Histological changes in facial melasma after treatment with triple combination cream with or without oral tranexamic acid and/or microneedling: A randomised clinical trial

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Abstract

Background: Melasma is an acquired dyschromia with several histologic alterations in the epidermis, basement membrane and upper dermis. The treatment of melasma is challenging due to the irregular response and chronicity of the disease. To date, there are no curative strategies, largely due to the limited understanding of the intrinsic effects of each treatment.

Objectives: The objective of the study was to evaluate the histological changes promoted by triple combination cream, with or without complementary treatment with microneedling and oral tranexamic acid, in the treatment of melasma.

Methods: A factorial, randomised, controlled and evaluator-blinded clinical trial was performed involving 64 women with facial melasma, divided in four groups, who underwent 60 days of treatment with triple combination cream alone (control group) or combined with two monthly microneedling sessions (microneedling group), TA 250 mg twice daily (tranexamic acid group), or both tranexamic acid group and microneedling group. The participants underwent biopsy of the area with melasma at inclusion (D1) and D60. The primary outcomes were the variation (D1 × D60) between the variables: Thickness of the epidermis and stratum corneum, stratum corneum compaction and solar elastosis; melanin density in the epidermis and upper dermis; proportion between the extension of the nonintact and intact basement membrane zone; mast cell count in the upper dermis; melanocyte count in the basal layer, pendulum melanocyte count and melanocyte area; immunostaining density of vascular endothelial growth factor; stem cell factor and keratinocyte growth factor.

Results: One participant in the TG discontinued tranexamic acid due persistent headache; and herpes simplex occurred in three patients after microneedling. The groups showed a 24% (CI95%: 17–35%; $P < 0.01$) reduction in epidermal melanin density. There was no change in dermal melanin density or the area of melanocytes after treatment. There was an overall 25% (CI95%: 7–42%; $P < 0.01$) reduction in the number of pendulum melanocytes, especially in the microneedling and tranexamic acid group, that presented a 41% (CI95%: 7–73%; $P < 0.01$) reduction. The extension of the nonintact basal membrane relative to the intact basal membrane decreased after treatment, especially in microneedling group and microneedling and tranexamic acid group. There was an increase of 13% (CI95%: 5–21%; $P = 0.02$) in epidermal thickness and 6% (CI95%: 0–22%; $P = 0.04$) thinning of the stratum corneum in the groups. All groups showed stratum corneum compaction. Solar elastosis improved only in the microneedling group and microneedling and tranexamic acid group. Vascular endothelial growth factor immunostaining increased 14% (CI95%: 4–24%; $P = 0.03$) in the groups; and stem cell factor increased only in microneedling group. There was no change in the number of mast cells, CD34 and keratinocyte growth factor immunostaining.

Limitations: The site of biopsy may not represent all of the facial melasma and the immunohistochemical sensitivity of the cytokines does not have a stoichiometric relationship with proteins.

Conclusion: A greater thickness of the epidermis is associated with melasma bleaching. Dermal melanin seems to have no impact on melasma prognosis. Damage to the skin barrier and stimulus of angiogenesis should be avoided in the treatment of melasma. Microneedling complements the topical treatment of melasma by improving patterns of skin photoaging. Oral tranexamic acid complements the topical treatment of melasma by inhibiting the stem cell factor.

Key words: Melasma, microneedling, tranexamic acid, triple formula

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Plain Language Summary

The clinical study evaluated women with facial melasma and the changes caused in their skin after different treatments: triple formula and sunscreen with or without microneedling and tranexamic acid. The results of this study showed that a greater thickness of the epidermis is associated with bleaching of melasma. The melanin that is deep in the skin seems to have no impact on melasma prognosis. Treatments that irritate or sensitize the skin should be avoided in the treatment of melasma. Oral tranexamic acid complements the topical treatment of melasma by inhibiting vascular endothelial growth factor that stimulates melanin production. Microneedling complements the topical treatment of melasma rejuvenating the skin.

Introduction

Melasma is an acquired and chronic dyschromia of the skin. The characteristic lesions are hyperchromic and symmetrical macules located exclusively in sun-exposed areas, especially in the convex regions of the face. Melasma mainly affects women of childbearing age with an intermediate skin phototype (Fitzpatrick types III to V). Facial melasma greatly compromises the quality of life of patients and is a frequent complaint in dermatological offices.[1-3]

Normal skin pigmentation depends on the homeostasis between produced and degraded melanin. Ultraviolet radiation acts as the main extrinsic stimulus of pigmentation by promoting upregulation of melanocortin-1 receptor and α-melanocyte-stimulating hormone (α-MSH), in addition to inducing a senescent phenotype in fibroblasts that results in the increased synthesis of skin aging-associated secreted proteins. Skin aging-associated secreted proteins include melanogenic cytokines such as stem cell factor, keratinocyte growth factor and melanocyte growth factor.[4-6] Furthermore, in melasma skin, the function of the autophagic system, which is responsible for the degradation of damaged melanosomes, seems to be impaired.[7] Vascular proliferation, collagen disorder in the upper dermis, mast cell infiltrate and structural damage in the basement membrane zone are characteristic of melasma skin.[8]

The treatment of melasma is challenging due to the irregular response and chronicity of the disease. To date, there are no curative strategies, largely due to the limited understanding of its pathophysiology and the intrinsic effects of each treatment. Standard therapy for melasma consists of rigorous sun protection and the use of sunscreen combined with topical depigmenting agents. In first line treatment, the combination of 4% hydroquinone, 0.05% tretinoin and 0.01% fluocinolone acetonide, known as triple combination (or triple formula) cream, is used for a certain amount of time to lighten the lesions. However, its lightening effect is limited and relapses are frequent.[9,10] Thus, complementary treatments are proposed to improve clinical outcomes.

