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Original Article

Reflectance confocal microscopy in the treatment monitoring of androgenetic alopecia topical combination therapy

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Abstract

Androgenetic alopecia (AGA) is the most common type of non-scarring alopecia. While global photography is the most commonly employed to track therapeutic efficacy, reflectance confocal microscopy (RCM) is a non-invasive imaging tool that may offer novel insights in tracking treatment progression for AGA. Ten patients with androgenetic alopecia initiating topical combination therapy elected to undergo treatment monitoring of this formulation using global photography and/or reflectance confocal microscopy. All patients had evidence of follicular miniaturization at baseline, and rimmed dermal papillae at both baseline and the end of the study. One patient had follicular miniaturization at baseline but not at the end of the study; however, this patient did not exhibit any changes in their Hamilton Norwood Score. Two patients exhibited inflammatory cells in the epidermis of the frontal scalp and mid-scalp at baseline on RCM that were no longer visible on RCM after 12 weeks of treatment. Of these two patients, one patient had a Hamilton Norwood score that did not improve after treatment, even though inflammatory cells were no longer present post-treatment. RCM offers a novel, non-invasive option for monitoring sub-clinical treatment progress in patients with AGA that can uncover novel insights in patients' presentation and response to treatment earlier than with global photography.

Key words
Androgenetic alopecia; Reflectance confocal microscopy; Noninvasive diagnosis; Treatment monitoring.

Introduction

Androgenetic alopecia (AGA) is the most common type of non-scarring alopecia. It affects up to 50% of both sexes by age 50.[1,2] Although AGA may begin as early as puberty, its frequency increases with age and it is genetically influenced.[3] Hair loss is often accompanied by psychosocial comorbidities such as anxiety or depression and can have a detrimental effect on patients' quality of life. First line treatment for androgenetic alopecia in men includes topical minoxidil and oral finasteride. Topical minoxidil was FDA approved in the US for hair loss in the 1988, but the full mechanism of action of the substance is not fully known. More recently, topical finasteride has been further investigated due to its potential reduction in systemic side effects compared to oral preparation.

Prolonged time between start of treatment and clinically appreciable results can be distressing to patients. Standard topical minoxidil strengths

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of 2% and 5% take on average 4 to 6 months for visible results, with peak hair regrowth occurring at 1 year.[4] However improvement in hair density and diameter is detectable by trichoscopy as early as 8 weeks in studies of topical minoxidil and combined preparations of topical minoxidil and finasteride.[5] Additionally, other characteristics of AGA that can be identified with Reflectance Confocal Microscopy (RCM) suggests that there may be subclinical findings that can measure treatment progress even in the presence of visible results or lack thereof.[9] Reflectance confocal microscopy (RCM) is a non-invasive imaging tool that has predominantly been utilized in the diagnosis and evaluation of skin cancers. Global photography is the most common method of therapeutic evaluation for AGA in clinical studies. In this preliminary case series, we explored the utility of reflectance confocal microscopy (RCM) with global photography as tools for monitoring the efficacy of topical AGA therapy.

Methods

Participants in this study were men with AGA who had not been previously treated for AGA in the past 6 months and were initiating therapy with a topical formulation (provided by Hairstim) consisting of finasteride 0.1%, minoxidil 6%, spironolactone 0.5%, and tretinoin 0.0125%. Patients applied the compounded formulation once to twice daily.

A total of 10 male patients with androgenetic alopecia elected to undergo treatment monitoring of this formulation using global photography and/or reflectance confocal microscopy. Patient ages ranged from 20 to 64 years old (average age of 41 years old) and skin phototypes I through IV. Hair images were taken with reflectance confocal microscopy (RCM) and digital global photography at baseline and 12 weeks. RCM images were taken with the handheld Vivascope 3000 at one to two sites of the most clinically significant hair loss. The handheld Vivascope 3000 was chosen as it is more ergonomically designed and suitable for curved surfaces such as the scalp in comparison to Vivascope 1500, with the advantage of not requiring any adhesive on the scalp. Global photography images were taken from standardized frontal and vertex angles using a digital camera with artificial intelligence providing facial feature guidelines for consistent photo capturing. The photographs were scored by 2 experts using the Hamilton-Norwood scale.

