideal vertical ratio is 1:2,3.
On the other hand, for the facial transverse analysis, the face may be transversely divided into five equal parts (*Figure 3*).
- The central fifth of the face is delineated by the medial canthi of the eyes; normally the alar base width should be roughly equal to the intercanthal width.
- The medial fifth of the face is measured from the lateral to the medial canthi of the eyes. Bigonial width is slightly greater than biocular width (distance from the left to the right lateral canthus). Bigonial width should be approximately 70-75% of bizygomatic width.
- The lateral or outer fifth of the face is measured from the lateral canthus of the eyes to the helix of the ear.

As we would further explain in detail, evaluation of the lips in relation to lower facial third is mandatory to obtain harmonic results.
Lip enhancement can be achieved through several methods. Hyaluronic acid injection is the most popular technique nowadays. This filler is safe, reliable and versatile.

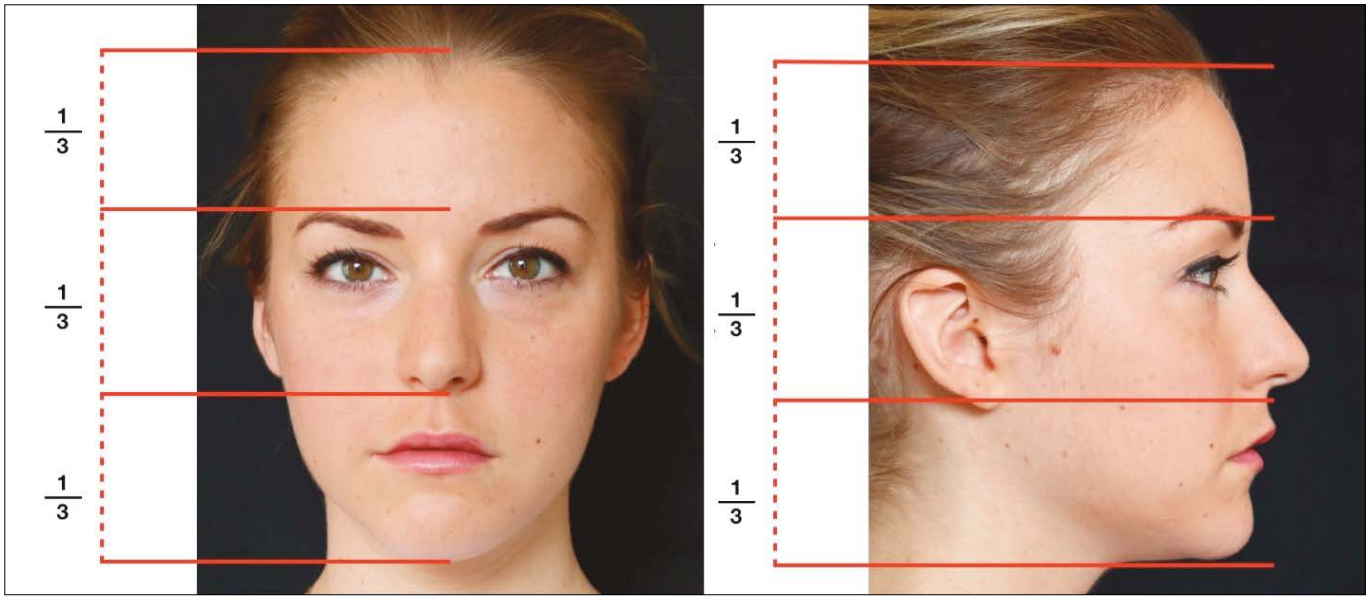

Figure 1 *- The face is divided vertically into thirds: upper third, middle third and lower third; all equal in vertical height.*

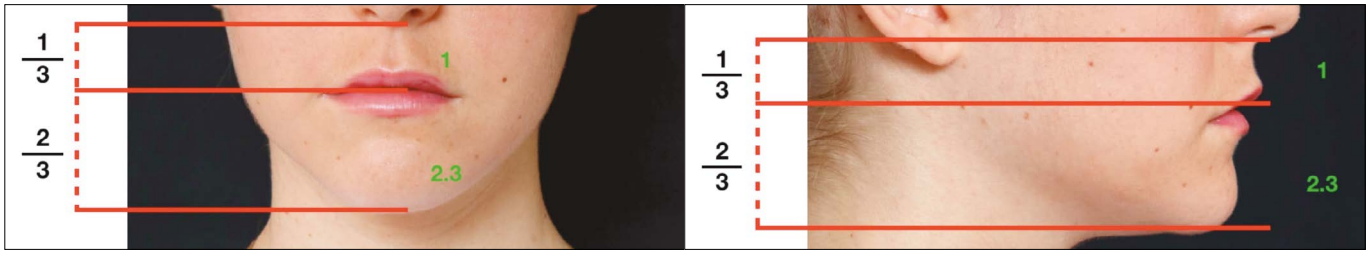

Figure 2 *- The lower third of the face is also divided vertically into thirds (in frontal and lateral view): the distance from the nasal base to oral rim corresponding to the distance from the oral rim to chin. The ideal vertical height ratio of the upper third to the lower two third is 1:2.3.*

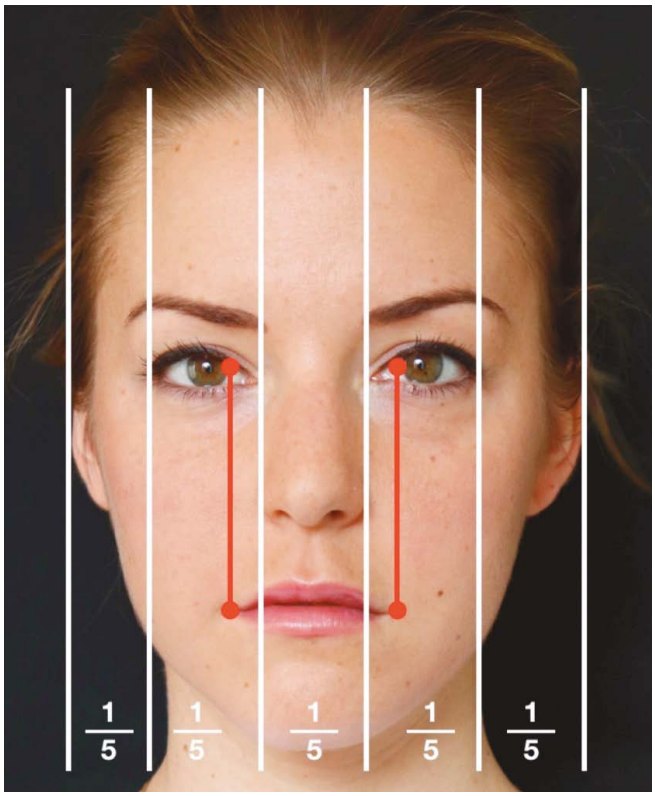

Figure 3 *- In frontal view, the face can be divided in five equal parts. Normally, the mouth width at rest is approximately equal to the distance between the medial iris margins.*

All these features contributed to increase its use in lip enhancement procedures.
Despite filler choice, approaching this delicate region can often lead to unpleasant and unnatural results, leaving both physicians and patients unsatisfied. An accurate evaluation of the patient's facial features as well as a standardized injection method could help decrease bad outcomes. In this paper we describe an innovative and complete clinical evaluation of the labial region in relation to the lower third of the face: we named it "*liplush analysis*". Liplush analysis is a template that could guide physicians to obtain harmonic and pleasant lips through the recognition of specific key points situated in the lower third of the face.

Materials and methods

We enrolled a total of 180 Caucasian women (mean age 33.66, range 20-50 years) who underwent lip redefinition and augmentation with hyaluronic acid between September 2018 and April 2019. Hyaluronic acid allergy represents the only exclusion criteria. All injections were performed by the same operator. Antiviral medication was administered in patients with a known history of HSV I infection. Topical anaesthetics were applied 15 minutes before starting the treatment. This included combination of lidocaine and prilocaine. Infraorbital and mental nerve blocks were either intraoral and transcutaneous. All procedures were performed either in public or private practice. All patients underwent treatment with the same hyaluronic acid (HA CPM 22,5 mg/ml – 3.0 mg/ml lidocaine – 0.6 ml for the contour and HA CPM 25.5 mg/ml – 3.0 mg/ml – 0.6 ml for the shape). Filler injection is performed using retrograde threading and vertical technique with a 0.5 inches 30-gauge needle. The majority of patients underwent redefinition and augmentation of both upper and lower lip: some cases required injection of a single area. The amount of hyaluronic acid used in each patient varied from a minimum amount of 1 ml to a maximum of 2 ml. All patients were evaluated with liplush analysis protocol before receiving the treatment. Injection procedures were performed according to the measurements described.

Liplush analysis

The core of liplush analysis relies on the identification of 9 specific key points (*Figure 4*):

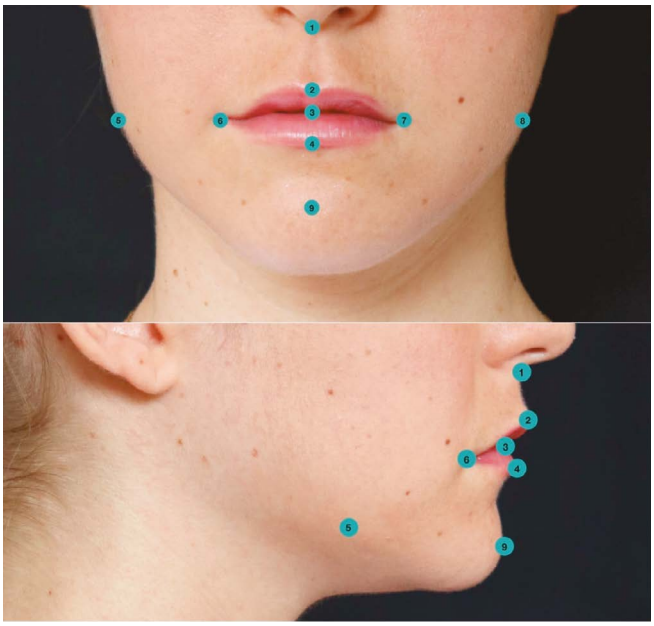

Figure 4 *- In frontal and lateral view (in this case right lateral view), the ten "liplush points" are identified.*

1) Sn (subnasale): the deepest midline point where the base of the nasal columella meets the upper lip.
2) Ls (labrale superius): the midline point representing the mucocutaneous vermilion border of the upper lip.
3) St (stomion): the most anterior midline point of contact between the upper and lower lip; if the lips are held apart in repose, a superior and inferior point in repose may be distinguished.
4) Li (labrale inferius): the midline point representing the mucocutaneous vermilion border of the lower lip.
5) Go' right (gonion): the most lateral point on the mandibular (gonial) angle; it is close to skeletal gonion.
6) Ch right (cheilion): the point located at lateral oral commissure (the angle of the mouth).
7) Ch left (cheilion): same as in point 6.
8) Go' left (gonion): same as in point 5.
9) Pog' (Pogonion): the most prominent midline point of the soft tissue chin pad.

The distance between points 2 and 3 represents the upper lip vermillion while the distance from poins 3 and 4 corresponds to the lower vermillion (*Figure 5*).
The ideal ratio between these two values is 1:1.6[8].
The distance between each labial commissure (points 6 and 7) and each gonion (points 5 and 8) should be inferior to the width of the mouth (*Figure 6)*. The ratio is 0.6:1.
Studying the relationship between the aforementioned parts provides us with valuable information on mouth width. This is a valid tool to plan the proximity level of injections to the labial commissure: if we inject close to the oral commissures, the mouth will appear wider.

After having identified the previous key points our evaluation continues by noting the following parameters, directly related to the core points listed:
- LIP CURVATURE
- LIP CURL
- LIP INCLINATION
- LIP PROMINENCE

Lip curvature

The contour of the upper lip at rest in frontal view mainly depends on the differential height between the mid-philtrum, the vermillion border at superior tubercle level and the bilateral oral commissure (*Figure 7*). The mid-philtrum is measured from subnasale (Sn - point 1) to stomion superior (Sts - point 3).
The commissure height is measured from each oral commissure (Ch right and left - points 6 and 7) perpendicular to a horizontal line drawn through subnasale point (Sn - point 1). The mid-philtrum height and bilateral oral commissure heights should be approximately equal in adults with the lips lightly in contact. The mid-philtrum height may be approximately 1-3 mm shorter than the commissure height with the lip at rest. The distance between vermillion border at superior tubercles level and the nasal base has to be shorter than mid-philtrum height. Height difference between these key points increases as the lip curvature becomes more represented. While evaluating lip curvature it's also necessary to establish the dentolabial relationship; the ideal proportion is obtained when the upper lip covers approximately the

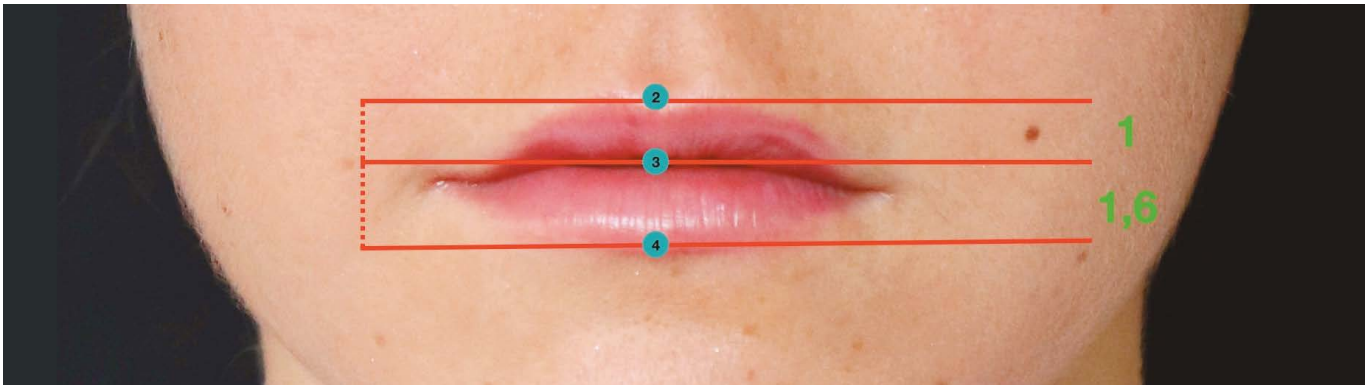

Figure 5 *- The ideal vertical height ratio of the upper lip vermilion to the lower lip vermilion is 1:1.6.*

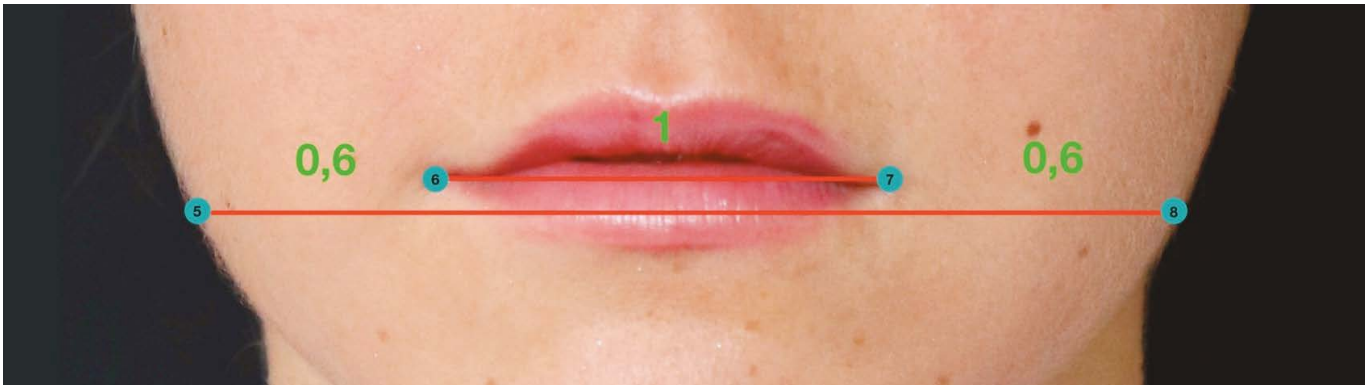

Figure 6 *- The ratio of the distance between each labial commissure and each gonion is 0.6:1.*

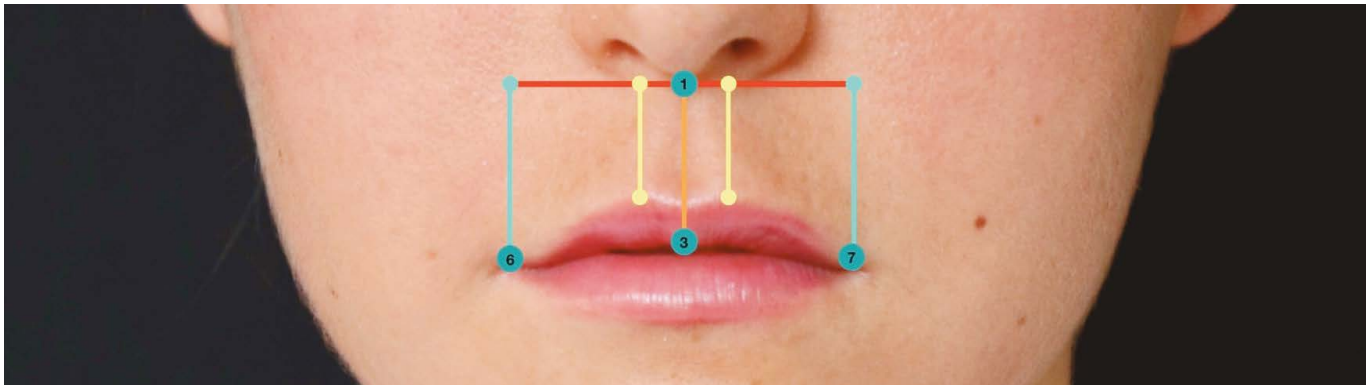

Figure 7 *- Lip curvature is represented by the distance of mid-philtrum and bilateral oral commissures from a horizontal line drawn through subnasale point.*

upper two-thirds of the maxillary incisor with 2-4 mm maxillary incisor exposure (*Figure 8*).
The interlabial separation is no more than 2-3 mm (an interlabial gap greater than 4 mm is usually and indicator of an incomplete lip seal) and corresponds to the distance between Stomion Superior and Inferior.

Lip Curl

By lip "curl" we mean the mild sagittal anterior curvature or "curl" of the upper lip in lateral view. Evaluation of upper lip curl may be performed by drawing a line from subnasale (Sn - point 1) to labial superius (Ls - point 2) (*Figure 9*). The depth of resulting concavity below this line should range from 1 mm to 3 mm.
This is an important parameter that frequently allows to understand if a lip has been previously treated.

