Evaluation of Melasma reduction using the **ME LINE** protocol

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INTRODUCTION

The pigmentation system of normal skin is confined to the epidermis. The only cell capable of synthesizing melanin is the melanocyte. It is found in the basal layer of the epidermis and is neuroectodermal in origin. It constitutes up to 5% of epidermal cells. Keratinocytes obtain melanin from melanocytes, as well as providing the necessary micro-environment for their survival, proliferation, differentiation and migration through the production of various ligands that interact with melanocyte receptors. By way of its dendrites, each melanocyte interacts with around 36 keratinocytes in what is known as the "epidermal melanin unit."

Melasma or chloasma is a circumscribed, patchy and symmetrical form of acquired hypermelanosis. It affects photo-exposed areas. The various studies into the histopathological findings in melasma have demonstrated a genuine increase in the number of melanocytes in the basal layer, as well as the number of dendrites and the degree of pigment transfer into keratinocytes. These studies have also identified increased melanocyte metabolism (increased number and size of mitochondria, Golgi apparatus, rough endoplasmic reticulum and ribosomes). (Sánchez NP, Pathak MA, Sato S, Fitzpatrick TB, Sánchez JL, Mihm MC. "Melasma: A clinical, light microscopic, ultrastructural and immunofluorescence study". J Am Acad Dermatol 1981; 4: 698-710. Kang WH, Yoon KH, Lee ES, Kim J, Lee KB, Yim H y cols. "Melasma: Histopathological characteristics in 56 Korean patients". J Am Acad Dermatol 2002; 146: 228-237.).

Clinically speaking, the condition manifests itself in the form of irregular brown macules of varying degrees of darkness, over the forehead, temples, top lip and cheeks. It is more common in women, particularly those of Hispanic (Victor FC, Gelber J, Rao B. Melasma: a review. J Cutan Med Surg. 2004;8:97-102.) and Oriental ethnicity, living in regions with strong sun exposure.

i. Objective.
A clinical study was planned to demonstrate the effectiveness of a topical treatment based on depigmenting active ingredients, in conjunction with the co-adjuvant use of a semi-occlusive mask peel in patients with Melasma to assess their improvement and possible side effects.

ii. Sample.
22 volunteers were recruited consecutively between 01-02-2016 and 15-02-2016. A melasma frequency of 1% was assigned to the Spanish population (p1%, q99%, z1.96), minimum n = 14.

Exclusion criteria:
- Diagnosis of systemic pathology (auto-immune diseases)
- Chronic use of medication or treatments
- Pregnancy/breastfeeding
- Minors
- Specific contraindications regarding product to be applied (e.g. allergy)
- Contraindications regarding the procedure, e.g.:
· Active skin infections (especially herpes simplex)
· Presence of lesions in application area (e.g. purpura)
· Recent sun exposure
· Photosensitivity
· Immediate social engagements

-MASI score less than 16 or greater than 31
-High carotene intake

Inclusion criteria:
- Presence of non exclusion criteria
- Agreement not to undergo any other aesthetic treatments (from 30 days prior to the first session until the end of follow-up).
- Agreement not to make any dietary changes.
- Signature of informed consent form.

MATERIAL AND METHODS

i. Pre-Clinical Studies
ii. Application Protocol

Application technique for topical products

The use of two topically applied products is indicated: **ME LINE Caucasian Skin Day**, which is a solution to be applied in the morning to the pigmented facial areas only; and **ME LINE Caucasian Skin Night**, which is a cream to be applied in the evening to the whole face.

ME LINE Caucasian Skin Day contains ingredients that regulate and control melanocyte activity, working to block melanin synthesis. This is achieved through the combined actions of kojic acid, phytic acid and tranexamic acid, which inhibit the activity of the enzyme tyrosinase, as well as working to control free radical activity, thus reducing a powerful stimulus for pigment synthesis. The product also contains lactobionic acid, which helps maintain cutaneous hydration levels and boosts the skin's barrier function.

