Clinical, Cosmetic and Investigational Dermatology

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ORIGINAL RESEARCH

Efficacy and Safety of Nicotinamide 10%, Associated with Magnesium Ascorbyl Phosphate 5% and Hyaluronic Acid 5%, Compared to Hydroquinone 4% in Women with Facial Melasma: A Randomized, Double-Blind, Controlled Clinical Trial.

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Background: Nicotinamide has demonstrated efficacy in the treatment of melasma. Topical antioxidants and humectants may enhance its performance. Currently, there is no controlled trial on the combination of 10% nicotinamide, 5% magnesium ascorbyl phosphate, and 5% hyaluronic acid, a dermo-cosmetic compound, in comparison to 4% hydroquinone for the treatment of melasma. This study aimed to explore the tolerability and efficacy of the association of the combined product *versus* hydroquinone.

Methods: A randomized, double-blind trial involving women with facial melasma was conducted. Participants were instructed to apply the combined product (NIC group) twice daily or 4% hydroquinone for 60 days (HQ group) at night and placebo in the morning. Evaluations were performed at inclusion, after 14 and 60 days of treatment, measuring the modified Melasma Area and Severity Index (mMASI), Melasma Quality of Life Scale (MELASQoL), and colorimetric luminosity. The Global Aesthetic Improvement Scale (GAIS) was assessed by a blinded evaluator.

Results: Both interventions led to a progressive improvement in mMASI, MELASQoL, and GAIS, without a difference between them on D14 and D60 ($p>0.2$). For NIC, the mean reduction (95% CI) in mMASI was 16% (8–24%) on D14 and 32% (23–41%) on D60, while for HQ, it was 10% (7–24%) on D14 and 43% (34–52%) on D60. Reduction in colorimetric luminosity was greater in the HQ group at D60 ($p=0.01$). No serious side effects were identified. Of the initially included 50 patients, one was lost to follow-up in the HQ group on D60, and one withdrew consent from the NIC group, both unrelated to treatment.

Conclusion: The association of 10% nicotinamide, 5% magnesium ascorbyl phosphate, and 5% hyaluronic acid was safe and well-tolerated, although its overall clinical efficacy was numerically inferior to 4% hydroquinone. This regimen can be considered for patients with poor tolerability to hydroquinone.

Clinical Trial Registration: #RBR-4mkfmr8.

Keywords: melasma, hyperpigmentation, nicotinamide, hydroquinone, antioxidants

Introduction

Melasma is a chronic acquired hyperpigmentation of the skin, characterized by the presence of brownish macules in sun-exposed areas, particularly on the face. While it can affect both genders and various ethnicities, it is more prevalent in women during childbearing age, especially those with intermediate Fitzpatrick skin types (III to V).[1,2] The development of melasma is influenced by hormonal and external factors such as sun exposure, cosmetics, and photosensitizing medications, alongside genetic susceptibility.[3,4]

Received: 16 April 2024
Accepted: 27 September 2024
Published: 3 October 2024

The standard treatment for melasma typically involves a combination of sun protection with depigmenting agents, with hydroquinone (HQ) being the most widely used and studied topical bleaching. HQ is a potent tyrosinase inhibitor, however, it also exhibits melanocytotoxic activity. While it yields satisfactory results, it may sensitize the skin of some patients and lead to conditions such as hypochromia *in confetti* and ochronosis, especially after prolonged use, and in higher concentrations.[5]

Sunlight exposure and the release of inflammatory cytokines contribute to disrupting the balance between oxidant and antioxidant agents, leading to increased oxidative stress, which contributes to sustained melanogenesis in melasma.[6–8] Numerous studies have underscored the effectiveness of both topical and oral antioxidants in mitigating hyperpigmentation.[5,9–13]

Nicotinamide (NIC), or niacinamide, aside from playing a role in energy metabolism and DNA synthesis regulation, exhibits notable biological effects, including depigmenting and photoprotective actions. NIC can prevent photocarcinogenesis and protect against UV-induced immunosuppression, leading to improvement in epidermal homeostasis and cellular bioenergetics in oxidative stress-exposed skin. In addition, it delays the transfer of melanosomes from melanocytes to keratinocytes.[14–18] Also restores the skin barrier by increasing protein and ceramide synthesis and keratinocyte differentiation.