In 2015, Lima *et al*. published a treatment protocol for patients with recalcitrant facial melasma, in which he combined sunscreen and triple combination cream with two microneedling sessions (moderate injury protocol) separated by an interval of one month.[11] The histopathological analysis revealed that microneedling performed according to the described protocol decreases melanin density, restructures the basement membrane zone and improves solar elastosis in the upper dermis of the melasma skin.[12]

Tranexamic acid is a derivative of lysine that binds to plasminogen and inhibits its conversion into plasmin. Tranexamic acid promotes the lightening of skin with melasma, although the mechanisms are not yet fully understood. *In vitro* studies suggest that blocking the conversion of plasminogen (which is present in keratinocytes and basal cells) decreases the interaction between keratinocytes and melanocytes. In addition, the decrease in plasmin inhibits the production of arachidonic acid and prostaglandins, which are pro-melanogenic substances, by keratinocytes. In the dermis, tranexamic acid reduces the synthesis of vascular endothelial growth factor, which modulates angiogenesis. Tranexamic acid is able to stimulate autophagy pathways by increasing the synthesis of mitogen-activated protein kinases, extracellular signal-regulated kinase 1/2, beclin-1, autophagy-related protein 12 and LC3-I/II, in addition to reducing the synthesis of the mTOR complex.[13] In addition, tranexamic acid indirectly decreases the circulation of α-MSH. Finally, tranexamic acid has a structure similar to that of tyrosine and, for this reason, can compete with tyrosinase, inhibiting its activity.[14,15]

The objective of this study was to evaluate the histopathological changes promoted by the standard treatment for melasma, with or without complementary microneedling and oral tranexamic acid treatments.

Methods

This was a factorial, randomised, controlled and evaluator-blinded clinical trial, that included 64 female participants with a clinical diagnosis of facial melasma, who had been untreated for at least one month, except for the use of sunscreen. The participants were followed up in the dermatology outpatient clinics of Paulista School of Medicine, Federal University of São Paulo (EPM/UNIFESP, for its acronym in Portuguese), and Botucatu School of Medicine, São Paulo State University (FMB/UNESP, for its acronym in Portuguese). The exclusion criteria were the presence of other facial dermatoses, pregnancy, breastfeeding, anticoagulant use and immunosuppression.

The research project was approved by the Research Ethics Committee of UNIFESP under number 1310/2017 and all patients signed an informed consent form. The study was registered with the Brazilian Registry of Clinical Trials (ReBEC: ensaiosclinicos.gov.br – RBR-23snwx). The

clinical, quality of life and colorimetric efficacy data for this study, which was conducted between February and September 2018, were published in Cassiano *et al*.[16]

Subjects eligible after clinical evaluation were randomised in blocks by computer simulation and allocated consecutively into four groups: The control group, microneedling group, tranexamic acid group (TG) and microneedling and TG (microneedling and tranexamic acid group). For the first eight weeks (D60), the control group received an oral placebo; tinted sunscreen (Capital Soleil SPF 50, tinted, Vichy), to be applied every 3 h during the day and triple combination cream (Tri-Luma®), to be used at night. The microneedling group underwent two microneedling sessions (Dr. Roller™ 1.5 mm; Derma Rolling System; Gyeonggi Province, Korea) according to the Lima protocol (moderate injury)[11] under topical anaesthesia (4% lidocaine cream; Dermomax®) with an interval of 30 days between each session, in addition to tinted sunscreen every 3 h, triple combination cream at night and oral placebo. The TG received 250 mg tranexamic acid 12/12 h, in addition to sunscreen and triple combination cream. The microneedling and tranexamic acid group underwent the two microneedling sessions (according to the previously described protocol) and received 250 mg tranexamic acid 12/12 h, in addition to sunscreen and triple combination cream.

At all visits (D1 and D60), the participants were photographed in a standardised way, assessed for their melasma severity (Modified Melasma Area and Severity Index) and quality of life (Melasma Quality of Life Scale). Colorimetry was performed, with results expressed as the individual typological angle.[17]

On D1, the melasma area was biopsied with a 3-mm punch; biopsy was repeated on D60 in the same region up to a maximum distance of 1 cm. The biopsies were processed in paraffin. The histological sections were stained with haematoxylin-eosin, Fontana-Masson, periodic acid Schiff and toluidine blue. Immunohistochemical staining was performed with the antibodies anti-Melan-A (Dako, pure), (anti-vascular endothelial growth factor; Dako, diluted 1:50), anti-CD34 (Dako, pure), (anti-stem cell factor; Abcam, diluted 1:50) and (anti- keratinocyte growth factor; Abcam, diluted 1:100).

The slides were photographed (3DHistech, Budapest, Hungary) in triplicate at ×40 magnification in representative interfollicular areas and without artefacts and were saved as TIFF files. Using ImageJ 1.51e software, the variables were quantitatively evaluated by one trained dermatologist that was blinded to the time of biopsy (D1 or D60) and treatment group.

The primary outcomes were the variation (D1 × D60) between the variables: Thickness of the epidermis and stratum corneum (haematoxylin-eosin); melanin density in the epidermis and upper dermis (Fontana Masson); proportion between the extension of the nonintact and intact basement membrane zone (periodic acid Schiff); mast cell count in the upper dermis (toluidine blue); melanocyte count in the basal layer, pendulum melanocyte count (basal layer melanocytes that project toward the dermis) and mean melanocyte area (Melan-A) and staining density of vascular endothelial growth factor, CD34, stem cell factor and keratinocyte growth factor in the upper dermis using the colour deconvolution method.[18] Stratum corneum compaction (0, absent; 1, mild/focal to moderate and 2, intense) and solar elastosis (0, absent; 1, mild to moderate and 2, intense) were evaluated qualitatively after haematoxylin-eosin staining.[8]

The sample size was calculated to detect a difference of more than 20% between the groups. A dropout rate of 10% was considered. A power of 0.8 and an alpha value of 0.05 were adopted, resulting in a sample of 64 patients. Qualitative data are represented as percentages. The normality of the quantitative data was assessed by the Shapiro–Wilk test; these data are expressed as the mean and standard deviation or as median and interquartile range (p25–p75), if indicated.[19] IBM SPSS 25v software was used to compare the histological differences among the groups at D60 compared to D1 using generalised linear mixed effects models and *post hoc* Sidak correction. The link function (e.g., log or identity) and probability distribution (e.g., gamma, normal, binomial negative and ordinal logistic) were modelled to achieve a lower Akaike Information Criteria. Data were analysed as intention to treat and missing data were imputed by the mixed model.

