J. Cosmet. Sci., 71, 263–290 (September/October 2020)

Evaluating the Safety and Efficacy of a Topical Formulation Containing Epidermal Growth Factor, Tranexamic Acid, Vitamin C, Arbutin, Niacinamide and Other Ingredients as Hydroquinone 4% Alternatives to Improve Hyperpigmentation: A Prospective, Randomized, Controlled Split Face Study

BRANDON D. KALASHO, ARDALAN MINOKADEH, SANDY ZHANG-NUNES, RICHARD A. ZOUMALAN, NIMA L. SHEMIRANI, ANDREA R. WALDMAN, VADIM PLETZER, and CHRISTOPHER I. ZOUMALAN, *Zoumalan Plastic Surgery, Beverly Hills, CA (B.D.K., R.A.Z., C.I.Z.), Skin Care and Laser Physicians of Beverly Hills, Los Angeles, CA (A.M.), Department of Ophthalmology, Keck School of Medicine of USC, Los Angeles, CA (S.Z.-N.), Eos Rejuvenation, Beverly Hills, CA(N.L.S.), Albert Einstein College of Medicine, Montefiore Medical Center, New York, NY (A.R.W.), KlinConsult, Wellington, New Zealand (V.P.)*

Accepted for publication April 13, 2020.

Synopsis

Hyperpigmentation is a common concern of patients in aesthetic practice. There are various treatment options, but topical depigmenting agents such as hydroquinone (HQ) are usually a first-line option. Given HQ's side effects and potential controversy over its long-term use from prior animal studies, there is a consumer demand for non-HQ topical formulations that provide similar efficacy, but with a reduced adverse reaction profile to HQ. There is increasing evidence to support the use of selective growth factors, tranexamic acid, niacinamide, arbutin, and Vitamin C in improving hyperpigmentation. This study sought to determine whether a non-HQ topical formulation, composed of the aforementioned ingredients, could provide similar or improved efficacy to topical HQ, but with a reduced adverse reaction profile. This single-center, prospective, randomized, controlled split face study investigated the safety and efficacy of a proprietary product SKNB19 compared with hydroquinone 4% (HQ4%) in treating hyperpigmentation. Eighteen adult subjects with facial pigmentation were randomly assigned to have one side of their face treated with SKNB19 twice a day (morning and night application) and the other treated with HQ4% applied nightly. Patients used a 5-point scale to self-assess their overall appearance, and a 4-point scale to assess redness, irritation, and tolerability to the skin-brightening creams. A Wilcoxon signed-rank test was used to test whether there was a statistical

Address all correspondence to Christopher I. Zoumalan at research@zoumalanmd.com.

difference between the two treatments. Three-dimensional imaging was performed before treatment was administered and again 1 month following treatment initiation using a Canfield Vectra 3D imaging system. Five independent reviewers comprising two dermatologists, two facial plastic surgeons, and one oculoplastic surgeon graded and performed a qualitative comparative assessment of each side of the face using the before and after images. A Wilcoxon signed-rank test was used to test whether there was a statistical difference in overall appearance between SKNB19- and HQ4%-treated sides. SKNB19-treated hyperpigmentation had a statistically significant improvement in the overall appearance of hyperpigmentation and was shown to be 28.5% better than HQ4%-treated skin in the patient self-assessment and 27% better than HQ4%-treated skin in the independent reviewer assessment. On pair-wise comparison, the independent reviewer assessment also showed that 88.2% of the SKNB19-treated sides appeared equal or better than the HQ4%-treated sides. One patient dropped out of the study because of severe intolerance to HQ4%. No patients experienced intolerance to SKNB19, and all were able to continue its use without adverse effects. SKNB19-treated hyperpigmentation also had a statistically significant reduction in irritation when compared with HQ4%-treated hyperpigmentation. Patients reported a reduction in redness when using SKNB19 as opposed to HQ4%, but these figures did not reach statistical significance. This study supports that SKNB19, a recently developed non-HQ proprietary product, is safe and effective in improving hyperpigmentation. SKNB19 significantly improved the appearance of hyperpigmentation when compared with HQ4% in both patient self-assessment and independent reviewer assessment. SKNB19 exhibited a lower adverse reaction profile and was significantly better tolerated than HQ4%. SKNB19 should be considered as a safe and effective non-HQ alternative for the management of hyperpigmentation.

INTRODUCTION

Hyperpigmentation is a common concern of patients in aesthetic practice, and it can result from conditions such as melasma, postinflammatory hyperpigmentation (PIH), and solar lentigines (1). There are various treatment options available for hyperpigmentation, but the first-line treatment includes topical depigmenting agents such as hydroquinone (HQ2–4%) but may also include the use of tretinoin, azelaic acid, superficial peeling agents, and/or lasers (1).

Despite many possible treatment options, the results with these treatments are often temporary, especially in melasma, as the discoloration generally returns with continued exposure to the sun (2). Hydroquinone (HQ) is the most widely used and studied among possible treatment options. HQ's ability to lighten hyperpigmentation stems from its competitive inhibition of the enzyme tyrosinase, which prevents the conversion of tyrosine to dihydroxyphenylalanine (DOPA), ultimately halting melanin synthesis (3). Although very effective and dosed at different strengths, HQ can cause an irritant dermatitis in some individuals, and chronic use can lead to exogenous ochronosis (4,5). Its use has been historically controversial because of some animal studies that have shown toxicity to DNA, renal, and liver cells (6–8). Because of such concerns, its use as a cosmetic additive has been banned in the European Union, and only available as a prescription (9).

Given the aforementioned issues associated with HQ, there is a consumer demand for non-HQ topical formulations that provide similar efficacy, but with a reduced adverse reaction profile. Although various ingredients have been evaluated, tranexamic acid (TA) has been shown through various randomized, controlled trials to have similar efficacy to HQ, but with a milder adverse reaction profile (10–13).

There is a growing interest in the use of growth factors in improving hyperpigmentation. Various growth factors such as epidermal growth factor (EGF), tumor necrosis factor alpha, interleukins 1 and 6 (IL-1 and IL-6), Dickkopf 1 (DKK1), and transforming growth factor (TGF- β1) have been implicated in reducing melanocytic activity in previous studies (14–17).

Our study will evaluate a new topical formulation, SKNB19, consisting of synthetic recombinant human EGF and TA as the key ingredients in a topical formulation. Both EGF and TA are implicated in improving hyperpigmentation (11,13,17–20). The cream also contains other synthetic recombinant human growth factors along with vitamin C, arbutin 3%, and niacinamide 5%, all of which have been individually shown to improve hyperpigmentation (21–25). In this prospective, randomized split study, the efficacy and safety of this topical cream is evaluated and compared with that those of hydroquinone 4% (HQ4%).

METHODS

STUDY DESIGN AND POPULATION

This single-center, prospective, randomized controlled study investigates the safety and efficacy of the proprietary product SKNB19 compared with HQ4% in treating hyperpigmentation. Both SKNB19 and HQ4% used in the study are manufactured by MD Medical Designs, Inc. (Beverly Hills, CA). HQ4% is mixed in a premanufactured anhydrous topical gel consisting of the following inactive ingredients: dimethicone, caprylyl methicone, PEG-12 dimethicone/PPG-20 crosspolymer, butyrospermum parkii (shea) butter, polysilicone-11, tocopheryl acetate, and butylated hydroxytoluene (BHT) (PCCA WO6 Anhydrous Topical Gel, Houston, TX). Tocopheryl acetate and BHT are not active ingredients and are serving as preservatives in this formula.

SKNB19 comprises synthetic recombinant human EGF (0.001%) and TA (3%) as the key ingredients in a topical formulation. Other active ingredients include water-soluble vitamin C (5%), arbutin (3%), and niacinamide (5%). The active ingredients are mixed in a moisturizing cream base consisting of the following inactive ingredients: water, caprylic/capric triglyceride, isononyl isononanoate, C12-15 alkyl benzoate, silica, cetearyl alcohol, glyceryl stearate, PEG-100 stearate, ceteareth-20, polysorbate 60, hydroxyethyl acrylate/sodium acryloyldimethyl taurate copolymer, xylitylglucoside, anhydroxylitol, xylitol, phenoxyethanol, ethylhexylglycerin, titanium dioxide, citric acid, citrus aurantium bergamia (bergamot) fruit oil, citrus grandis (grapefruit) peel oil, citrus aurantium dulcis (orange) peel oil, and citrus tangerina (tangerine) peel oil.

Subjects older than 18 years with pigmentation on the face, as determined by clinical examination, were included in this study. Subtypes of hyperpigmentation that met inclusion criteria were melasma, PIH, and/or solar lentigines. Each patient was required to be free of using any whitening creams, retinol-based products, or treatments to correct their hyperpigmentation (i.e., lasers and peels) for the past 6 mo. In addition, patients were required to be out of direct sun during the duration of the treatment. Female patients who were pregnant, planning to be pregnant, or breast feeding were also excluded from the study. Written informed consent was obtained from all study subjects before enrollment. The protocol for the study was conducted according to the Declaration of Helsinki and the Health Insurance Portability and Accountability Act.

In this split study, participants were assigned based on a computer-generated randomization protocol such that one side of the face with hyperpigmentation was treated with SKNB19 twice a day (morning and night application) and the other side was treated with HQ4% applied nightly only. Our early experience using SKNB19 before the onset of this

study showed that it was well tolerated for use twice daily, whereas HQ4% was better tolerated nightly. All patients were instructed to apply a zinc oxide–based sunscreen to both sides of the face with sun protection factor 30 that was provided to them.

ASSESSMENTS

We assessed hyperpigmentation at baseline (before treatment) and at 1 mo after treatment initiation using both patient self-assessment and independent reviewer assessments. Patients used a 5-point scale to self-assess their overall appearance, in each case a higher score denoted a better outcome. Patients also used a 4-point scale to assess redness, irritation, and tolerability to the skin-brightening creams (Table I). For the patient self-assessment, a Wilcoxon signed-rank test was used to test whether there was a statistical difference between the two treatments in overall appearance, irritation, redness, and tolerability.

Three-dimensional imaging was performed before treatment and again 1 mo following treatment initiation using a standardized Canfield Vectra 3D imaging system (Canfield Scientific Inc., Parsippany, NJ). The study used five independent reviewers comprising two dermatologists, two facial plastic surgeons, and one oculoplastic surgeon. Each reviewer was blinded to the study treatment, assessed the images pre- and posttreatment, and graded the appearance of each side on overall appearance (see Supplementary Figures 1–8 for independent reviewer grading scale). The grading scale was as follows: –1 indicating worsened appearance, 0 indicating no change, 1 indicating mild improvement, 2 indicating moderate improvement, and 3 indicating significant improvement (Table II).

In addition, the independent reviewers also performed a qualitative comparative assessment of the sides treated with each cream and noted the "better overall" side. A Wilcoxon signed-rank test was used to test whether there was a statistical difference in overall appearance between SKNB19- and HQ4%-treated sides.

RESULTS

Eighteen patients (16 females and two males) met the inclusion criteria and were enrolled in the study. The mean age of the patients was 38.8 (±9.7) years (range: 23–61 years).

Table I
Patient Self-Assessment Survey Scale

Score	–1	0	1	2	3
Overall appearance	Worsened	No change	Mild improvement	Moderate improvement	Significant improvement
Irritation	—	None	Mild irritation	Moderate irritation	Significant irritation
Redness	—	None	Mild redness	Moderate redness	Significant redness
Tolerability	—	No issues	Mild issues	Moderate issues	Severe intolerability

Table II
Independent Reviewer Assessment Scale

Score	−1	0	1	2	3
Overall appearance	Worsened	No change	Mild improvement	Moderate improvement	Significant improvement

PATIENT SELF-ASSESSMENT

After 1 mo of treatment, the patients assessed the two products from baseline on overall appearance, irritation, redness, and tolerability using a Likert scale (Table I). One patient dropped out of the study after 2 wk because of severe irritation, redness, and intolerability from HQ4% use. This patient's last observation for HQ4% was carried forward to the 1-mo assessment.