Vitamin C (VC) is a potent antioxidant and is the most abundant in human skin. In the cytoplasm of keratinocytes, melanocytes, and fibroblasts, enzymes related to VC participate in the neutralization of free radicals and reduction of solar erythema and melanogenesis.[13,19] Topical VC also prevents oxidative damage from UVA radiation and particulate hydrocarbon pollutants, reversing epithelial damage, collagen and lipid synthesis in the skin barrier.[20,21]

L-ascorbic acid is the most biologically active form of VC. However, the hydrophobic structure from the stratum corneum reduces its penetration, and pH above 3.5 reduces the L-ascorbic acid stability. In this context, the industry challenge is to develop stable formulations and VC derivatives that have efficient transepidermal delivery to maximize the concentration of active vitamin C in the skin, like magnesium ascorbyl phosphate (VCPMG).[21]

Melasma is characterized by a local defective skin barrier function.[22,23] Topical hyaluronic acid (HA) enhances epidermal hydration, and binds to extracellular matrix molecules and cell surface receptors, thereby regulating cellular behavior by controlling the tissue's macro and microenvironments. Humectants, such as HA, contribute to the restoration of the skin barrier and facilitate the penetration of active substances through the skin.[24]

It is important to explore safe and effective alternatives to HQ. To date, no randomized controlled study has evaluated the response to the combination of NIC + VCPMG + HA in the treatment of facial melasma Therefore, we propose a study that compares this combination with HQ.

Materials and Methods

A multicenter, randomized, double-blinded clinical trial involving 50 women with facial melasma was conducted at UNIFESP (São Paulo - SP), FMB-Unesp (Botucatu - SP), and Corium Clinic (Presidente Prudente - SP). Participants were recruited from dermatological patients between May and August 2023. The diagnosis of facial melasma was established through clinical and dermoscopic examination by an experienced dermatologist. The study protocol received approval from the Institutional Review Board, all participants signed informed consent, and the protocol was registered in the Brazilian Registry of Clinical Trials (https://ensaiosclinicos.gov.br/rg/RBR-4mkfmr8).

Inclusion criteria comprised women aged between 18 and 60 years old, presenting moderate to severe facial melasma (Melasma Area and Severity Index - mMASI≥4), without treatment for at least 45 days, except for sunscreen use during the washout period. Pregnant and lactating women and individuals with hypersensitivity or previous side effects to the studied medications were not included.

Participants were instructed to apply, on the facial spots, a gel cream containing 10% NIC + 5% VCPMG + 5% HA (NIC group) twice daily or to use only 4% HQ gel cream at night and an identical placebo in the morning (HQ group) for 60 days.

The treatment protocols were randomized in blocks through computer simulation, and the participants were consecutively allocated. The researchers had no access to the randomization list (central randomization). At the inclusion (D0), each participant received a brown envelope containing a numbered metal tube, with numbers ranging from 1 to 50, and they were randomized into two groups:

- NIC Group: received broad-spectrum tinted sunscreen (Episol color, SPF 70) and two tubes (morning and night) containing 10% NIC + 5% VCPMG + 5% HA gel cream, applied twice daily for 60 days.

- HQ Group: received the broad-spectrum tinted sunscreen, a tube with 4% HQ gel cream for nightly use, and another identical tube containing a topical placebo for morning use for 60 days.

Clinical and demographic data were evaluated at the inclusion. At D0, D14, and D60, standardized photographs, colorimetric measurements, assessments of clinical severity using mMASI, and evaluations of the quality of life through the Melasma Quality of Life Scale (MELASQoL) were performed.[1,25]

Colorimetry was assessed by calculating the difference in colorimetric luminosity (Dif*L) between the skin affected by melasma and the adjacent unaffected skin (<2 cm distance), measured using the CR-400 Chroma Meter (Konica Minolta). The site was selected as the area of maximum melasma pigmentation on D0. To maintain consistency in the location for future evaluations, the assessed area was marked in a standardized clinical photograph. The assessment of luminosity differences between affected and unaffected skin is a highly accurate measure. Evaluating the contrast between these areas effectively reflects the treatment's impact on the patient's daily life. A cell cycle from the basal layer to the stratum corneum takes approximately 28 days. Therefore, 60 days (final assessment) ensures at least 2 cell cycles, making it possible to evaluate the response to therapies instituted for melasma.