The secondary outcome was the correlations between primary outcomes and the variation (D1-D60) of the clinical variables: Modified melasma area and severity index, colorimetry channel a*, colorimetry, colorimetry L* channel and individual typological angle. The correlations were estimated by Spearman's rho coefficient, since not all variables had Gaussean distribution and were represented as a heat map in which similar correlation patterns were grouped using the cluster method.[20] Significance was set at $P \leq 0.05$.

Results

The clinical trial was composed by 64 participants: 16 (25%) in the control group, 16 (25%) in the microneedling group and 16 (25%) in the TG and 16 (25%) in the microneedling and tranexamic acid group [Figure 1]. One participant (1.5%) discontinued tranexamic acid after persistent headache and herpes simplex occurred in three patients after microneedling. One participant (1.5%) from microneedling and tranexamic acid group refused the second biopsy.

At inclusion, the groups were homogeneous in terms of demographic characteristics, modified melasma area and severity index, quality of life (Melasma Quality of Life Scale) and individual typological angle. There was a predominance of adult participant with an intermediate skin phototype,

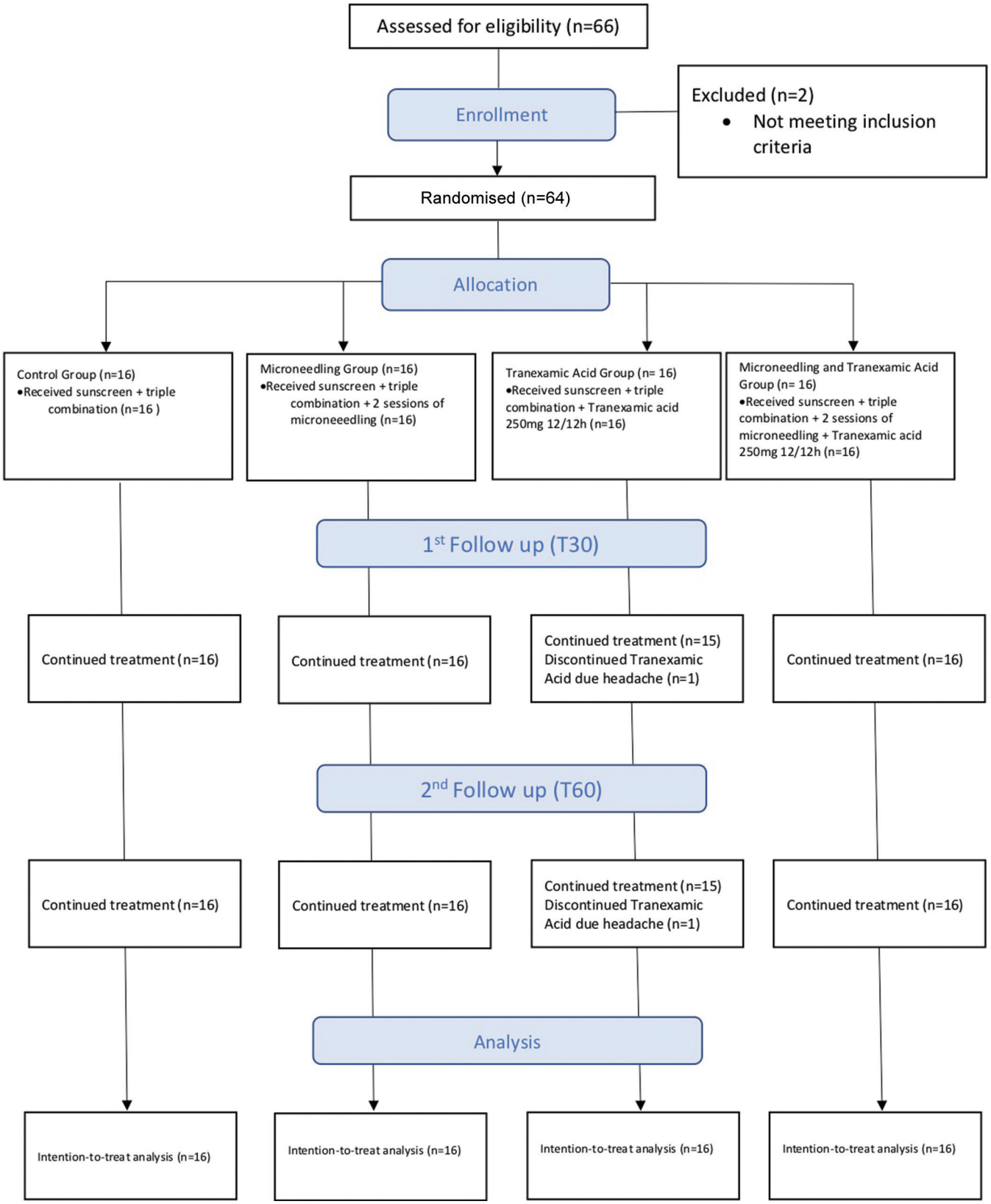

Figure 1: Participant flow CONSORT 2010

multiple pregnancies and a remarkable family history of melasma [Table 1]. According to the clinical distribution, there was a predominance of centrofacial (62.5%), followed by mixed (25%) and peripheral (12.5%) melasma type. Figure 2 shows clinical images from each group at D1 and D60.

The variables analysed with histological staining and immunostaining are shown in Tables 2 and 3. Figure 3 shows the correlation between the variations (D60-D1) of the variables. The groups showed a 24% (CI 95%: 17–35%; $P < 0.01$) median reduction in epidermal melanin density, but there was no change in dermal melanin density after treatment [Figure 4]. The reduction in epidermal melanin was weakly correlated with the colorimetry variation (rho-individual typological angle = –0.293), but not with the modified melasma area and severity index variation (rho = 0.142).

The amount and area of melanocytes did not change with treatment. However, there was a 25% (CI 95%: 7–42%; $P < 0.01$) mean reduction in the number of pendulum melanocytes [Figure 5], especially in the microneedling and tranexamic acid group, that presented a 41% (CI 95%: 7–73%; $P < 0.01$) reduction of these cells. The variation in pendulum melanocytes was also correlated with the improvement in the colorimetry results (rho-individual typological angle = 0.291).

