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The Value of Trichoscopy in the Follow-up of Treatment Response in Patients With Androgenetic Alopecia

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ABSTRACT

Introduction: Although trichoscopy has been used successfully in the diagnosis of androgenetic alopecia (AGA), its utility in monitoring the therapeutic outcome is not clear.

Objectives: To investigate the value of trichoscopy in the follow-up of treatment response in patients with AGA.

Methods: Ninety-five patients with AGA were included. Trichoscopic images were obtained on a 1 cm^2 circular area in the left frontoparietal scalp pre- and post-treatment in all and in 61 patients, respectively. Two doctors evaluated those images who are less experienced (doctor A) and experienced (doctor B) in hair diseases and trichoscopy. Terminal, vellus and total hair count (THC, VHC, ToHC) and terminal to vellus hair (T/V) ratio were determined. These numerical data were also calculated by TrichoScan analysis of the same area. The agreement between trichoscopy and TrichoScan data was assessed.

Results: The agreement between doctor A and TrichoScan was moderate for THC and ToHC pre-treatment and good post-treatment; poor for VHC and T/V ratio pre- and post-treatment. The agreement between doctor B and TrichoScan was excellent for THC, VHC and ToHC; good for T/V ratio pre- and post-treatment.

Conclusions: Trichoscopy, when performed by a doctor experienced in hair diseases and trichoscopy, can be used as a sensitive method in monitoring the treatment response in patients with AGA which is based on the determination of THC, VHC, ToHC and T/V ratio. If it is to be performed by a less experienced doctor, it can be preferred as a sensitive follow-up method using THC and ToHC.

Introduction

Androgenetic alopecia (AGA), characterized by the progressive miniaturization of genetically susceptible hair follicles under the impact of androgen hormones, is the most common form of non-cicatricial alopecia [1-3]. It causes significant psychosocial impairment, hence necessitating an effective treatment [1,2]. Besides hair transplantation, there are also several non-surgical treatment options for managing AGA, including topical minoxidil, oral anti-androgens, 650-900 nm low-level laser therapy, and platelet-rich plasma [1,2,4]. Any non-surgical treatment aims to stop hair loss, transform vellus hairs into terminal hairs and stimulate hair regrowth. It is possible to achieve these goals with the currently available non-surgical treatments [1,2]. However, their effects are not permanent as AGA is, by nature, a chronic and progressive disease for which long-term non-surgical treatments may be required, even for a lifetime [1]. Therefore, the treatment response should be monitored regularly using objective methods to develop and maintain an effective treatment plan and to prevent the financial loss of an unbeneficial therapy.

Several methods can be used in the follow-up of treatment response in patients with AGA, such as scalp biopsy, global photographing of the scalp, trichogram, phototrichogram, and TrichoScan [2,5-7]. However, most of them are of little use to the clinician because they are time-consuming, costly, or difficult/impractical to apply [5-7]. Among these methods, TrichoScan is still the most objective method performed [5,6]. It combines epiluminescence microscopy with automatic digital image analysis and is able to quantitatively analyze hair growth and hair loss [6]. On the other hand, it is a paid software that only works with certain handheld and video dermoscopes, limiting its widespread use [5]. So, there is a need for other cheaper, easy, and reliable tools for monitoring the therapy of AGA.

Trichoscopy is a non-invasive, simple, and easy method that has been successfully utilized for the diagnosis of AGA [7,8]. The main diagnostic trichoscopic features of AGA are hair diameter diversity, increased proportion of thin and vellus hairs, a large number of single-hair pilosebaceous units, yellow dots and peripilar sign [1,8]. Trichoscopy was also used to assess the therapeutic outcome in many studies conducted to evaluate or compare the efficacy of different treatment modalities in AGA [9-13]. Hair diameter and hair density were the two most frequently analyzed trichoscopic parameters for that purpose in those studies [9-11]. Yet, although it is a frequently preferred method, the reliability of trichoscopy in monitoring the treatment response needs to be clarified. Only a few studies assessed the compatibility of trichoscopic analysis results with that obtained with TrichoScan, the most objective method currently available [12,13]. However, in those studies, the number of patients was low and not all hair growth parameters that are necessary for assessing the treatment response in patients with AGA were evaluated.

Objectives

Herein, we aimed to investigate the value of trichoscopy in the follow-up of treatment response in a large number of patients with AGA, by evaluating all necessary hair growth parameters and by comparing with TrichoScan analysis.