The primary outcome was the change in mMASI from baseline at each visit. Secondary outcomes included differences in MELASQoL, Dif*L, and GAIS (Global Aesthetic Improvement Scale). GAIS is a 5-point scale used to evaluate the overall improvement in a patient's appearance after a specific treatment or procedure, ranging from "worse" to "very much improved".[26] These assessments were conducted by a blinded investigator not involved in the initial evaluations or in the follow-up assessments.

Additionally, participants were questioned during follow-up assessments on day 14 and day 60 regarding product tolerability, compliance, adverse effects, and the daily frequency of sunscreen applications. They also provided a subjective improvement evaluation at D14 and D60, categorizing the evolution of melasma as worsening, neutral, mild improvement, moderate improvement, significant improvement, and exceptional improvement.

The sample size was calculated to detect a minimum of a 10% difference in mMASI reduction between the groups, assuming an equivalent standard deviation. We set the power at 90% and alpha at 5%, accounting for up to a 10% potential dropout rate.[27] The dropout criteria were loss to follow-up (failure to attend assessments on Day 14 and/or Day 60) or withdrawal of consent.

All outcomes were analyzed on an intention-to-treat basis.[28] The absolute reduction in scores (D14-D0 and D60-D0) and the difference in the categorical scores (eg, GAIS and subjective perception of improvement) between groups were assessed using the Mann–Whitney test. The frequency of irritative adverse effects was compared between the groups using Fisher's exact test.[29,30] Significance was set at a p-value <0.05.[31]

Results

The main clinical and demographic variables of the groups at inclusion are presented in Table 1, and no differences were observed between the groups ($p > 0.1$). Out of the initially enrolled 50 patients, there was one loss to follow-up on day 60 in the HQ group, and one withdrawal of consent in the NIC group, both of which were unrelated to the treatment. The patient in the HQ group did not attend the follow-up appointment due to difficulties with transportation logistics to the research center. The patient in the NIC group withdrew consent due to personal reasons. The study flowchart according to CONSORT 2010 is presented in Figure 1.

The main outcomes are summarized in Figure 2. Both groups showed progressive improvement in clinical outcomes (Figure 3) and quality of life, with no significant difference between the groups ($p > 0.2$). Within the NIC group, the mean reduction (95% CI) in mMASI was 16% (8-24%) on D14 and 32% (23–41%) on D60, while for the HQ group, it was 10% (7-24%) on D14 and 43% (34–52%) on D60, respectively.

Additionally, both groups demonstrated reductions in MELASQoL (95% CI) on D14 and D60, with a mean reduction of 18% (12–23%) on D14 and 30% (22–39%) on D60 in the NIC group. The HQ group showed reductions of 20% (10–31%) on D14 and 33% (22–46%) on D60.

Table 1 Clinical and Demographic Data of the Participants (n = 49)

Variables	NIC	HQ
n	24	25
Age (years), mean (SD)	44.0 (5.7)	44.8 (7.1)
Skin phototype, n (%)		
II	2 (8%)	3 (12%)
III	10 (42%)	9 (36%)
IV	11 (46%)	8 (32%)
V	1 (4%)	5 (20%)
Age of onset, mean (SD)	31.4 (6.1)	28.3 (8.9)
Family occurrence, n (%)	17 (71%)	20 (80%)
mMASI, mean (SD)	6.2 (3.1)	7.2 (4.4)
MELASQoL, mean (SD)	44.7 (17.9)	50.7 (15.3)
Dif*L, mean (SD)	5.0 (2.1)	4.7 (2.7)

Note: Dif*L: difference between colorimetric luminosity (*L) from the melasma to the adjacent unaffected skin.
Abbreviations: mMASI, modified Melasma Area Severity Index; MELASQoL, Melasma Quality of Life Scale.

Reductions in luminosity difference (colorimetry) were observed in the NIC group, from 7% (3–22%) on D14 to 17% (0–34%) on D60. In the HQ group, the reductions were from 18% (6–43%) on day 14 to 27% (13–43%) on D60.

The GAIS score did not differ between the groups on both D14 and D60. Any improvement was evidenced in 5 (21%) and 11 (46%) participants in the NIC group on D14 and D60, respectively, and 11 (44%) and 18 (75%) in the HQ group.