Overall appearance. After 1 mo of SKNB19 treatment, of the 18 patients, 18 (33.3%) saw a mild improvement, six (33.3%) patients saw a moderate improvement, and six (33.3%) patients saw a significant improvement in the overall appearance of their hyperpigmentation relative to baseline. After 1 mo of HQ4% treatment, of 18 patients, one (5.6%) saw worsening in overall appearance, three (16.7%) saw no change, 11 (61.1%) saw a mild improvement, and three (16.7%) saw a moderate improvement in their hyperpigmentation relative to baseline (Figure 1, Table III). Of the 18 patients, 15 (83.3%, Confidence Interval (CI): 66.1–100%) rated the SKNB19-treated side as having better overall appearance than the HQ4%-treated side. Of the 18 patients, three (16.7%, CI: 0.0–33.9%) rated both the SKNB19- and HQ4%-treated sides as having the same overall appearance. None of the patients rated the HQ4%-treated side as having better overall appearance (Figure 2). Differences in pair-wise scores were statistically significant ($p = <0.001$). After 1 mo of treatment, patients reported a 100% improvement (mild, moderate, or significant) in the overall appearance of SKNB19-treated skin sections and 77.8% improvement in HQ4%-treated skin sections. Relative to HQ4%, this is a 28.5% improvement with SKNB19.

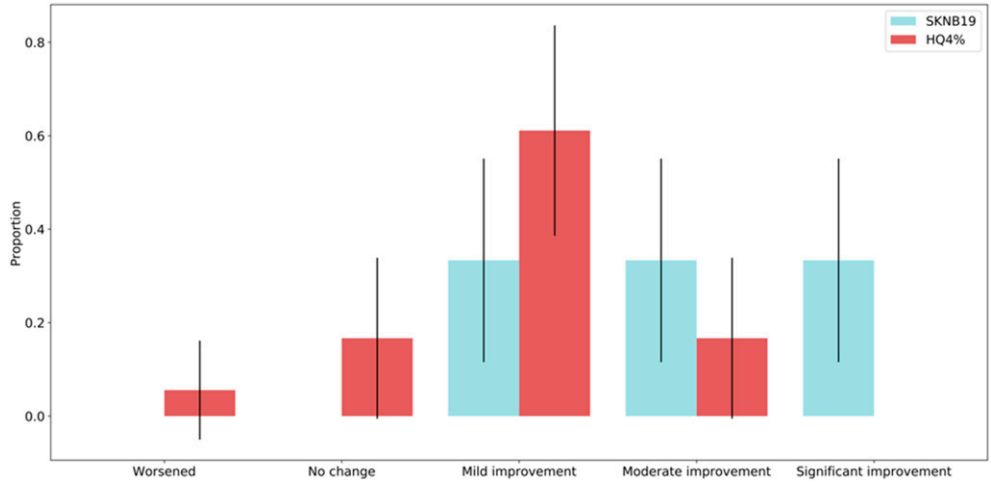


Figure 1. Patient self-assessment: Distribution of overall appearance scores.

Table III
Patient Self-Assessment: Distribution of Overall Appearance Scores

Treatment	Score	Proportion (%), $N = 18$ patients	95% Margin of error
SKNB19	Worsened	0 (0.0%)	±0.0%
	No change	0 (0.0%)	±0.0%
	Mild improvement	6 (33.3%)	±21.8%
	Moderate improvement	6 (33.3%)	±21.8%
	Significant improvement	6 (33.3%)	±21.8%
HQ4%	Worsened	1 (5.6%)	±10.6%
	No change	3 (16.7%)	±17.2%
	Mild improvement	11 (61.1%)	±22.5%
	Moderate improvement	3 (16.7%)	±17.2%
	Significant improvement	0 (0.0%)	±0.0%

Irritation. After 1 mo, 100.0% of patients reported no irritation on the SKNB19-treated side. By contrast, of the 18 patients, 13 (72.2%) reported no irritation, four (22.2%) reported mild irritation, and one (5.6%) reported significant irritation on the HQ4%-treated side (Figure 3). On pair-wise comparison, five of the 18 (27.8%, CI: 7.1–48.5%) patients reported less irritation with the SKNB19-treated side, whereas none reported less irritation with the HQ4%-treated side. Differences in pair-wise scores were mildly statistically significant ($p = 0.048$).

Redness. After 1 mo, of the 18 patients, 16 (88.9%) reported no redness and two (11.1%) reported mild, transient redness on the SKNB19-treated side, whereas 13 (72.2%) patients reported no redness, three (16.7%) reported mild redness, one (5.6%) reported moderate redness, and one (5.6%) reported significant redness on the HQ4%-treated side (Figure 4). On pair-wise comparison, of the 18 patients, five (27.8%, CI: 7.1–48.5%) patients reported less redness with the SKNB19-treated side, whereas two (11.1%, CI:

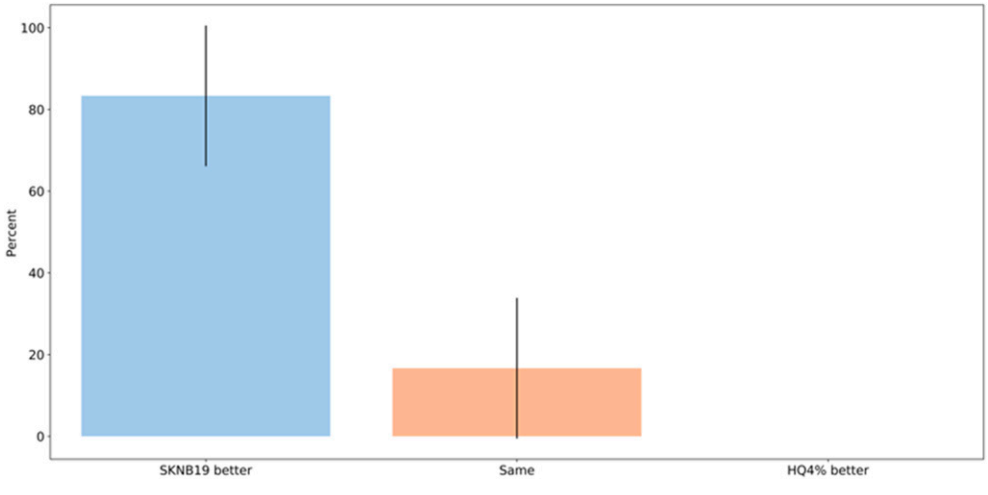


Figure 2. Patient self-assessment: Bar plot demonstrating the proportion of overall appearance scores for which SKNB19 was better, same, or worse compared with HQ4%. In 83.3% of cases, SKNB19 was rated as having better overall appearance than HQ4%. In 16.7% of cases, SKNB19 and HQ4% were rated as having the same overall appearance.

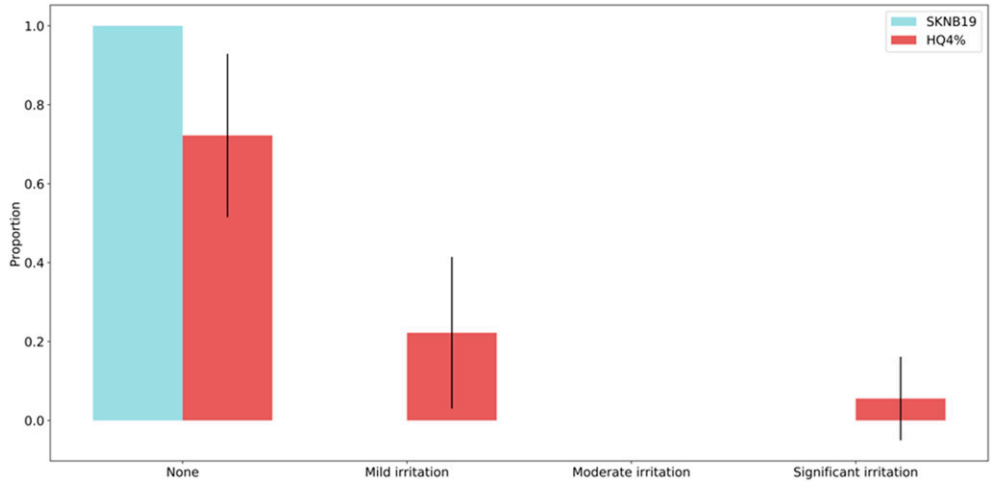


Figure 3. Patient self-assessment: Distribution of irritation scores. After 1 mo of HQ4% treatment, 27.8% of skin sections had mild or significant irritation compared with 0% with SKNB19.

0.0–25.6%) reported less redness with the HQ4%-treated side. Differences in pair-wise scores did not reach statistical significance ($p = 0.19$).

Tolerability. After 1 mo of SKNB19 treatment, 100.0% of patients reported no tolerability issues. After 1 mo of HQ4%, of the 18 patients, 13 (72.2%) reported no tolerability issues, four (22.2%) reported mild tolerability issues and one (5.6%) reported severe intolerability (Figure 5).

On pair-wise comparison, of the 18 patients, five (27.8%, CI: 7.1–48.5%) reported better tolerability to treatment with the SKNB19-treated side than the HQ4%-treated side, whereas none reported better tolerability to treatment with the HQ4%-treated side. Differences in pair-wise scores were mildly statistically significant ($p = 0.048$).

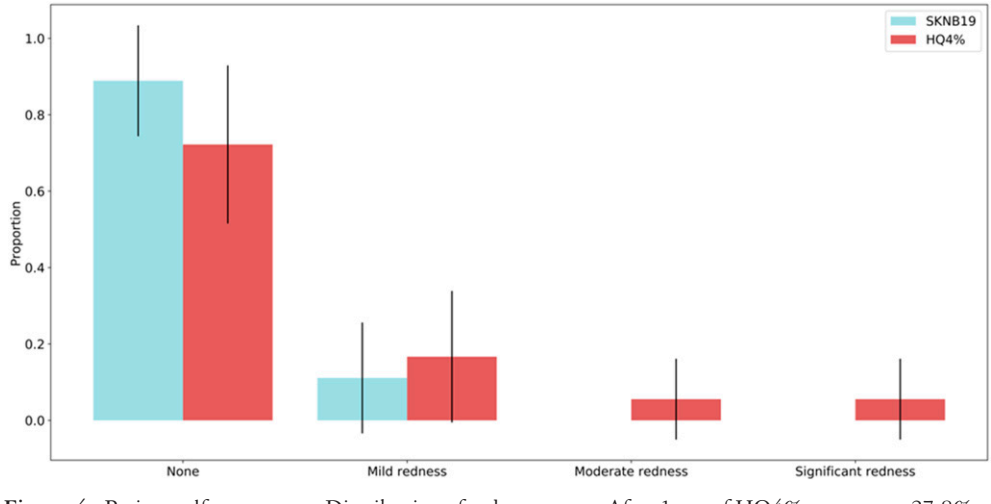


Figure 4. Patient self-assessment: Distribution of redness scores. After 1 mo of HQ4% treatment, 27.8% of skin sections had mild, moderate, or significant redness compared with 11.1% with SKNB19.