Lip inclination

This parameter is important both for upper and lower lip assessment.
Upper lip inclination may be evaluated by measuring the nasolabial angle. This value is obtained by drawing a line tangent to the nasal columella (columella tangent) and a line tangent to the upper lip (upper lip tangent) through labial superius (Ls - point 2). This angle will depend on the inclination of the nasal columella and the upper lip. To better assess the inclination of the upper lip, the nasolabial angle may be separated by a true horizontal line passing through subnasale (Sn - point 1) into upper and lower component parts. Average value for upper lip tangent to true horizontal plane is about 85° (*Figure 10*).
Lower lip inclination may be carved by measuring the mentolabial angle, which is formed by the lower lip and soft tissue chin.
The mentolabial angle may be divided in two parts by a true horizontal line drawn through sublabiale. The upper component angle constitutes the lower lip inclination value; this angle is therefore obtained by crossing the line tangent to the lower lip through labial inferius (Li - point 4) and the true horizontal line through sublabiale (*Figure 11*).
During this step it is advisable to notice mentolabial sulcus too. It forms the transition zone from the lower lip to the soft tissue chin; its morphology is an important aesthetic parameter of the lower face, strongly related to the aesthetic appearance of the lips. The depth of the mentolabial fold may be measured from the depressor point of the fold with a line drawn from the labial inferius (Li - point 4) point to the pogonion (Pog - point 9); in women the average value of this is 4 mm (*Figure 12*).

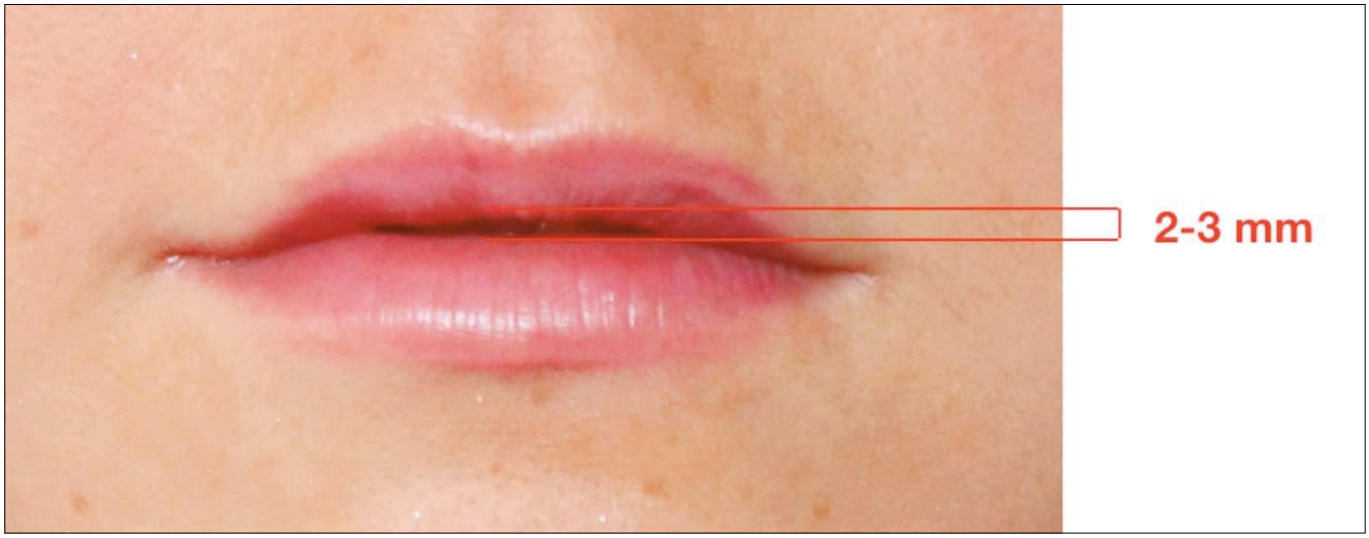

Figure 8 *- The dentolabial "ideal" relationship results when the upper lip cover upper two-thirds of the maxillary incisor with 2-4 mm maxillary incisor exposure. The interlabial separation is no more than 2-3 mm.*

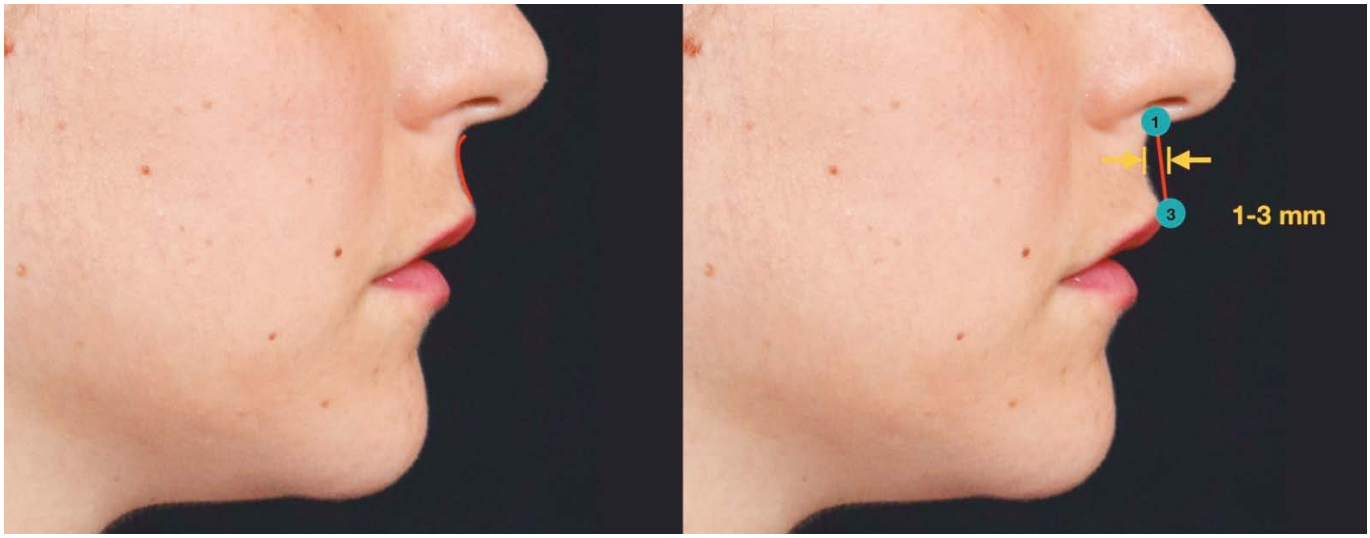

Figure 9 *- Lip curl is evaluated by drawing a line from subnasale (Sn) point to the labial superius (Ls) point; the depth of the upper concavity from this line should measure between 1 mm and 3 mm.*

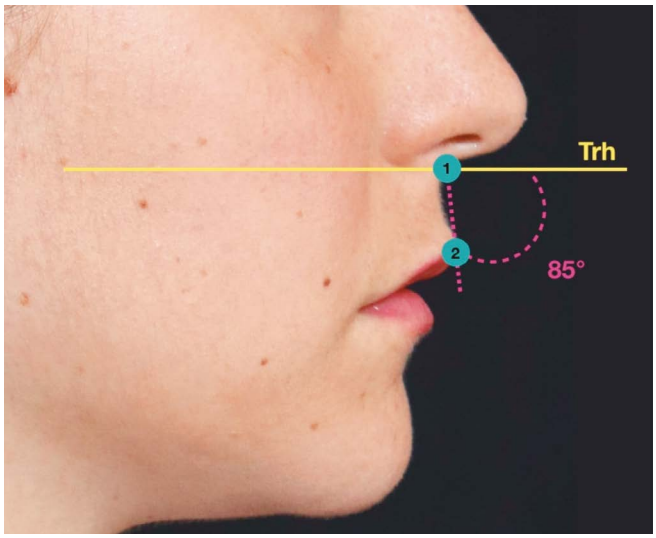

Figure 10 *- Upper Lip inclination is obtained through the lower component of the nasolabial angle; average values for upper lip tangent to true horizontal plane is about 85°.*

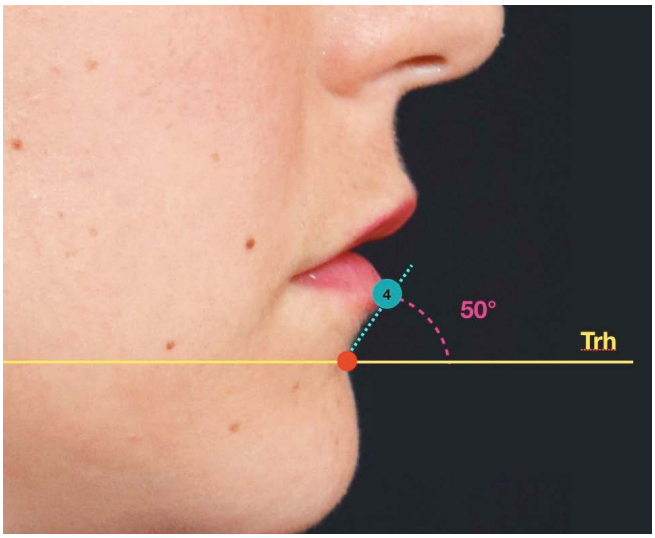

Figure 11 *- Lip inclination of the lower lip is evaluated through the upper component of the mentolabial angle; average values for upper lip tangent to true horizontal plane is about 50°.*

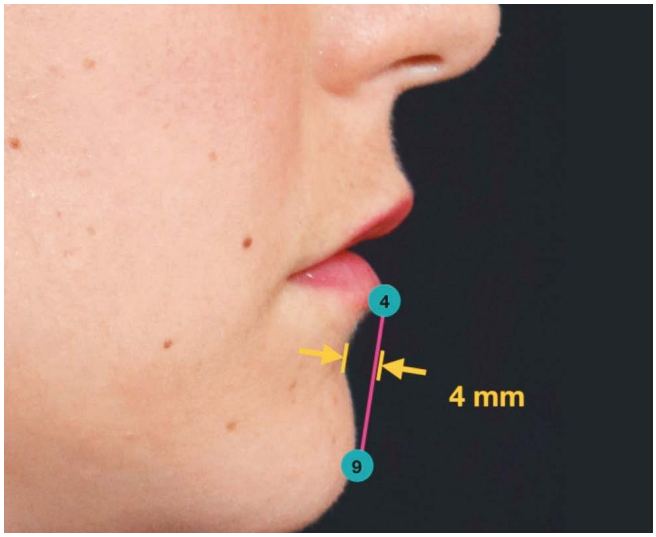

Figure 12 *- Mentolabial sulcus is evaluated from the depressor point of the fold with a line draw from the labial inferius (Li) point to the pogonion (Pog); the average value of this is 4 mm.*

Lip prominence

Lip prominence evaluation must be conducted within the contexct of a whole facial assessment. Nose prominence as well as chin protrusion have to be taken into account. Several methods have been published in literature to establish the relationship between these anatomical components.

We will use S-Line (Streiner Line) (*Figure 13*); this line is drawn from the mid-columella to pogonion (Pog - point 9); the lips should lie on this line. If the lips lie behind this line the lips are retrusive, and if they lie in front of this, they are protrusive. To achieve a complete analysis, it is important to study the lips also in a dynamic setting. Anterior oral seal should be evaluated by asking the patient to swallow and observing the behaviour of the lower lip. Signs of excessive contraction of the mentalis muscle could appear in this phase. When swallowing, the lips should normally form a seal (anterior oral seal) in front of the anterior teeth. When this is not possible, adaptive patterns of the lip and tongue activity may occur to obtain an anterior oral seal.

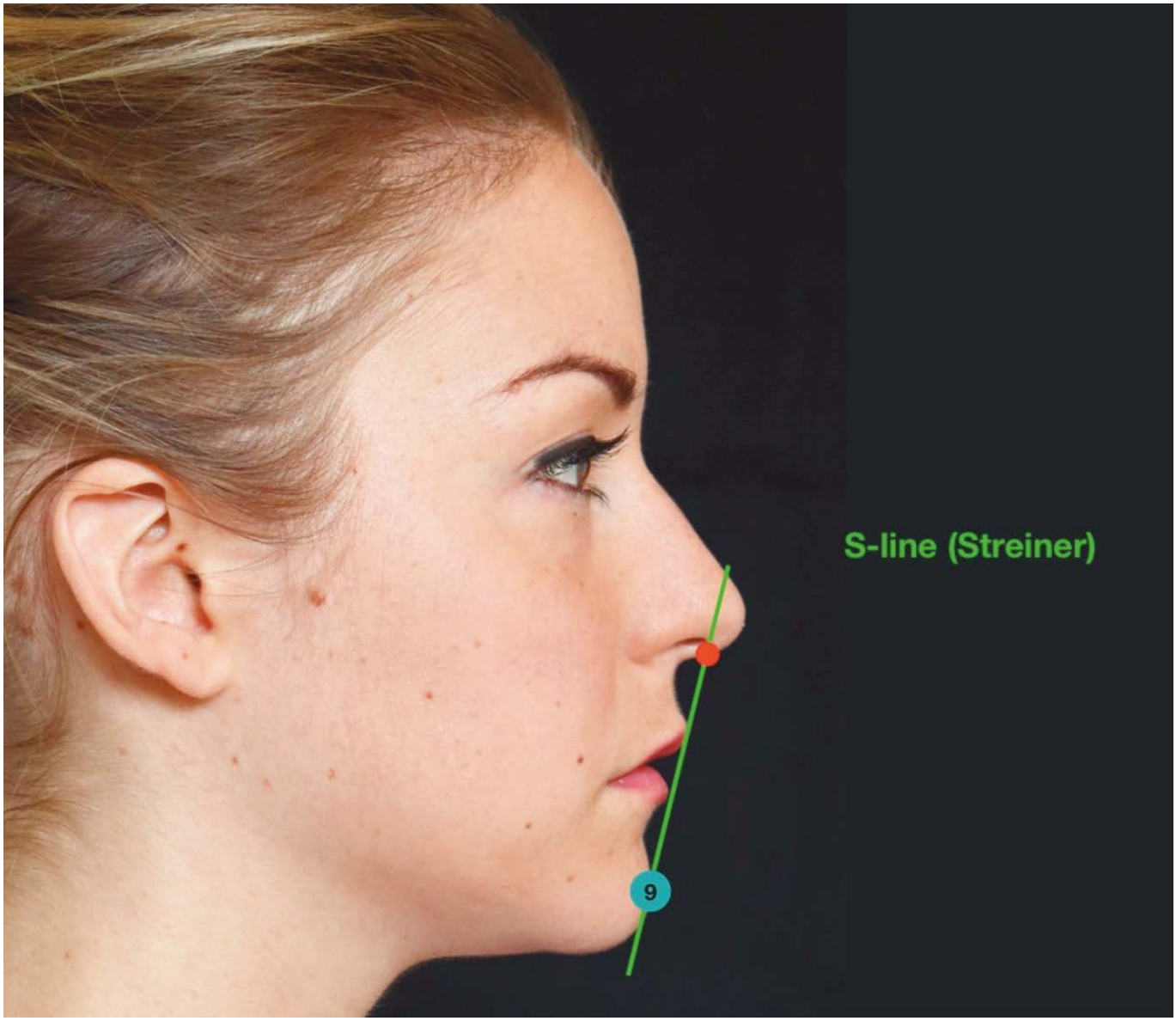

Figure 13 *- S-line is drawn from the mid-columella to pogonion (Pog); the lips should ideally lie on this line.*

Patients with mild lip incompetence often maintain a lip seal by slight circumoral contraction; this can be sometimes difficult to detect. Revealing signs are the flattening of the mentolabial fold and, with more severe lip incompetence, the appearance of corrugation over the anterior surface of the chin where the fibers of the mentalis muscle, responsible for lip elevation, are inserted.

Results

The subjects rinsed their lips with water and were seated in a study room with controlled temperature (23 +/- 2°C) and relative humidity (45 +/- 5%) for a least 20 minutes before clinical measurements.
During measurements, we prohibited any activity that might affect lip characteristics such as a conversation and drinking.

Frontal and side photographs of the lower third and lips area were taken with a digital camera (EOS 200D, Canon, Tokyo, Japan). Subjects were instructed to relax the entire face so as not to change the lip shape. Photographs were taken under fixed condition of light, illumination, position of the strobe and distance between the subject and the camera. Photographs were captured in manual mode (shutter speed, 1/100; aperture value, 13.0; ISO speed, 250, image quality, RAW; white balance mode, colour temperature (5200K); lens, EF100 mm f/2.8 Macro USM (Canon); and image size, 4752 x 3168 pixel.
Before treatment, all patients filled a questionnaire made of eight questions with five possible answers (value from 1 to 5) related to their own perception of lips' appearance in relation with the rest of the face (maximum score of 40). The same questionnaire was proposed fifteen days after medical procedure.
The scale ratings ranged from 1 (not like) to 5 (like). An overall score superior to 35 was considered an index of patient satisfaction (*Table 1*).

1) Shape of the upper lip in frontal view				
1	2	3	4	5
2) Volume of the upper lip in frontal view				
1	2	3	4	5
3) Shape of the lower lip in frontal view				
1	2	3	4	5
4) Volume of the lower lip in frontal view				
1	2	3	4	5
5) Projection of the upper lip in lateral view				
1	2	3	4	5
6) Projection of the lower lip in lateral view				
1	2	3	4	5
7) Harmony of the lips in the lower third in frontal view				
1	2	3	4	5
8) Harmony of the lips in the lower third in lateral view				
1	2	3	4	5

Table 1 *- Liplush questionnaire. Eight questions with five possible answers (value from 1 to 5) related to patients' perception of lips appearance in relation with the rest of face.*

Before surgery, the average result of the overall questionnaire score was 19.5%. After surgery, 88.9% (n=160) patients achieved a score higher than 35 (mean 38.7); 7.2% (n=13) had a score between 35 and 30. 3.9% (n=7).

CASE 1
In the frontal view we find an interlabial separation of about 2-3 mm with a reduced height of the upper lip compared to the lower lip. Furthermore, the curvature of the upper lip is poorly represented.
In lateral view the upper lip lacks curl representation. Upper lip inclination, in relationship with the true horizontal line, results in a wider angle, exceeding physiologic 85°; while lower lip inclination, in relationship with the true horizontal line, results correct according to the parameters. Lip prominence, related to nose and chin, deserves correction. Labiomental fold results in the normal value range . At rest, lips keep a normal position without excessive muscular contraction (*Table 2*).
In this case both upper and lower lips have been treated. 0.6 ml of HA CPM 22.5 mg/ml – 3,0 mg/ml lidocaine were used for the contour and 1.0 ml of HA CPM 25.5 mg/ml – 3.0 mg/ml for the shape (*Figure 14*).

CASE 2
In the frontal view, the ratio between upper and lower vermilion is correct; the lips are in contact without an interlabial gap, curvature is poorly represented with a slight asymmetry; in lateral view lip curl and inclination are slightly higher and do not need to be treated.
Lip prominence, related to nose and chin, deserves correction.
The labiomental fold is deeper and more accentuated.