ME LINE Caucasian Skin Night reinforces the treatment, firstly by inhibiting melanin synthesis and deposition thanks to its depigmenting active ingredients, and secondly through its continuous controlled exfoliation activity, with a view to boosting epidermal turnover and thus reducing the number of affected keratinocytes, to promote general skin tone uniformity over the entire skin area treated.

Semi-occlusive mask peel application technique
First of all, a pre-peel solution (**ME LINE 00 PREP**) is applied, whose primary active ingredients are lactobionic acid (10%), lactic acid (10%) and ferulic acid (10%), as a vehicle solution and exfoliant agent, with a view to achieving superior penetration of the mask's main active ingredients.

1. Procedure
· Cleanse and de-grease skin in-depth with a view to eliminating any make-up residues or sebum with the potential to interfere with the peel's final result.
· Dry the skin.
· Pour the contents of the solution ampoule into a suitable container and apply a uniform layer over the whole face using a brush.
· Leave on to act for 3 minutes.

2. Application of Controlled Chemical Dermo-abrasion Mask
· Apply a uniform medium thickness layer – as even as possible – using a brush.
· Wait for 15 minutes.
· Apply a second layer to areas of skin with more lesions. The first session lasts 30-45 minutes in total.

3. Cleanse and eliminate the cream mask using a gentle lipid-rich soap.

Post-treatment care
24 hours after the treatment, pruritus, erythema and a pulling sensation appear. In cases where the product has been left on for longer, slight swelling may appear, lasting 1-3 days. 48 hours after the treatment, fine desquamation appears, lasting 3-4 days. During and after this time-frame, a moisturizing product (**ME LINE 03 MOIST**) should be used with a view to repairing and hydrating skin, aiding in its recovery. Similarly, and with a view to protecting skin against ultraviolet radiation, the treatment should be accompanied by SPF 30+ sun protection, **ME LINE 04 B.B.**

From day 4 following application of the controlled dermabrasion product, the patient starts to apply the at-home treatment products, which are used once a day.

iii. Measurements

MELASMA AREA AND SEVERITY INDEX (MASI)		
Forehead	F	30%
Right malar	RM	30%
Left malar	LM	30%
Chin	C	10%

% area affected (A), score from 0 to 6
Pigment darkness (D), score from 0 to 4
Color homogeneity (H), score from 0 to 4

MASI Score = 0,3 (DF + HF) AF
\+ 0,3 (DMR + HMR) AMR
\+ 0,3 (DML + HML) AML
\+ 0,1 (DC + HC) AC

A:
0: not involved
1: <10%
2: 10-29%
3: 30-49%
4: 50-69%
5: 70-89%
6: 90-100%

D y H:
0: absent
1: mild
2: moderate
3: marked
4: maximum

Range: 0-48

Numerical value	0	1	2	3	4	5	6
Pigment darkness (D) scale from 0 to 4	Absent	Mild	Moderate	Marked	Very marked		
Pigment homogeneity (H) scale from 0 to 4	No pigment	Specks	Patches less than 2 cm	Patches greater than 2 cm	Homogeneous		
Area involved	None	Less than 10%	11-29%	30-49%	50-69%	70-89%	90-100%

Severity	Mild	Moderate	Severe
Total score	less than or = 15	between 16 and 31	greater than or = 32

The Melasma Area and Severity Index (MASI) was created by Kimbrough and Green in 2004, in an attempt to standardize the subjective assessment of melasma. It is calculated by dividing the face into four areas: forehead (f), right malar region (rm), left malar region (lm) and chin (c).

Kimbrough-Green CK, Griffiths CEM, Finkel LJ, Hamilton TA, Bulengo-Ransby SM, Ellis CN, et al. Topical retinoic acid (tretinoin) for melasma in black patients. Arch Dermatol 2004;130:727-33.

Skin scanner (VisioScan or similar)
This type of technology provides parameters, which, in conjunction with standard inspection of the patient, form the basis of the subjective assessment of the condition. The scanner provides: standard photographs, polarized light photographs and colorimetric photographs (melanin and hemoglobin).