The extension of the nonintact basal membrane relative to the intact basal membrane decreased after treatment, especially in the groups that underwent microneedling. Microneedling group presented a 37% (CI 95%: 4–69%; $P = 0.01$) reduction and the microneedling and tranexamic acid group 49% (CI 95%: 19–77%; $P < 0.01$) [Figure 6].

There was an increase of 13% (CI 95%: 5–21%; $P = 0.02$) in epidermal thickness, especially in TG and 6% (CI 95%: 0–22%; $P = 0.04$) thinning of the stratum corneum in the groups. However, the control group and TG had greater thinning of the stratum corneum than the groups that underwent microneedling. All groups showed stratum corneum compaction; however, it was most pronounced in the microneedling group, which increased compaction by 62% (CI 95%: 20–95%; $P = 0.03$). Solar elastosis improved only in the groups that underwent microneedling: Microneedling group reduced solar elastosis by 16% (CI 95%: 1–33%; $P = 0.02$) and microneedling and tranexamic acid group by 27% (CI 95%: 11–41%; $P < 0.01$). The increased epidermal thickness was the variable that was most correlated with the variation in modified melasma area and severity index (rho = 0.264).

Mean vascular endothelial growth factor immunostaining increased 14% (CI 95%: 4–24%; $P = 0.03$) in the groups [Figure 7]. The variation in vascular endothelial growth factor immunostaining was correlated with the variation in stem cell factor (rho = 0.444) and both showed the greatest variation in the microneedling group. There was no difference in the number of mast cells or immunostaining by CD34 and keratinocyte growth factor.

Discussion

The previously published clinical outcomes of this study showed an additive effect of both tranexamic acid and microneedling when combined with triple combination cream in the treatment of melasma, without significant adverse effects. In addition, microneedling seems to be associated with reduced relapses after 120 days of follow-up.[16]

The histological changes in melasma skin are quite pronounced and the disease should not be understood as merely an increase in epidermal melanin synthesis caused by hyperfunctioning melanocytes. Skin with melasma exhibits complex changes in the epidermis, dermis, basement membrane and cutaneous barrier that directly influence melanin production and lesion chronicity.

Table 1: Baseline data of the groups (*n*=64)

n	CG	TG	MG	MTG	Total	P-value
	16	16	16	16	64	-
Age (years)[a]	44.6 (6.6)	47.6 (8.9)	44.7 (10.2)	46.1 (7.0)	45.7 (8.2)	0.71
Skin phototype[b]						
II	2 (12)	2 (12)	4 (25)	3 (18)	11 (17)	0.60
III	5 (31)	6 (37)	5 (31)	5 (31)	21 (32)	
IV	7 (43)	4 (25)	6 (37)	5 (31)	22 (34)	
V	2 (12)	4 (25)	1 (6)	3 (18)	10 (15)	
Sun exposure[b]	7 (43)	6 (37)	6 (37)	4 (25)	23 (35)	0.73
Number of pregnancies[c]	2 (1-4)	2 (2-3)	3 (2-4)	2 (2-3)	2 (2-3)	0.78
Age at onset (years)[a]	32.0 (7.0)	29.8 (10.3)	31.6 (11.0)	32.8 (7.3)	31.5 (8.9)	0.81
Family history[b]	7 (43)	12 (75)	9 (56)	13 (81)	41 (64)	0.10
Modified melasma area and severity index[a]	4.4 (3.3)	5.9 (4.5)	6.7 (3.8)	5.9 (4.6)	5.7 (4.1)	0.49
MELASQoL-BP[c]	60 (46–68)	56 (50–62)	49 (27–54)	53 (35–62)	55 (42–63)	0.09
Colorimetry (ITA)[a]	8.0 (15.1)	10.2 (12.5)	13.1 (15.1)	13.7 (16.2)	11.2 (14.6)	0.67

[a]Mean (standard deviation), [b]*n* (%), [c]Median (p25-p75), ITA: Individual typological angle, CG: Control group, TG: Tranexamic acid group, MG: Microneedling group, MTG: Microneedling and tranexamic acid group