	Liplush Analysis	Liplush Analysis
Frontal View	RATIO UPPER/LOWER VERMILION	Inferior Low heigh of the upper lip compared to the lower lip.
	LIP CURVATURE	Poorly represented
Lateral View	LIP CURL	Reduced
	UPPER LIP INCLINATION	Reduced (angle > 85°)
	LOWER LIP INCLINATION	Correct
	LIP PROMINENCE	Reduced

Table 2 *- Liplush analysis of case 1, before treatment, in frontal and lateral view.*

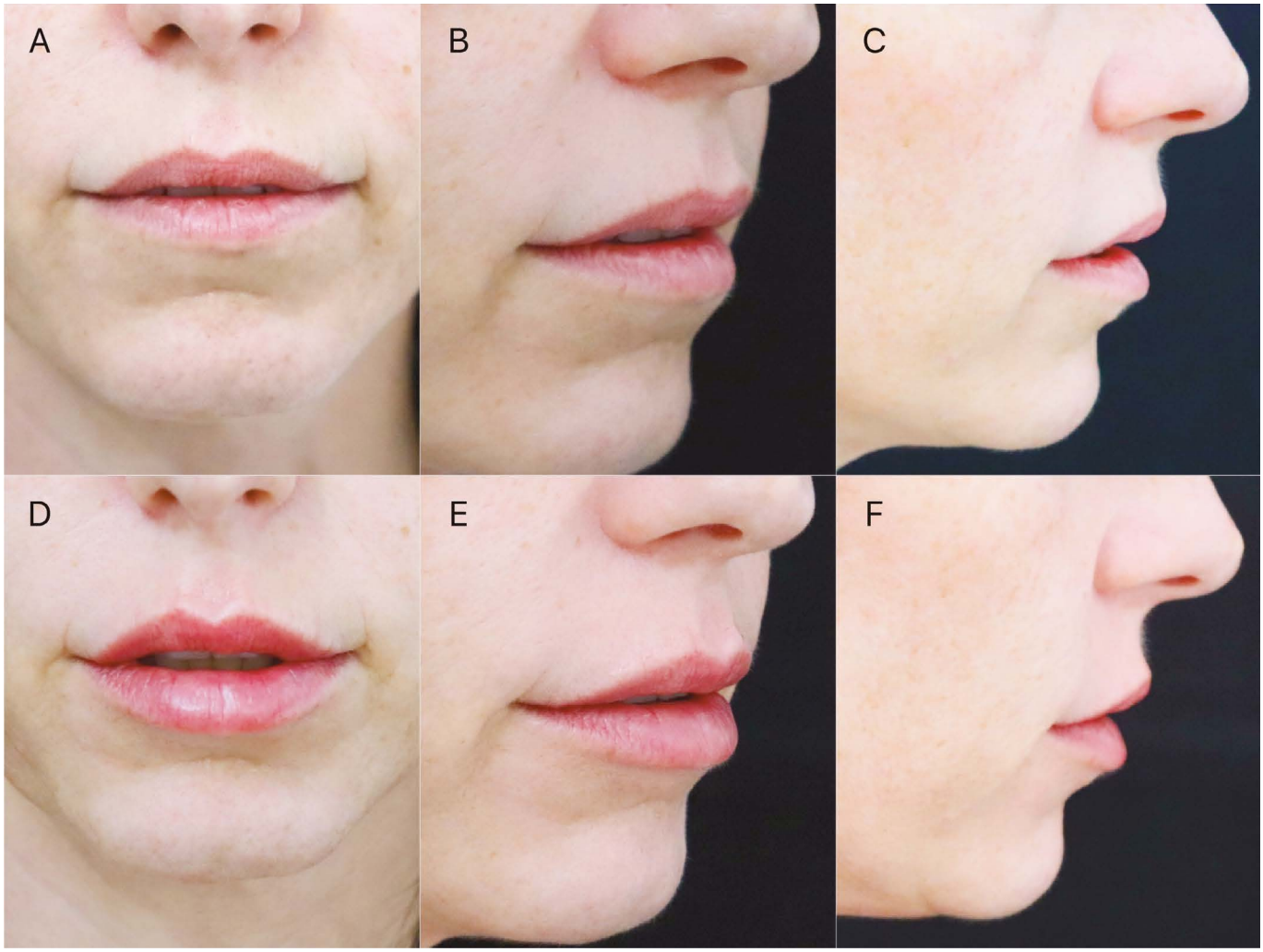

Figure 14 *- S Case 1. Pre-treatment (A, B and C) and post-treatment (D, E and F) photographs of patient who underwent lip filler infiltration according to liplush analysis.*

At rest, lips keep a normal position without excessive muscular contraction (*Table 3*). In this case both upper and lower lips have been treated and 0.8 ml of HA CPM 22.5 mg/ml – 3.0 mg/ml lidocaine were used for the contour and 0-6 ml of HA CPM 25.5 mg/ml – 3.0 mg/ml were used for the shape (*Figure 15*).

Discussion

In the last twenty years many papers concerning lip analysis and treatment techniques have been published[9]. The number of publications has grown in parallel to the popularity of lip enhancement procedures.

	Liplush Analysis	Liplush Analysis
Frontal View	RATIO UPPER/LOWER VERMILION	Correct
	LIP CURVATURE	Poorly represented
Lateral View	LIP CURL	Correct
	UPPER LIP INCLINATION	Correct
	LOWER LIP INCLINATION	Correct
	LIP PROMINENCE	Reduced

Table 3 *- Liplush analysis of case 2, before treatment, in frontal and lateral view.*

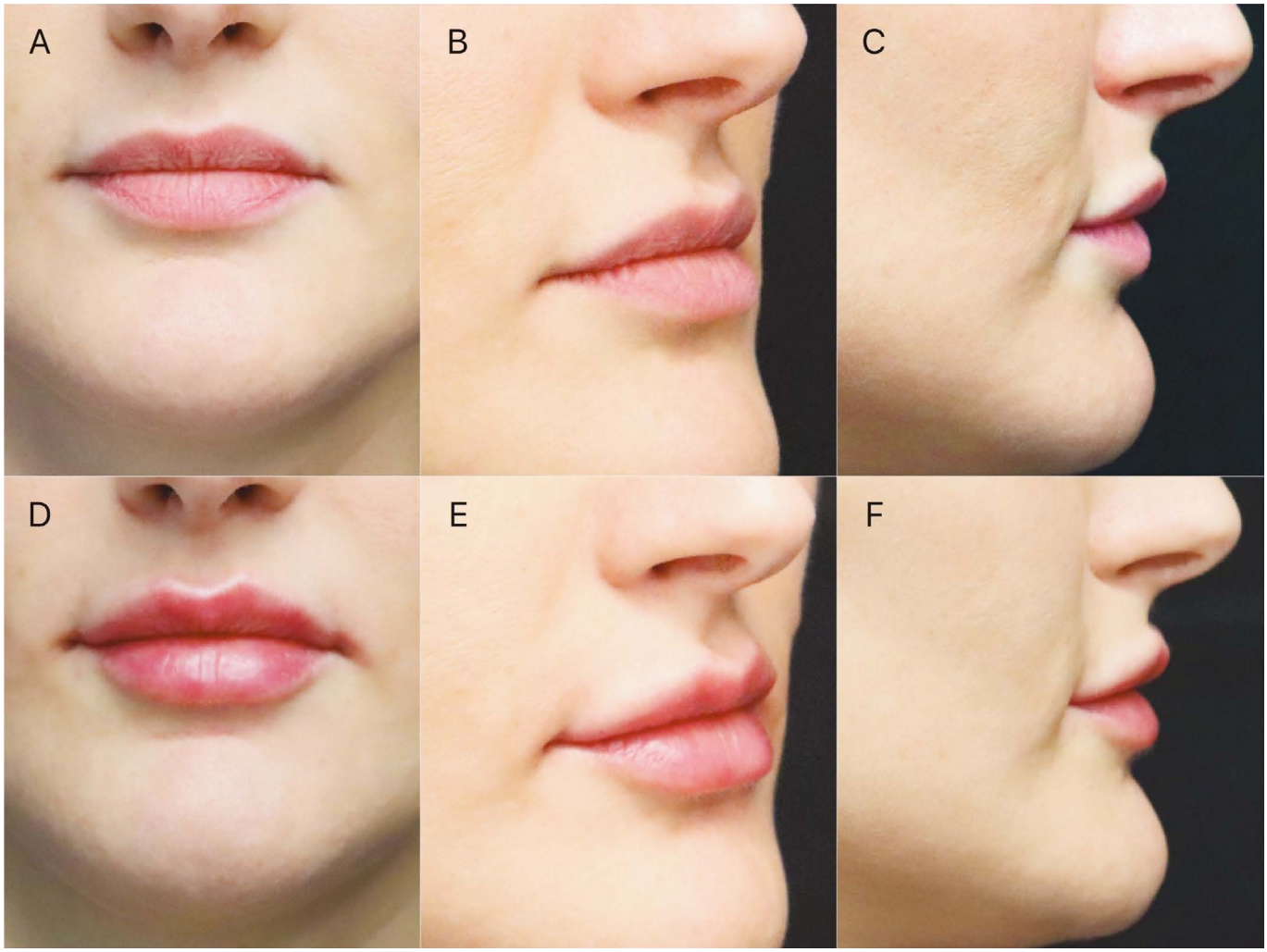

Figure 15 *- Case 2. Pre-treatment (A, B and C) and post-treatment (D, E and F) photographs of patient who underwent lip filler infiltration according to liplush analysis.*

However, something is missing: even if performed by the best practitioners with the use of high-quality materials the results obtained are not always as good as expected. Predicting how the patient's lips could become the "ideal lips" thanks to our injections is extremely challenging.
In the current literature, we can find many information concerning filler options for lip augmentation. An interesting review and meta analysis from 2015 summarizes all the materials used for soft tissue augmentation, including their origin, composition, degree of permanence after injection, major brands and whether published studies exist regarding the use in lip augmentation[10]. Both biologic (animal or non-animal) substance and non-biologic substances have been used. This meta analysis partially failed to examine the effectiveness of each type of filler material due to the lack of a universal method to assess results. Many studies, in fact, do not have quantifiable methods of efficacy assessment; otherwise, if they report any scale, such scales are employed differently. Despite the many options available among filler materials, hyaluronate seems to be the preferred filler[11]. The advantages of using hyaluronic acid in lips are several and this is also the choice of the Authors[12]. According to our experience, we do not recommend permanent materials because of the higher risk of developing complications as infections, lumpiness and granulomas.
The best injecting technique is another aspect discussed in literature. The interesting work of Sarnoff et al.[8] describes the lip enhancement technique dividing the procedure in 6 clear steps. The possible pitfalls and tricks of each phase are highlighted. For example, they suggest pinching the skin with non-dominant hand during retrograde threading to recreate sculpted philtrum columns: this keeps the filler in a line and prevents it from spreading laterally. Sometimes augmentation is not sufficient: additional treatments may be necessary to treat those patients that have pronounced radial "lipstick bleed lines" in the cutaneous portion of the upper lip. The use of fraxel laser or neuromodulators to optimize the result should be carefully evaluated.
The technique chosen is undoubtedly important but nowadays it is clear that the perfect lip is not just a matter of increasing volume indiscriminately. A complete and detailed pre-treatment analysis is very important[13]. Many variables need to be considered; nonetheless, parameters such as sex, race and age must be respected. Some works regarding the perception of beauty have been published as well. The aim is to understand in detail which characteristics are considered attractive in lips and in

the face in general[14]. A visible transition line or border between the vermilion and skin, a V-shaped Cupid's bow, a full lip tubercle and vermilion, an ascendant line in oral commissures all represent positive aesthetic characteristics related to the lip region. Such traits must be maintained to achieve aesthetic improvement; as a result, a good knowledge of the entire lower third is required. Efforts have been made in trying to find a method to classify patients according to their lips features in order to yield a standardized scale. These are some examples of validated scales: Medicis lip fullness scale (MLFS); the lip fullness grading scale (LFGS); the perioral lines scale (POLS) and oral commissures severity scale (OCSS)[15-16-17]. Despite this huge background, in fact, we did not find in literature a simple and reproducible method that can provide the physician with a standardized tool to evaluate lips themselves and in relation with the whole facial unit. An enlarged lip, in fact, is not beautiful if its shape is not attractive and the majority of anthropometric measurements fail to measure the shape of the lip, which can be a major determinant of the ultimate aesthetic outcome.
The Liplush analysis could be helpful to introduce a standardized assessment method for both novice injectors and expert physicians. Furthermore, our results in terms of patient satisfaction support this statement. We think that this evaluation can increase the result and treatment quality perception by the patient.

Conclusion

Lip enhancement and redefinition is one of the most frequently requested procedures in cosmetic practice. Perception of beauty certainly varies depending on cultural and ethnic influences. However, we were able to define certain parameters of the lips and lower third of the face that seem to add attractiveness. This scientific approach is appealing to correct proportions of inferior third of the face and to suit to the different shape of lips; moreover, it is helpful in preoperative consultation, as it can serve as a guiding tool in aesthetic lip treatment.

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Conflict of interest

The authors declare that they have no conflict of interest.

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aesthetic medicine

Original Article

Prospective clinical study and ultrasound assessment in patients with bruxism treated with botulinum toxin

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Abstract

Introduction: Bruxism (BRX) can be defined as clenching and/or grinding of the teeth in a particularly intense or involuntary way. It occurs interchangeably during sleep or while awake due to repeated contraction and/or hypertrophy of the masticatory muscles.
Treatments are mainly intended to limit induced temporomandibular joint (TMJ) damage and are manifold: irreversible occlusion, bite splints, pharmacological and/or cognitive therapies. However, botulinum toxin type A (BoNT-A) is especially effective.
The aim is to assess the attenuation or disappearance of BRX-related symptoms after injection of BoNT-A due to relaxation of the masticatory muscles (especially the masseter muscles).
Materials and Method: this is a clinical, prospective and longitudinal study on 43 adult female patients aged between 24 and 67 (37.0 ± 9.6). It was carried out from September 2018 to October 2019.
Assessment controls were performed before, two weeks and four months after the first treatment with BoNT-A, and two weeks and five months after the second treatment. Digital photographs were taken at each control visit, the Smith-Knight Tooth Wear Index was assessed and orthopantomography (OPG) was performed. Bigonial diameters were measured with a digital caliper. The masseter muscles were assessed bilaterally, at rest and during contraction, by ultrasound.
Results: after BoNT-A treatment, 26% of patients were free of BRX, whereas considerable improvements were observed in the remaining 74%. Adverse effects were mild and of short duration.
Conclusion: BoNT-A treatment was able to prevent lesions on orofacial structures (teeth, jaw muscles, TMJ), and at the same time relieve pain and associated symptoms induced by repeated muscle contraction in BRX.

Keywords

Bruxism, tooth wear, botulinum toxin type A, temporomandibular disorders, masseter muscle

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Introduction

Bruxism (BRX) is defined as a dysfunctional disorder of regular mouth movements together with the habits of clenching and/or grinding teeth due to the contraction of one or more muscle groups involved in mastication[1,2]. BRX induces an overload of the stomatognathic system, the temporomandibular joint (TMJ) in particular, considered the most contributing risk factor to TMJ instability[3]. The involvement of masticatory muscles and ligaments means that many patients report tension or pain during mastication and/or when getting up in the morning. Chronic pain and abnormal jaw mobility often occur together with tooth wear; in BRX, dental restorations, including dental implants, are not effective[5]. Headaches are common, with atypical pain distribution[6]. Some patients perceive this condition as an aesthetic alteration and focus their concern on the squarer shape of their faces. New diagnostic criteria are shown in *Table 1*.

Based on each patient, degenerative changes of TMJ in BRX result in different adaptive responses[7,8]. These may be classified according to three types:

1) ***TMJ Dysfunction*** and pain stemming from the masticatory muscles. This would be classified as myofascial pain.

2) ***Intrinsic Changes of TMJ***. There is joint anterior disc displacement, with or without reduction. In its evolution, this may result in joint luxation and condyle dislocation.

3) Finally, joint disorders cause chronic degeneration with or without symptomatic inflammation, resulting in cases of ***Osteoarthritis***.

BRX is closely associated with psychological factors and personality traits, stress being one of the most significant ones. Sleep BRX is usually associated with psycho-emotional disorders, conditioned by poor dental occlusion[9]. Some clinical studies, particularly in the United States, indicate that stress is the main reason for BRX patients seeking medical-psychological advice[8]. There are multiple BRX treatments, including bite splints, irreversible occlusion procedures, and pharmacological and/or cognitive-behavioral therapies[9,10]. They are all intended to reduce the side effects of BRX on biological structures involved.

Due to their different degrees of efficacy, dental splints are more a symptomatic treatment than an etiological one[9]. Cognitive-behavioral therapies show efficacy in the long term, resulting in high withdrawal rates[9,10]. Regarding the pharmacological treatment of BRX, the use of benzodiazepines and tricyclic antidepressants generates a high rate of dependency and their long-term efficacy is limited.

In the last years, good results have been obtained with BoNT-A injections in the masticatory muscles, particularly the masseter muscle, when they are hypertrophied or show a strong dynamic contraction[10–14]. BoNT-A reduces excessive muscle contraction, both at rest and during mastication. The relaxing effect of BoNT-A may be observed within two to four days after the initial injection; muscle relaxation may last up to six months, although it may be prolonged if sequential treatments of BoNT-A are regularly administered. In our preliminary study we already indicated that ultrasound measurements at the control visit four months after the first treatment, compared with initial measurements, served as predictive values for administering the second injection[15].

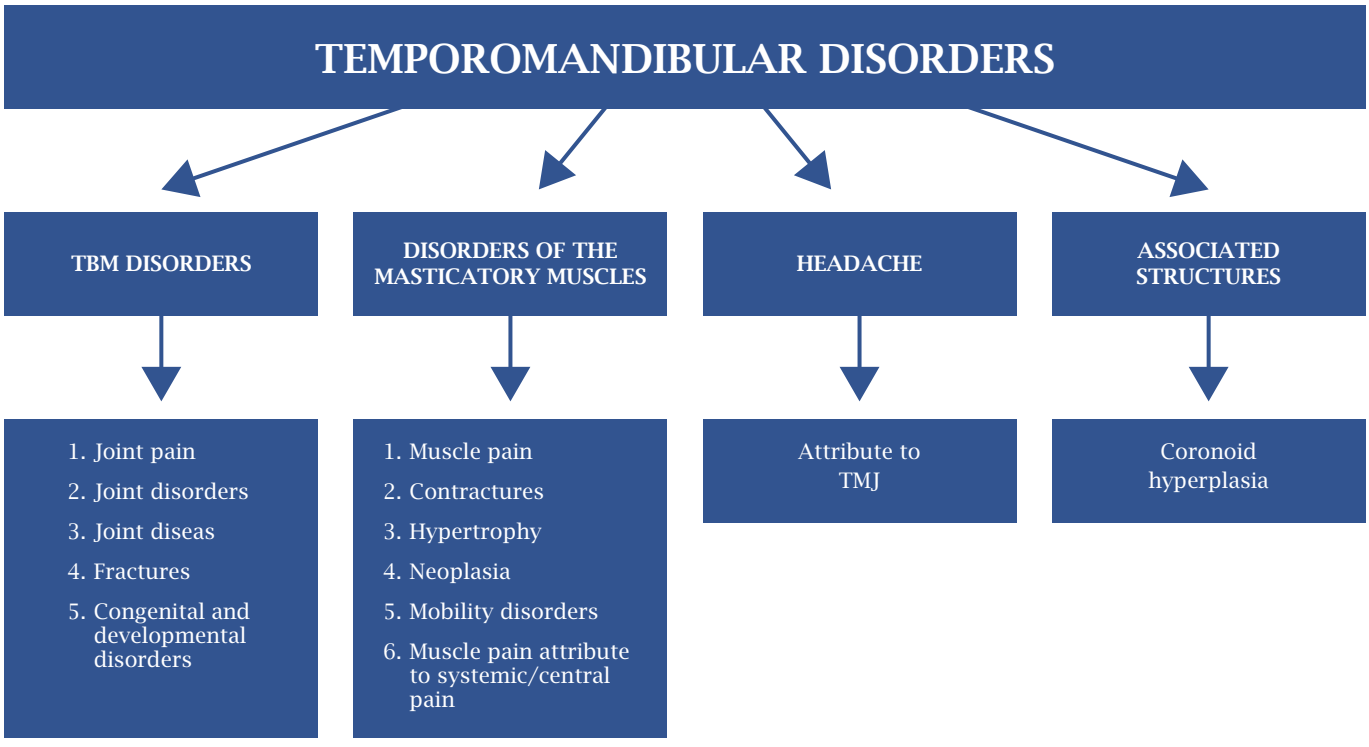

Table 1 *- Diagnostic criteria for temporomandibular disorders.*

Purpose

The purpose of the study is to assess the clinical benefit of muscle relaxation induced by BoNT-A injections in 43 patients with BRX. For this reason, controls were performed before, two weeks and four months after the first treatment session and two weeks and five months after the second treatment. Muscle hypertrophy patterns were assessed by ultrasound, considering the possible adverse effects attributable to the procedure.