Methods

Patients

The study was approved by local Institutional Review Board (Project No: KA18/324), and informed consent was obtained from all patients.

This prospective study included patients between 16 and 65 years of age who presented to our clinic with the complaint of hair loss and were diagnosed with AGA between October 2018 and August 2019. The diagnosis of AGA was made by the characteristic patterned alopecia (diffuse hair thinning over the central scalp with preservation of the frontal hairline in females and frontotemporal recession and/or loss of hair over the vertex in males) along with the trichoscopic presence of >20% hair diameter diversity. The hair-pull test, trichoscopy, and laboratory examination (total blood cell count, blood levels of ferritin, and thyroid-stimulating hormone) were performed; the triggering factors (high fever, major surgical intervention, drugs, heavy diet, emotional stress) were questioned to exclude telogen effluvium, diffuse lichen planopilaris and diffuse alopecia areata. The Hamilton-Norwood (H-N, stages I-V) and Ludwig (stages I and II) classifications were used to determine the grade of AGA in male and female patients, respectively [14].

Male patients received 5% minoxidil topical solution or foam and female patients received 2% minoxidil topical solution twice daily for treatment. All patients were invited to a follow-up visit 3 months after the start of treatment.

Trichoscopic Analysis

An examination area was determined at the intersection of vertex and left temporal region of each patient. A cardboard template with a circle-shaped hole of approximately 1 cm^2 in its middle was placed on the area to be examined (Figure 1A) and the hairs in that area were cut to a length of 0.5–1 mm. Another template with a 0.562 cm^2 circle-shaped hole, which is equivalent to the area analyzed by TrichoScan, was divided into four equal sections horizontally and longitudinally by using two strings and was placed on the examination area (Figure 1B). Digital trichoscopic photographs (FotoFinder Systems GmbH, 2007, Medicam 800 HD) with a 20 fold

magnification were obtained from the whole examination area and from each section (Figure 1C). A dot-shaped tattoo was placed in the center of the examination area using a tattoo device (Mei-cha) to ensure that all the assessments were made from the same area. The whole procedure took 12-15 minutes. The same procedure was reperformed on the same examination area at 3-month follow-up.

Two doctors who are less experienced (doctor A) and experienced (doctor B) in hair diseases and trichoscopy evaluated trichoscopic images on their own desktop computers. While doctor A was a research assistant in the 4th year of dermatology residency, doctor B was an associate professor of dermatology who has been dealing with hair diseases and trichoscopy for more than 10 years. Doctor A evaluated the trichoscopic images twice with 1 month interval. Both doctors were blinded to the Trichoscan data, and their own and each other trichoscopic analysis results.

Doctor A and doctor B calculated the total number of hairs by counting and summing the hairs in the four sections separately. The diameter of each hair was determined with the help of a guide ruler image on the screen. As defined by the TrichoScan software, hairs that are 0.04 mm and thicker were considered as terminal hairs, while those thinner than 0.04 mm were defined as vellus hairs. To facilitate counting and to reduce any possible errors, vellus hairs were marked with red dots and terminal hairs with yellow dots using the free-of-charge Microsoft Paint program (Figure 1D). Both doctors then determined terminal hair count (THC), vellus hair count (VHC), total hair count (ToHC) and terminal to vellus hair (T/V) ratio.

TrichoScan Analysis

TrichoScan analysis (TrichoScan® HD, Professional Version 3, FotoFinder Systems GmbH, 2007) was carried out on the same examination area determined for trichoscopy at the same session of obtaining trichoscopic images.

To create an adequate contrast between the hair in the examination area and the scalp, after cutting the hairs, a black hair dye (Goldwell Topchic Permanent Hair Color)