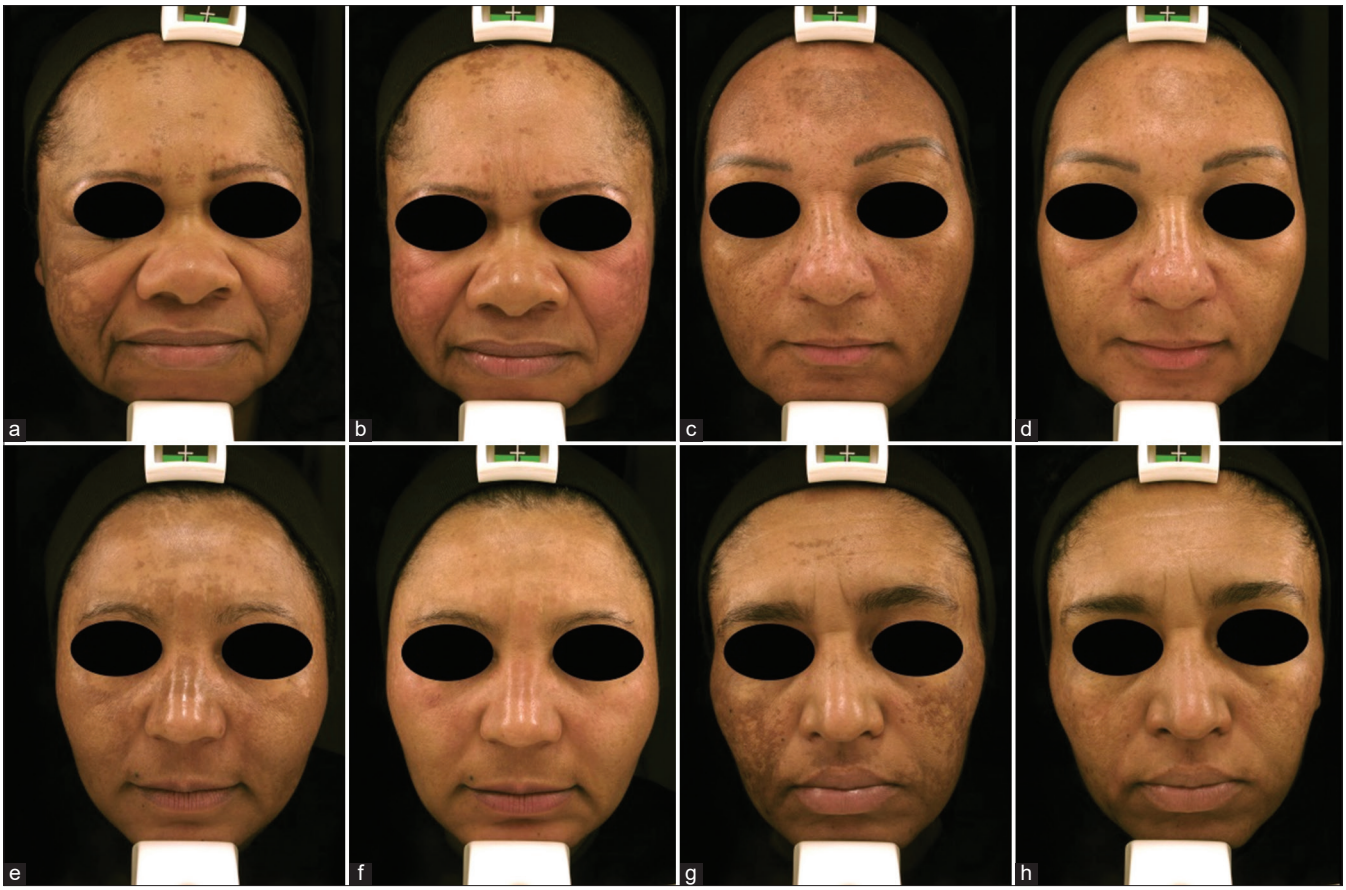

Figure 2: Clinical images from each group in D1/D60. (a and b): control group, (c and d): microneedling group, (e and f): tranexamic acid group, (g and h): microneedling and tranexamic acid group

The decrease in epidermal melanin density in all groups reinforces the bleaching action that occurs regardless of the proposed therapy. However, the difference in bleaching among the different groups, measured by the variation in modified melasma area and severity index, was not correlated with the decrease in epidermal melanin density. This is because unlike modified melasma area and severity index, which strongly depends on the extent of the diseased area, skin biopsy is a spot evaluation, which may limit its significance. However, there was also no correlation between colorimetric variation and epidermal melanin density variation. There is a lack of studies validating the correlation of epidermal melanin density with non-invasive treatment methods.

In addition, dermal melanin levels did not change with skin lightening. Thus, the real role of melanophages in the chronicity of melasma should be questioned, especially because their density is hundreds of times lower than that of epidermal melanin; this goes against the clinical classification of dermal and epidermal melasmas, which has never been histologically supported. However, melanophages are markers of photoaging and are also more prominent in more pigmented skin.[21-23]

The basement membrane is synthesised by fibroblasts and keratinocytes and plays an important role in maintaining homeostasis between the dermis and epidermis. In melasma, the lamina densa (type IV collagen) shows discontinuations, lower density and thinning. The lamina lucida, in turn, shows loss of anchoring fibrils. The alteration of the basement membrane zone facilitates the traffic of dermal cytokines to melanocytes, which stimulates melanin production.[24] In the study, only the groups that underwent microneedling showed improved solar elastosis in the upper dermis; additionally, these groups showed greater restructuring of the basal layer than the non-microneedling groups. The decrease in melasma relapses seems to be directly related to the improvement of photodamage, which was promoted by the microneedles. In other microneedling studies for the treatment of melasma, basement membrane zone repair occurred early.[12,25]

The role of pendulum melanocytes in melasma and its pathogenesis is unknown. They are present in photoaged skin but are more abundant in melasma. Pendulum melanocytes have no melanogenic role because they are not connected to the epidermis and, in experimental studies, can be induced by Type A ultraviolet radiation.[26] In the present sample, the reduction in pendulum melanocytes was the histological marker most associated with individual typological angle variation. Microneedling was found to be effective for

Table 2: Histological and immunohistochemical variables according to group (*n*=64)

Variable	CG	TG	MG	MTG	Total
Epidermal melanin (%)					
D1	52.0 (12.6)	52.8 (21.4)	52.2 (15.5)	47.6 (18.5)	51.1 (17.0)
D60	42.7 (15.4)[a]	32.1 (20.9)[a]	36.7 (17.5)[a]	40.2 (23.7)[a]	38.1 (19.6)[a]
Dermal melanin (×100, %)					
D1	4.2 (2.6)	4.6 (5.3)	3.0 (1.7)	2.9 (3.2)	3.7 (3.5)
D60	4.2 (2.8)	3.1 (2.8)	2.6 (1.6)	4.5 (3.5)	3.6 (2.8)
Basal layer melanocytes[b]					
D1	9.6 (2.7)	8.6 (1.8)	8.8 (1.4)	8.9 (2.3)	8.9 (2.1)
D60	9.7 (2.8)	9.0 (2.2)	8.4 (2.2)	7.7 (2.2)	8.7 (2.4)
Pendulum melanocytes[b]					
D1	1.7 (1.7)	1.8 (2.2)	1.5 (1.5)	2.3 (1.7)	1.8 (1.8)
D60	1.3 (1.6)	1.7 (1.5)	1.0 (1.4)	0.9 (1.1)[a]	1.2 (1.4)[a]
Area of melanocytes (μm^2)					
D1	249.1 (59.1)	297.3 (47.7)	294.8 (63.8)	261.8 (72.3)	275.2 (64.5)
D60	259.8 (51.7)	276.4 (64.9)	276.5 (53.5)	241.5 (62.6)	263.2 (59.1)
Mast cells[b]					
D1	7.2 (3.4)	5.4 (2.9)	7.1 (4.4)	6.4 (2.3)	6.6 (3.3)
D60	6.1 (2.3)	6.7 (3.2)	7.3 (2.6)	7.4 (2.4)	6.9 (2.7)
BMZ discontinuities (%)					
D1	16.3 (14.5)	12.5 (15.8)	14.8 (9.8)	22.2 (13.6)	16.4 (13.8)
D60	12.3 (9.4)	13.2 (10.7)	10.7 (12.8)[a]	12.2 (12.8)[a]	12.1 (11.3)[a]
Thickness of the stratum corneum (μm)					
D1	56.7 (28.8)	54.6 (24.3)	54.2 (30.6)	49.0 (23.9)	53.6 (26.6)
D60	44.0 (26.1) [a]	48.7 (25.2)[a]	47.6 (32.3)	53.0 (26.8)	48.3 (27.3)[a]
Thickness of the epidermis (μm)					
D1	230.6 (53.0)	241.5 (52.8)	275.5 (91.0)	263.2 (74.7)	252.7 (70.3)
D60	255.0 (58.2)	284.1 (74.9)[a]	279.9 (47.7)	271.7 (66.3)	273.0 (62.1)[a]
Compaction of the stratum corneum (intense)					
D1	5 (31%)	4 (25%)	3 (19%)	4 (25%)	16 (25%)
D60	8 (53%)	8 (50%)	8 (50%)[a]	5 (33%)	29 (47%)[a]
Solar elastosis (moderate)					
D1	10 (63%)	11 (70%)	13 (88%)	14 (%)	48 (75%)
D60	9 (60%)	10 (63%)	7 (44%)[a]	5 (33%)[a]	31 (50%)[a]