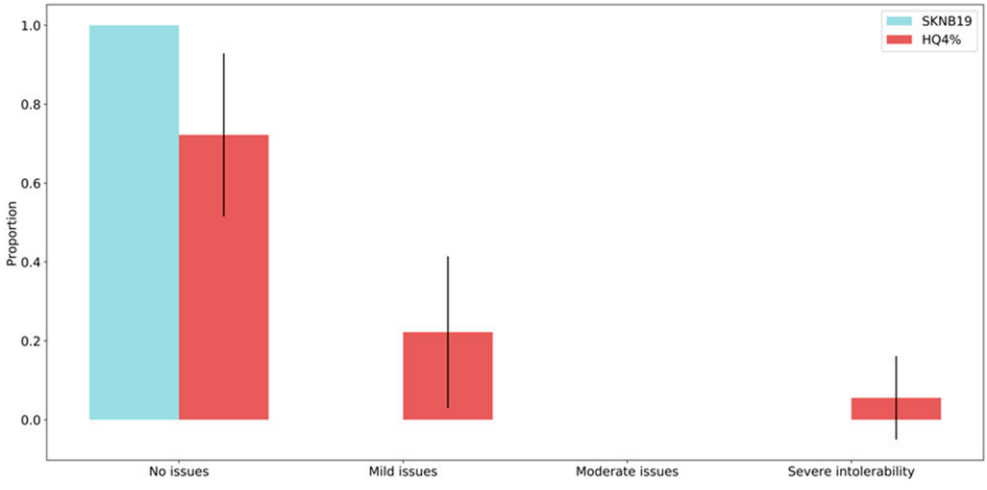


Figure 5. Patient self-assessment: Distribution of tolerability scores. After 1 mo of HQ4% treatment, 27.8% of skin sections had mild or significant tolerability issues compared with 0% with SKNB19.

INDEPENDENT REVIEWER ASSESSMENT

Seventeen patients (15 females and two males) were included in the independent reviewer assessment. The mean age of the patients was 39.7 ± 9.1 (range: 27–61) years. One patient dropped out of the study because of intolerability to HQ4%. Five independent evaluators reviewed 17 patients, for a total of 85 ratings that were taken into analysis. After 1 mo of treatment, evaluators reported an improvement (whether mild, moderate, or significant) in the overall appearance in 70 of 85 cases (82.4%) on the SKNB19-treated side and in 55 of 85 cases (64.7%) on the HQ4%-treated side (Figure 6, Table IV). Relative to HQ4%, this is a 27% improvement.

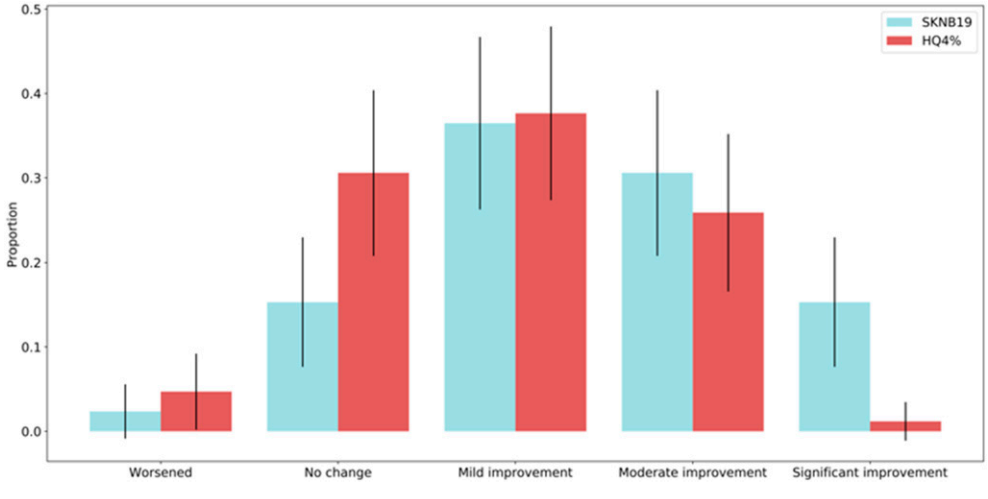


Figure 6. Independent reviewer assessment: Distribution of scores.

Table IV
Independent Reviewer Assessment: Distribution of Evaluator Scores for Overall Appearance

Treatment	Score	Score counts (%), $N = 85$	95% Margin of error
SKNB19	Worsened	2 (2.4%)	±3.2%
	No change	13 (15.3%)	±7.7%
	Mild improvement	31 (36.5%)	±10.2%
	Moderate improvement	26 (30.6%)	±9.8%
	Significant improvement	13 (15.3%)	±7.7%
HQ4%	Worsened	4 (4.7%)	±4.5%
	No change	26 (30.6%)	±9.8%
	Mild improvement	32 (37.6%)	±10.3%
	Moderate improvement	22 (25.9%)	±9.3%
	Significant improvement	1 (1.2%)	±2.3%

On pair-wise comparison, of the 85 SKNB19-treated cases, 31 (36.5%, CI: 26.3–46.7%) were rated as being equal in overall appearance by the independent evaluators compared with the HQ4%-treated cases, and 44 (51.8%, CI: 41.2–62.4%) were rated as having better overall appearance by the independent evaluators than the HQ4%-treated cases. Of 85 the HQ4%-treated cases, 10 (11.8%, CI: 5.0–18.6%) were rated as having a better overall appearance (Figure 7, Table V) than the SKNB19-treated cases. In summary, on pair-wise comparison, the independent reviewer assessment showed that evaluators gave higher scores to SKNB19-treated sides than HQ4%-treated sides ($p < 0.001$). Most notably, 88.2% of the SKNB19-treated sides appeared to be equal to or better than the HQ4%-treated sides. Figures 8-17 present the before and after results of five patients that were included in the study.

DISCUSSION

Hyperpigmentation is a dermatologic condition that causes an unappealing appearance in a variety of individuals. The current mainstay of treatment is topical HQ, which is

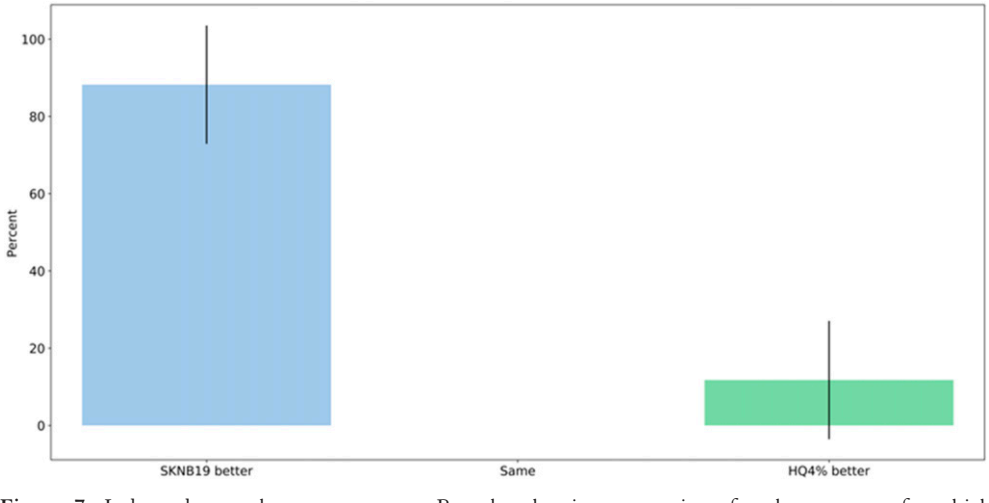


Figure 7. Independent evaluator assessment: Bar plot showing proportion of evaluator scores for which SKNB19 was better, same, or worse compared with HQ4%.

Table V
Independent Reviewer Assessment: Proportion of Evaluator Scores for which SKNB19 Was Better, Same, or Worse Compared with HQ4%

Side	Proportion (%)	95% Margin of error
SKNB19 better	44/85 (51.8%)	±10.6%
Same	31/85 (36.5%)	±10.2%
HQ4% better	10/85 (11.8%)	±6.8%

available in over-the-counter 2% and prescription 4% formulations. Despite possessing excellent skin-lightening properties, HQ's usage remains controversial, and studies suggest potentially carcinogenic and toxic effects affecting a variety of tissues. Therefore, the aim of this study was to determine if the use of a potential non-HQ alternative formulation provides equal or greater efficacy with a reduced adverse reaction profile.

Figure 8. Three-dimensional photographic comparison of bilateral facial hyperpigmentation (forehead/mid-face/cheek areas) in a 38-year-old woman prior at baseline visit (before treatment).

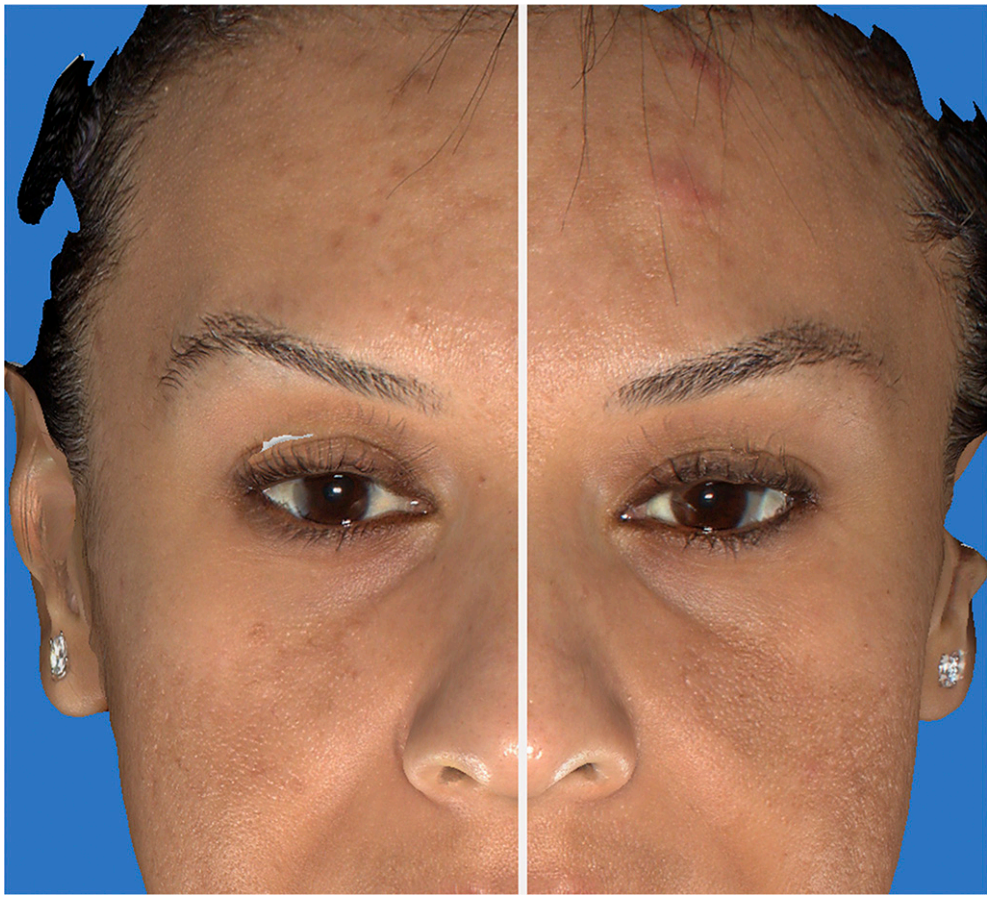


Figure 9. Three-dimensional photographic comparison of bilateral facial hyperpigmentation (forehead/midface/cheek areas) after 4 wk of twice-daily application HQ4% (right side of face) and SKNB19 (left side of face) SKNB19-treated hyperpigmentation shows a noticeable improvement in hyperpigmentation (forehead/midface/cheek area) compared with the side treated with HQ4%.

In our study, those patients suffering from hyperpigmentation treated with SKNB19 had a statistically significant improvement in the overall appearance of hyperpigmentation and shown to be 28.5% better than HQ4%-treated skin based on mild to significant improvement ratings. SKNB19-treated patients also had a statistically significant reduction in irritation when compared with HQ4%-treated patients with hyperpigmentation. Although patients reported a reduction in redness when using SKNB19 as opposed to HQ4%, these figures did not reach statistical significance. One patient experienced severe intolerance to HQ and was unable to continue the full duration of the treatment. No patients experienced intolerance to SKNB19, and all were able to continue its use without adverse effects.