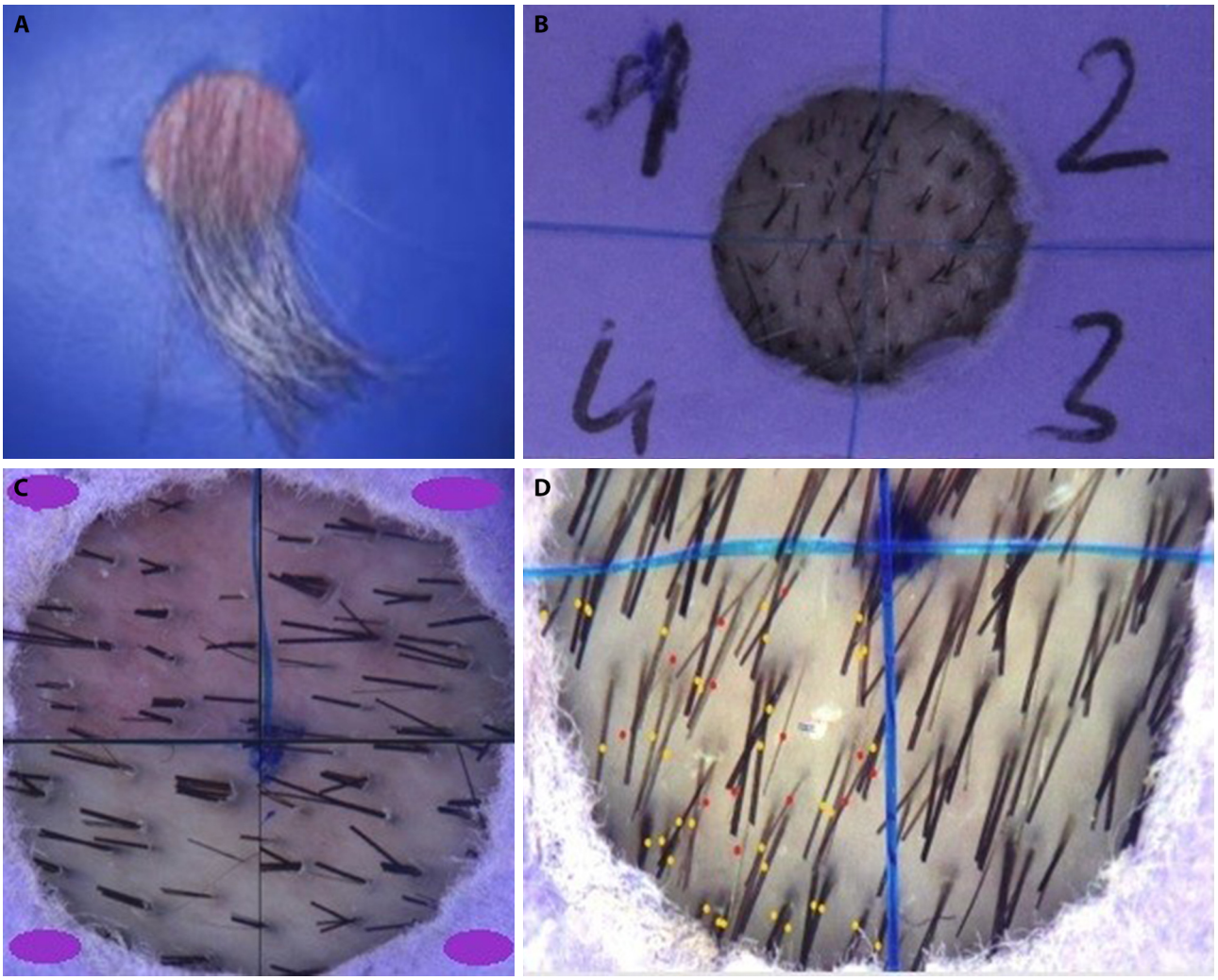

Figure 1. (A) A cardboard template with a circle-shaped hole of approximately 1 cm^2 in its middle placed on the area to be examined. (B) A template with a circle-shaped hole equivalent to the area analyzed by TrichoScan divided into four equal sections by using two strings and placed on the examination area. (C) Digital trichoscopic photographs with a 20-fold magnification obtained from the examination area. (D) Vellus hairs marked with red dots and terminal hairs with yellow dots using Microsoft Paint program on trichoscopic images.

and a hair color cream (Rondo Coiffeur 6% Creme Oxyd) were mixed at 1:1 ratio and was applied to the examination area. The mixture was gently cleaned using alcohol gauze after 15 minutes. THC, VHC, ToHC and T/V ratio were calculated with TrichoScan analysis. The same procedure was repeated at 3-month follow-up.

Assessments

The numerical data, namely THC, VHC, ToHC and T/V ratio obtained by doctor A and doctor B with trichoscopy before treatment and at the end of the 3rd month of treatment were compared with the TrichoScan analysis results. The inter-observer agreement was calculated by comparing the trichoscopic analysis results obtained by doctor A with that of doctor B. The initial examination results of doctor A were used for these assessments. The trichoscopic analysis results determined by doctor A twice were compared in order to determine the intra-observer agreement.