[a]*P* (before×after) <0.05, [b]Units per field, CG: Control group, TG: Tranexamic acid group, MG: Microneedling group, MTG: Microneedling and tranexamic acid group, BMZ: Basement membrane zone

lightening melasma, reducing solar elastosis and reducing pendulum melanocytes, suggesting that the reversal of factors associated with photoaging is a valid strategy in the treatment of melasma.[12,27]

All groups showed epidermal thickening after the treatments and it is known that microneedling promotes keratinocyte proliferation.[25] With a thicker epidermis, there is a greater distribution of melanin and, therefore, photochromophores have a greater capacity to absorb radiation. Thus, solar radiation has less direct action on melanocytes, thus reducing melanin synthesis.

The stratum corneum is thinner and more compacted in melasma skin.[8] The stratum corneum was further thinned and compacted after treatment with triple formula cream.[26] However, the cutaneous barrier, which is already deficient in melasma, must be preserved.[28] For this reason, topical alternatives that are less abrasive than the retinoic acid in triple formula cream should be proposed. Skin resurfacing with peels or lasers should also be performed sparingly to avoid melasma relapse after further thinning of the epidermis and inflammatory stimulation of the superficial dermis. Over time, the integrity of the stratum corneum effectively blocks ultraviolet radiation and its thinning may permit ultraviolet radiation damage, thus perpetuating the disease.

Hypervascularisation is not just an epiphenomenon of melasma; it also participates in the maintenance of hyperpigmentation. In melasma skin, there is an increase in the number and calibre of vessels and this increase is induced by several angiogenic factors, including vascular

endothelial growth factor and type B ultraviolet radiation. Vascular endothelial growth factor stimulates the release of arachidonic acid, which ultimately stimulates melanogenesis. In addition, vascular endothelial growth factor can release cytokines and soluble factors such as plasminogen, which also affect melanin production.[29] In the present study, there was a correlation between the variations in vascular endothelial growth factor and stem cell factor, which were stimulated in the microneedling group but not in the microneedling and tranexamic acid group.

Furthermore, vascular endothelial growth factor increased in all groups after the treatments. In fact, a previous histological study of the triple formula identified an increase in the number of vessels in the skin after 24 weeks of use.[26] Therefore, the increase in vascular endothelial growth factor may be related to the topical treatment that all groups received. New topical with a corticosteroid-like effect but without angiogenic action should be proposed as depigmenting agents for melasma.

Stem cell factor is a growth factor that binds to the tyrosine kinase receptor, leading to the activation of multiple transduction factors. It is related to tissue remodelling and fibrosis. In melasma, photostimulated fibroblasts and capillary endothelium participate in the activation of melanocytes through the secretion of soluble stem cell factor from the

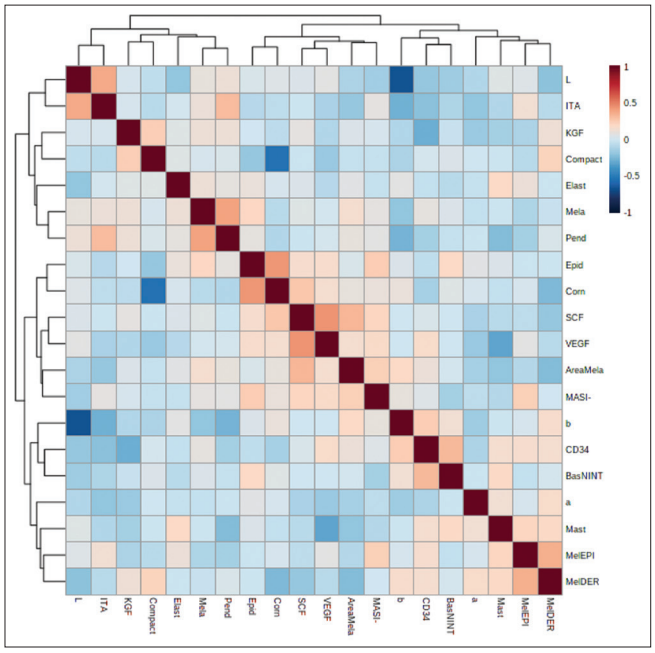

Figure 3: Correlations between the variations (D60-D1) in the histological and immunohistochemical findings and the main clinical variables (n = 64). MelDERM: Dermal melanin; MelEPI: epidermal melanin; Mast: Mast cells; a: Colorimetry channel a* (erythema); BasNINT: Nonintact basal layer; b: Colorimetry channel *b (blue) MASI: Melasma area severity index; AreaMela: Melanocyte area; VEGF: Vascular endothelial growth factor; SCF: Stem cell factor; Corn: Stratum corneum thickness; Epid: Epidermal thickness; Pend: Pendulum melanocytes; Mela: Melanocytes; Elast: Solar elastosis; Compact: Stratum corneum compaction; KGF: Keratinocyte growth factor; ITA: Individual typological angle; L: Colorimetry L* channel (luminosity)