Colorimetry
Due to skin's structure, composed of several translucent layers, measurement of its color is completely different from color measurements of other materials. Skin modifies light. Environmental light and the light emitted by measurement devices penetrate skin to different depths and are not absorbed or reflected in the same way. The primary components influencing skin's color are: melanin (pigmentation), which appears grey or brown in the superficial layers of skin, and hemoglobin (the red component of blood), which appears red or sometimes blue and is found in skin's deep layers. If the light emitted by the shadow penetrates skin deeply, the red component of skin's color will be overestimated. These physical properties of skin make measuring its color a very complex enterprise. The different light sources of the measurement devices, the different pressures on skin's surface and the different measurement areas, in addition to skin's unique properties make it impossible to determine skin's true color using traditional measurement techniques and their results are not fully comparable.

To measure skin's color, we will use the Skin-Colorimeter® Probe CL 400, Courage -Khazaka, GmBH. The raw data from the probe are corrected using a special color matrix, bringing them even closer to normal values. The measured skin color is expressed as an x-y-z value, and can be converted to a related value (L*a*b).

L* regards the white-black axis, shine, and a* and b* are the coordinates in the color space. a* expresses erythema values on the red-green axis and b* shows the skin's position on the blue-yellow axis. The L* value (shine) is inversely proportional to pigmentation. L* is related to pigmentation. The a* value is proportional to redness (erythema/microcirculation). In addition, the "Individual Typology Angle" ITA is automatically calculated (the classification of an individual's skin color). The ITA° formula is: [Arctangent ((L*-50)/b*)]180/∏. Using the ITA° data, it is possible to classify skin into different types:

ITA° >55°: "very fair"
55° >ITA°> 41°: "fair"
41° >ITA°> 28°: "intermediate"
28° >ITA°> 10°: "dark"

Colorimeter technical details
Skin-Colorimeter® CL 400
Dimensions: 13 cm
Measurement area nucleus: Ø 5
Area illuminated: approx. 17 mm Ø
Cable length: approx. 1.3 m
Weight: 85 g
Measurement technique: reflection
Light: 8 white LEDs in circular arrangement
Wavelength range of light emitted: 440-670 nm
Units: xyz
RGB, L*a*b* index values (due to skin's unique structure and the special light source, the values do not correspond exactly to ISO standards and are thus expressed as index values). Calibration to skin colors using a special correction matrix.
Relative error: ± 5 %

Subjective patient scale
This scale is to be used by the PATIENT to describe the IMPROVEMENT seen on the face: it comprises 5 categories.

Score	Guide
5	Very good
4	Good
3	Fair
2	Poor
1	Very poor

Assessor's subjective scale
The subjective scale used by the ASSESSOR to describe the patient's skin's condition will comprise 5 categories (although intermediate scores such a 2-3 or 4-5 may also be used if considered appropriate by the assessor):

Puntaje	Guía
5	Muy Bueno
4	Bueno
3	Regular
2	Malo
1	Muy Malo

Statistical processing
- A single database will be generated, in which the first row corresponds to the number of variables and each subsequent row repre sents the data for each patient in the study.
- The final database will be revised using SPSS software or similar.
- In line with traditional practice, for continuous quantitative variables, we have used the mean as the central tendency index and the standard deviation as the dispersion index.
- Statistical significance (p): 0.05
- CI: 95%
- Mean comparison using Student Test: paired samples, 2-tailed test.

RESULTS

Initial sample: 22 subjects
Lost to follow-up: 1. Not convenient to attend follow-up. No adverse effects.
Final total sample (n): 21

1. MASI scale variable

	Before MASI scores	**After MASI scores**
Mean	25.50	12.79
Std. Dev. (SD)	7.65	6.17
Std. Err. of Mn. (SEM)	2.05	1.65
P:	<0.0001	
CI95 Diff.:	12.71 (11.00 - 14.43).	
T: 16.0047		
Df: 13		
Std. err. diff: 0.794		

2. Colorimetry variables
3. Variable "L*"
P value. Two-tailed P value: 0.0012
Confidence interval: mean "Group One" – "Group Two" = -356.57 (CI95% -544.32 to -168.82)

	Group One	Group Two
Mean	6030.71	6387.29
SD	251.15	236.59
SEM	67.12	63.23

Variable "a"
P value. Two-tailed P value: 0.2898.
Confidence interval: mean of "Group One" – "Group Two" = 65.07 (CI95% confidence interval of this difference -62.31 to 192.46

	Group One	Group Two
Mean	1513.29	1448.21
SD	220.47	130.93
SEM	58.92	34.99

Variable "ItA".
Individual Typology Angle (ITA), for the classification of an individual's skin color. The ITA° formula is: [Arctangent ((L*-50)/b*)]180/∏.