Results

The results of topical treatment showed improvement in patients with varying baseline severities of hair loss. Two of the 5 cases showed an average decrease of 1 or more points on the Hamilton Norwood scale at week 12 **(Table 1)**. Two patients underwent no change in Hamilton Norwood scoring during the duration of the study and one had a change of 0.5 point from baseline **(Table 1)**. No severe adverse effects were reported.

The presence of confocal characteristics attributed to AGA in prior studies were inspected: hair follicular miniaturization, inflammatory cell infiltration, dilated blood vessels, follicular hyperkeratosis, rimmed dermal papillae **(Table 1)**. All patients had evidence of follicular miniaturization at baseline, and rimmed dermal papillae at both baseline and the end of the study. Case #1 represented the only case where there was no hair follicle miniaturization after treatment, however, this case underwent no change in Hamilton Norwood scoring. Neither fibrosis nor scarring were observed in any case. Interestingly, two of the patients exhibited inflammatory cells in the epidermis of the

frontal scalp and mid-scalp at baseline **(Figure 1)**.

This inflammation was no longer conspicuous in subsequent RCM imaging of the same locations after 12 weeks of treatment. However, even though one of these patients no longer exhibited inflammatory infiltrate after treatment, case #4, they also underwent no change in Hamilton Norwood Scoring after treatment. Demodex was found in the follicular openings of the mid scalp at baseline imaging of case #3, but was no longer observed at final confocal imaging **(Figure 2)**.

Table 1 Hamilton-Norwood Scores and Reflectance confocal microscopy findings of all cases at baseline and 12-week study endpoints.

Case	*Age*	*Hamilton-Norwood Scores (Reviewer 1/ Reviewer 2)*			*RCM Findings (baseline/week 12)*			
		Baseline	*Week 12*	*Average change from baseline at week 12*	*Hair follicle miniaturization*	*Inflammatory cell infiltration*	*Blood vessel dilation*	*Follicular hyperkeratosis*
1	31	2/2	2/2	0	yes/no	no/no	no/no	yes/yes
2	64	5/5	4/4	1	yes/yes	yes/no	yes/no	no/no
3	20	6/2	5/5	1	yes/yes	no/no	no/no	yes/yes
4	25	2/2	1/1	0	yes/yes	yes/no	no/no	no/no
5	46	2/1	1/1	0.5	-	-	-	-
6	29	2/2	1/1	1	-	-	-	-
7	25	5/6	4/4	1.5	-	-	-	-
8	62	6/6	5a/5	0	-	-	-	-
9	56	5a/5a	5a/5a	0	-	-	-	-
10	55	5a/4	4/4	0.5	yes/yes	no/no	no/no	no/no

Cases 1, 2, 3, 4 and 10 underwent RCM imaging prior to treatment and 12 weeks following start of treatment consisting of a compounded formulation of minoxidil, finasteride, spironolactone and tretinoin.

Discussion

This preliminary study is the first to utilize RCM with global photography in the evaluation of the efficacy of 4 topical ingredients in the treatment of AGA; namely finasteride 0.1%, minoxidil 6%, spironolactone 0.5%, and tretinoin 0.0125%. The primary method of measuring therapeutic efficacy of alopecia in clinical studies is through global photographic evaluation. In the current study, we implemented global photography as well as another emerging imaging tool in the study of alopecia, reflectance confocal microscopy (RCM). This non-invasive imaging tool captures grayscale horizontal images from the stratum corneum down through the superficial papillary dermis, offering a painless and safer alternative to scalp biopsies. Image contrast is provided by differences in the size and refractive indices of cellular organelles, with structures including melanin, keratin, and collagen acting as endogenous chromophores. Thus far, alopecia studies utilizing RCM have primarily focused on scarring subtypes such as scalp discoid lupus, lichen planopilaris, frontal fibrosing alopecia, and folliculitis decalvans. In these studies, RCM findings corresponded with known histopathology features.