Materials and method

This is a prospective and longitudinal study on 43 female patients between 24 and 67 years (mean age of 37.0 ± 9.6). They all reported pain and/or tension of a greater or lesser degree and more noticeable after waking up; some complained of mild-to-moderate pain during mastication. Six (14%) patients also stated they were unhappy with the squarer shape of their faces.
The study was conducted between September 2018 and November 2019 at Clínica Alcolea (Hospitalet de Llobregat, Barcelona) and Clínica Mona Lisa (Barcelona). Regarding the circadian cycle in relation to BRX, only one (2%) patient stated being aware of suffering it while awake; 11 (26%) patients experienced it during sleep (sometimes mentioned by their partners, and due to the feeling of tension when waking up); the remaining 31 (72%) presented with both awake and sleep BRX.
For this study, the following criteria were considered (*Figure 1*):

1) Tooth wear and excessive clenching or grinding of the teeth
2) Hypertrophy of the masseter muscles during voluntary contraction
3) Pain and radiation to neighboring structures in degrees, fatigue during mastication or rigidity tension when moving the jaw after waking up
4) Tooth hypersensitivity
5) Audible clicks or snaps, with or without locking of TMJ
6) Dental impression on the side of the tongue or cheeks, or without bleeding

Patient Assessment

Results were assessed at each control visit before, two weeks and four months after the first treatment with BoNT-A, and two weeks and five months after the second treatment. At each control visit, the following was systematically performed:

1) Taking of photographs with a digital camera (Canon®, D2000, Canon Inc, Tokyo, Japan) at rest and during contraction.
2) Assessment of the Tooth Wear Index (TWI). In 1984, Smith and Knight defined this concept by which all four visible surfaces (buccal, cervical, lingual and occlusal-incisal) of all teeth present are scored for wear, irrespective of how it occurred22 (*Table 2*).
3) Orthopantomography (OPG), which allows to diagnose and estimate:
   a. Potential degenerative bone disorders.
   b. Other more non-specific pathological disorders.
   c. Grade of pathological disorders found.
   d. Middle- or long-term efficacy of therapeutic measures taken.
   e. Other TMJ disorders (fractures, cysts, tumors, inflammation, aplasia, hypoplasia, hyperplasia and degenerative disorders)[23].
4) MRIs, the most reliable imaging diagnostic method. However, of the 43 patients that participated in the study, only five had an MRI due to the high cost of the test, the discomfort of the procedure and the fear of excessive radiation.
5) Modified bigonial diameter obtained using a digital caliper, which does not measure the distance between both angles of the jaw, but the mean between the points at rest and of maximum contraction of the masseter muscles. This measurement was well correlated with the one obtained by ultrasound for each masseter muscle (*Figure 2*).
6) Ultrasound measurements of each masseter muscle (and/or temporal muscle in case of treatment). Each masseter muscle was measured at rest and during maximum contraction[24]. An ultrasound machine (Sonosite® Micromaxx, Sonosite Inc, Irvine, CA, USA) with a 7-12- MHz multifrequency transducer was used. Ultrasound measurements were performed with the patient in a seated position and without touching the skin of their faces with the probe, with the transducer located 2 cm above and parallel to the edge of the jaw.

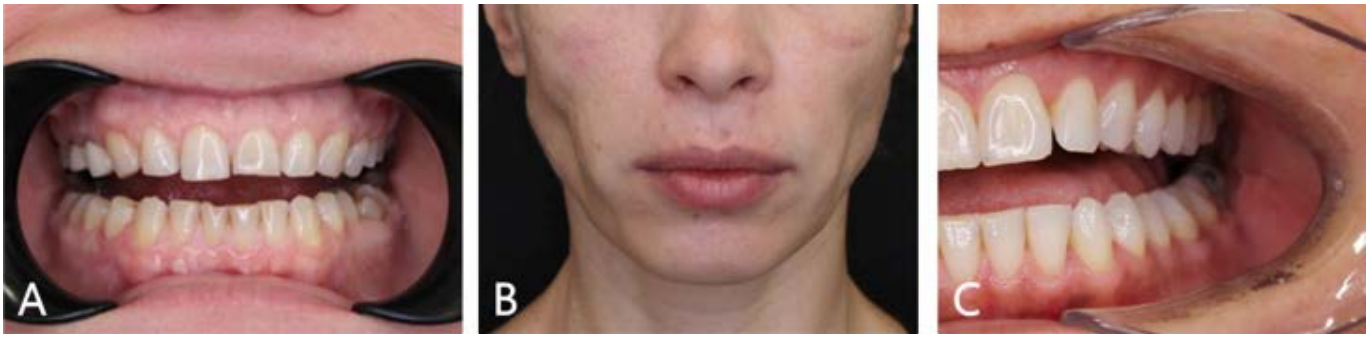

Figure 1 *- Usual findings in BRX. A. Visible tooth wear secondary to bruxism. B. Muscle hypertrophy during forced contraction of the masseter muscles shaped as a square jaw. C. Typical dental impression on the tongue.*

Grades	Surface	Criteria
0	B/L/O/I/C	Without loss of enamel characteristics Without loss of contour
1	B/L/O/I/C	Mild oss of enamel characteristics Minimum loss of contour
2	B/L/O/I/C	Loss of enamel with dentin exposure on less of 1/3 of the surface Loss in enamel by simple dentin exposure Defect with less than 1 mm of depth
3	B/L/O/I/C	Loss of enamel with dentin exposure on less of 1/3 of the surface Loss in enamel with substantial dentin loss Defect with 1-2 mm of depth
4	B/L/O/I/C	Complete loss of enamel and secondary pulp exposure Pulp or secondary dentin exposure Defect with higher than 1 mm of depth and pulp exposure secondary to dentin loss

Table 2 *- Grades and criteria used to estimate Smith and Knight's Tooth Wear Index of tooth surfaces: B, buccal; L, lingual; O, occlusal; I, incisal; C, cervical.*

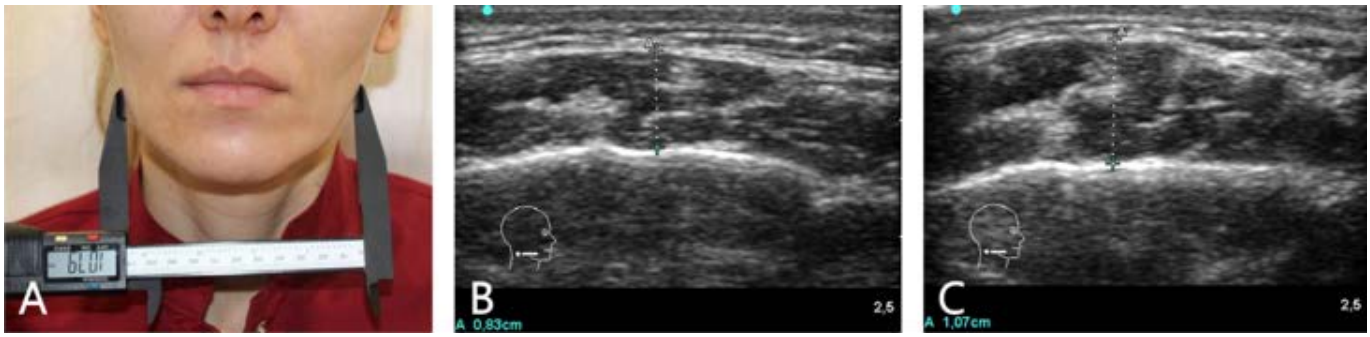

Figure 2 *- Study patient no. 6 (36 years old) A. Modified bigonial diameter obtained using a digital spreading caliper. B. Measurements taken by ultrasound of the right masseter muscle at rest and during contraction of the same patient.*

Injection Technique

Before administering the injection, the BoNT-A (Azzalure®, Galderma SA, Madrid) was reconstituted with 1 ml of saline. Each vial, containing 125 Speywood units (s.U.). BoNT-A, was bilaterally applied to each masseter muscle using an insulin syringe with an integrated 12-mm 30 G needle (Braun®, Melsungen, Germany).

The units of BoNT-A injected for each patient were estimated based on previously taken ultrasound measurements of each masseter muscle both at rest and during contraction. Total doses ranged from 35-85 s.U. per patient (57.5 ± 13.5). All three points of the lower third of each masseter muscle was injected with 7.5-12 s.U. based on the contraction previously shown by each muscle (*Figure 3*). Out of 43 treated patients, 11 (26%) required an additional injection of 5-7.5 s.U. of BoNT-A on one or two points of the masseter muscle, at the control visit, based on residual muscle activity measured by ultrasound.

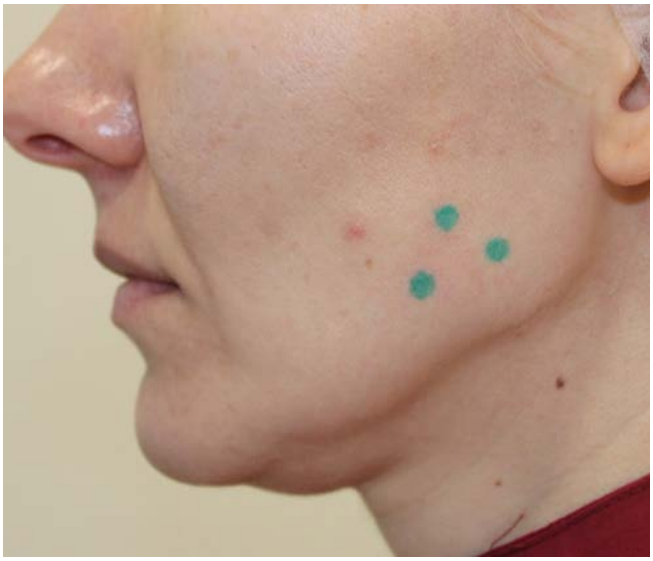

Figure 3 *- Marking of guide points for the injection of the left masseter muscle of study patient no. 6, based on the layout of the muscle fascicles.*

Result Assessment

The treatment, adverse effects and patient satisfaction were assessed according to the following scale: 0, No improvement; 1, Mild improvement; 2, Moderate improvement; 3, Significant improvement; 4, Free of bruxism. Adverse effects were quantified according to a 0-4 scale: 0, None; 1, Mild; 2, Moderate; 3, Severe; and 4, Very Severe. Patient satisfaction was also assessed on a 0-4 scale, where 0: Not satisfied, and 4: Very satisfied.

Statistical Analysis

An SPSS (v. 20) software for Windows was used. Variables were mean, minimum and maximum, range, percentage (%) and standard deviation (SD). Confidence intervals were set and a multivariate analysis was conducted for related samples (Student's t-test). Results were considered statistically significant ($p<0.05$).

Results

With the exception of age, data analysis showed small typical deviations with little dispersion from the arithmetic mean, indicating that it was representative of the sample. Under this premise, and in order to reduce the number of variables without losing information, a Student's t-test was performed. Ultrasound scans showed that thickness measurements of the masseter muscles at rest (1.22 mm ± 0.23) and during contraction (1.47 mm ± 0.26) before and two weeks after treatment (at rest: 1.04 mm ± 0.22; during contraction: 1.24 mm ± 0.26) were statistically significant ($p<0.05$). However, there were no differences between the control measurements two weeks and four months after treatment (at rest: 1.08 mm ± 0.19; during contraction: 1.26 mm ± 0.21) (Figure 4). The same process was carried out with the bigonial diameter variable: there was a significant difference between the values at rest before treatment (122.4 mm ± 6.5) and two weeks after treatment (118.9 mm ± 6.2), as well as during contraction (127.2 mm ± 6.8 and 123.0 mm ± 6.3, respectively), however there were no significant differences between results two weeks and four months after treatment (119.8 mm ± 6.0 and 123.3 mm ± 6.3) (*Figure 4*).

All patients underwent a second control four months after the first session; comparing these results with those obtained two weeks after the first treatment: although there was a decrease in bigonial diameter measurements (118.9 mm ± 6.5 at rest and 122.6 mm ± 6.3 during contraction), the difference was not significant[15]. When ultrasound measurements of the masseter muscle four months after the first session were compared to those five months after the second session, no statistically significant differences were found either (*Figure 5*). As expected, ultrasound and bigonial diameter measurements showed a similar evolution in their comparisons. Of all patients treated, 11 (26%) were free of bruxism; the remaining 32 (74%) obtained great improvement, with a satisfactory response to BoNT-A. When asked whether they would recommend the treatment or not: 41 (95%) patients

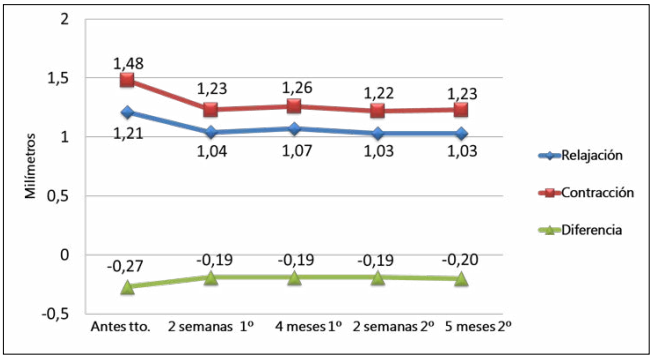

Figure 4 *- The chart shows the mean evolution of ultrasound measurements of both masseter muscles at rest and during contraction before and two weeks and four months after the first treatment with BoNT-A, and two weeks and five months after the second treatment.*

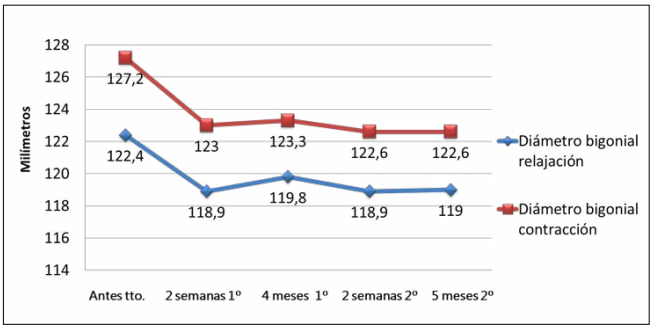

Figure 5 *- Evolution of bigonial diameter measurements before, two weeks and four months after the first treatment with BoNT-A, and two weeks and five months after the second treatment. Notice how the reduction achieved after the first treatment with BoNT-A varies very little throughout the study.*

reported they would, despite having experienced some adverse effects. Two patients stated that they would not recommend it, one due to moderate pain during the injection and ecchymosis that lasted one week; however, she admitted experiencing a considerable improvement of her initial condition. The other patient presented with muscle fatigue during mastication for 21 days, with full recovery. However, neither of these patients withdrew from the study. A 95% degree of satisfaction was reported, including both satisfied and very satisfied patients. It is remarkable that bigonial diameter and masseter muscle values were slightly better at control visits conducted five months after the second treatment than four months after the first treatment; although not statistically significant, patients could be treated at intervals longer than five months.

Adverse effects

In general, side effects after treatment with BoNT-A were mild and temporary (*Table 3*). No patients reported paresthesia or changes in facial expression. *Table 4* shows the correlation between patient satisfaction and clinical assessment: the fact that six (14%) patients reported being satisfied or very satisfied with the treatment, despite having experienced an adverse effect, is significant.

Adverse effects	N (%)	Duration (days)
Pain	7 (16%)	< 2
Ecchymosys	6 (14%)	4 - 10
Edema	3 (7%)	< 2
Muscle fatigue	7 (16%)	5 - 20

Table 3 *- Adverse effects.*

BRX treatment with TB-A		Patient satisfaction		
		Satisfied	Not satisfied	Total
Assessment clinic	No adverse effects	35 (81%)	1 (2,5%)	36 (84%)
	with adverse effects	6 (14%)	1 (2,5%)	7 (16%)
	total	41 (95%)	2 (5%)	43 (100%)

Table 4 *- Contingency table correlating patient satisfaction with clinical assessments. Notice that mild-to-moderate adverse effects do not have an impact on patient satisfaction regarding results.*

Photographic Results

Photographs taken were highly demonstrative of facial shape changes.
Figure 6 shows a series of three photographs of study patient no. 24 taken at control visits, in which the effect of relaxation four months after the first treatment (B) and five months after the second treatment (C) can be observed. A decrease in thickness of the left masseter muscle before and up to five months after the second treatment is also visible.
The patient was free of BRX and very satisfied with the more oval shape of her face. *Figure 7* is an OPG (A) from the same patient showing degenerative changes of the medial surfaces of both condyles due to disc flattening and displacement. Based on the Tooth Wear Index, the patient was classified as Grade 2, with enamel loss and dentin exposure on a third of tooth surface (B).
Figure 8 shows relaxation of the masseter muscles with BoNT-A, which spreads to the entire orofacial area. In this case, TMJ is normal and the Tooth Wear Index is minimum (*Figure 9*).
It is noteworthy that there is a strong correlation between the photographs, OPG, the Tooth Wear Index and the measurements obtained by ultrasound in all study patients.