There were no serious adverse effects related to the treatments. In the NIC group, seven cases of mild itching (29%) were reported on day 14, characterized by itching lasting a few minutes after application, without redness. In comparison, the HQ group noted nine cases (36%, p = 0.85) of irritation or dryness. By day 60, the NIC group observed three cases

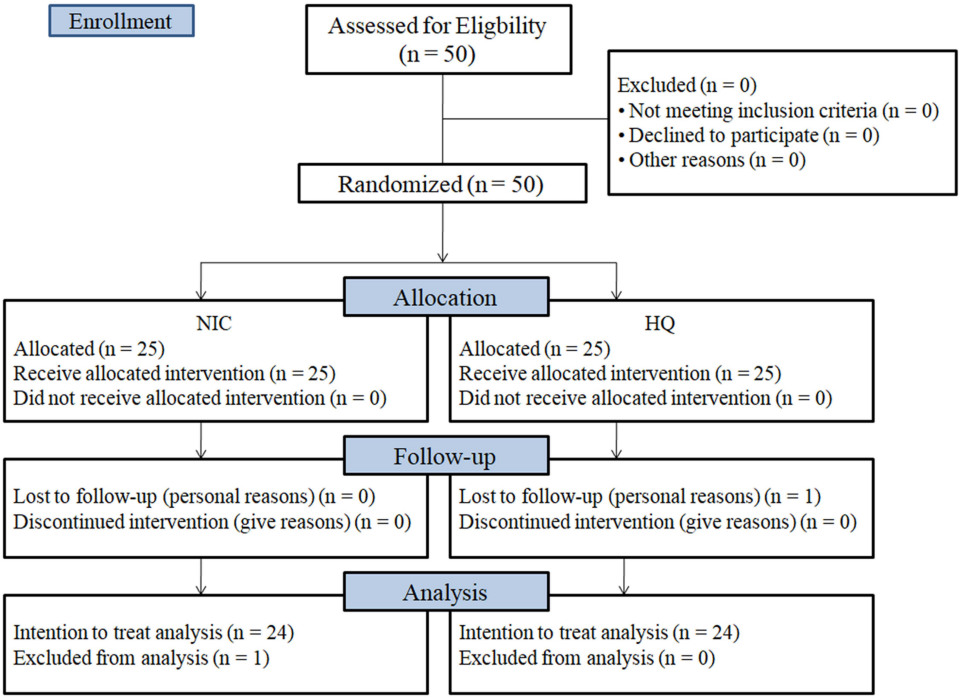

Figure 1 Study flowchart according to CONSORT 2010.