Table 3: Results (mean and standard deviation) of the percentage density of immunohistochemical staining of the upper dermis according to group (n=64)

Marker	CG	TG	MG	MTG	Total
SCF					
D1	14.4 (8.0)	12.3 (4.7)	10.7 (12.2)	12.2 (4.4)	12.4 (5.6)
D60	14.8 (5.3)	12.8 (4.6)	15.5 (11.9)[a]	11.9 (5.1)	13.7 (6.0)
CD34					
D1	21.1 (11.3)	20.3 (9.5)	20.4 (11.3)	18.0 (9.0)	19.9 (10.2)
D60	19.0 (8.3)	19.5 (8.8)	18.1 (7.9)	13.5 (5.9)	17.6 (8.0)
VEGF					
D1	18.2 (6.8)	17.6 (4.7)	15.9 (4.8)	15.5 (5.4)	16.8 (5.5)
D60	21.2 (6.9)	18.7 (7.5)	18.2 (4.7)[a]	15.1 (5.1)	18.3 (6.4)[a]
KGF					
D1	4.5 (2.8)	3.8 (3.0)	3.3 (1.4)	3.6 (2.8)	3.8 (2.6)
D60	4.7 (4.1)	3.2 (2.6)	3.9 (1.8)	5.0 (4.5)	4.2 (3.4)

[a]*P* (before×after) ≤0.05, SCF: Stem cell factor, VEGF: Vascular endothelial growth factor, KGF: Keratinocyte growth factor, CG: Control group, TG: Tranexamic acid group, MG: Microneedling group, MTG: Microneedling and tranexamic acid group

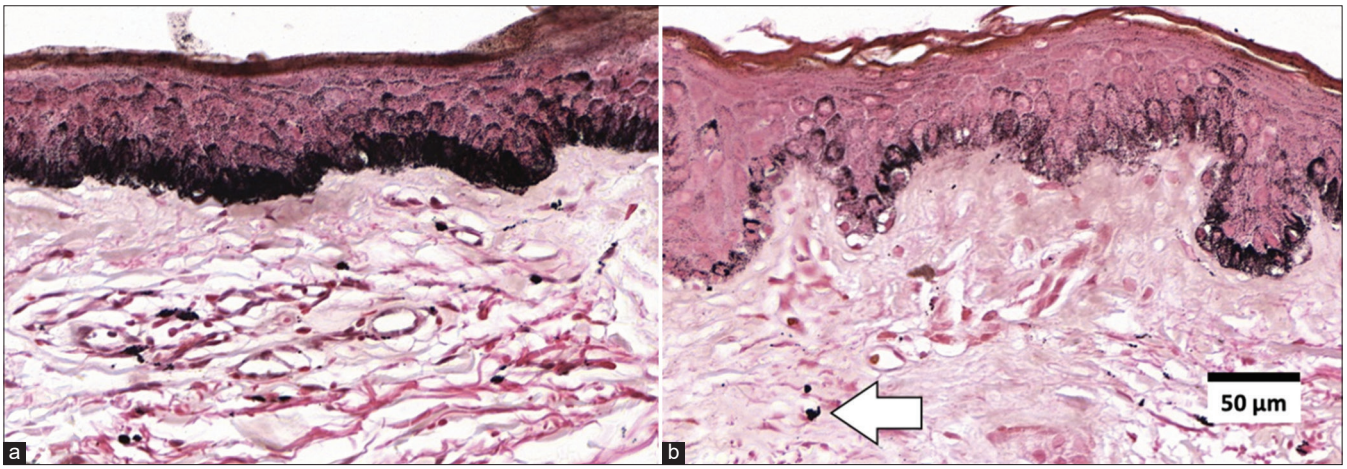

Figure 4: Skin with melasma before (a) and after (b) 60 days of standard treatment combined with microneedling (Fontana-Masson, ×40). Important reduction in the density of epidermal melanin and an increase in epithelial thickness. Detail (arrow), melanin in the superficial dermis

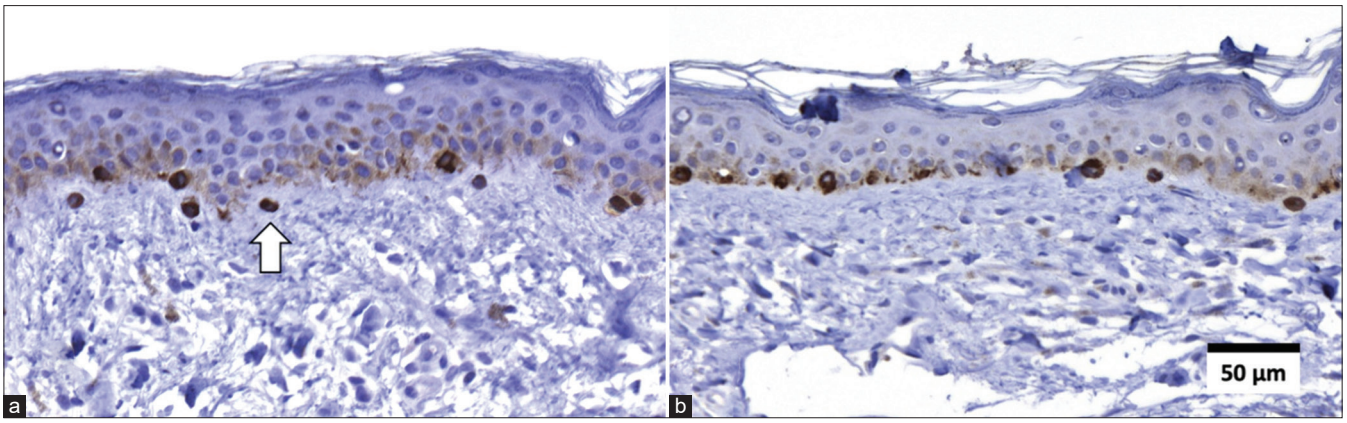

Figure 5: Skin with melasma before (a) and after (b) 60 days of standard treatment combined with tranexamic acid and microneedling (Melan-A, ×40). Maintenance of melanocyte density in the basal layer but reduction in pendulum melanocytes. Detail (arrow), pendulum melanocytes