Given the potential difficulty in evaluating the clinical differences and changes in hyperpigmentation within the patient's baseline and 1-mo images, we recruited five independent physicians who can evaluate and treat hyperpigmentation in their daily practice to assess the before and after images. Our results showed the assessments were very similar

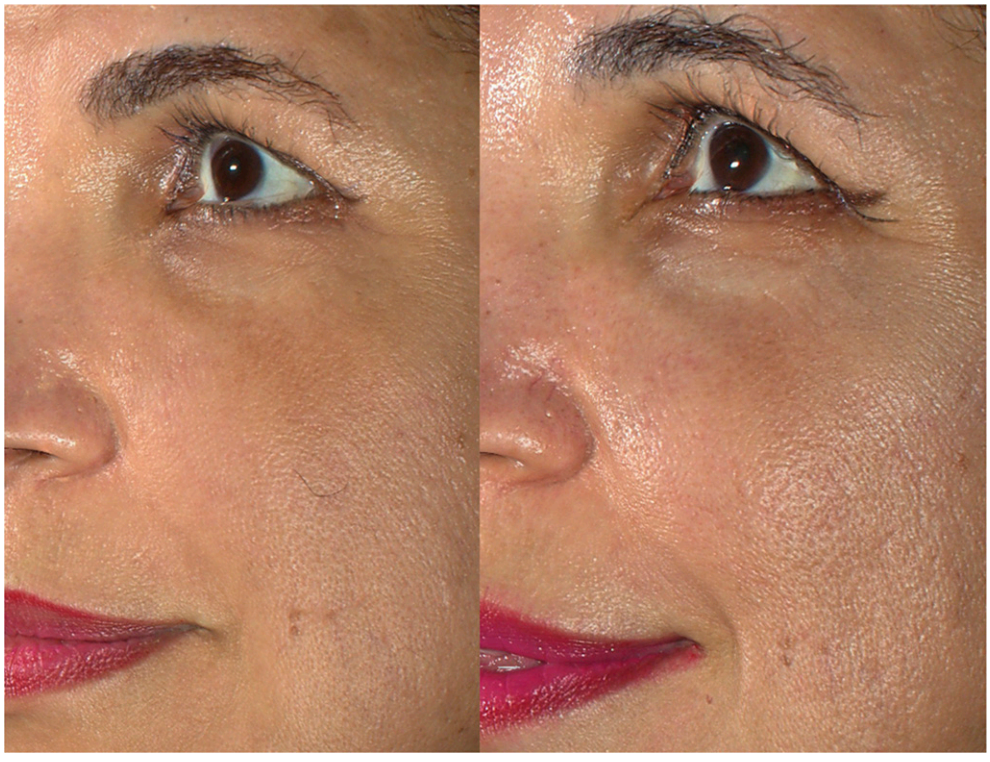


Figure 10. Three-dimensional photographic comparison of left-sided facial hyperpigmentation (lower eyelid and midface area) before (left) and after (right) 4 wk of twice-daily application HQ4% in a 53-year-old woman.

between the five reviewers with a strong interreliability. The reviewers' pair-wise comparison data showed that 88.2% of the SKNB19-treated side appeared either the same or better than the HQ4%-treated side. This study supports SKNB19 as an alternative, non-HQ product that can be used to improve skin hyperpigmentation, such as that seen in melasma, PIH, and solar lentigines.

SKNB19's ability to effectively improve hyperpigmentation can be attributed to its unique blend of ingredients that all have demonstrated safety and efficacy from prior peer-reviewed medical journal publications, which will be described in more detail in the following. TA has emerged as an effective treatment for hyperpigmentation including melasma. The mechanism of action for the reduction in hyperpigmentation is due to TA's ability to decrease tyrosinase activity in melanocytes. Topical TA formulations have been shown to be effective in the treatment of hyperpigmentation and melasma; 2% emulsion, 3% cream, 5% solution, and 5% liposomal cream have all been clinically studied (11,13,18–20). Topical TA applications demonstrate equal efficacy in reducing melasma when compared with HQ alone, topical HQ plus dexamethasone, and intradermal injections of TA (26). Kim et al 2016 investigated the mechanism of action of topical TA, finding that skin biopsies treated with TA showed a decrease in the melanin content in the epidermis. A decrease in vascular endothelial growth factor and a downregulation of endothelin were also reported (20).

Figure 11. Three-dimensional photographic comparison of right-sided facial hyperpigmentation (lower eyelid and midface area) before (left) and after (right) 4 wk of twice-daily application SKNB19 in a 53-year-old woman. SKNB19-treated hyperpigmentation shows a noticeable improvement in hyperpigmentation compared with the side treated with HQ4% (Figure 10).

EGF has been previously demonstrated to decrease inflammation-induced melanogenesis. Melanocytes respond to EGF through the extracellular signal–regulated kinases, which serve to reduce melanin synthesis through downregulation of microphthalmia-associated transcription factor (27). EGF is also able to limit hyperpigmentation by reducing tyrosinase activity and reducing melanogenesis (17). A recent randomized controlled study found that topical EGF improved melasma in their 15-patient cohort (28).

There is increased interest in other growth factors that have been shown to improve pigmentation in animal models. For instance, TGF-β1 has been shown to reduce melanocytic activity in a rat model study. A recent topical product using TGF-β1 along with other ingredients has been shown to improve melasma (29). IL-6 shows promise as well by inducing depigmentation in a prior animal study by Choi et al (30). Last, Yamaguchi et al have extensively studied DKK1, which is an inhibitor of Wnt signaling. DKK1 has been shown to be expressed in high mRNA levels by fibroblasts in the dermis of the human skin on the palms and soles and inhibits the function and proliferation of melanocytes in the palmoplantar epidermis (31,32). Although there is potential for DKK1's targeted pathway in reducing pigmentation in the palmoplantar epidermis, future studies will need to further investigate DKK1's role in hyperpigmentation.

Niacinamide, also known as vitamin B3, is a water-soluble vitamin that has been shown to reduce melanosome transfer (33), provide photoprotection (34), and possess anti-inflammatory properties (35–37), all of which can be attributed to its efficacy in the treatment of hyperpigmentation. A double-blinded, left–right randomized clinical trial in which patients with melasma were randomly treated with either niacinamide

Figure 12. Three-dimensional photographic comparison of left-sided facial hyperpigmentation (cheek and under eye area) before (left) and after (right) 4 wk of twice-daily application HQ4% in a 27-year-old woman.

Figure 13. Three-dimensional photographic comparison of right-sided facial hyperpigmentation (cheek and under eye area) before (left) and after (right) 4 wk of twice-daily application SKNB19 in a 27-year-old woman. The subject reported moderate irritation and redness at the 4-wk visit with the HQ4%-treated side (Figure 12). SKNB19-treated hyperpigmentation shows a noticeable improvement in hyperpigmentation compared with the side treated with HQ4% (Figure 12).

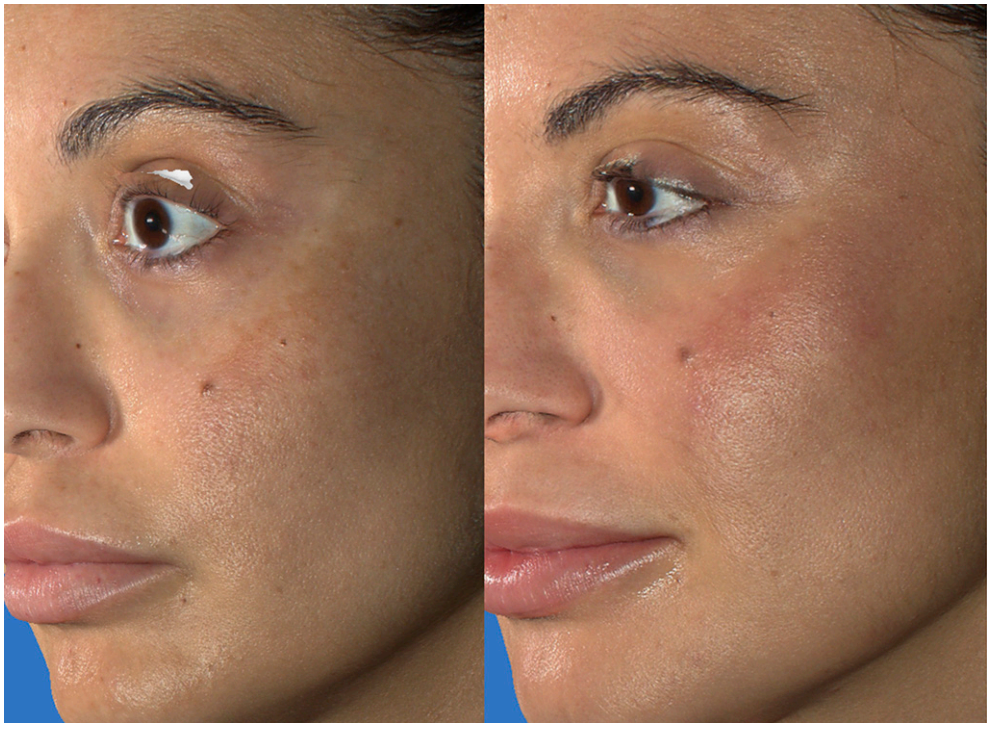


Figure 14. Three-dimensional photographic comparison of left-sided facial hyperpigmentation (cheek area) before (left) and after (right) 4 wk of twice-daily application HQ4% in a 35-year-old woman.

4% or HQ4% on either side of their face demonstrated that niacinamide was an effective alternative to HQ. Posttreatment biopsies indicated a significant reduction in the amount of epidermal melanin and inflammatory infiltrate in the niacinamide treated side. Although no statistically significant difference was found using a colorimetric assessment, HQ4% demonstrated a higher frequency of moderate adverse effects relative to niacinamide (18% vs. 7%, respectively). No significant side effects were observed following treatment with niacinamide, allowing it to be used for longer time periods (25).

Vitamin C and arbutin also provide skin-lightening benefits. Vitamin C, also known as ascorbic acid, contains antioxidant properties capable of hindering melanin synthesis by reducing *o*-dopaquinone to DOPA (21). Vitamin C has been shown to improve photoaging and hyperpigmentation in studies when used in combination with other skin-lightening agents (22,23). Vitamin C is readily oxidized, and in our formulation, we used a form which helped maintain its stability more effectively. Arbutin is a *b-D*-glucopyranoside derivative of HQ that can be extracted from bearberry, pear, cranberry, or blueberry plants (38). Arbutin is an inhibitor of tyrosinase and can inhibit melanosome maturation (24,39).

There are some limitations to our study that should be mentioned. The duration of study was only 1 mo, which could be perceived as being too short for appropriate evaluation. Despite the 1-mo follow-up, our study showed that there was a significant improvement in the overall appearance of hyperpigmentation using SKNB19 when compared with

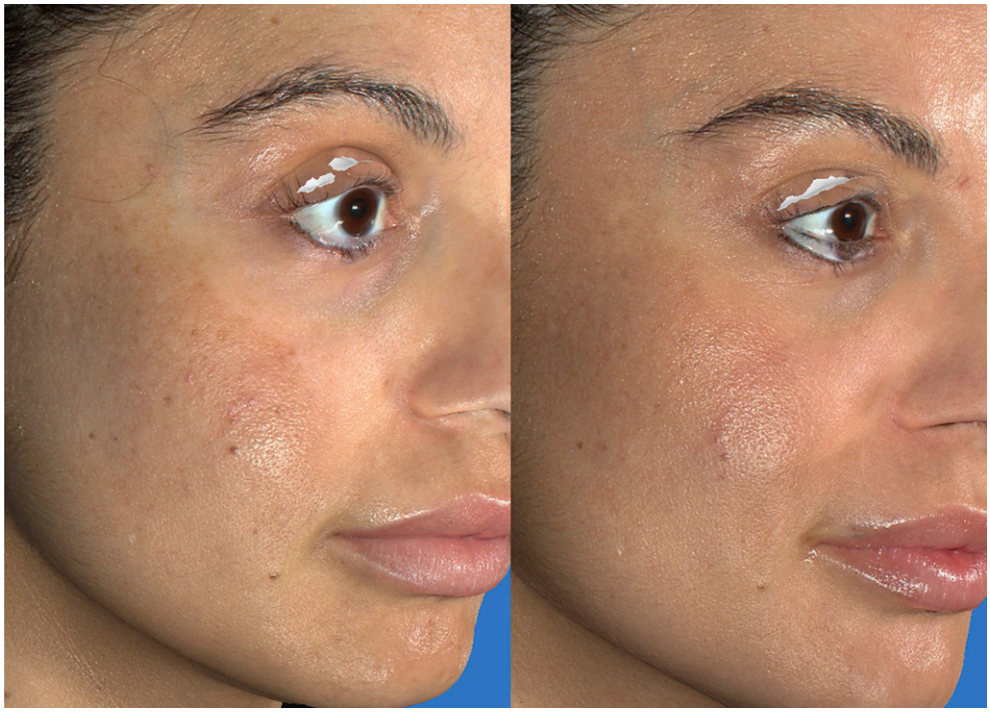


Figure 15. Three-dimensional photographic comparison of right-sided facial hyperpigmentation (cheek area) before (left) and after (right) 4 wk of twice-daily application SKNB19 in a 35-year-old woman. The subject reported moderate irritation and redness at the 4-wk visit with the HQ4%-treated side (Figure 14). SKNB19-treated hyperpigmentation shows a noticeable improvement in hyperpigmentation compared with the side treated with HQ4% (Figure 14).