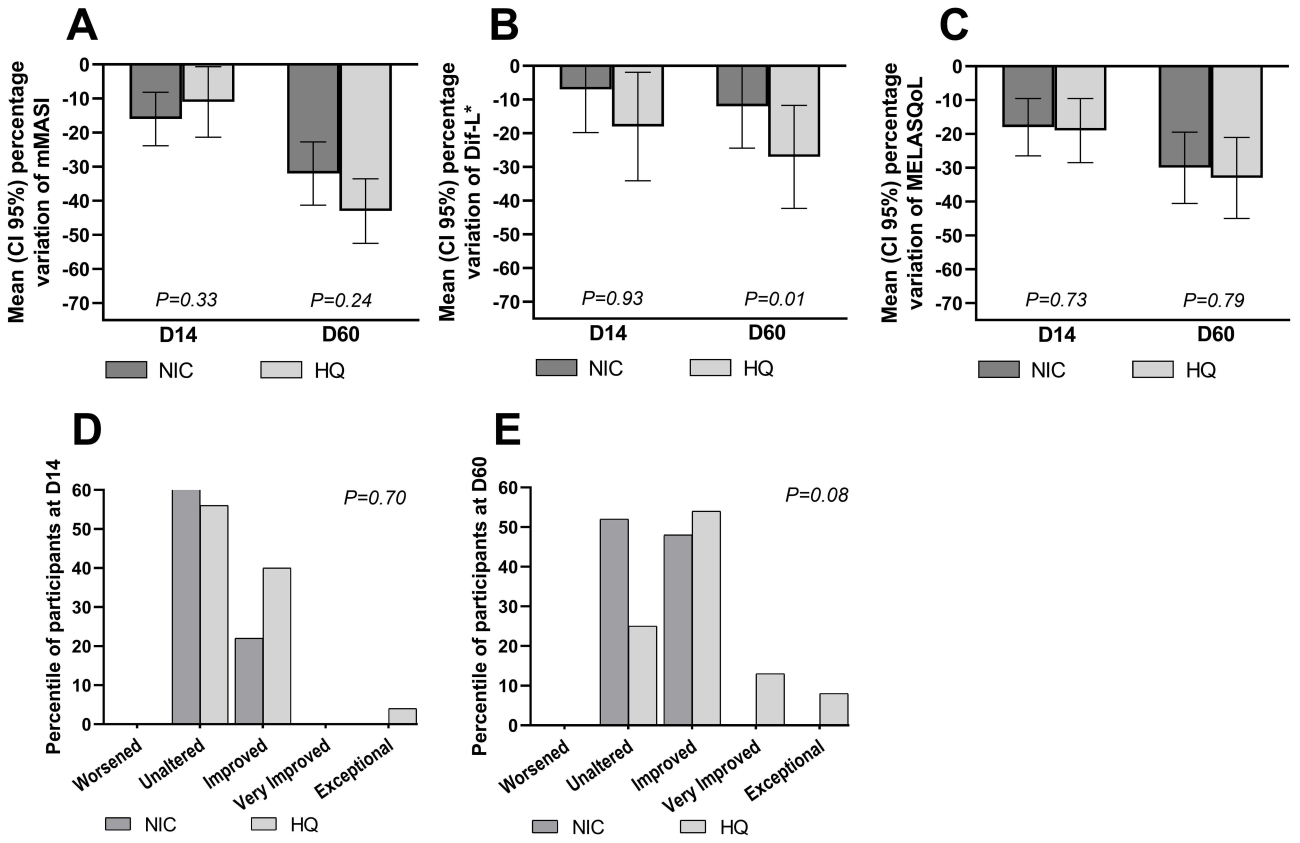

Figure 2 Main outcomes for D14 and D60. (**A**) Percentage reduction in mMASI score; (**B**) Percentage reductions in colorimetric difference between healthy and affected skin; (**C**) percentage reduction in MELASQoL; (**D**) and (**E**) GAIS scores at D14 and D60. GAIS is a 5-point scale used to evaluate the overall improvement in a patient's appearance after a specific treatment or procedure, ranging from "worse" to "very much improved". These assessments were conducted by a blinded investigator.

(13%, p = 0.11) of mild acne (fewer than 5 inflammatory lesions on the cheek) and three additional cases (13%) of mild itching, again lasting a few minutes post-application and without redness. In contrast, the HQ group recorded nine cases (38%, p = 0.10) of irritation or dryness. Importantly, all reported cases resolved spontaneously, with no need for intervention or treatment interruption (Figure 4).

All participants who experienced no adverse effects used the treatment for at least six days per week, and all applied sunscreen at least twice a day.

According to participants' subjective evaluations, on D14, perceived improvement was reported by 71% of NIC group participants and 76% of HQ group participants (p = 0.15); on D60, both groups showed improvement in 92% of cases (p = 0.78).

Discussion

A consistent improvement was observed in clinical measures, quality of life scores, and colorimetric measurements in women with melasma treated with 4% HQ gel cream for 60 days. Similarly, those treated with 10% NIC + 5% VCPMG + 5% HA gel cream up to day 60 also showed progressive favorable results. The participants of the study represented a usual sample of Brazilian adult women with melasma, regarding clinical and epidemiological data from previous studies.[10,11,32–38]

In this trial, the topical combination of NIC + VCPMG + HA reached numerically inferior clinical results compared to HQ on D14 or D60. In a prior study comparing the 4% NIC and 4% HQ, where participants applied one product to each side of the face, a significant improvement was observed in 44% of patients using NIC and 55% using HQ after two months. However, side effects were more prevalent with HQ (29%) compared to NIC (18%), indicating the better tolerability of NIC and a higher safety profile.[15] This study also assessed histopathological characteristics of melasma-affected skin before and after the use of 4% NIC, revealing a significant reduction in melanin content in the epidermis, as well as decreased inflammatory infiltrate and solar elastosis.[15]