P value. Two-tailed P value = 0.0011.
Confidence interval: mean "Group One" - "Group Two" = -11.00 (CI95% -16.70 to -5.30)

	Group One	Group Two
Mean	38.00	49.00
SD	7.95	6.25
SEM	2.13	1.67

DISCUSSION

The evaluation of the results of any treatment aimed at offering a solution to a condition or pathology that is primarily aesthetic in nature must take into account both statistical significance and clinical significance. That is, a good esthetic treatment must provide results that are significant, measurable and repeatable, as well as allowing the patient to perceive these differences. If there are no objectively measurable differences before and after the treatment, or if the patient does not perceive these changes, the treatment in question is bound to fail.

The severity of melasma can be clinically determined according to the skin surface affected, its color, the homogeneity of the lesions and the time over which it has developed. It is classified as mild, moderate or severe. For the quantitative assessment of melasma severity, the Melasma Area and Severity Index (MASI) is used, a clinimetric method allowing for greater precision in the determination of the disorder's severity in a more systematic manner.

In melasma treatment, the general objective is to lighten the intensity of the hyperpigmentation and reduce the affected area. The specific objectives are: reducing hyperpigmentation to the patient's satisfaction, both in terms of its severity and its spread; avoiding recurrence; improving quality of life; educating the patient to avoid risk factors and carrying out a more in-depth assessment of each patient, looking for endogenous factors causing the recurrence of the lesions with the potential to be modified. One of the main factors in achieving a satisfactory response to treatment is compliance with same. In this vein, it is possible to achieve compliance rates close to 90% when there is a favorable doctor-patient relationship and effective therapy is given leading to a high degree of satisfaction. Patient education is the key to achieving this. During the process of educating the patient, it is important to emphasize certain aspects, such as the chronic nature of the condition, the need for lifestyle and occupational changes, modifications to clothing and the need to avoid sun exposure, even on cloudy days. If the doctor does not manage to convince the patient to modify his/her habits mentioned above, the patient will not fulfil the necessary preventive measures, leading to a failure of therapy, chronic persistence of the condition, lesion recurrence, the seeking of multiple treatments and, by consequence, forms of melasma that are difficult to treat.

When evaluating the results of a melasma treatment, there are 3 areas or variables that must be carefully considered: melanin load, load of other pigments and clinical impact. In this paper, these three areas are represented by the variables: "MASI", "L*", "ITA" and "a".

First of all, it is very important to consider the MASI variable.

This is the main variable used in this paper. It is a validated, globally accepted scale, which is agreed upon by the entire medical community.

It takes into account the extent of the lesions, the heterogeneity of the affected areas and the pigment load. It generates very high statistical significance (<0.0001), with a difference between the means of 12.71 points. Given that the MASI variable is made up of a 48-point scale, this difference could be interpreted as an improvement well above 20%.
Secondly, we have the results for the L* variable, which are consistent with those for the MASI variable. The greater the L* value, the less pigmented the skin. The pre-treatment L* value is 6030.71 while the post-treatment value is 6387.29. Once again, the difference is very significant. P value: 0.0012.