HQ4%. Nevertheless, future studies should use a larger patient population and follow the cohort for a longer period of time to uncover whether longer use of this product may result in potentially even better outcomes.

Another limitation is the difference of application frequency between the two products investigated. It would have been ideal if both HQ4% and SKNB19 were applied twice daily. In our clinical practice, HQ4% is recommended to be used once a day because of its tolerance issues. Although it would have been ideal to dose the two products twice daily, we wished to stay consistent with our clinical practice patterns. In considering our protocol for our study, increasing HQ4%'s dose to twice daily may have increased the degree of improvement in the treated areas of hyperpigmentation, but this increase in frequency would potentially increase the chances of HQ4%-related adverse effects. We wanted to establish a study methodology that was most consistent with our clinical practice patterns. Nevertheless, this creates a potential limitation to HQ4%'s full potential and creates a bias in our study, which we are aware of, but given that SKNB19 could be tolerated twice daily, we wished to investigate SKNB19 at this ideal frequency interval.

Another area of limitation in our study is the differences in formula bases, or excipient bases, that were used between the two products. Although we were effectively evaluating the active ingredients between SKNB19 and HQ4%, each had differing excipient bases, which can arguably affect stability and absorption efficacy of the active ingredients and

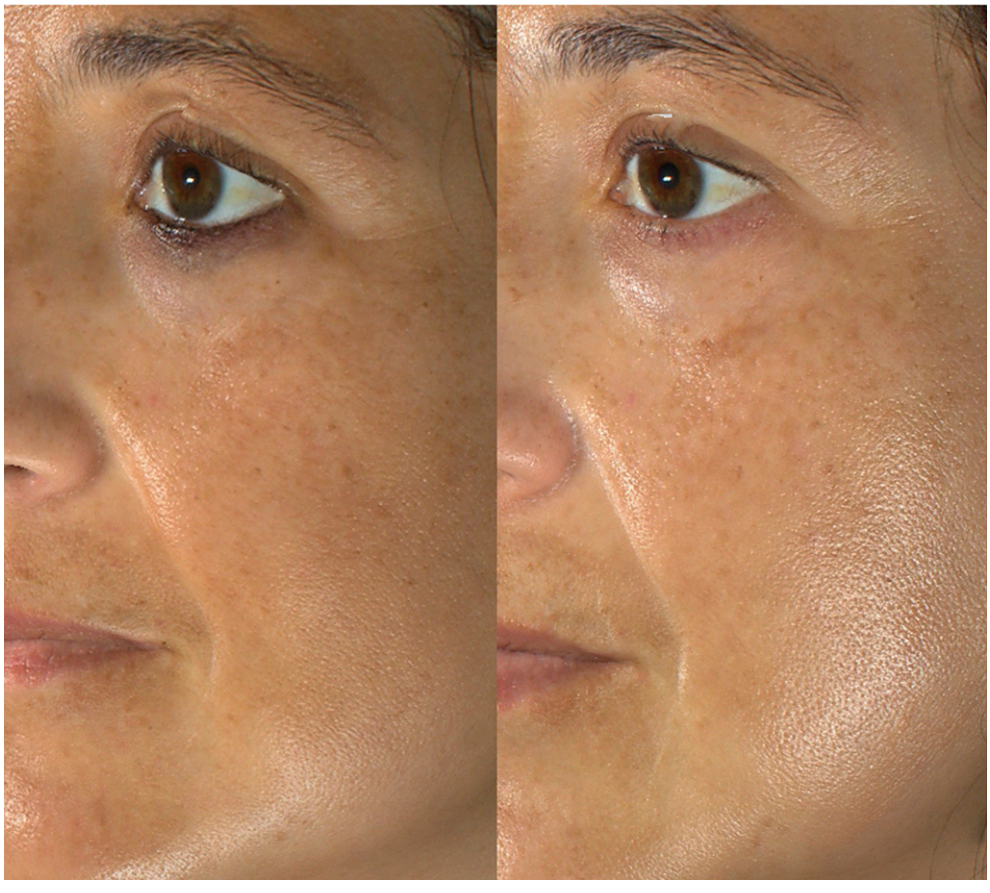


Figure 16. Three-dimensional photographic comparison of left-sided facial hyperpigmentation (cheek and midface) before (left) and after (right) 4 wk of twice-daily application HQ4% in a 53-year-old woman.

lead to some possible degrees of efficacy bias. The HQ4% formulation that we used in the study has been provided to our patients for several years in our clinical practice, and its base has been found to be very tolerable compared with other bases that we had previously used. In our practice, we usually discontinue HQ4% after 1 mo of use. By contrast, when developing SKNB19, we wanted to have an excipient base that was more consistent with a moisturizer feel because this product was developed for a longer duration of use in contrast to HQ4%. Nevertheless, future studies may need to consider using the same excipient base for both products tested.

There are certainly other well-described methods for measuring the degree of hyperpigmentation, either through different grading scales such as Melasma Area and Severity Index (MASI) score or, for instance, chromameters. In our clinical study, we decided on a relatively straightforward grading scale that can readily be used by physicians of various specialties who manage hyperpigmentation. In contrast to our grading scale, the MASI scale has been shown to have a high rate of intra- and interrater variability and may not be practical in the hands of many clinicians managing hyperpigmentation (40). We found our scale to work very well for our five independent evaluators, and it had a high rate of interevaluator reliability. Nevertheless, there has been recent literature supporting the

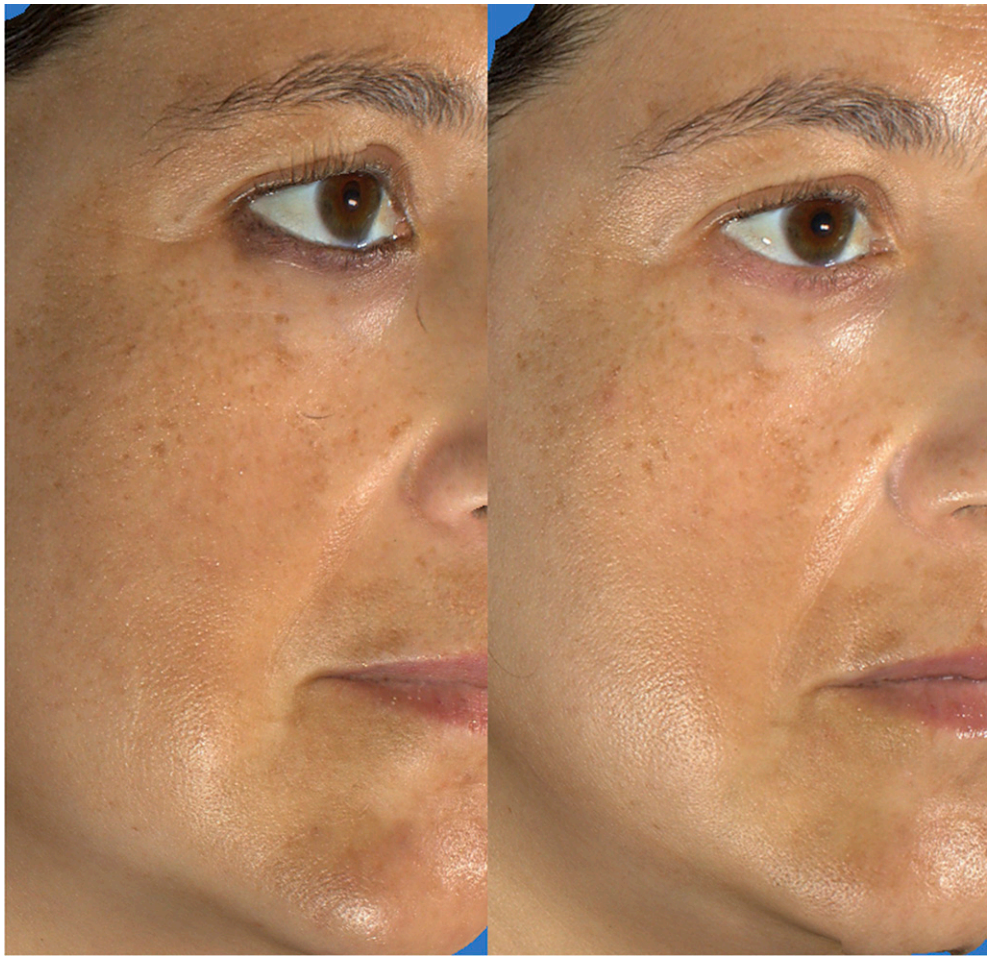


Figure 17. Three-dimensional photographic comparison of right-sided facial hyperpigmentation (cheek and midface) before (left) and after (right) 4 wk of twice-daily application SKNB19 in a 53-year-old woman. SKNB19-treated hyperpigmentation shows a noticeable improvement in hyperpigmentation more so when compared with the side treated with HQ4% (Figure 16).

efficacy of a modified MASI scale, which we may consider using in future studies (40,41). Furthermore, chromameters can be quite effective in providing excellent objective data for the clinician treating hyperpigmentation. In our study, we used 3D imaging, thus providing our independent evaluators high-resolution images to effectively evaluate the hyperpigmented areas. Future studies may incorporate the use of a chromometer in further evaluating the efficacy SKNB19.

CONCLUSION

In our study, we show that a recently developed proprietary product, SKNB19, has increased efficacy in treating hyperpigmentation relative to the current standard treatment HQ4%. In addition to improving the appearance of hyperpigmentation as seen through both patient-reported and independent reviewer assessments, the product also demonstrated

better tolerability, as well as reduced redness and irritation relative to HQ4%. This product has been found to be safe and effective for use in hyperpigmentation.

REFERENCES

(1) N. A. Vashi and R. V. Kundu, Facial hyperpigmentation: causes and treatment, *Br. J. Dermatol.*, **169**(Suppl 3), 41–56 (2013).

(2) D. Rigopoulos, S. Gregoriou, and A. Katsambas, Hyperpigmentation and melasma, *J. Cosmet. Dermatol.*, **6**, 195–202 (2007).

(3) A. K. Gupta, M. D. Gover, K. Nouri, and S. Taylor, The treatment of melasma: a review of clinical trials, *J. Am. Acad. Dermatol.*, **55**, 1048–1065 (2006).

(4) S. Yanagishita-Nakatsuji, K. Fukai, A. Ohyama, A. Umekoji, J. Sowa-Osako, and D. Tsuruta, Probable allergic contact dermatitis from hydroquinone presenting as leukomelanoderma: report of two cases, *J. Dermatol.*, **44**, e330–e331 (2017).