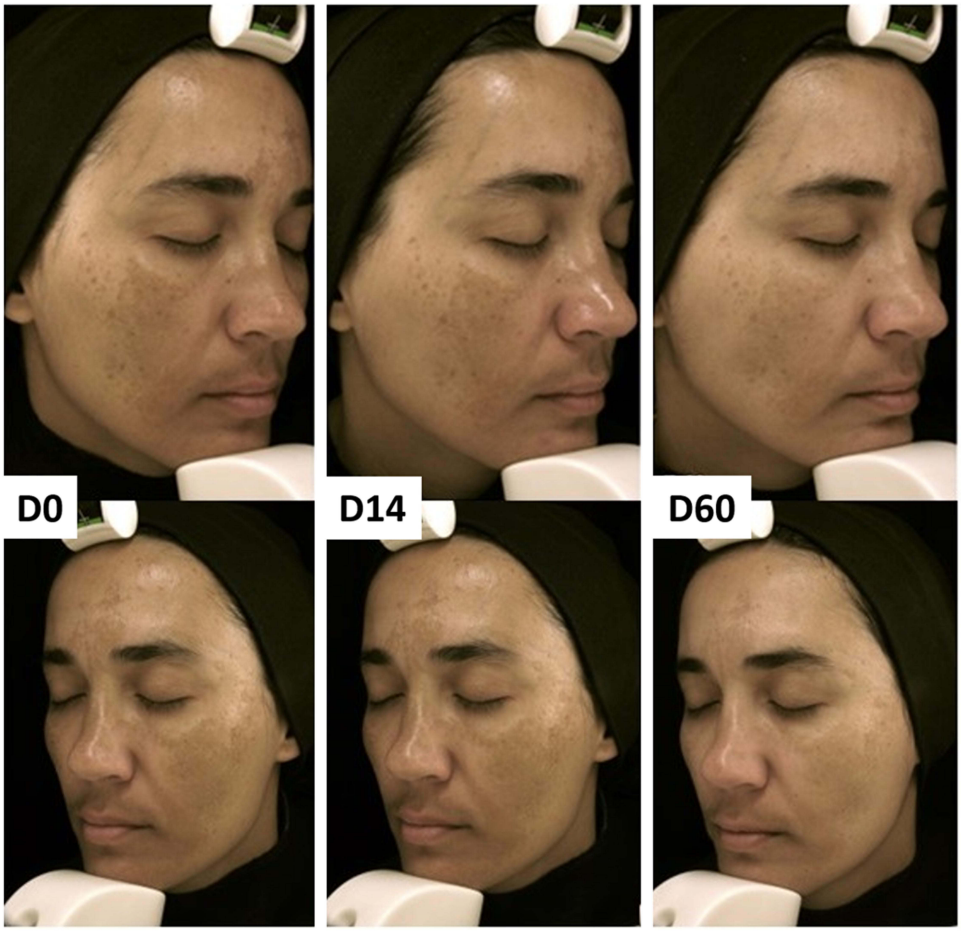

Figure 3 Facial melasma treated with topical 10% nicotinamide + 5% magnesium ascorbyl phosphate + 5% hyaluronic acid. Standardized photography from the inclusion, after 14 and 60 days.

The efficacy of topical NIC in skin depigmentation was evaluated through a randomized clinical trial involving 18 Japanese women. NIC 5% was applied to one side of the face, while a moisturizer was applied to the contralateral side. The results after eight weeks demonstrated a reduction in pigmentation in the intervention side compared to the control side.[39] In our sample, the topical combination of NIC + VCPMG + HA promoted 32% mMASI reduction in eight weeks, with excellent tolerability. This may be particularly relevant for melasma patients from sunny regions or with sensitive skin, who may have reduced tolerability to treatments with retinoids and hydroquinone.