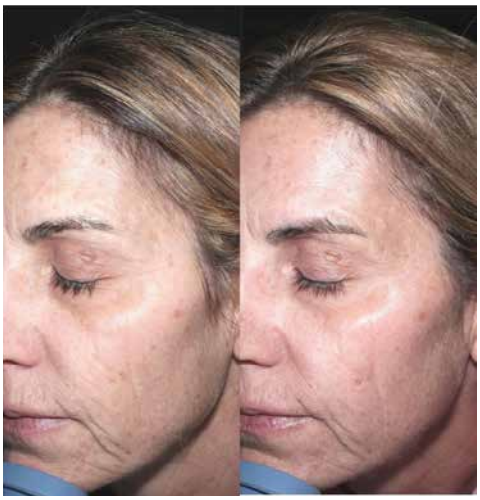
Lastly, the results for the "ITA" variable are in concordance with the MASI and L* values, confirming the correlation between the different variables measured. The greater the ITA value, the lighter the skin. We move from a pre-treatment ITA value of 48.00 to a post-treatment ITA value of 49.00. According to the ITA classification, this represents a shift from "intermediate" skin to "fair" skin. Once again, this difference is very significant. P value: 0.0011.

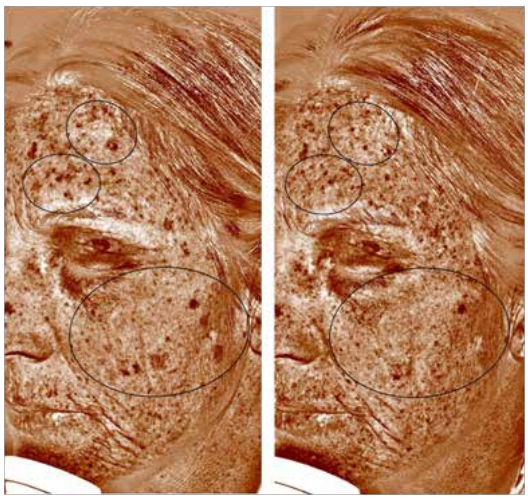
On the other hand, it is very important to take into account variations in skin's other pigments, which may occur concomitantly and mask the results. Traditionally, human skin color is attributed to 4 pigments: melanin, oxyhemoglobin, deoxyhemoglobin and carotenoids. In this paper, the study design takes into account any excess of or change in carotene intake (inclusion and exclusion criteria). Nonetheless, hemoglobin was not accounted for. That is why variable "a" is so important, which measures changes in the red spectrum. Should there be significant changes in this variable, the changes observed con be attributable to optical effects or changes in pigments other than melanin. However, variable "a" is stable. There is no significant variation in the p value, as expected.

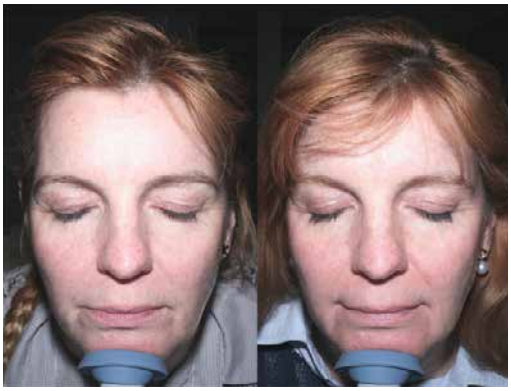
The combined analysis of these 4 variables reveals positive, significant and coherent changes in all 3 variables related to the reduction in the melanin load, in the absence of changes in the hemoglobin load. The results are highly consistent. With regard to the analysis of the different ingredients used in the treatment of this condition, it is important to consider the fact that there are different ways of classifying depigmenting agents. They can be grouped by chemical origin into: phenolic and non-phenolic agents; by their action mechanism into: tyrosinase inhibitors (hydroquinone, mequinol, kojic acid, azelaic acid, vitamin B6, licorice, arbutin); melanin synthesis inhibitors (ascorbic acid, glutathione); non-selective melanogenesis inhibitors (indomethacin, corticosteroids), melanocyte selective toxicity inducers (acetyl-cisteaminylphenol, N-acetylcysteine, isopropylcatechol, mercurial agents), agents promoting the absorption of the depigmenting ingredients (retinoic acid, alpha hydroxy acids). In line with their galenic formulation, topical depigmenting agents are found as monotherapies (single agents) or combination therapies (two or three depigmenting agents combined), with a view to boosting the depigmenting effect while minimizing adverse events.