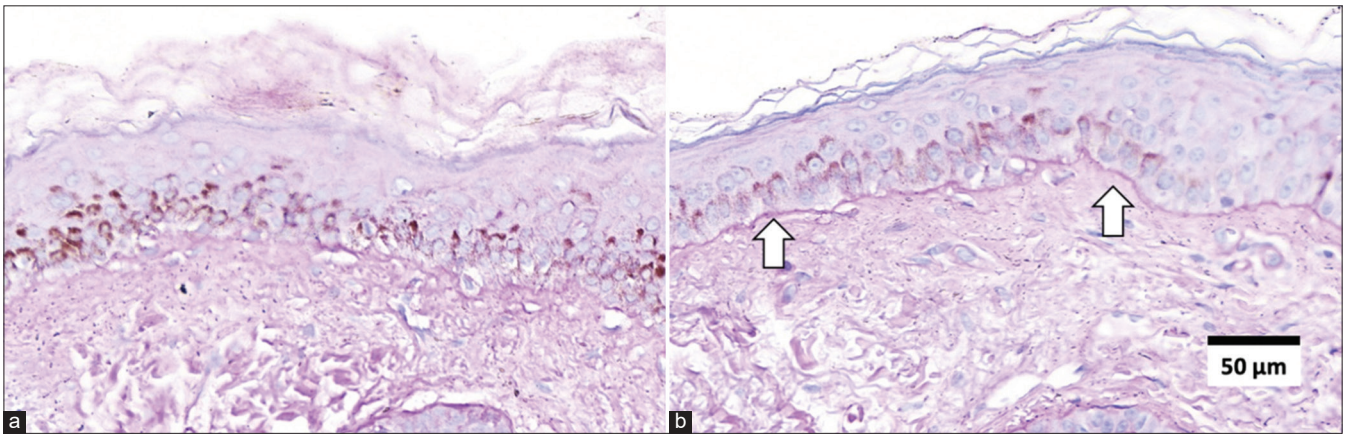

Figure 6: Skin with melasma before (a) and after (b) 60 days of standard treatment combined with microneedling (periodic acid Schiff, ×40). Repair of the basement membrane zone after treatment. Detail (arrow), thick and continuous basal membrane zone

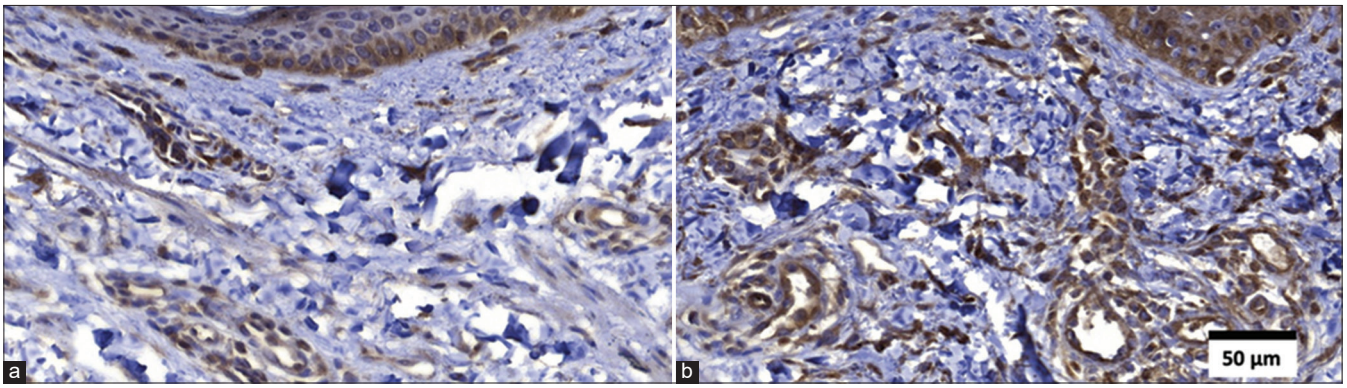

Figure 7: Skin with melasma before (a) and after (b) 60 days of standard treatment combined with tranexamic acid and microneedling (vascular endothelial growth factor, ×40). Increased staining of the upper dermis after treatment

dermis towards the epidermis.[6,30,31] In the present study, stem cell factor staining was increased in the microneedling group because tissue repair occurred after microneedling injury. However, stem cell factor staining did not increase in the microneedling and tranexamic acid group, which also underwent microneedling. Thus, we suggest that tranexamic acid plays a role in the inhibition of stem cell factor secretion by fibroblasts and dermal vessels. In addition, the injury caused by microneedles must not be very intense, since tissue remodelling includes the presence of stem cell factor, which can worsen the hyperchromia.

This study has limitations related to the histological sampling method, which may not represent all of the facial skin

affected by melasma and to the evaluation of cytokines, whose immunohistochemical sensitivity does not have a stoichiometric relationship with proteins. Furthermore, the randomised patients were not stratified by Wood's lamp classification. However, these limitations did not prevent the identification of differences among the groups, nor did it prevent the study from revealing information on melasma presents possible new lines of future research in the clinical approach to this dermatosis.

Conclusion

Melasma is a multifactorial disease that affects different skin compartments. Triple combination cream, microneedling and tranexamic acid promoted distinct epidermal and dermal histological changes. A greater thickness of the epidermis is associated with melasma bleaching. Dermal melanin change seems to have no impact on melasma prognosis. Damage to the skin barrier and stimulation of angiogenesis should be avoided in the treatment of melasma. Microneedling complements the topical treatment of melasma by improving patterns of skin photoaging. Oral tranexamic acid complements the topical treatment of melasma by inhibiting the stem cell factor.

Declaration of patient consent

The authors certify that they have obtained all appropriate patient consent.

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Conflicts of interest

There are no conflicts of interest.

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