Statistical Analysis

Statistical analysis was conducted using IBM SPSS (Statistical Package for the Social Sciences software version 25, SPSS Inc.) and MedCalc v16.0 (MedCalc Software). Descriptive statistics for numerical variables were expressed as mean ± SD or median (range), where appropriate; number of cases and percentages were used for categorical data.

The agreement between trichoscopy and TrichoScan data for doctor A and doctor B before and after treatment was calculated with the concordance correlation coefficient (CCC) (excellent [>0.99], good [0.95-0.99], moderate [0.90-0.94], poor [<0.90]) with a 95% confidence interval (CI). Intra- and inter-observer agreement was determined by intra-class correlation coefficient (ICC) (excellent [0.95-1.00], high [0.85-0.94], moderate [0.70-0.84], unacceptable [0.00-0.69]) which was calculated by using the 'Two-Way Mixed Effect Model"; $P < 0.001$ was considered significant.

Whether there was a difference between pre- and post-treatment measurements was analyzed using t-test for dependent samples when the parametric test assumptions were met, and wilxocon test when the assumptions were not met. The probability of type I error in all analyzes was determined as 0.05.

Results

The study included 95 patients with AGA (76 males and 19 females). The general characteristics of the patients are listed in Table 1. Of the 95 patients, 34 patients (35.8%) were lost to follow-up; thus, we could not perform trichoscopic and TrichoScan analyses at the 3rd month of treatment.

The agreement between doctor A and TrichoScan was moderate for THC and ToHC pre-treatment and good post-treatment; poor for VHC and T/V ratio both pre- and post-treatment. The agreement between doctor B and TrichoScan was excellent for THC, VHC, and ToHC and good for T/V ratio both pre- and post-treatment. The CCC values and respective 95% CIs regarding the agreement between trichoscopy and TrichoScan data for doctor A and doctor B pre- and post-treatment are given in Table 2.

After 3 months of treatment, doctor A, doctor B, and TrichoScan analysis detected a significant increase in THC and ToHC and no significant change in VHC. The hair counts and T/V ratio determined by doctor A, doctor B, and TrichoScan analysis, and the p values regarding the change in mean hair counts post-treatment are given in Table 3.

Intra-observer agreement was excellent for VHC, ToHC, and T/V ratio and high for THC pre-treatment; excellent for all four parameters post-treatment. Inter-observer agreement was excellent for THC and ToHC; high for VHC and T/V ratio both pre- and post-treatment. The ICC and respective P values regarding intra- and inter-observer agreement pre- and post-treatment are given in Table 4.

Conclusions

Any successful treatment in AGA should increase the number of terminal hairs while reducing the number of vellus hairs and increasing the hair density. Therefore, a helpful method

Table 1. General characteristics of 76 male and 19 female patients with androgenetic alopecia.

General characteristics	Males	Females
Mean age±SD, years	29.5±5	38±13
H-N stage of AGA, N (%)		
Stage I	8 (10.5)	
Stage II	20 (26.3)	
Stage III	13 (17.1)	
Stage III vertex	17 (22.4)	
Stage IV	11 (14.5)	
Stage IV A	1 (1.3)	
Stage V	6 (7.9)	
Ludwig stage of AGA, N (%)		
Stage I		12 (63.2)
Stage II		7 (36.8)
Mean duration of AGA, years	6.2	4.8
Previous treatments, N (%)		
No treatment	58 (76.3)	16 (84.2)
Topical minoxidil spray/foam	12 (15.8)	2 (10.5)
Oral biotin	4 (5.2)	0 (0)
Oral finasteride	1 (1.3)	0 (0)
PRP injections	1 (1.3)	0 (0)
Oral multivitamin complex	0 (0)	1 (5.2)
Oral biotin	4 (5.2)	0 (0)

AGA = androgenetic alopecia; H-N = Hamilton-Norwood; PRP = platelet-rich plasma; SD = standard deviation.

Table 2. The concordance correlation coefficient values along with the level of agreement regarding the agreement between trichoscopy and TrichoScan data for doctor A and doctor B pre- and post-treatment.