(5) S. N. Mishra, R. S. Dhurat, D. J. Deshpande, and C. S. Nayak, Diagnostic utility of dermatoscopy in hydroquinone- induced exogenous ochronosis, *Int. J. Dermatol.*, **52**, 413–417 (2013).

(6) F. W. Kari, J. Bucher, S. L. Eustis, J. K. Haseman, and J. E. Huff, Toxicity and carcinogenicity of hydroquinone in F344/N ratsand B6C3F1 mice, *Food Chem. Toxicol.*, **30**, 737–747 (1992).

(7) T. Tsutsui, N. Hayashi, H. Maizumi, J. Huff, and J. C. Barrett, Benzene-, catechol-, hydroquinone- and phenol-induced cell transformation, gene mutations, chromosome aberrations, aneuploidy, sister chromatid exchanges and unscheduled DNA synthesis in Syrian hamster embryo cells, *Mutat. Res.*, **373**, 113–123 (1997).

(8) M. H. Yu and S. O. Lee, Hydroquinone stimulates cell invasion through activator protein-1-dependent induction of MMP-9 in HepG2 human hepatoma cells, *Food Chem. Toxicol.*, **89**, 120–125 (2016).

(9) V. M. Sheth and A. G. Pandya, Melasma: a comprehensive update: part II, *J. Am. Acad. Dermatol.*, **65**, 699–714 (2011).

(10) N. Atefi, B. Dalvand, M. Ghassemi, G. Mehran, and A. Heydarian, Therapeutic effects of topical tranexamic acid in comparison with hydroquinone in treatment of women with melasma. *Dermatol. Ther (Heidelb).*, **7**, 417–424 (2017).

(11) M. Banihashemi, N. Zabolinejad, M. R. Jaafari, M. Salehi, and A. Jabari, Comparison of therapeutic effects of liposomal tranexamic acid and conventional hydroquinone on melasma, *J. Cosmet. Dermatol.*, **14**, 174–177 (2015).

(12) M. S. Janney, R. Subramaniyan, R. Dabas, S. Lal, N. M. Das, and S. K. Godara, A randomized controlled study comparing the efficacy of topical 5% tranexamic acid solution versus 3% hydroquinone cream in melasma, *J. Cutan. Aesthetic Surg.*, **12**, 63–67 (2019).

(13) B. Ebrahimi and F. F. Naeini, Topical tranexamic acid as a promising treatment for melasma, *J. Res. Med. Sci.*, **19**, 753–757 (2014).

(14) M. Martínez-Esparza, C. Ferrer, M. T. Castells, J. C. García-Borrón, and A. Zuastiand, Transforming growth factor beta1 mediates hypopigmentation of B16 mouse melanoma cells by inhibition of melanin formation and melanosome maturation, *Int. J. Biochem. Cell Biol.*, **33**, 971–983 (2001).

(15) M. Brenner and V. J. Hearing, Modifying skin pigmentation - approaches through intrinsic biochemistry and exogenous agents, *Drug Discov. Today Dis. Mech.*, **5**, e189–e199 (2008).

(16) H. Kim, H. R. Choi, D. S. Kim, and K. C. Park, Topical hypopigmenting agents for pigmentary disorders and their mechanisms of action, *Ann. Dermatol.*, **24**, 1–6 (2012).

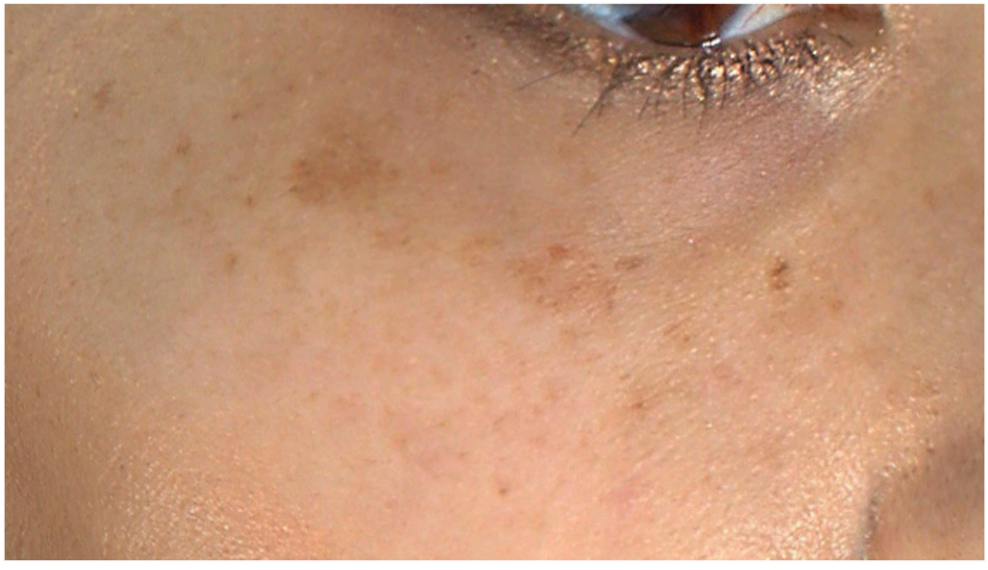
(17) G. H. Park, Y. Rhee do, H. R. Moon, C. H. Won, M. W. Lee, J. H. Choi, K. C. Moon, and S. E. Chang, Effect of an epidermal growth factor-containing cream on postinflammatory hyperpigmentation after Q-switched 532-nm neodymium-doped yttrium aluminum garnet laser treatment, *Dermatol. Surg.*, **41**, 131–135 (2015).

(18) D. Steiner, C. Feola, N. Bialeski, F. A. Silva, A. C. P. Antiori, F. Addor, and B. B. Folino, Study evaluating the efficacy of topical and injected tranexamic acid in treatment of melasma, *Surg. Cosmet. Dermatol.*, **1**, 174–177 (2009).

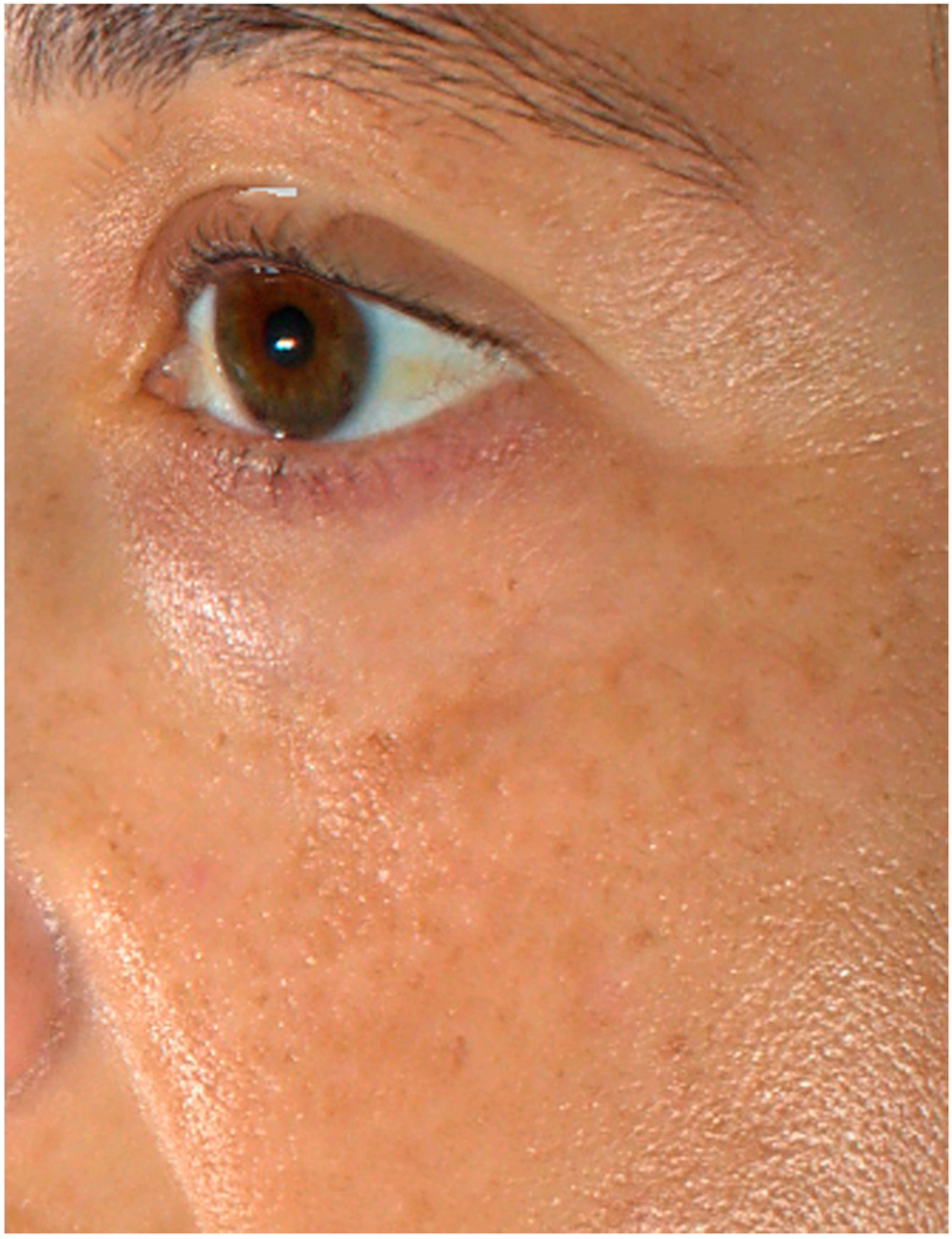
(19) P. Kanechorn Na Ayuthaya, N. Niumphradit, A. Manosroi, and A. Nakakes, Topical 5% tranexamic acid for the treatment of melasma in Asians: a double-blind randomized controlled clinical trial, *J. Cosmet. Laser Ther.*, **14**, 150–154 (2012).

(20) S. J. Kim, J. Y. Park, T. Shibata, R. Fujiwara, and H. Y. Kang, Efficacy and possible mechanisms of topical tranexamic acid in melasma. *Clin. Exp. Dermatol.*, **41**, 480–485 (2016).