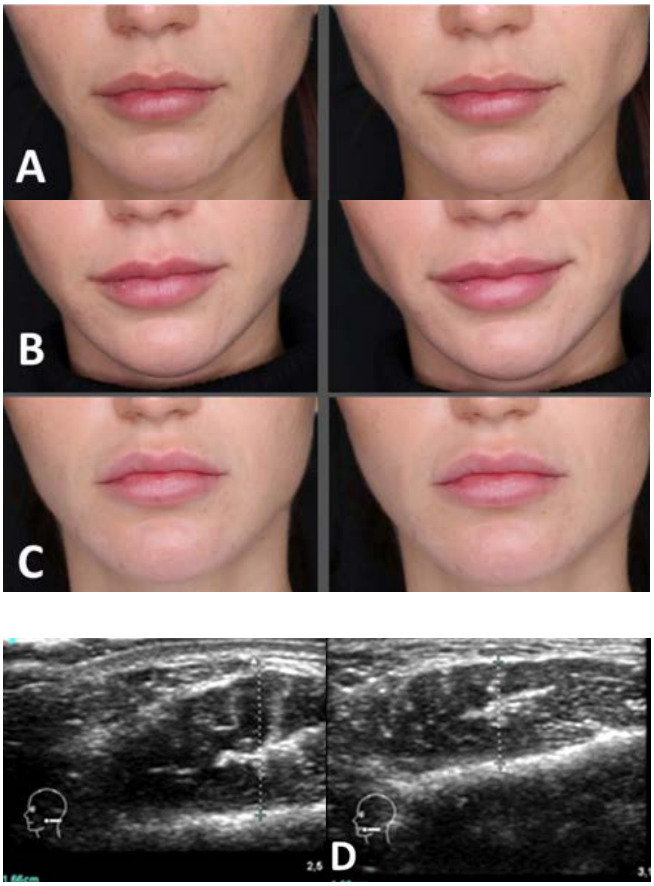

Figure 6 *- Study patient no. 26 (29 years old) treated with BoNT-A. The masseter muscles at rest and during contraction can be observed. A. Before treatment. B. Result four months after first treatment. C. Five months after second treatment. D. Notice the thickness reduction of the left masseter muscle at maximum contraction. Left: before treatment. Right: five months after the second treatment.*

Discussion

According to published data, this study shows that BRX treatment with BoNT-A is efficacious[22–25]. It has been rigorously conducted, both regarding the follow-up of potential adverse effects and the dosage and

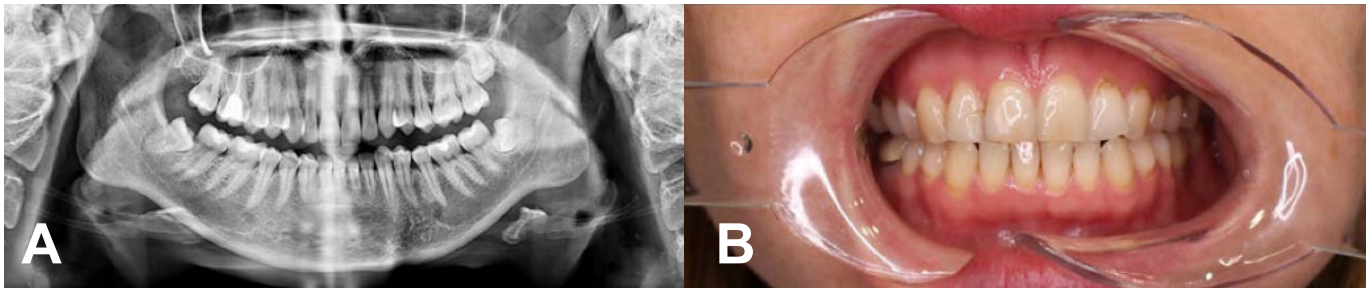

Figure 7 *- Patient no. 26. A. Normal joint disc in OPG. B. Grade 1 of tooth wear, with mild loss of surface enamel and minimum loss of contour.*

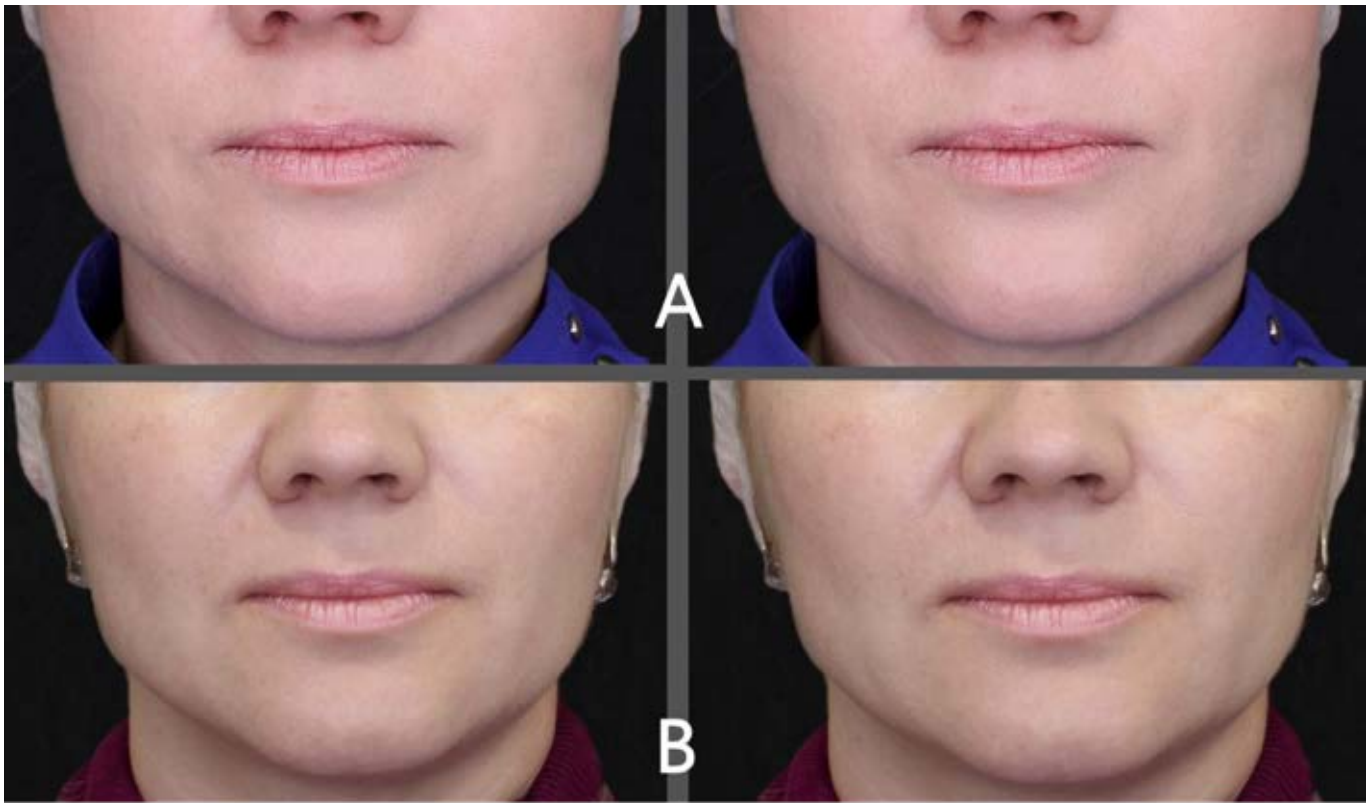

Figure 8 *- Study patient no. 40 (40 years old) treated with BoNT-A. A. Masseter muscles at rest and during contraction before treatment. B. Result four months after first treatment. There is relaxation of the masseter muscles as well as in the area around the mouth.*

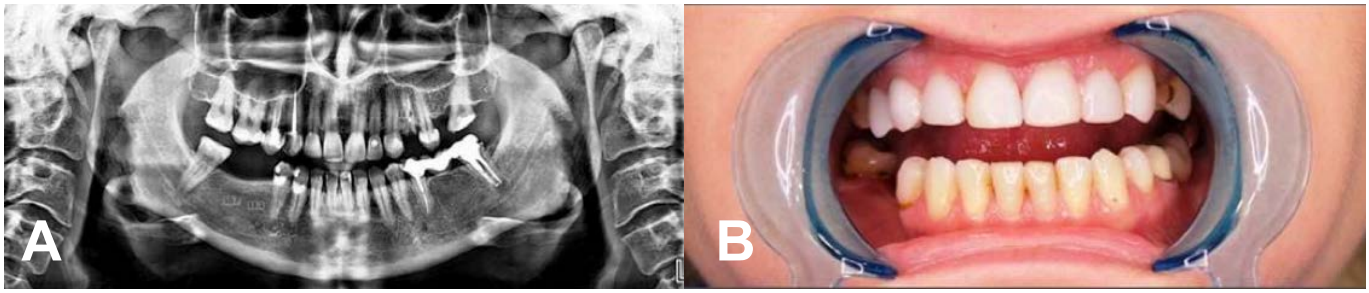

Figure 9 *- Study patient no. 40 (40 years old) treated with BoNT-A. A. Masseter muscles at rest and during contraction before treatment. B. Result four months after first treatment. There is relaxation of the masseter muscles as well as in the area around the mouth.*

Sleep BRX, associated with an important psycho-emotional component and often related with dental occlusion, is particularly harmful in dental attrition and TMJ[1-4]. Our research revealed that 26% of patients suffered from sleep BRX and 72% presented with awake and sleep BRX, of whom 84% had a significant improvement and the remaining 16% was free of BRX after treatment with BoNT-A.

BoNT-A should be considered a first-choice treatment for BRX due to the good results reported, which have been validated in this study, and the lack of relevant adverse effects[2-8,19,26,27]. BRX is a pathology that begins in adolescence and is highly prevalent in this stage; therefore early treatment would make sense in order to prevent any injuries to the TMJ. Although early treatment of BRX is advisable, we are aware of the need to provide additional thorough information both to minors and their parents or legal guardians, as well as of the fact that this population group has not been studied enough.

In stomatological practice, the usual complaints attributed to BRX include masticatory pain and teeth grinding, often mentioned by their partners. More than pain or anxiety, the main reason for consulting an aesthetic doctor is usually hypertrophy of the masseter muscles, together with a perception of the square shape of their faces[24,25].

Regarding the use of either tricyclic antidepressants and/or anxiolytics or dopaminergic agonists for the treatment of BRX, the former have associated analgesic properties[11-13], whereas the latter, through an increase of dopamine levels, help restore the modulation of the dopaminergic pathways in basal ganglia[27]. Despite the lack of rigorous studies supporting their use, both types are usually prescribed due to the high prevalence of BRX among adolescents[10,13].

Cognitive-behavioral therapies have a limited short-term effect, with a high rate of withdrawal before the achievement of visible muscle relaxation results[10]. Many of the studies conducted have very little evidence and are associated with a low-quality methodology[9,10]. Likewise, if the above-mentioned treatments are compared to the controlled and selective use of BoNT-A, the latter represents a better option for treating BRX due to both its efficacy and fast response. Evidently, when the condition occurs concomitantly with anxiety and/or depression, the risk/benefit ratio should be properly assessed before prescribing antidepressants and anxiolytics to adolescents.

BoNT-A is very easy to administer on patients who do not require any other medications. Injections may be administered every six months, during which time patients are free of BRX symptoms. Several studies have reported that BoNT-A injections are effective in controlling involuntary orofacial movements, reducing the adverse effects associated with the motor muscles of the jaw, and in decreasing any related pain[28,29].

Ultrasound assessments of results obtained after BoNT-A treatment four months after the first injection and five months after the second one enable the prediction of when to schedule each patient's visit, so as to prevent loss of efficacy[15]. When ultrasound values are near those reached at control visits, patients may wait 1-2 months until the next injection; if values are near the ones observed prior to treatment, it is recommended the injection is administered within a month.

Mean doses of BoNT-A used (57.5 ± 13.5 s.U.) in our patients are lower than those used in the aesthetic treatment of the upper third of the face. The possibility of BoNT-A migration is very limited since it is injected in muscles with volume, which are larger and thicker compared to flat muscles of the face. However, caution and proper dosage adjustment is advisable in order to prevent early fatigue during mastication, although said effect is usually mild, of limited duration and resolves in a few days.

We are aware that the beneficial effects on the Tooth Wear Index and TMJ disorders require lengthy follow-up periods, since changes in OPG and/or MRIs are only visible after some time.

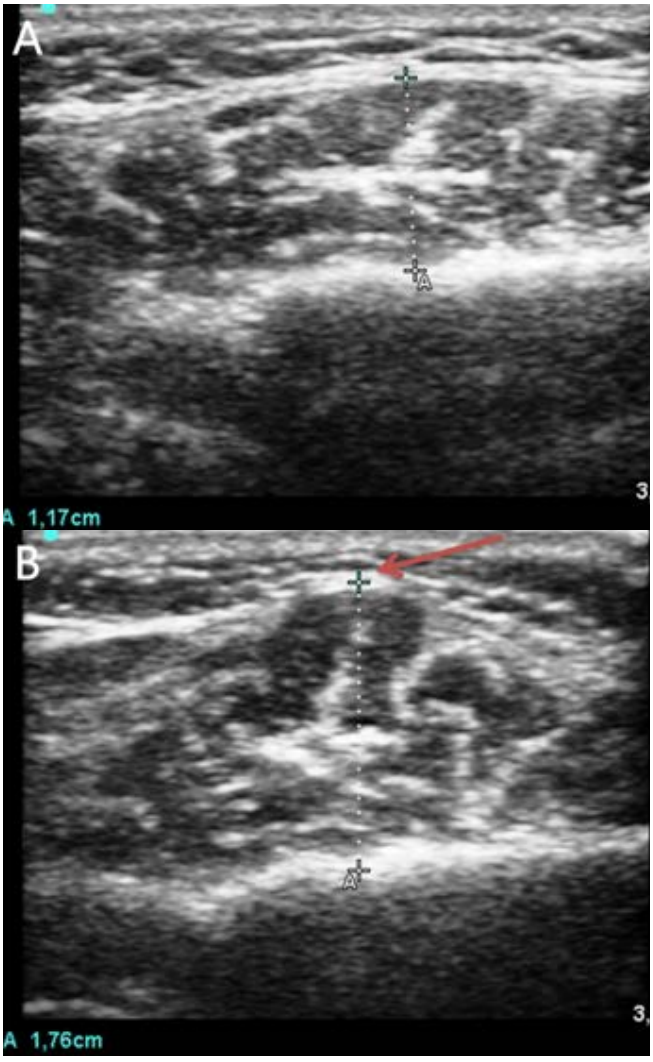

Figure 10 *- Study patient no. 26 (29 years old). Measurements of the right masseter muscle before treatment. A. At rest. B. Notice that at maximum contraction, a dome-shaped elevation of the middle fascicle of the masseter muscle occurs.*

Conclusions

Measurements of the masseter muscles obtained by ultrasound and digital caliper of bigonial diameter are well correlated with a decrease in muscle thickness and relief of BRX-associated symptoms.
All patients treated in this study had a significant improvement (74%) or were free of BRX (26%).
Results obtained in this study after one year of follow-up validate those reached at six months, indicating that ultrasound measurements may predict when to repeat the next treatment with BoNT-A.
Related adverse effects were mild and resolved a few days later. There were no changes in facial expression.
However, it is recommended that new studies with a larger number of patients and a longer duration are conducted.

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aesthetic medicine

Original Article

A Prospective Post-Marketing Pilot Study for the Assessment of Tolerability and Efficacy of a Compositum Containing Trichloroacetic Acid and Hydrogen Peroxide for the Treatment of Papulopustular and or Cystic Acne

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Abstract

Introduction: the multifactorial etiopathogenesis of Acne vulgaris led to multiple treatment options. PRX-T33® is a commercially available class I medical device combining trichloroacetic acid (TCA), hydrogen peroxide (H2O2), and kojic acid, with high skin tolerability for the label indications. The PRX-T33® formulation allows the TCA to reach the dermis, where it stimulates keratinocyte and fibroblast growth factor activity without damaging the superficial layers. Moreover, the antiseptic activity of H2O2 and TCA could reduce/prevent the formation of acne lesions.

Material and Methods: a nine-week, single-center, prospective, open-label, uncontrolled post-marketing study was performed to evaluate the clinical efficacy and tolerability of PRX-T33® for active acne treatment. Qualitative and quantitative analyses (tolerability grading, Tutakne scoring, lesion counting, digital and structural imaging, pH-metry, corneometry, sebometry) were performed in a cohort of 21 patients aged 18-35, of both genders, affected, from at least six months, by active acne.

Results: a significant decrease in the number and severity of lesions following PRX-T33® treatment was observed ($p<0.05$), with limited and transient adverse reactions and side effects. Patients' subjective evaluation supported the objective data.

Conclusion: PRX-T33® was safe, well-tolerated, and effective in the treatment of active acne.

Keywords

Active acne, chemical peeling, PRX-T33®, trichloroacetic acid, peroxide hydrogen

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Introduction

Acne vulgaris, or acne, is one of the most common skin disorders, affecting the pilosebaceous follicles, with either non-inflammatory or inflammatory skin lesions, such as comedones, papules, pustules, nodules, and cysts[1]. The peak incidence occurs during adolescence when it affects about 90% of the adolescent community, and it often persists in adulthood, especially among women, with an incidence of 8% between 25 and 35 years of age, and 3% between 35 and 45 years of age[2]. Acne is undoubtedly a disease with profound psychological effects and a strong psychosocial impact, which can lead to the onset of signs and symptoms of depression and substantial, negative effects on the quality of life (QoL)[3].

The etiopathogenesis of acne is multifactorial, immune-mediated, and androgentriggered, involving four key factors with interrelated mechanisms: 1) inflammation, 2) increased sebum production, 3) hyperkeratinization of the follicular infundibulum, and 4) Cutibacterium acnes infection[4], with a family history in about 40% of cases[5].

The multitude of pathogenic factors has led to the development of a wide range of options for the treatment of acne, including topical retinoid, azelaic acid, and benzoyl peroxide, as well as oral isotretinoin, all indicated as monotherapy or associated to hormone therapy and to systemic or topical antibiotics. However, antibiotics, even if effective in treatment and control of lesions, can induce bacterial resistance if used for long periods[6]. Physical modalities, such as laser therapy and chemical peeling, are also used for the treatment of active acne. In particular, chemical peeling, a procedure widely used for skin regeneration and tissue remodeling, has revealed its effectiveness not only for the treatment of acne scars but also for active acne[7]. In more detail, superficial chemical peeling is a relatively simple and safe procedure that causes a controlled injury of the skin, whose depth is determined by the concentration of the agent(s) applied by the type of vehicle, buffering, and duration of treatment[8]. The selection of the appropriate chemical peel for active acne depends mainly on the patient's skin type and acne activity.

Nevertheless, peelings implicate a downtime, as long as skin is exfoliating and healing, which temporarily exacerbates the patient's blemishes and potentially his/her distress.

PRX-T33® is a class I medical device, commercially available since 2011, with peeling effect that combines trichloroacetic acid (TCA), hydrogen peroxide (H2O2), and kojic acid. The peculiar formulation of PRX-T33® allows TCA, considered as the golden standard in chemical peels, to permeate beneath the epidermis without damaging the superficial layers, and hence without causing frost and subsequent skin desquamation. The amount of TCA that reaches the dermis stimulates fibroblast activity through growth factors released by keratinocytes and induces new collagen synthesis[9]. In this way, it prevents the development of acne lesions into permanent scars. PRXT33® is currently indicated for the treatment of cutaneous elastosis, post-acne scarring, for the prevention and treatment of atrophic scars in general. H2O2 acts synergistically with TCA on fibroblastic activation[10-12], and both play an antiseptic role on the local bacterial flora as well. Kojic acid decreases melanin production and hence can contribute to preventing possible post-inflammatory hyperpigmentation.

Post-marketing surveillance performed by the manufacturer (GPQ SRL, Milano, Italy) since December 2011 revealed the high cutaneous tolerability of PRX-T33® for the officially approved label indications.

Based on current literature on the peculiar formulation and consequent potential synergic activity of TCA and H2O2[10-12], we hypothesized that PRX-T33® could be effective in the treatment of acne papules, pustules and or cysts, both through a very mild superficial exfoliation, acting as unplugging agent of glandular secretory ducts, and the dehydration and antiseptic activity of acne lesions. PRX-T33® could also be able to prevent additional skin infections.

To test our hypothesis, we designed a single-center, prospective, open-label, uncontrolled post-marketing study (clinical protocol WQM0001) to evaluate the clinical efficacy and tolerability of PRX-T33® for the treatment of active acne with papulopustular and or cystic acne. The study has been performed from December 2013 to June 2015, and results support a safe, well-tolerated, and effective use of PRX-T33® in active acne.