Parameters	Pre-treatment CCC (95% CI)	Level of agreement	Post-treatment CCC (95% CI)	Level of agreement
Doctor A				
THC	0.9443 (0.9179-0.9624)	Moderate	0.9755 (0.9602-0.9849)	Good
VHC	0.8199 (0.7433-0.8752)	Poor	0.8974 (0.8366-0.9364)	Poor
ToHC	0.9412 (0.9131-0.9604)	Moderate	0.9631 (0.9403-0.9773)	Good
T/V ratio	0.8161 (0.7395-0.8718)	Poor	0.8719 (0.7989-0.9195)	Poor
Doctor B				
THC	0.996 (0.9944-0.9975)	Excellent	0.9991 (0.9985-9994)	Excellent
VHC	0.990 (0.9862-0.9938)	Excellent	0.9948 (0.9914-0.9968)	Excellent
ToHC	0.9971 (0.9956-0.9980)	Excellent	0.999 (0.9984-0.9994)	Excellent
T/V ratio	0.9734 (0.9607-0.9821)	Good	0.9875 (0.9793-0.9925)	Good

CCC = concordance correlation coefficient; CI = confidence interval; THC = terminal hair count; ToHC = total hair count; T/V ratio = terminal to vellus hair ratio; VHC = vellus hair count

Table 3. The hair counts and terminal to vellus hair ratio determined by doctor A, doctor B and TrichoScan analysis, and the P values regarding the change in mean hair counts post-treatment.

Parameters	Pre-treatment	Post-treatment	P
Doctor A			
THC, mean±SD	96.38±22.506	103.34±24.993	0.001
VHC, mean±SD	36.81±14.162	37.11±12.232	0.856
ToHC, mean±SD	133.16±25.699	140.46±28.065	<0.001
T/V ratio, median (range)	2.81 (0.88-7.50)	2.97 (1.14-5.66)	
Doctor B			
THC, mean±SD	98.16±21.254	102.59±23.474	0.022
VHC, mean±SD	34.24±13.175	35.08±12.191	0.569
ToHC, mean±SD	132.40±24.880	137.67±28.065	0.025
T/V ratio, median (range)	3.1 (1.12-8.15)	3.03 (1.16-6.16)	
Trichoscan			
THC, mean±SD	98.61±21.304	103.11±23.593	0.027
VHC, mean±SD	33.99±13.342	34.69±11.984	0.635
ToHC, mean±SD	132.65±25.172	137.80±26.379	0.029
T/V ratio, median (range)	3.22 (1.11-8.67)	3.09 (1.20-6.39)	

SD = standard deviation; THC = terminal hair count; ToHC = total hair count; T/V ratio = terminal to vellus hair ratio; VHC = vellus hair count

to be used in monitoring the treatment outcome in patients with AGA must be able to objectively analyze the hair growth parameters, namely THC, VHC, ToHC, and T/V ratio. In the present study, when the experienced doctor in hair diseases and trichoscopy determined all those four hair growth parameters by trichoscopy, the results were similar to those

Table 4. The intra-class correlation coefficient and respective P values regarding intra- and inter-observer agreement pre- and post-treatment.

Parameters	Pre-treatment ICC (P value)	Level of agreement	Post-treatment ICC (P value)	Level of agreement
Intra-observer agreement				
THC	0.879 (< 0.001)	High	1.000 (< 0.001)	Excellent
VHC	0.997 (< 0.001)	Excellent	0.999 (< 0.001)	Excellent
ToHC	0.999 (< 0.001)	Excellent	1.000 (< 0.001)	Excellent
T/V ratio	0.988 (< 0.001)	Excellent	0.997 (< 0.001)	Excellent
Inter-observer agreement				
THC	0.972 (< 0.001)	Excellent	0.987 (< 0.001)	Excellent
VHC	0.899 (< 0.001)	High	0.948 (< 0.001)	High
ToHC	0.972 (< 0.001)	Excellent	0.980 (< 0.001)	Excellent
T/V ratio	0.884 (< 0.001)	High	0.928 (< 0.001)	High

ICC = intra-class correlation coefficient; THC = terminal hair count; ToHC = total hair count; T/V ratio = terminal to vellus hair ratio; VHC = vellus hair count

determined by TrichoScan analysis. On the other hand, only THC and ToHC determined by the less experienced doctor were consistent with the Trichoscan analysis measurement. The change in mean hair counts post-treatment detected by both doctors, particularly by the experienced doctor, using trichoscopy resembled the change detected by TrichoScan. In other words, both trichoscopy and TrichoScan determined a similar treatment outcome. Considering the repeatability and reproducibility of the trichoscopic analysis, the intra- and inter-observer agreement were either excellent or high for all hair growth parameters studied.