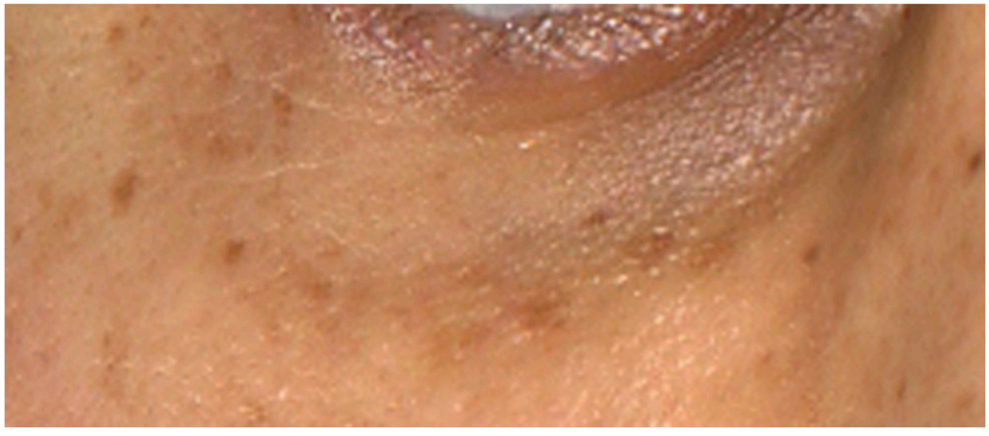
(21) P. E. Grimes, Management of hyperpigmentation in darker racial ethnic groups, *Semin. Cutan. Med. Surg.*, **28**, 77–85 (2009).
(22) L. E. Espinal-Perez, B. Moncada, and J. P. Castanedo-Cazares, A double-blind randomized trial of 5% ascorbic acid vs. 4% hydroquinone in melasma, *Int. J. Dermatol.*, **43**, 604–761 (2004).
(23) P. K. Farris, Topical vitamin C: a useful agent for treating photoaging and other dermatologic conditions, *Dermatol. Surg.*, **31**, 814–818 (2005).
(24) Z. D. Draelos, Skin lightening preparations and the hydroquinone controversy. *Dermatol. Ther.*, **20**, 308–313 (2007).
(25) J. Navarrete-Solís, J. P. Castanedo-Cázares, B. Torres-Álvarez, C. Oros-Ovalle, C. Fuentes-Ahumada, F. J. González, J. D. Martínez-Ramírez, and B. Moncada, A double-blind, randomized clinical trial of niacinamide 4% versus hydroquinone 4% in the treatment of melasma, *Dermatol Res Pract*, **2011**, 379173 (2011).
(26) M. Taraz, S. Niknam, and A. H. Ehsani, Tranexamic acid in treatment of melasma: a comprehensive review of clinical studies, *Dermatol. Ther.*, **30**, 1–8 (2017).
(27) W. J. Yun, S. H. Bang, K. H. Min, S. W. Kim, M. W. Lee, and S. E. Chang, Epidermal growth factor and epidermal growth factor signaling attenuate laser-induced melanogenesis, *Dermatol. Surg.*, **39**, 1903–1911 (2013).
(28) A. Lyons, J. Stoll, and R. Moy, A randomized, double-blind, placebo-controlled, split-face study of the efficacy of topical epidermal growth factor for the treatment of melasma, *J. Drugs Dermatol.*, **17**, 970–973 (2018).
(29) W. O. Pratchyapurit, Combined use of two formulations containing diacetyl boldine, TGF-β1 biomimetic oligopeptide-68 with other hypopigmenting/exfoliating agents and sunscreen provides effective and convenient treatment for facial melasma. Either is equal to or is better than 4% hydroquinone on normal skin. *J. Cosmet. Dermatol.*, **15**, 131–144 (2016).
(30) H. Choi, S. Ahn, B. G. Lee, I. Chang, and J. S. Hwang, Inhibition of skin pigmentation by an extract of *Lepidium apetalum* and its possible implication in IL-6 mediated signaling, *Pigment Cell Res.* **18**, 439–446 (2005).
(31) Y. I. Yamaguchi, A. Morita, A. Maeda, and V. J. Hearing, Regulation of skin pigmentation and thickness by Dickkopf 1 (DKK1), *J. Invest. Dermatol. Symp. Proc.*, **14**, 73–75 (2009).
(32) Y. Yamaguchi, T. Passeron, H. Watabe, K. Yasumoto, F. Rouzaud, T. Hoashi, and V. J. Hearingand, The effects of dickkopf 1 on gene expression and Wnt signaling by melanocytes: mechanisms underlying its suppression of melanocyte function and proliferation, *J. Invest. Dermatol.*, **127**, 1217–1225 (2007).
(33) T. Hakozaki, L. Minwalla, J. Zhuang, M. Chhoa, A. Matsubara, K. Miyamoto, A Greatens, G. G. Hillebrand, D. L. Bissett, and R. E. Boissyand, The effect of niacinamide on reducing cutaneous pigmentation and suppression of melanosome transfer, *Br. J. Dermatol.*, **147**, 20–31 (2002).
(34) H. L. Gensler, Prevention of photoimmunosuppression and photocarcinogenesis by topical nicotinamide, *Nutr. Canc.*, **29**, 157–162 (1997).
(35) A. R. Shalita, J. G. Smith, L. C. Parish, M. C.S Sofman, and, D. K. Chalker, Topical nicotinamide compared with clindamycin gel in the treatment of inflammatory acne vulgaris, *Int. J. Dermatol.*, **34**, 434–437 (1995).
(36) A. Wozniacka, M. Wieczorkowska, J. Gebicki, and A. Sysa-Jedrzejowska, Topical application of 1-methylnicotinamide in the treatment of rosacea: a pilot study, *Clin. Exp. Dermatol.*, **30**, 632–635 (2005).
(37) D. Levine, Z. Even-Chen, I. Lipets, O. A. Pritulo, T. V. Svyatenko, Y. Andrashko, M. Lebwohl, and A. Gottlieb, Pilot, multicenter, double-blind, randomized placebo-controlled bilateral comparative study of a combination of calcipotriene and nicotinamide for the treatment of psoriasis, *J. Am. Acad. Dermatol.*, **63**, 775–781 (2010).
(38) W. Zhu and J. Gao, The use of botanical extracts as topical skin-lightening agents for the improvement of skin pigmentation disorders, *J. Invest. Dermatol. Symp. Proc.*, **13**, 20–24 (2008).
(39) V. D. Callender, S. St Surin-Lord, E. C. Davis, and M. Maclin, Postinflammatory hyperpigmentation: etiologic and therapeutic considerations, *Am. J. Clin. Dermatol.*, **12**, 87–99 (2011).
(40) I. Majid, I. Haq, S. Imran, A. Keen, K. Aziz, and T. Arif, Proposing melasma severity index: a new, more practical, office-based scoring system for assessing the severity of melasma, *Indian J. Dermatol.*, **61**, 39–44 (2016).
(41) D. A. Abou-Taleb, A. K. Ibrahim, E. M. Youssef, and A. E. Moubasher, Reliability, validity, and sensitivity to change overtime of the modified melasma area and severity index score. *Dermatol. Surg.*, **43**, 210–217 (2017).



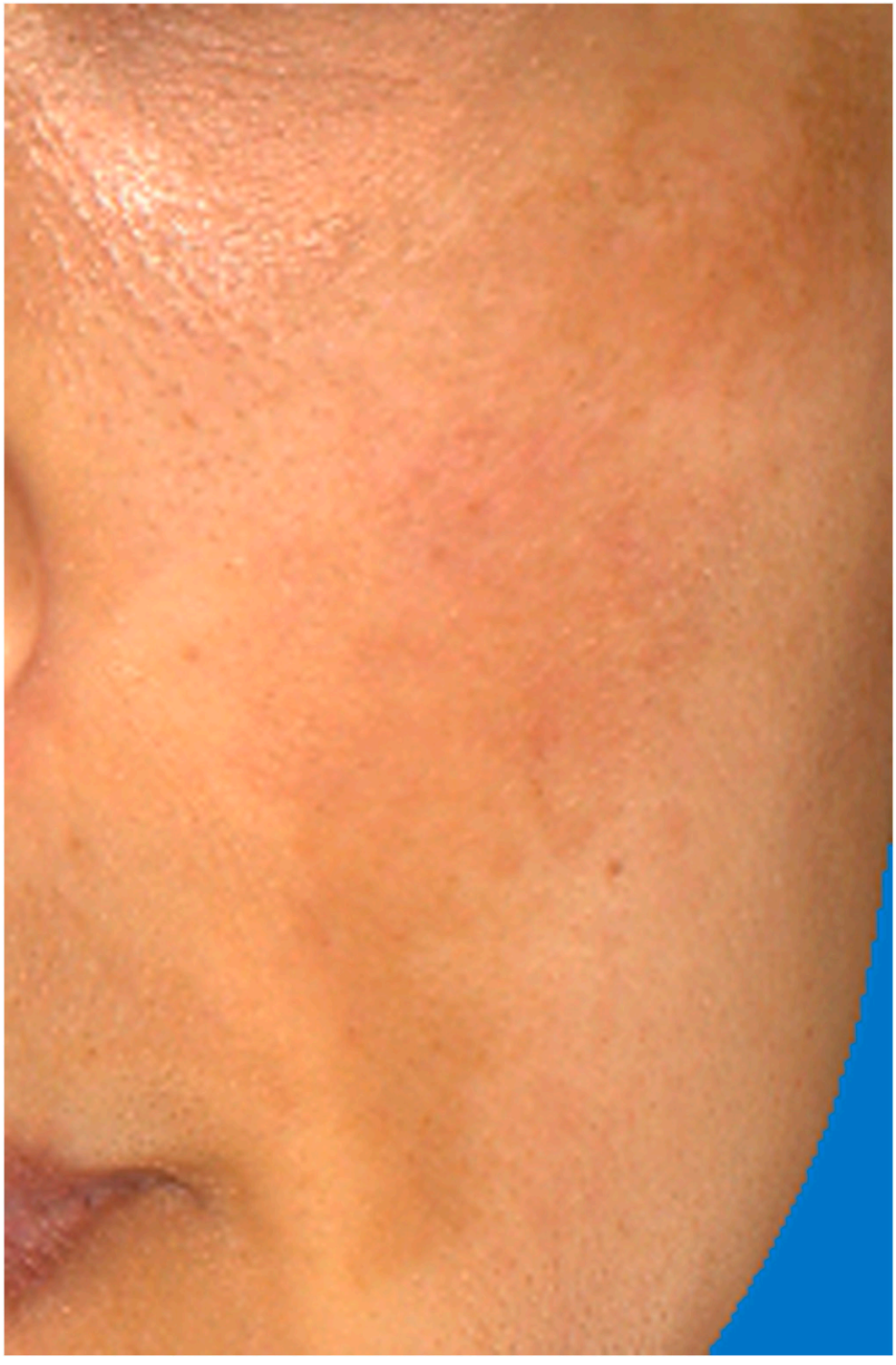
Supplementary Figure 1. Mild lower eyelid hyperpigmentation.



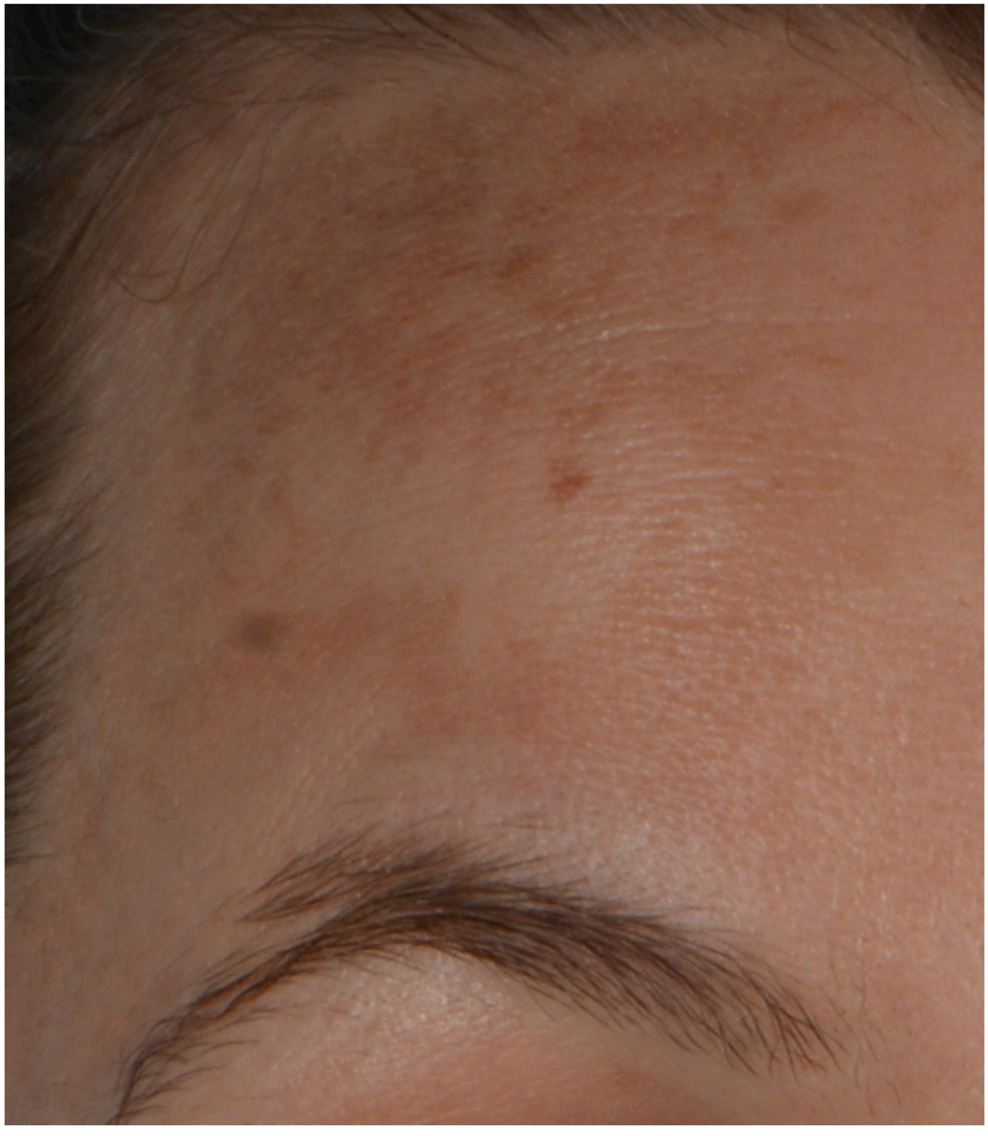
Supplementary Figure 2. Mild cheek and midface hyperpigmentation.