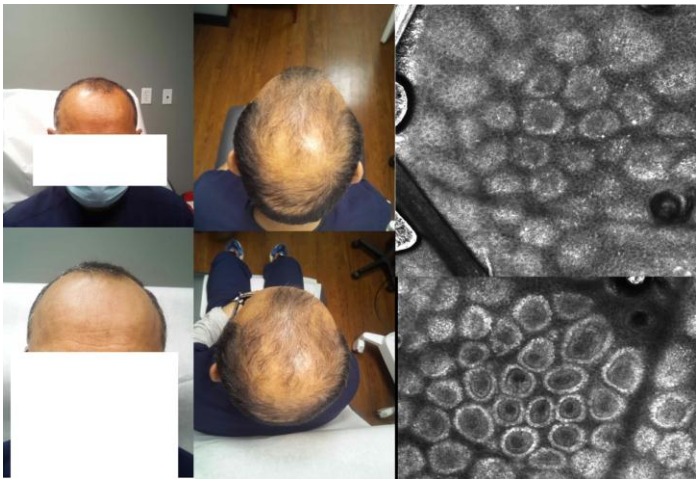

Figure 1 Top row shows clinical photographs and confocal image taken at baseline of case 2. Inflammatory cells are visible in RCM imaging of the frontal scalp at the basal layer of the epidermis. Bottom row shows clinical photographs and confocal image taken at 12 weeks following treatment initiation with quadruple topical formula. Confocal imaging taken again at the frontal scalp does not show evidence of inflammation.

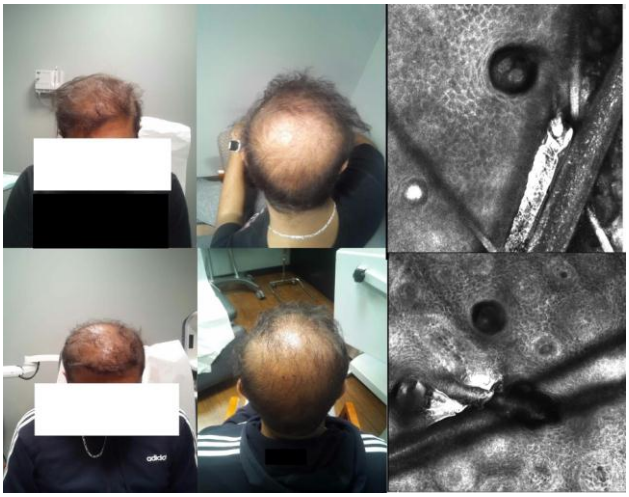

Figure 2 The above patient improved 1 point on the Hamilton Norwood between baseline (top row) and 12 weeks (bottom row). Confocal imaging of the mid-scalp region exhibited demodex at baseline (top right). Demodex were not observed at 12 weeks follow up (bottom right).

The histology of AGA is characterized by miniaturized hair follicles, and reduction in the sizes of dermal papillae, hair bulb and hair shaft diameter. In approximately 40% of patients with AGA, mild peri-infundibular lymphocytic infiltrate and perifollicular collagen deposition are present. [1] Ardigò *et al.* conducted a study of real time assessments of alopecia utilizing RCM, which included 30 patients with androgenetic alopecia. The study results showed dermal papillae rimming, follicular hyperkeratosis and miniaturized hair to be associated with AGA.[9] Dermal papillae rimming, or hyperpigmentation of the dermal-epidermal junction, as well as diffuse dermal fiber thickening are features seen in AGA which can be attributed to increased UV damage in areas of balding. In the current study, dermal papillae rimming was found in all patients monitored with RCM at baseline, and at follow up, even in patients who had clinical improvement at the end of the study.

RCM investigation of AGA has also revealed inflammatory cell infiltration and dilated vessels in the skin surrounding miniaturized follicles.[10] Inflammatory cell infiltration was noted in two cases and subsequently resolved at the end of the current study It was unclear if cutaneous vasodilation occurred in the current study. However, topical minoxidil has been proven to increase cutaneous blood flow in the scalp. This would be an interesting topic of future study, as there are early investigations in the application of RCM for quantification of blood cell flow.[11] Of particular interest, in two of the patients who underwent no change in Hamilton Norwood scoring after 12 weeks, RCM revealed that case 1 demonstrated no hair follicle miniaturization after treatment, and case #4 displayed a lack of inflammatory cell infiltration after treatment. Thus, RCM offers a novel, non-invasive option that can uncover findings of treatment related improvement before they are visually detectable by global photography in patients with AGA.

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