Methods

The class 1 medical device PRX-T33® (GPQ SRL, Milano, Italy) was tested in a singlecenter, prospective, open-label, uncontrolled post-marketing study (clinical protocol WQM0001, Version 6, dated June 10, 2013), conducted from December 2013 to June 2015 at the Servizio ambulatoriale di Medicina Estetica dell'Ospedale Generale San Giovanni Calibita Fatebenefratelli Isola Tiberina, Rome, Italy (Principal Investigator: Dr. E. Bartoletti; Study Sponsor: GPQ SRL). The clinical study was performed following the Good Clinical Practice guidelines and has been approved by the Ethical Committee of the Institution. All patients enrolled in the study gave assigned consent to participate in the study and to the inclusion of material pertaining to themselves; they acknowledge that they cannot be identified via the paper and have been fully anonymized by the authors. All mandatory laboratory health and safety procedures were compiled within the course of any experimental work conducted.

The objectives of the study were: 1) Assess the tolerability of PRX-T33® treatment on the drying of acne lesions in patients with papulopustular and or cystic acne; 2) evaluate the effectiveness of the above treatment with qualitative and quantitative methods; 3) assess the patient's quality of life following the proposed treatment.

The study lasted nine weeks for each patient from the time of enrolment, including eight weeks of effective treatment with PRX-T33® and one week of post-treatment check-up.

Reaction	Grading
No reaction	0
Weakly positive reaction (usually characterized by mild erythema and / or dryness in most of the treatment site	1
Moderately positive reaction (usually distinct erythema or dryness, possible spread outside the treatment site	2
Strongly positive reaction (severe erythema, often widespread, with edema and / or eschar formation)	3

Table 1 *- The classification of the degree of irritation on human skin based on what is described in Annex C of UNI EN ISO 10993-10: 2010 "Biological evaluation of medical devices", for the assessment of the tolerability of the treatment[12].*

Selection of Study Subjects

The clinical study enrolled patients between 18-35 years of both sexes, affected from at least six months, by acne papules, pustules, and or cysts on both sides of the face. Other inclusion criteria were: Subjects not treated with other topical acne medications for at least one month or with systemic drugs for at least three months. Exclusion criteria: Subjects with documented allergy to kojic acid, active cutaneous and or herpetic eruptions, seborrheic dermatitis, pregnant or breastfeeding women, and subjects affected by neoplastic or autoimmune diseases. The three study objectives were: 1) Evaluation of tolerability of treatment with PRXT33® on drying of acne lesions in patients affected by acne papules, pustules and or cysts; 2) evaluation of treatment efficacy by qualitative and quantitative methods; 3) assessment of the QoL of patients following the PRX-T33® treatment.

Treatment Protocol

PRX-T33® treatment was performed weekly for a total of 8 weeks. The first and second application of PRX-T33® was applied exclusively on half of the face showing the highest number of lesions to assess the tolerability of this medical device. Subsequently, once the tolerability of the product was verified, patients were treated weekly with PRX-T33® for a total of 8 weeks. Each molecule of PRX-T33® contains a unique combination of 33% TCA, hydrogen peroxide (H2O2) and 5% kojic acid; making it not as "aggressive" as pure TCA.
The product was applied over clean skin through a silicon spatula. An amount of 0.5 mL of PRX-T33® was enough for a surface of 6.30-8.50 cm2. Residual PRX-T33® was eliminated with a water-soaked swab. Pustules were slightly scratched with a sterile 21G needle and gently drained, followed by the application of PRX-T33® with a swab.
In case of contact of PRX-T33® with blood from the drained pustules, the area was immediately washed with sterile saline solution.

Initial Tolerability and Evaluation

Tolerability was graded from 0 (no reaction) to 3 (markedly positive reaction) according to appendix C of the UNI EN ISO 10993-10:2010 "Biological evaluation of medical devices" (*Table 1*)[12]. The treatment was considered well tolerated when the degree of irritation was between 0 and 2. Conversely, the appearance of severe irritation (grade 3) was indicative of a non-tolerant patient.

Evaluation of Treatment Efficacy

Treatment efficacy was evaluated through an objective assessment of the lesions: number and sites of acne lesions (papules, pustules, and nodules), according to the Tutakne scoring system (grading the lesion severity from 1 to 4)[13], at basal level (pretreatment visit 1 [T0]), and at each treatment visit (*Table 2*). Digital photographs of acne lesions were taken with the Canon PowerShot G6 digital camera (Canon, Inc, Tokyo, Japan), while structural photographs were taken with the 3D in vivo optical skin imaging system Antera 3D (Miravex, Dublin, Ireland) at T0, at treatment visit #3 (T1); at treatment visit #5 (T2); at follow-up visit, one week after the last treatment of the study (T3). Subsequently, the efficacy of PRXT33® topical administration on the number and the severity of the lesions was assessed through the comparison of images at T0 and T3 through both the Tutakne scoring system of acne severity (grading from 1 to 4)[13].

Grade	Signs
Grade 1	Comedones, occasional papules
Grade 2	Papules, comedones, few pustules
Grade 3	Predominant pustules, nodules, abscesses
Grade 4	Mainly cysts, abscesses, widespread scarring

Table 2 *- Acne vulgaris grading score, using a grading system which classifies acne vulgaris into four grades[13].*

H-metry, Sebometry, and Corneometry Assessments

pH-metry, sebometry, and corneometry were performed at basal level (T0) and follow-up (T3) in different areas of the face, using a Derma Unit SSC 33 (Courage +Khazaka electronic GmbH, Köln, Germany). Sebometry values were expressed as micrograms/cm2, while cutaneous hydration values were expressed as corneometry units.

Quality of Life

The QoL questionnaire used included eleven items and was based on a validated Visual Analog Scale (VAS) graded from 0 to 10 a week after the end of the treatment (T3)[14].

Statistical Analysis

The sample size of the study was calculated assuming initial average grading of 2.5 at the Tutakne scoring assessment[13], and a 50% improvement of grading at follow-up, with a standard deviation value of 1.25. Based on this hypothesis, a sample size of 20 subjects was enough to guarantee power for the study of 0.8 (80%), with a statistical significance level alpha of 5% (0.05).
The Student's t-test was performed on quantitative paired data collected before and after the treatment in each patient. The non-parametric Wilcoxon signed-rank test for paired data was applied in case of deviation from a normal distribution for evaluation and comparison of lesion number and grading before and after the treatment according to the Tutakne method. P values <0.05 were considered statistically significant. Patients' age and the number of papules, pustules, and nodules were presented as mean ± standard deviation; other data were presented as absolute frequency and number of individuals.

Results

Twenty-one consecutive patients were enrolled in the study aged 18-35 (male subjects: mean age 21 years, range 18-24; female subjects: mean age 23 years, range 18-33; and male subjects: n=7). Two of the 21 patients dropped out (one after visit 4, one at follow-up), and consequently, the skin tolerability data were collected for all the 21 patients, while the final efficacy data were collected and evaluated for 19 patients. There were two dropouts, one patient stopped after the fourth treatment session because she was not satisfied with the result obtained so far, another patient ended all the treatment sessions but was not returned for the final inspection visit, one week after the last session.

Tolerability and Safety Data

The treatment with PRX-T33® induced only limited and transient adverse reactions and side effects. After the first application of PRX-T33®, 90.5% of patients (n=19/21) showed a cutaneous irritation level graded 0-2, 14.3% (n= 3/21) showed a grade 3 irritation, 42.9% (n= 9/21) grade 2, and 52.4% (n=11/21) had grade 1.
After the second application, 81.0% of patients (n= 15/19) showed an irritation level graded 0-2, 42.8% (n= 8/19) had grade 1 and 38.1% (n=7/19) had grade 2, while 19.0% of patients (n=3/19) showed a grade 3 cutaneous irritation only in one of the first two sessions performed, for which they continued and ended the treatment, without showing any sign of adverse reaction in the subsequent sessions. Only one patient (4.7%) had a grade 3 reaction in both the first two sessions but completed the treatment without the onset of adverse reactions. However, all patients, including the two subjects showing a grade 3 irritation in both the first two treatment visits, completed the 8-week treatment without any adverse event.
Regarding the side effects, the erythema/redness shows a high percentage compared to the other effects, but a significant decrease (from 71% to 26%) was observed at the final control visit (post-treatment) compared to the previous visits. As a second side effect, exfoliation has a high percentage, but also, in this case, there is an 8% difference between the second session and the final follow-up visit (T3) (*Table 3*).

Efficacy Data

We observed a decrease in the average number of acne lesions after three visits, including the T0 visit. A Student's t-test performed on quantitative paired data revealed an average of 15.2 acne lesions at T0, decreased to an average of 8.5 acne lesions at T3, with a statistically significant difference (p= 0.007, CI 95%) (*Figure 1*).

Visit	Erythema / Redness		Inflammation		Exfoliation		Other	
	n	%	n	%	n	%	n	%
2	15	71	1	5	4	19	1	5
3	14	67	2	10	6	29	1	5
4	15	71	2	10	4	19	1	5
Last	5	26	1	5	2	11	0	0

Table 3 *- Side effects detected along the study.*

The non-parametric Wilcoxon signed-rank test for paired data revealed a statistically significant lower severity of acne lesions, established according to the Tutakne method, at T3 vs. T0 (p= 0.007, CI 95%).
Digital photography imaging supported the quantitative analysis, showing a clear gradual decrease of acne lesions at different time points of treatment with PRXT33® vs. T0 (*Figure 2*). In addition, digital images of each patient at T3 vs. T0 were compared through the Tutakne scoring system of acne severity, and the results indicate that 60% of patients obtained an improvement at T3 vs. T0, 25% of patients was not affected by the treatment with PRX-T33®, and 5% of patients showed a worsening of the condition (the percentage range refers to the Tutakne scoring system). While 100% of male subjects showed an improvement after the treatment with PRX-T33®, female subjects showed variable outcomes.

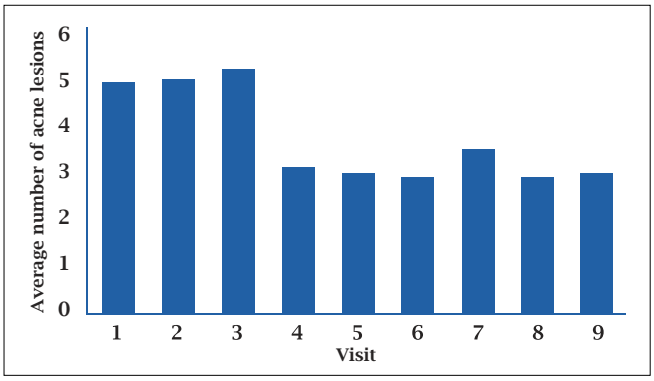

Figure 1 *- Average number of acne lesions detected at basal level (pre-treatment visit #1), at each treatment visit (visits #2 to #8), and at the follow-up (visit #9, one week after the last topical administration of PRXT33®). Data are reported as mean ± SEM. *: p<0.05 vs. data collected at visit #1. 42x23mm (300 x 300 DPI).*

pH-metry, Sebometry, and Corneometry Data

Sebometry results revealed an overall decrease of the values at T3 vs. T0, except for the central forehead area and of left nose wing, while pH-metry revealed no significant change of pH values at T3 vs. T0. Finally, corneometry results revealed an overall increase of cutaneous hydration at T3 vs. T0 in all the different areas of the face, from an average of 47.5 corneometry units at T0 to an average of 51.7 corneometry units at T3 (p= 0.011) (*Figure 3*).
The objective qualitative and quantitative analysis of acne lesions, we had been performed at different time points, reflected the patients' subjective criteria.

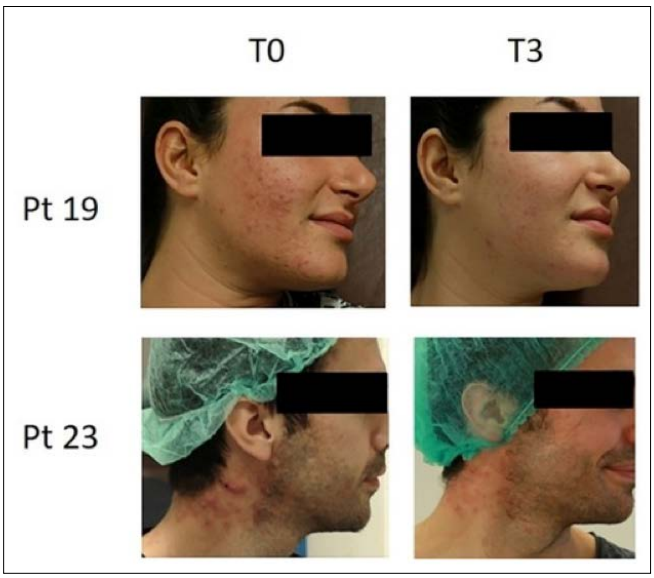

Figure 2 *- Digital photography imaging of two representative patients performed at different time points of treatment with PRX-T33®. T0: basal level at pre-treatment visit 1; and T3: follow-up visit, one week after the last topical administration of PRX-T33®. 47x40mm (300 x 300 DPI).*

Quality of Life Data

The QoL questionnaire filled by patients at one week after the last treatment (T3), revealed a positive evaluation of overall results (average evaluation: 70.63), as patients would be available to repeat the treatment

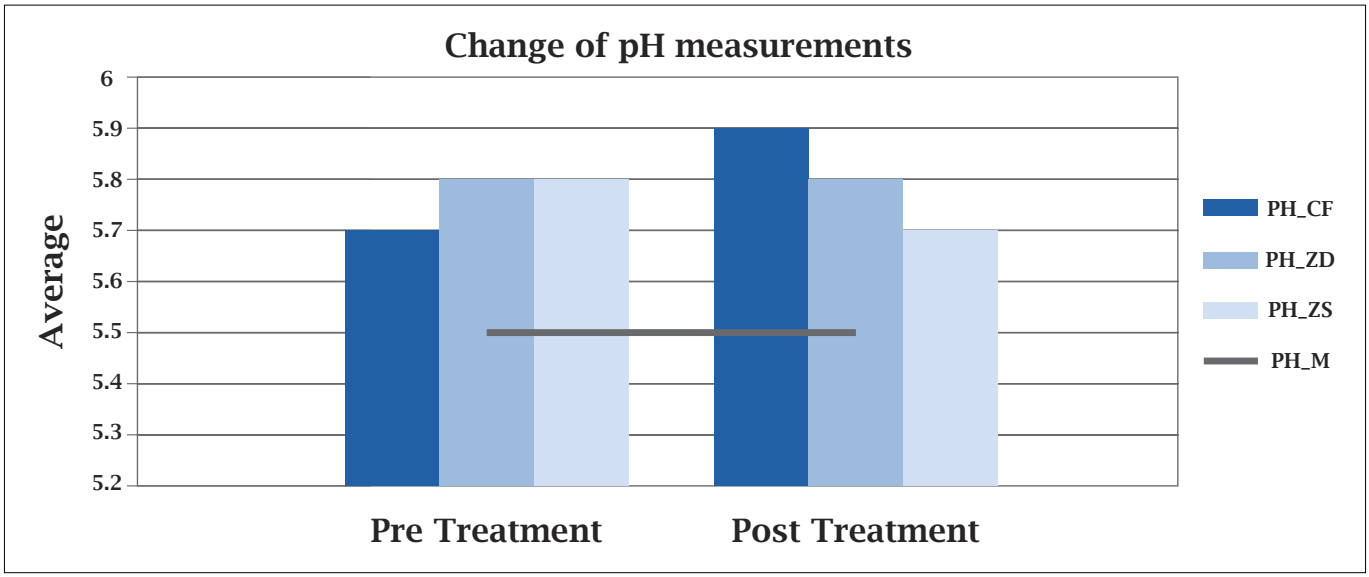

Figure 3 *- Changes in pH values. Abbreviations: PH_CF, pH center front; PH_ZD, pH right cheekbone; PH_ZS, pH left cheekbone; PH_M, pH chin. 38x23mm (300 x 300 DPI)*

(85.37) and would recommend the treatment to others (84.79). Besides, according to the questionnaire data, the treatment resulted well tolerated, without any significant effect on patients' social activity. The side effects did not influence their social life.
Patients tolerated, on average, side effects such as flaking and dry skin. The questions about burning and redness also received an average positive rating.

Discussion

The treatment targets in active acne are the reduction of inflammation, a quick decrease in the number of acne lesions, and the improvement of skin texture. Chemical peeling is a widely used and generally safe method for the treatment of a variety of skin conditions[15]. Among others, chemical peels are revealing to be beneficial in the treatment of active acne as they target multiple pathogenetic factors of the disease[7].
However, the number of studies is still relatively low, and the efficacy and safety of such treatment options are yet to be well evaluated through an extensive review of clinical data[10]. Moreover, no consensus has been reached so far for optimal treatment timing, duration, and concentration of the different chemical agents[8]. The selection of a chemical peel method for active acne should be individualized based on the skin type, history of acne, concurrence of other skin diseases, and previous treatments. PRX-T33® chemical peel is an improved formula of the TCA peel, able to penetrate deep inside, stimulate fibroblast proliferation and activity in the dermis, and tissue repair avoiding atrophic scar development. TCA applied on the skin surface induces a local reaction through the Skin Stress Response System, which acts in the dermis through proopiomelanocortin and its derived peptide hormone9. At the same time, PRX-T33® is endowed with antiseptic activity[6] and able to inhibit the production of melanin, brightening the treated area of the face. These properties are based on the positive and encouraging results obtained in our study. The assessment of the severity of acne continues to be a challenge for dermatologists. The study we performed as pilot study to evaluate the tolerability and effectiveness of PRX-T33® on active acne in subjects aged 18-35 included both qualitative and quantitative analysis of acne lesions through various measurements (grading and counting) based on clinical examination and photographic documentation, as described elsewhere, in order to obtain an accurate and reproducible evaluation. Most importantly, the qualitative and quantitative analysis of acne lesions before and after the treatment with PRX-T33® was consistent with the results from the QoL questionnaire filled by the patients at the end of the study, revealing a positive impact both on clinical progression of the disease, both on the psychological well-being.
In conclusion, the overall results of our study support the efficacy of PRX-T33® in the treatment of active acne with papules, pustules, and or cysts. The treatment proved to be safe, well-tolerated, and manageable, thus encouraging the setup of a specific protocol for this new therapeutic indication.

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Conflict of Interests

The authors report no conflict of interest.

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aesthetic medicine

Original Article

Factors Related to Skin Moisturizing Functions in Adults: A Cross-Sectional Study

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Running head: Related factor for skin moisturizing function

Abstract

Background: Skin problems can develop regardless of age. It is highly important to maintain skin moisturization. Previous studies have suggested a relationship between lifestyle and skin.
Aims: Skin problems can develop regardless of age. In this context, it is highly important to maintain proper skin moisturization. This study clarified the relationship between lifestyle and skin moisturizing functions.
Methods: This study employed a cross-sectional design in which 86 adult participants were recruited from a university in Japan. Self-administered questionnaires were distributed to determine and analyze lifestyle characteristics related to two skin moisturizing function parameters, including transepidermal water loss (TEWL) and stratum corneum (SC) hydration. Lifestyles factors included diet, exercise, and rest. Multiple linear regression analyses were conducted using TEWL/SC hydration as dependent variables while adjusting for confounders.
Results: Based on the lifestyle factors listed above, multiple regression analyses were conducted on variables that satisfied the inclusion criteria.
We found that exercise (β=0.34, $p<0.001$) and rest (β=0.35, $p<0.001$) were positively associated with SC (adjusted R^2 of 0.26), while there were no lifestyle factors associated with TEWL in this study; the adjusted R2 was 0.02.
Conclusions: Participants with high scores for exercise and resting habits had high SC values. However, TEWL was not highly associated with lifestyle. Results suggest that skin condition may be improved through lifestyle interventions designed to promote exercise and rest.