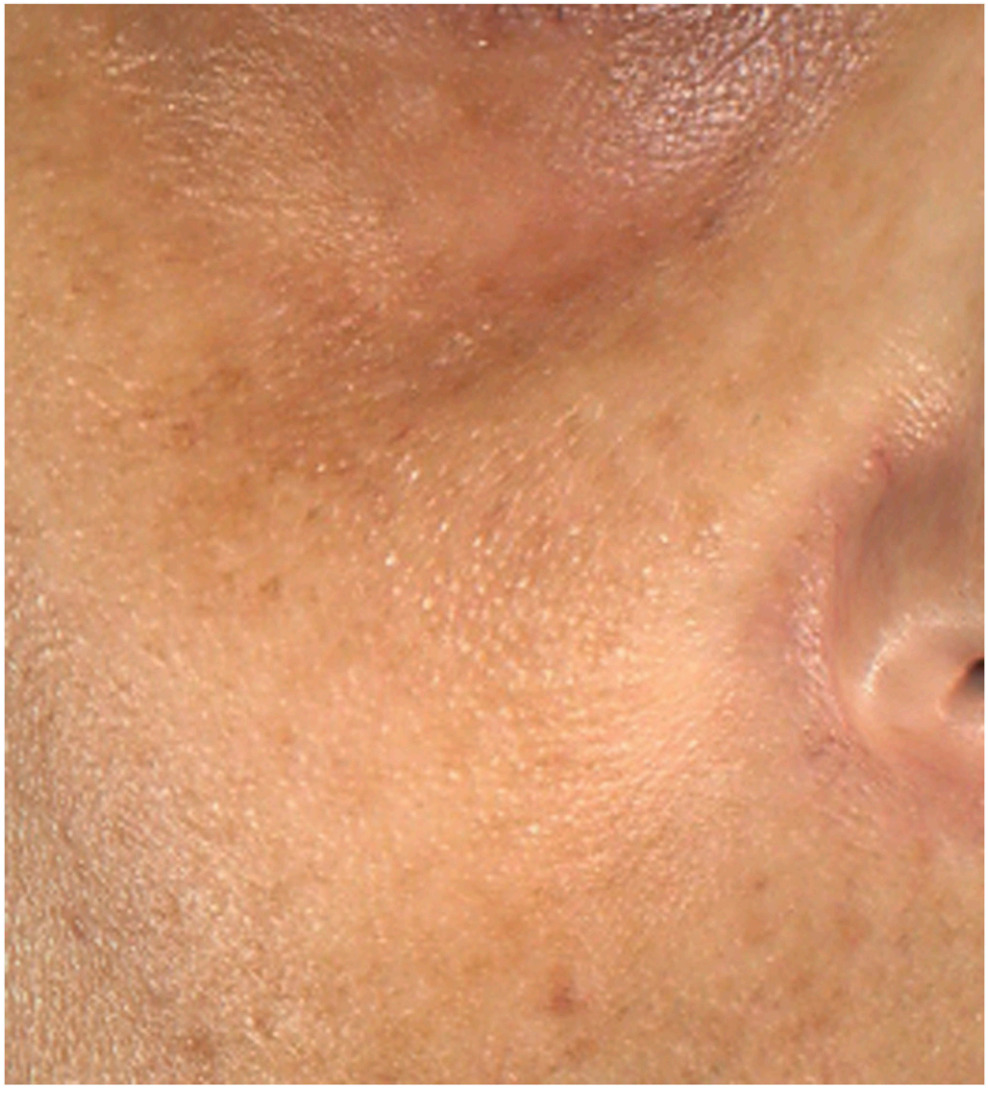
Supplementary Figure 3. Moderate lower eyelid hyperpigmentation.



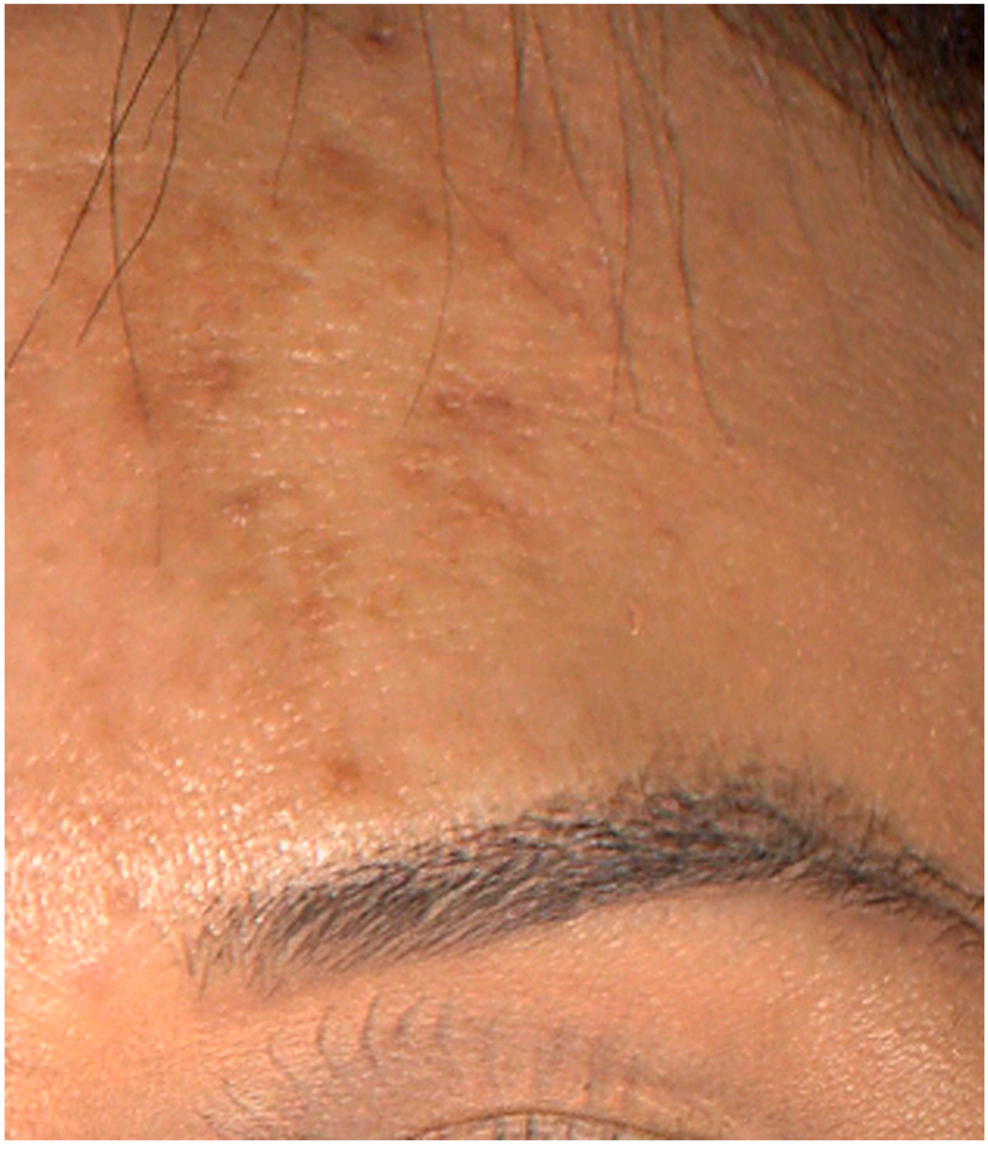
Supplementary Figure 4. Moderate cheek and midface hyperpigmentation.



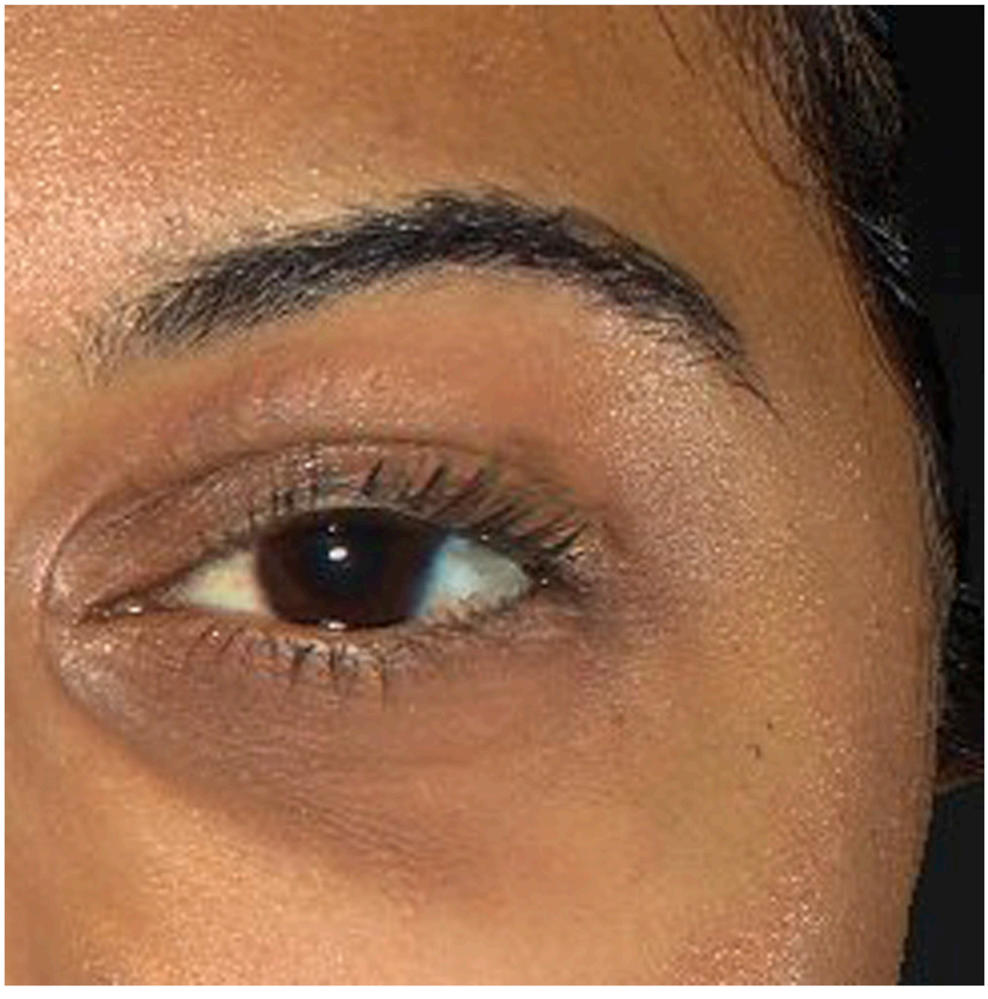
Supplementary Figure 5. Moderate forehead hyperpigmentation.



Supplementary Figure 6. Moderate lower eyelid and midface hyperpigmentation.



Supplementary Figure 7. Severe forehead hyperpigmentation.



Supplementary Figure 8. Severe lower eyelid hyperpigmentation.