Previous studies used both handheld and video dermoscopes to assess the efficacy of different treatment modalities for AGA [9-13,15-24]. With a handheld dermoscope, some studies evaluated changes in the trichoscopic findings of AGA during the treatment period, including hair diameter diversity, single-hair pilosebaceous units, yellow dots, and peripilar sign [15,16]. In others, trichoscopic images were saved on a computer after they were obtained with a handheld dermoscope equipped with a camera or by a video dermoscope [17,18]. Hair density was calculated manually as the number of hairs per cm^2 on the trichoscopic images. Hair diameter was measured with the help of different software programs to determine mean hair diameter or to define vellus and terminal hairs, which were counted manually thereafter [17]. The hair diameter to define terminal and vellus hairs varied between different studies [15-17]. Hairs less than 0.04 mm or 0.03 mm in diameter were counted as vellus hairs, while hairs thicker than 0.04 mm or 0.05 mm in diameter were counted as terminal hairs. The change from baseline in mean hair diameter and hair density and also THC and VHC at different time intervals were the most common primary efficacy outcome of the studied treatment modality [18-21]. Trichoscopic analysis was performed alone or with other follow-up methods, usually with global photographic assessment of hair growth by investigators and patients [18-24]. In our study, we performed a similar method described in some of the prior studies to determine hair growth parameters by trichoscopy [18-24]. We counted the hairs manually on the images we obtained and saved with the video dermoscope. In order to make the most accurate comparison, we chose the hair diameter used by the Trichoscan analysis to define vellus and terminal hairs. Trichoscopic analysis was not a time-consuming procedure in our study, which took a maximum of 15 minutes.

Bilgiç et al compared TrichoScan analysis with manual hair counting on trichoscopic images using the software "image analysis tool" [12]. Two independent dermatologists examined 90 images obtained from 30 male patients with AGA during the treatment period and determined only the hair density. The averages of those two ToHC measurements were compared with the results of the TrichoScan analysis, and the concordance was found to be unsatisfactory. They identified several hair fibers which were undetected by TrichoScan due to crossing and overlapping hairs, multiple hairs in the follicular unit, and the presence of unequable hair thickness throughout its length. In the study of Gassmuller et al [13], 3 evaluators manually outlined the length and thickness of hairs on 90 trichoscopic images obtained from 10 patients independently; the software "hair measure tool" automatically measured the hair density, thickness, and length. The comparative assessment showed a strong correlation between the evaluation of hair growth parameters using manual identification of hairs and TrichoScan analysis. Manual evaluators obtained higher values of hair density as very thin hairs were not analyzed by TrichoScan owing to the low camera resolution. The agreement between different evaluators was best for total hair density and parameters related

to hair length and worst for the parameters related to hair thickness. Considerable variability was noted in the same evaluator's results of manually identified hairs. It was suggested to be due to the tedious and time-consuming nature of the procedure, making it nearly impossible to repeatedly count hundreds of hairs without any error. In our study, the doctor's experience in hair diseases and trichoscopy affected the compatibility of trichoscopic evaluation of VHC and T/V ratio with TrichoScan analysis results. The less experienced doctor counted more vellus and fewer terminal hairs than TrichoScan, possibly because of identifying some terminal hairs or some other fibers as vellus hairs or as TrichoScan did not detect all vellus hairs. Nevertheless, the less experienced doctor determined a similar outcome at the 3rd month of treatment as did the experienced doctor and the trichoscan analysis; a significant increase in THC and ToHC, while no significant change in VHC.

To see how professional experience affects our results, we chose one of the researchers of our study as an experienced doctor who works mainly in the field of hair diseases and trichoscopy. Both doctors determined similar trichoscopic analysis results and treatment outcomes. However, while the trichoscopic measurements of the experienced doctor were quite consistent with the TrichoScan results, the hair growth parameters, particularly VHC and T/V ratio calculated by the less experienced doctor, needed to be in better agreement with the result of TrichoScan analysis. Based on our results, we believe that the education of young dermatologists is important to improve their knowledge and experience of trichoscopic examination. Thus, they will be able to perform trichoscopy more accurately and use it to assess the therapeutic outcome in AGA.

In conclusion, trichoscopy, when performed by a doctor experienced in hair diseases and trichoscopy, can be utilized as a sensitive method in monitoring the treatment response in patients with AGA, which is based on the determination of THC, VHC, ToHC, and T/V ratio. If it is to be performed by a less experienced doctor, it can also be used as a sensitive follow-up method, preferably using THC and ToHC parameters. In our opinion, encouraging trichoscopy training for young dermatologists will ensure a more successful and widespread use of trichoscopic examination in assessing the therapeutic outcome of AGA.

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