Keywords

Lifestyle, exercise, rest, skin physiological phenomena

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Introduction

Skin problems can develop regardless of age, and may range from acne in young people to dryness in the elderly. These conditions may cause mental anxiety and physical issues such as itchiness, thereby negatively affecting one's quality of life (QoL)[1,2]. The condition of the skin is related to the skin moisturizing function[3]. Therefore, maintaining and improving the skin moisturizing function may lead to the maintenance of the skin condition. The skin moisturizing function declines with age and skin aging begins in the 30s and 40s[4,5]. These results suggest that it is important to take preventive measures against skin dryness in one's 30s rather than in old age.

Transepidermal water loss (TEWL) and stratum corneum (SC) hydration are widely used as quantitative indicators for measuring the skin moisturizing function[4]. After using these indicators several authors have reported that particular lifestyle habits are associated with the skin moisturizing function. These include issues of daily skin moisturizing care, bathing habits, smoking, stress, dietary contents, and sleep quality[6-13]. While these studies produced valuable results, they tended to focus on specific issues such as meal contents or lifestyle aspects related to bathing habits or daily moisturizing care. As such, each produced results on the relationships between one aspect and skin moisturization. It is therefore unclear which lifestyle types are associated with overall skin moisturization levels.

However, since people have various habits in life, it is necessary to clarify the overall relationship between multiple factors and the skin moisturizing function. This study identified the relationship between multiple lifestyle habits and their impact on skin moisturizing function in order to obtain data for an intervention aimed at improving skin moisturizing function. The clarification of these relationships can also be used in the clinical setting to advise patients on general lifestyle changes that can prevent skin dryness.

Methods

Study design

This cross-sectional, observational study was conducted in Japan from July to October 2019.

Participants

To recruit various participants with a wide range of ages, recruitment targets were students and staff at X University. However, those with chronic skin diseases such as atopic dermatitis were excluded. A total of 86 participants without skin disorders were thus recruited. This study was conducted as part of a health checkup program to modify your lifestyle habits. All participation was voluntary.

Data collection

This study analyzed two skin moisturizing function parameters of TEWL and SC hydration based on data from the presence or absence of lifestyle habits that were associated with the skin moisturizing function in previous studies, and self-administered questionnaires concerning participant lifestyles. We confirmed that no responses had been omitted when collecting the questionnaires. All participants completed these in a room set to a temperature of 20-22ºC, with humidity levels measuring between 40-60%. Skin moisturizing function parameters were measured after 20 minutes of rest in the room where the questionnaire was answered. Participants were then given self-administered questionnaires asking for information about their sex, age, presence/absence of daily moisturizing care, presence/absence of outdoor activities, use of air conditioning, and lifestyle.

Lifestyle attributes were determined based on the Diagnostic Inventory of Health and Life Habit (DIHAL.2)[14]. Items on the DIHAL.2 are answered according to a 5-point Likert scale ranging from 1 (Not applicable) to 5 (Applies well). It further consists of four health subscales, including those on health, exercise, diet, and rest, which are assessed based on 47 total items and 12 factors. However, this study only used the exercise, diet, and rest subscales. Each scale was developed for use with junior high school students and adults. Reliability and validity were previously confirmed[14].

Skin moisturizing functions were evaluated based on TEWL and SC hydration. TEWL measurements were taken from the inner-center point of each participant's right forearm (an 8cm point on the palm side from the elbow fossa center). This was done due to the ease of obtaining stable values.10) All measurements were performed for 20 seconds, with the average value for the central 10 seconds used as data. The Tewameter TM300 was used for this purpose (Courage+Khazaka electronic GmbH, Köln, Germany).

SC hydration was measured from a point oriented 2cm from the TEWL measurement point to the end side. Each participant was measured three times, with the average value used for analysis.10) The CM825 was used to conduct these measurements (Courage+Khazaka electronic GmbH, Köln, Germany).

To influence the measurement index, we requested that participants adhere to the following three rules[10]:

1) Do not apply body cream or similar substances to the measurement site for at least 12 hours prior to the measurement.
2) Do not consume caffeinated beverages (e.g., coffee or tea) or smoke for at least three hours prior to study.
3) Do not engage in strenuous exercise for at least one hour prior to study.

All the participants had a shower the night before the measurement.

Ethical considerations

This study was conducted after gaining permission from the research ethics committee of O University Graduate School of Nursing (Approval No. 2019-29). Participants were informed that they had the right to decline participation and could withdraw from the study at any time. Further, participation was completely voluntary, and all participants received both oral and written information about the study purpose, contents, and extent. They were then assured that all responses were confidential.

Participant confidentiality was specifically protected by providing a code number for each participant prior to data collection and analysis. The collected questionnaires were also kept in a locked cabinet. As consent checkboxes were placed onto each questionnaire form, participant consent was indicated when each checked their respective boxes, which was done prior to submission.

Data analyses

Descriptive statistics were presented as means and standard deviations (SDs) for continuous variables, while numbers (%) were used for categorical variables.
Multiple linear regression analyses were conducted using TEWL/SC as dependent variables while controlling for confounders. The model included demographic characteristics and environmental factors (e.g., using settings or year as covariates). All analyses were concluded using SPSS statistics version 25 (IBM Corp, Armonk, NY).
The multicollinearity of predictors (including covariates) was explored using the variance inflation factor (VIF). VIF values were suitable for all variables, which were less than the values of multicollinearity (VIF values >10). Both age/gender and participant scores for diet, exercise, and rest were set as independent variables and input through the simultaneous entry method. Items from the questionnaire that were deemed significant based on the single regression analysis were also entered; input criteria were standardized $\beta > .200$ or $p < .05$.
Variables that were significantly based on the multiple regression analysis were then divided into two groups based on median score. An independent t-test was then conducted.

Results

Data from all 86 participants were included in the analysis of this study. Mean age (SD) was 33.8 (13.0) years, while 84% were women (*Table 1*). Most participants did not use a skin moisturizer, while more than 90% spent significant amounts of time in air-conditioned spaces. Only one participant had smoking experience.

Relationship between TEWL/SC hydration and questionnaire results

Single regression analyses were performed between TEWL/SC hydration and characteristics/DIHAL2 scores (*Table 2*). No factors were associated with TEWL, while SC hydration and DIHAL.2 lifestyle scores were positively associated. Multiple regression analyses were then conducted by selecting independent variables according to the input criteria (*Table 3*). Here, no factors were associated with TEWL; adjusted R^2 was .02. Further, no factors were negatively associated with SC hydration. However, exercise ($\beta = .30$, $p = .007$) and rest ($\beta = .35$, $p = .001$) were positively associated with SC hydration; adjusted R^2 was26.
Figures 1 and *2* show TEWL and SC hydration when respondent's exercise/rest scores were divided into groups based on the median. For SC hydration mean(SD), there was a significant difference between the two

Variables	Mean (SD)	n (%)[a]
Age	33.8(13.0)	
20-29		41 (47.7%)
30-		45 (52.3%)
Sex		
Male		14 (16.3%)
Female		72 (83.7%)
Moisturizer		
Use		27 (31.4%)
Do not use		59 (68.6%)
Sunscreen cream		
Use		45 (52.3%)
Do not use		41 (47.7%)
Smoking		
Current or past		1 (1.1%)
Never		85 (98.9%)
Outdoor activities (weekly)		
0 times		38 (44.2%)
1-2 times		35 (40.7%)
3-4 times		11 (13.0%)
5 or more		2 (2.3%)
Use of air conditioning		
Almost never		1 (1.1%)
Occasionally		1 (1.1%)
Sometimes		14 (16.3%)
Often		70 (81.4%)
Carbohydrate[b] intake		
0 times per day		0 (0%)
1 time per day		2 (2.3%)
2 times per day		25 (29.1%)
3 or more times per day		59 (68.6%)
Carbohydrate[b] intake per serving		
Hardly eat		1 (1.1%)
Half a bowl		19 (22.1%)
Bowl of rice		58 (67.4%)
More		8 (9.3%)
DIHAL2 scores	**Mean (SD)**	
Diet	44.81 (7.86)	
Exercise	25.77 (6.28)	
Rest	43.99 (8.34)	

Table 1 *- Participant Demographic Characteristics (N=86)*
Note: SD = standard deviation. [a]All values expressed as n (%), where the sum of percentages may be over 100% due to rounding off at the second decimal place. [b]Carbohydrates were items such as rice and bread.

	TEWL			SC		
Variables	**β**	**Std β**	***p-value***	**β**	**Std β**	***p-value***
Age	-0.01	-.13	*.252*	0.08	.16	*.144*
Sex	0.40	.11	*.325*	-2.80	-.15	*.156*
Moisturizer	0.08	.03	*.812*	-1.84	-.13	*.242*
Sunscreen cream	-0.13	-.05	*.658*	-2.21	-.17	*.129*
Use of air conditioning[a]	0.25	.08	*.468*	0.08	.01	*.962*
Outdoor activities (weekly)[b]						
1-2 times	-0.39	-.14	*.203*	0.402	.03	*.788*
3 times or more	-0.62	-.16	*.138*	-2.668	-.14	*.190*
Carbohydrate intake[c]	-0.01	-.00	*.983*	0.517	.04	*.743*
Carbohydrate intake per serving[d]	-0.35	-.15	*.171*	-1.92	-.12	*.268*
DIHAL scores	**β**	**Std β**	***p-value***	**β**	**Std β**	***p-value***
Diet	0.01	.07	*.524*	0.20	.24	*.029*
Exercise	0.03	.11	*.307*	0.47	.44	*<.001*
Rest	-0.01	-.03	*.787*	0.37	.46	*<.001*

Table 2 *- Single Regression Analysis Between TEWL/SC and Characteristics/DIHAL Scores (N=86)*
Note: TEWL = Trans-Epidermal Water Loss. SC = Stratum Corneum hydration. Std β = Standardized β. [a]: 0 = Almost no, occasionally, sometimes, 1 = Often. [b]: Reference category: Outdoor activities 0 times a week. [c]: 0 = 0 times a day, 1 times a day, 2 times a day, 1 = 3 or more times per day. [d]:0 = Hardly eat, Half a bowl, 1 = Bowl of rice, more.

	TEWL			SC		
Variables	**β**	**Std β**	***p-value***	**β**	**Std β**	***p-value***
Age	-0.21	-.20	*.098*	0.05	.09	*.386*
Sex	0.53	.14	*.223*	0.38	.02	*.835*
DIHAL scores	**β**	**Std β**	***p-value***	**β**	**Std β**	***p-value***
Diet	0.02	.14	*.285*	0.00	.00	*.985*
Exercise	0.05	.21	*.104*	0.32	.30	*.007*
Rest	-0.02	-.12	*.340*	0.28	.35	*.001*
Adjusted R^2	.02			.26		

Table 3 *- Factors Related to TEWL and SC (N=86)*
Note: TEWL = Trans-Epidermal Water Loss. SC = Stratum Corneum Hydration. Std β = Standardized β.

groups in regard to exercise and rest habit scores (Exercise: ≦Median 36.37(5.89) Median< 39.81(7.20). Rest: ≦Median 36.41(5.93) Median< 39.69(7.19). On the other hand, the differences in exercise/rest scores were not significant for TEWL mean(SD) (Exercise: ≦Median 4.79(1.45), Median< 5.24(1.28). Rest: ≦Median 5.16(1.38), Median< 4.84(1.38). Based on these results, a posteriori analysis of the test power of the multiple regression analysis for SC hydration was performed. Effect size f2=0.35, α=0.05, Total sample size=86, Number of predictors=5 and a posteriori analysis of the test power was performed with Power=0.99 and it was a high test power. Similarly, a posteriori analysis of test power was conducted for TEWL. Effect size f2=0.02, α=0.05, Total sample size=86, Number of predictors=5 and a posteriori analysis of the test power was performed with Power=0.13.

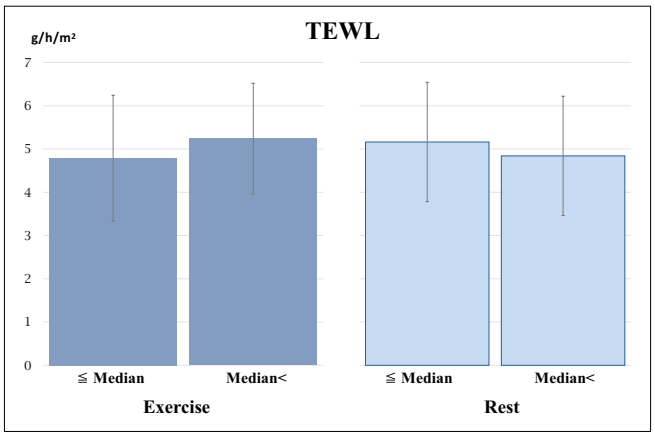

Figure 1 - There was no difference in TEWL when exercise/rest scores were divided based on median. (N=86). TEWL = Trans-Epidermal Water Loss. Independent t-test.

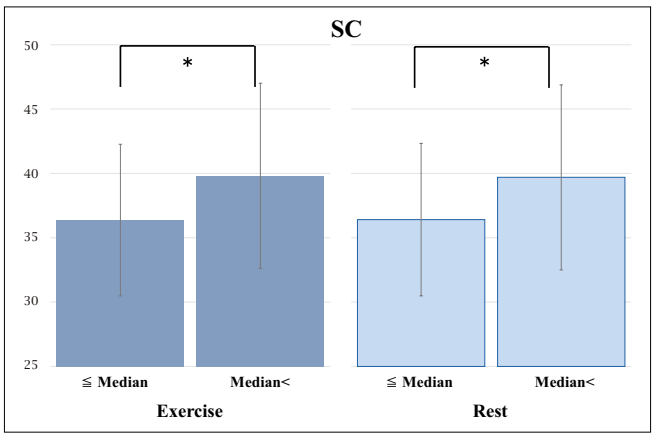

*Figure 2 - There was a significant difference in SC when exercise/rest scores were divided based on median. SC = Stratum Corneum Hydration. Independent t-test. *p<0.05.*

Discussion

This study investigated the relationships between lifestyle factors and skin moisturizing function based on two functional skin examinations and a self-administered questionnaire concerning participant lifestyles. Rest and exercise were associated with SC hydration. The adjusted R^2 for the results of multiple regression analysis in SC is not high at 0.26. However, it is significant that five lifestyle factors such as age, gender, rest, exercise and diet were able to explain 26% of the complex human skin moisturizing function.

Previous studies have demonstrated relationships between several individual lifestyle factors and the skin moisturizing function[6-13]. However, it remained unclear how the skin moisturizing function was impacted by overall lifestyle. This study found that the general lifestyle factors of rest and exercise significantly affected SC hydration.

This study's questionnaire inquired about sleep habits and stress avoidance behaviors as general rest habits. As such, participants with low rest scores were considered to have irregular sleep habits and/or stress. Notably, chronic stress leads to the secretion of glucocorticoids from the adrenal cortex , while stress due to insomnia leads to the secretion of glucocorticoids and affects the lamellar body of the skin[15,16]. In this study, it was also possible to observe that irregular sleep and stress promoted glucocorticoid secretion in those with low rest scores, thereby resulting in decreased skin function and reduced SC hydration.

Only a few studies have examined the relationship between the skin moisturizing function and exercise. One study reported that elderly subjects over 65 years who engaged in endurance exercise showed improvements to stratum corneum thickness and dermal collagen contents[17]. This is thought to be due to the exercise-induced mitochondrial biosynthesis. Decreased skin function has also been attributed to reduced mitochondria[18], while endurance exercise induces IL-15 and promotes mitochondrial biosynthesis[17,19]. In this study, it is possible that those with high exercise scores thereby promoted mitochondrial biosynthesis, thus improving the skin structure and allowing for increased SC hydration. Further, exercise can increase blood flow to the skin and induce sweating[20]. These factors may also be related to the skin moisturizing function. Interventions designed to improve skin health should thus include both rest and exercise, thereby increasing the skin moisturizing function.

While SC showed an association with some lifestyle habits, there was little association between TEWL and lifestyle habits. This may be due in part to the fact that the elderly were not included in the study. TEWL measures water transpiration from the body to the ex vivo body, which measures skin moisturizing function as well as the barrier function that prevents bacteria from entering the body from the ex vivo body. The barrier function of the skin relies on physical structures such as the lamellar body of the stratum corneum[21]. Changes in TEWL are associated with changes in skin structure due to chronic dermatitis and aging[22,23]. In older adults aged 65 years and more, endurance exercise improves the structure of the stratum corneum, so it is expected

that exercise habits will improve the structure of the stratum corneum and reduce TEWL[17]. However, the expected results may not have been obtained because the subjects in this study did not have skin disorders and did not include those older than 65 years of age. A similar study should be conducted in subjects with skin disorders and in the elderly to investigate changes in TEWL in presence of exercise habits.
This study also had some limitations. In the first place, its cross-sectional design could not prove any causality. In the second place , the study sample only included participants with no history of skin disease from one university in Japan, most of whom were women. Future studies are therefore needed to examine these issues among more diverse groups. Despite these limitations, this study provided evidence for the association between SC hydration and lifestyle habits such as rest and exercise in healthy adults.

Conclusions

Those with high scores for exercise and rest habits had higher SC hydration values and were in the healthy adults group . However, this study found no such associations between TEWL and lifestyle. These findings suggest that having an exercise habit, regular sleep habits, and stress management may increase SC hydration. Future longitudinal studies on exercise and resting habits are needed.
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Conflict of interest

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Disclosure

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Case Report

Insidious diagnosis of breast cancer in patient with previous MacrolaneTM breast infiltration: a case-report

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Running head: Breast cancer Macrolane filler

Abstract

Breast augmentation is one of the most performed aesthetic surgery. In addition to the silicone breast implants, hyaluronic acid base fillers represent a non-surgical alternative. There are different types of hyaluronic acid for this purpose, including MacrolaneTM. In addition to the classic complications associated with the mammary injection of these fillers, Macrolane may cause a well-known radiological ambiguity potentially leading to a delay in the diagnosis of an underlying breast cancer.

The patient underwent breast augmentation with hyaluronic acid and after several years from the procedure she noted the appearance of subcutaneous nodules and discontinuous mastodynia, attributed to previous Macrolane infiltrations: unfortunately the radiological images did not immediately show the underlying contextual cancer of the right breast.

Patient underwent therapeutic right mastectomy and prophylactic left mastectomy, because of the presence of BRCA1 mutation. Simultaneously we performed an immediate reconstruction with mammary implants and biological meshes. No complications arose in the follow up.

Several authors have already carried out studies on Macrolane focusing on its interference and delay in the diagnosis of malignant breast diseases. At present there is only one other case in literature reporting on a patient diagnosed with physical and instrumental examinations and delaying the diagnosis. We believe that the use of hyaluronic acid (Macrolane) fillers for breast augmentation should be avoided. In view of the complexity of these cases, a multidisciplinary approach is always advisable: we believe that a continuous dialogue between the Plastic surgeon, the Breast-dedicated Radiologist and the Oncologist is pivotal.