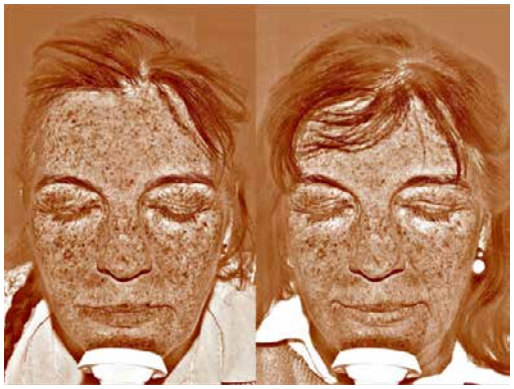
Generally speaking and in line with the medical consensus, melasma treatment is divided into two phases: the intensive phase and the maintenance phase. The intensive phase is thought to achieve satisfactory subjective and objective results within eight weeks, achieving a 50% reduction on the MASI scale with respect to the patient's baseline, followed by a six-month maintenance phase during which it is hoped that further reduction will be achieved. This paper specifically evaluates the intensive therapy phase. The activity of the ingredients applied daily represents a specific treatment for melasma control and must always be accompanied by a broad spectrum sun protection product with an SPF of at least 30 plus a synthetic, fragrance-free and non-abrasive skin cleanser.

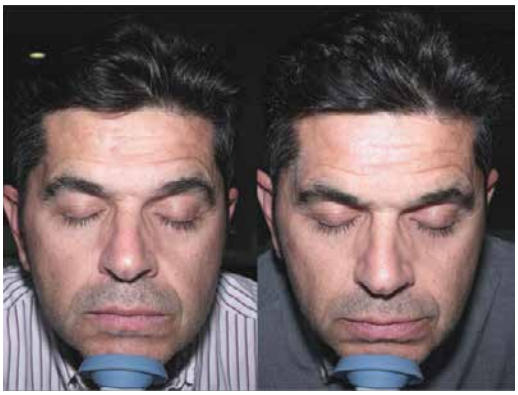
VISIBLE RESULTS

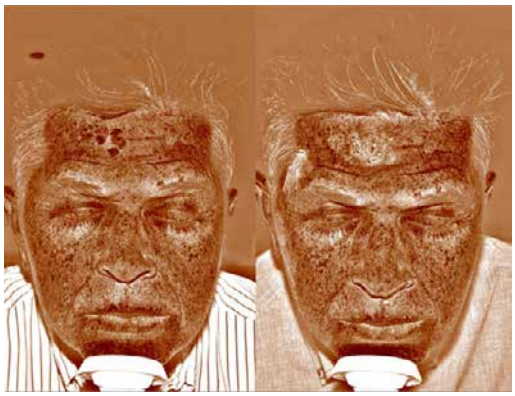


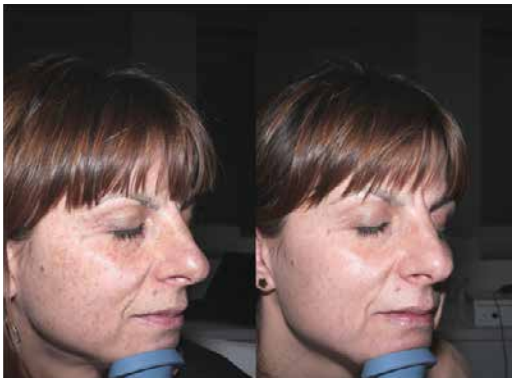


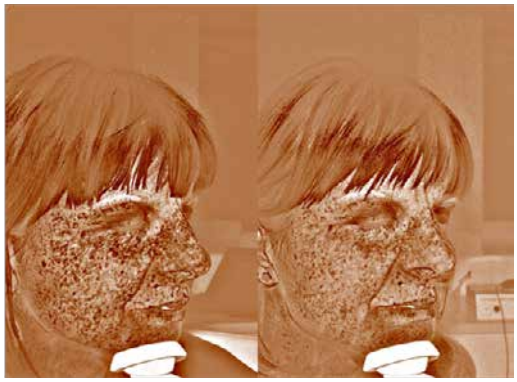












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