After 16 weeks of twice-daily application, 10% NIC resulted in a mean 36% reduction in mMASI in 14 Chinese patients with facial melasma, and no adverse effects were observed. However, the comparator group, which combined this regimen with biweekly salicylic acid peels, performed better.[40]

Another study aimed to investigate the role of interleukin 6 (IL-6) and endothelin-1 (ET-1) receptors in the increased dendricity of melanocytes in hyperpigmented facial lesions and the action of NIC in suppressing these ligands. The study observed that IL-6 and ET-1 increased melanocyte dendricity, consequently enhancing the transfer of melanosomes from melanocytes to keratinocytes. Therefore, this study demonstrated that NIC was effective in reducing the release of IL-6 and ET-1 from keratinocytes, inhibiting melanocyte dendricity, confirming its role in skin depigmenting.[41]

VCPMG is a stable, lipophilic, and esterified form of VC that remains stable at neutral pH.[21] An in vitro study has shown its potential to protect keratinocytes against UVA irradiation, possibly by increasing glutathione levels.[42] Additionally, other in vitro results demonstrated that it induces collagen synthesis to a similar extent as ascorbic acid, and contributes to skin depigmentation.[43,44] Further histologic studies on the topical combination of NIC + VCPMG + HA are warranted.

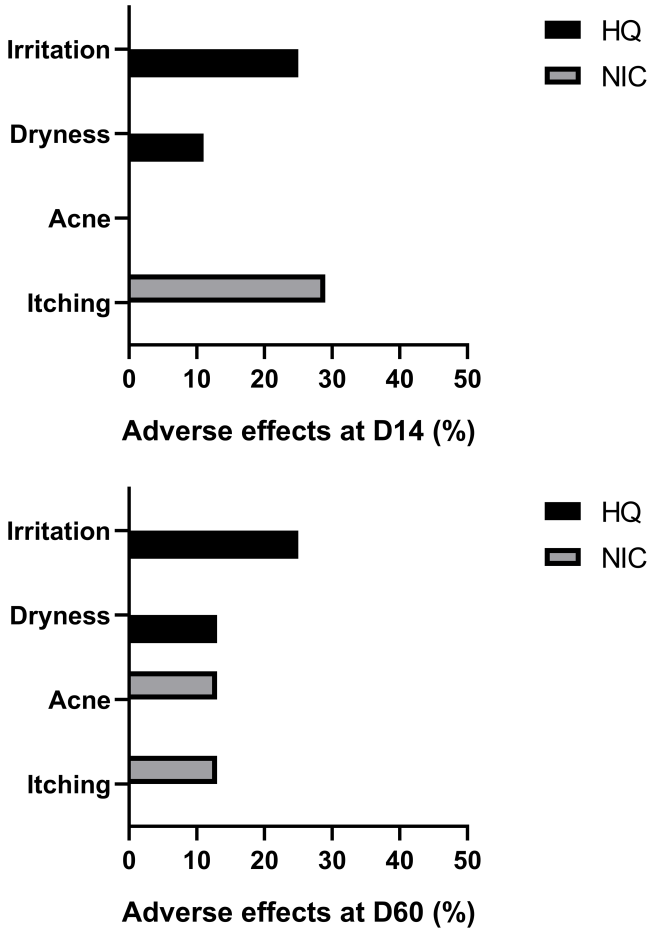

Figure 4 Frequency of the main adverse effects of the study according to the group at D14 and D60.

The time taken for a basal keratinocyte to reach the stratum corneum is approximately 28 days, and most trials for topic treatments for melasma assess clinical outcomes at 60-90 days. The evaluation conducted on Day 14 primarily aimed to identify adverse effects rather than assess potential early depigmenting effects of the treatments. The assessment of the isolated effects of the 5% VCPMG, or the 10% NIC in melasma demands specific designs.

Possible limitations of the study include the short duration of the intervention, modest sample size, lack of histopathologic analysis, as well as the assessment of relapses after stopping the treatments. The use of this combination in longer treatment regimens for melasma is warranted.

Conclusion

The topical combination of NIC + VCPMG + AH, was found to be safe and well-tolerated, although its overall clinical efficacy was numerically inferior to 4% hydroquinone. It can be considered as an alternative treatment for facial melasma, especially for patients with poor tolerability to HQ.

Data Sharing Statement

The raw data of this study is available under contact with the corresponding author.

Ethics Statement

The study was approved by the Research Ethics Committee of the UNIFESP (#6.142.230). All procedures in studies involving human participants were performed in accordance with the Declaration of Helsinki (as revised in 2013).

Informed-Consent Statement

Written informed consent was obtained from patients to publish their data and images in this paper.

Acknowledgments

To the patients in this study who gave written informed consent to the publication of their case details and images.

Funding

There is no funding to report.

Disclosure

The authors report no conflicts of interest in this work.

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