Keywords

Macrolane, Breast Cancer, Hyaluronic Acid, Ductal Carcinoma, Breast Reconstruction

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Introduction

The surgical procedures of breast augmentation and the use of fillers for cosmetic minimally-invasive procedures are among the main treatments increasingly required, according to the 2018 National Plastic Surgery statistics. Macrolane is a highly cross-linked, stabilized, non-animal and biodegradable hyaluronic acid-based gel (NASHA technology). After the European approval for its use in breast augmentation procedures in 2008, Macrolane was considered a valid non-invasive alternative to prosthesis mastoplasty[1].
Following the growing evidence of radiological ambiguity associated with its use as a breast filler[2-5], Macrolane was banned for this indication in 2012.
Currently in the literature there is only one case of breast cancer arising after mastoplasty with Macrolane, which masked an underlying cancer, interfering with physical and instrumental examinations and delaying the diagnosis[6].

We report a new case of breast cancer in a patient who had previously experienced bilateral mammary Macrolane injections, causing difficulties in diagnosis.

Case Report

A 54-year-old woman, non-smoker, with negative pharmacological and remote pathological anamnesis, but with family history of breast and pancreatic cancer, underwent aesthetic bilateral breast augmentation with hyaluronic acid (MacrolaneTM) in 2008 in another facility. The patient reported slow onset of discomfort and itching, followed by progressive appearance of subcutaneous nodules and discontinuous mastodynia. For these reasons in 2014 she was examined by her General Practitioner, who prescribed further imaging tests (two breast ultrasounds, a mammogram and an MRI between 2014 and 2018).

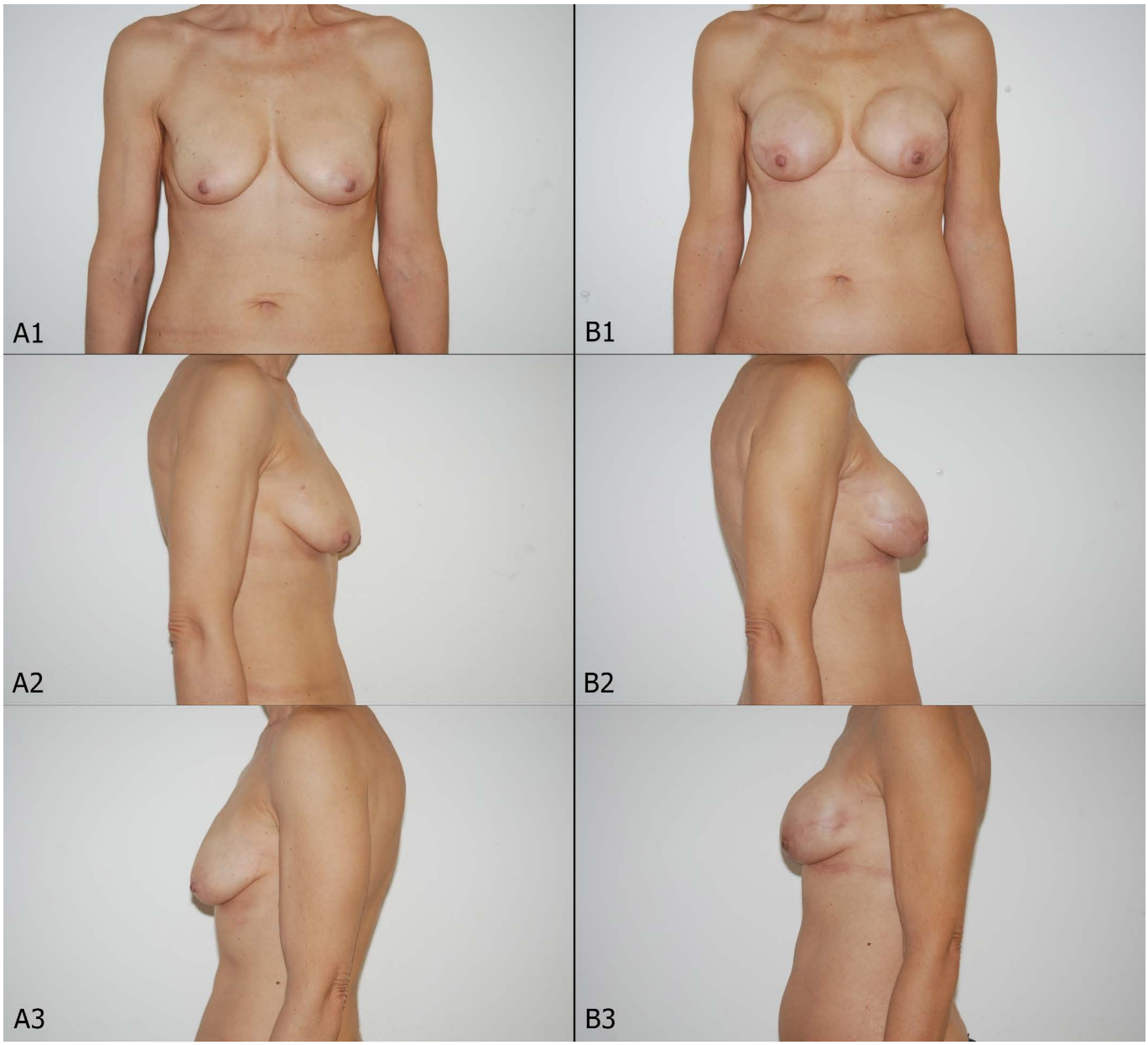

Figure 1 *- Preoperative photos (A1,2,3) and postoperative photos at five months (B1,2,3).*

These examinations showed an ambiguous and infrequent radiological imaging, so a more in-depth evaluation was required to rule out the presence of concurrent malignancy signs.
Therefore, with the deterioration of the local clinical and aesthetic conditions, the patient was referred to our Department. The clinical examination showed a slight mammary asymmetry with irregularity of the cutaneous surface and the presence of superficial and deep bilateral breast nodules (*Figure 1 - A1, A2, A3*).
The patient was unable to give us information about the quantities or the method of injection of Macrolane. MRI showed the presence of multiple adjacent and partially confluent areas with fluid contents; mammary bodies with a prevalence of fibro-glandular tissue; no clear focal lesions or areas with pathological enhancement after contrast administration. Consequently, ultrasound scan and mammogram were integrated.
Ultrasound confirmed the presence of fibro-glandular breast tissue with medium-high density and the presence of numerous non echogenic formations with regular contours bilaterally; no signs of periareolar ductal ectasia or suspicious lymph nodes in the axillary cavities, but some lymph-nodes with a non-specific reactive aspect. Mammogram (*Figure 2*) showed widespread signs of homogeneous thickening with unstructured fibrous opacities and numerous nodular opacities with poorly delimited margins. Moreover, it showed the presence of numerous micro-calcifications grouped in an area of about 20 mm in the upper-outer right quadrant, not reported in the last mammogram. A mammotome biopsy was performed in this area: the histological examination showed the presence of diffuse foci of intermediate-high grade ductal carcinoma in situ and a focus of infiltrating ductal carcinoma.
In addition, due to the positive family history for breast cancer in two first- degree relatives, mutation research for the BRCA gene was performed: BRCA1 gene was mutated. The case was subsequently discussed by a dedicated multidisciplinary team, which focused on the difficulty in interpreting the mammograms due to the presence of dense breast tissue.
For this reason, because of her family history and the presence of mutated BRCA1, the patient underwent bilateral nipple-sparing subcutaneous mastectomy, with an S-italic approach and contextual sentinel lymph-node biopsy in the right axilla.
After removal of the glands, the remaining hyaluronic acid cysts were identified in the subcutaneous tissue, on the fascia and in the pre-insertional anterolateral region of the Pectoralis Major muscle and removed (*Figure 3*). The patient underwent a pre-pectoral breast reconstruction with bilateral silicone implants (MENTOR® - Smooth Round Moderate Plus Profile Gel Breast Implant Cohesive I, n° 350-2501 BC: volume 250 cc, diameter 11.3 cm, projection 3.4 cm) with the use of biological mesh (Braxon® ADM) (*Figure 1 - B1, B2, B3*).
Definitive histological examination showed the presence of fibrocystic mastopathy with micropapillary apocrine metaplasia, on the left, and the presence of numerous foci of ductal carcinoma in situ (DCIS), with intermediate nuclear grade, necrosis and microcalcifications, on the right (*Figure 4 - A*). Sentinel lymph-node was free from infiltration [TNM-AJCC 2017 classification: pTis pN0 (sn) (0/4)]. The hyaluronic acid nodules appeared as aggregates of amorphous and weakly basophilic material (*Figure 4 - B, C*).

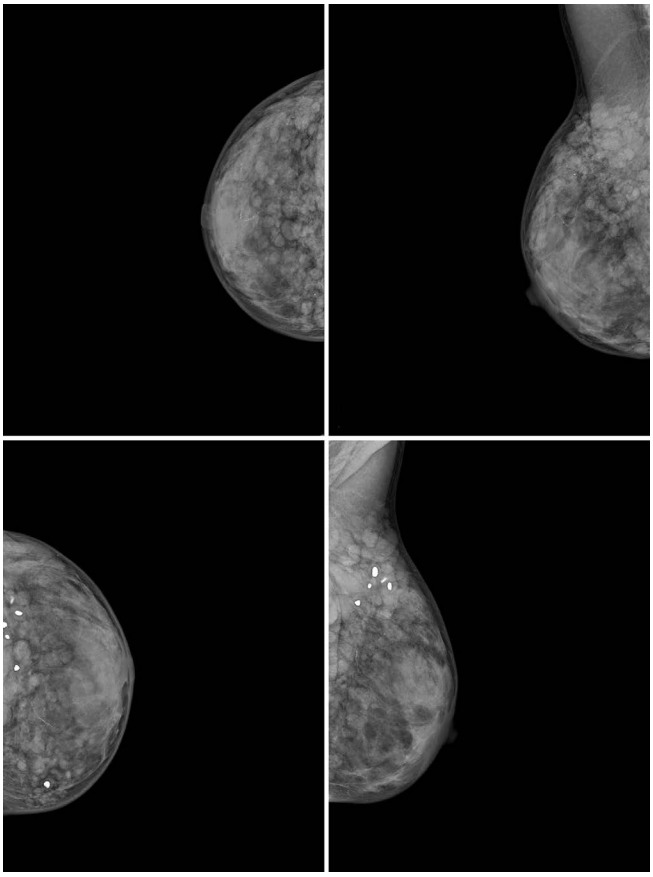

Figure 2 *- Bilateral mammogram.*

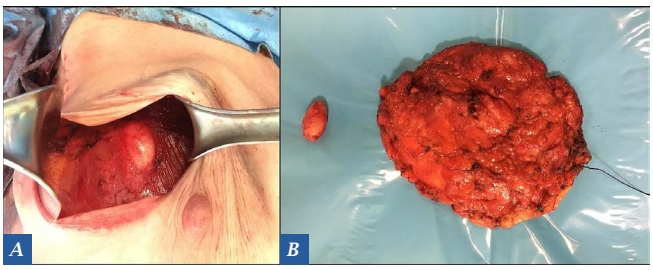

Figure 3 *- Intraoperative time: hyaluronic acid cyst between the fibers of the right Pectoralis Major Muscle (A); right mammary gland with multiple hyaluronic acid cysts and the excised cyst on the left (B).*

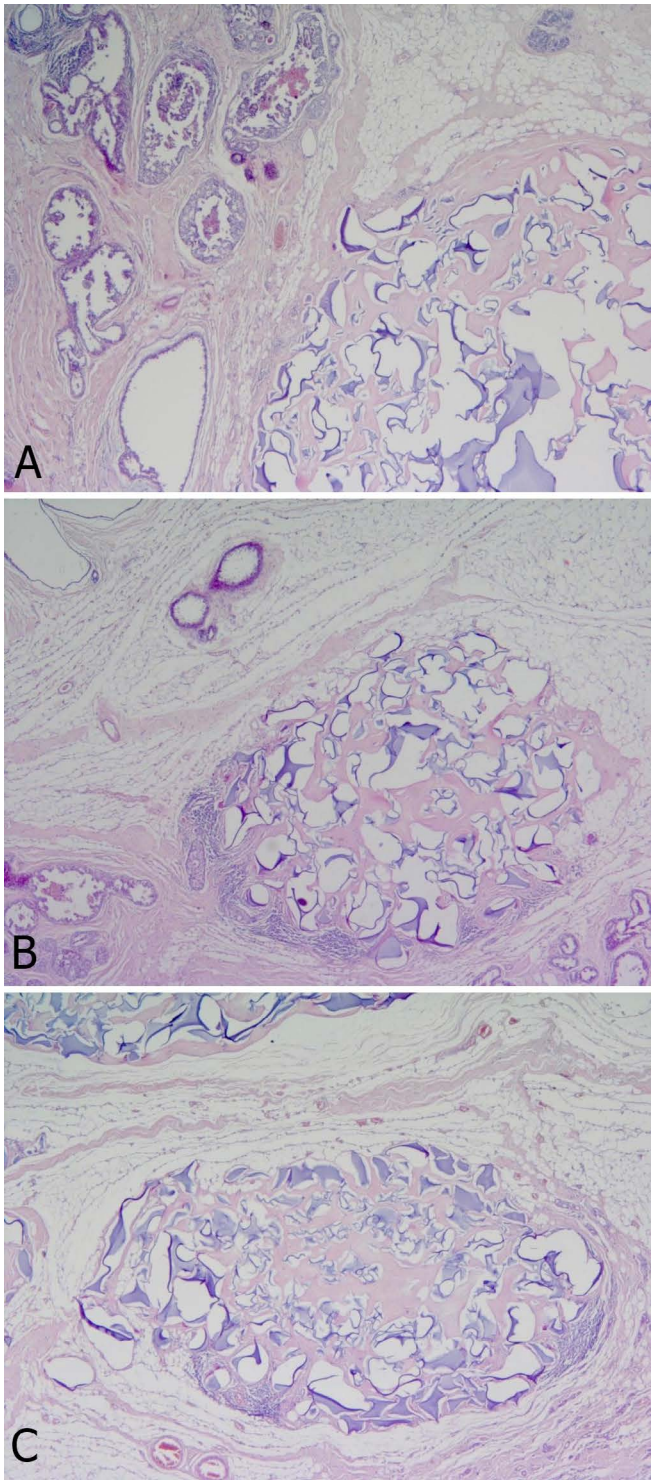

Figure 4 *- Histological slides: foci of ductal carcinoma in situ on the left and hyaluronic acid cyst on the right (A); hyaluronic acid cysts (B-C).*

Discussion

According to the 2018 National Plastic Surgery statistics, the surgical procedures of breast augmentation and the use of fillers for cosmetic minimally-invasive procedures are among the main treatments increasingly required.
Macrolane is a highly cross-linked, stabilized, non-animal and biodegradable hyaluronic acid-based gel, distributed by the Swedish Q-Med company. Since 2006 Macrolane has been available on the European market, as a resorbable filler for all body areas except for face and breast. In 2008 the European Community approved the use of Macrolane in breast augmentation procedures. Macrolane was considered a valid non-invasive alternative to prosthesis mastoplasty since it is resorbable and with the possibility of retreatment, its injection is fast, does not require general anesthesia and leaves no visible scars[1]. Furthermore, the allergic reactions and infections rates are lower compared to other breast augmentation techniques[7-8].
Following the growing evidence of radiological ambiguity associated with the use of Macrolane as a breast filler and after France banned this last indication in 2011[9], Q-med decided to temporarily suspend this indication in 2012. In the same year, this use was also prohibited in Italy[10].
Macrolane infiltrations can lead to various complications such as infections[11]: treatment involves the use of antibiotics and the possibility of evacuating collections of pus and hyaluronic acid. Despite the reabsorption capacity of Macrolane, its degradation mechanism and the consequences of repeated infiltration remain largely unknown[9]. The average reabsorption time is of about 18 months[12-17], but in the literature there are cases of persistence of Macrolane even beyond 24 months[2] and up to 4 years[18]. On the other hand, there are reports on cases of early Macrolane degradation before 6-12 months after infiltration[11]: therefore, aesthetic results are difficult to predict[3]. A single product infiltration is associated with longer degradation times: this is why it was recommended to inject Macrolane as a single implant below the mammary gland[11,19]. A single infiltration can also prevent other complications: firm breast and visible nodules, dislocation, radiological ambiguity. The superficial nodules can be treated with mechanical compression, whereas the deepest nodularities can be aspirated. In refractory or product dislocation cases, targeted injections of hyaluronidase are a good option[20].
The progressive limitation of the use of Macrolane for breast augmentation is a consequence of its interference with radiological examinations[2-6]. Macrolane is 98% water so its imaging characteristics will be similar to water. When there are multiple deposits in the breast, it is difficult to distinguish them from the glandular tissue. In addition, as Macrolane is a product not yet familiar to radiologists[21], it can mask breast conditions[19].
Sometimes the radiological images show solidified nodules and calcifications, so they can mimic breast cancer[6,9]: in these cases a biopsy must exclude malignant lesions[2,22-25]. In their report, Becchere et al. showed how Macrolane masked images of hypoechogenic lesions previously visualized in the preoperative time[26].
Finally, some authors hypothesize a possible increase in the risk of breast cancer following infiltration of hyaluronic acid-based fillers in the breast.
The infiltration procedure is advocated to be traumatic leading to inflammation of the breast tissue[3,5]. Moreover, hyaluronic acid has entered the debate as in some cancer patients there are increased levels of hyaluronic acid: currently, there are no data in the literature that correlate hyaluronic acid to the onset of cancer, nor data that correlate high levels of hyaluronic acid to a worse prognosis in cancer patients. The expression of

high levels of hyaluronic acid could be interpreted as an epiphenomenon, and therefore not the cause, but a manifestation of neoplastic pathology[27].

The use of Macrolane in breast augmentation has been associated with various complications, not related to the product safety or quality. Many authors have highlighted radiological ambiguities in different imaging tests, particularly in mammography. Many authors have carried out studies on this product and on its interference and delay in the diagnosis of malignant breast diseases[2,3,5,6,8,9,20,22,23,27-30]. After an accurate search in the online databases and literature, there is only one case reporting on a patient with breast cancer arising after mammoplasty with Macrolane, which masked an underlying cancer, interfering with physical and instrumental examinations and delaying the diagnosis[6].

As in the case described above so we found the same difficulties, even during the surgery time. Multiple evidences demonstrate that Macrolane remains in place for longer periods than those described in the literature, therefore its mechanism of degradation and reabsorption time remains unpredictable. Due to the clinical, radiological and therapeutic difficulties encountered in the management of patients with previous Macrolane breast infiltration, at present we believe that the use of hyaluronic acid (Macrolane) fillers for breast augmentation should be avoided. Furthermore, it is advisable to perform a complete and correct clinical and instrumental evaluation before any type of breast augmentation procedure, in order to exclude any underlying mammary malignancy: for this reason, a multidisciplinary approach through the Plastic surgeon, the Breast-dedicated Radiologist and the Oncologist is always advisable.

Acknowledgments

- Conflict of interest: the authors declare that they have no conflicts of interest to disclose.
- Statement of human and animal rights: this article does not contain ant studies with human participants or animals performed by any of the authors.
- Informed consent: Informed consent was obtained from all individual participants enrolled in the study.

Conflict of interest

The Authors declare that they have no conflict of interest.

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Courses and Congresses

Due to the Covid-19 related medical emergency, this page is suspended until further notice

aesthetic medicine