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LETTER TO THE EDITOR

Quantitative morphometric analysis of hair follicles in alopecia areata

KEYWORDS
Alopecia areata;
Hair follicle parameters;
Transverse sectioning;
Morphometry;
Image analysis

Transverse sectioning of scalp biopsies in alopecia areata (AA) gives a simultaneous overview of many follicles [1]. Quantitative assessment of hair or hair follicle morphology using image analysis is particularly valuable for the diagnosis and disease progress of hair disorders including AA. Computerized image analysis enables large number of hair to be measured in an automated fashion [2].

The aim of this study was to demonstrate hair density and possible changes in hair follicles that may occur during AA using image analysis. It was conducted on 20 patients presenting with alopecia areata, 16 males and 4 females. Their ages ranged between 16 and 40 years. The control group consisted of five individuals with no diseases of the scalp, three males and two females. Their ages ranged between 18 and 25 years. Four millimetre punch biopsies were taken from the scalp of patients and controls, after obtaining oral consent. Each specimen was embedded in paraffin, sectioned serially and horizontally by a microtome (5 μm thick) at the level of the sebaceous gland. The sections were stained with H&E stain and Mallory Trichrome stain.

The data were obtained by using Leica Quantimet Image Analyzer system. The Image Analyzer consisted of color video camera, color monitor, personal computer (PC) connected to 40× power light microscope and controlled by Leica computer software. This Image Analyzer was first calibrated by stage micrometer calibration slide to convert the measurement units produced by the Image Analyzer into a real world micrometer unit. Also, the Image Analyzer was calibrated to convert the color densometric measurement into an optical density unit standard by using a special densometric scale slide produced by Kodak film company, USA.

The following quantitative and morphometric parameters were measured for all hair follicles, terminal or vellus (Table 1; Fig. 1):

1. The number of hair follicles per unit area.
2. The diameter (μm), perimeter (μm), area (μm^2) and irregularity of hair follicles (unit).
3. The diameter (μm), perimeter (μm), area (μm^2), and roundness of hair shaft (unit).
4. Outer and inner root sheath diameters (μm).

Microsoft Office Excel 2003 was used for data management and analysis. Mean + standard deviation was calculated for quantitative values. For comparison between two groups the Student's *t*-test was performed. All tests were considered statistically significant when P was less than 0.05.

The total number of hair follicles in 4 mm punch biopsies taken from alopecia areata patients had a mean of 15.44 (±4.30 S.D.); while the number of hair follicles in control group had a mean of 24.56 (±3.97 S.D.). This difference is statistically significant, where P value is <0.01.

This was consistent with the findings of Whiting [1]. His study done on 287 AA patients and 22 controls revealed 14 and 35 terminal hair in 4 mm punch biopsies, respectively. The lower hair count in our controls with respect to the previous study may be due to racial variations between white population and African population.

Regarding the morphometric measurements, results of the present study showed significant decrease in hair follicle area and perimeter, hair shaft diameter and area in AA group as compared to the control group; whereas the hair follicle irregularity was significantly increased.

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Table 1 Statistical comparison of the morphometric measurements of hair follicles in control and alopecia groups using image analysis

	Control (n = 5)	Alopecia areata (n = 20)
Hair follicle diameter		
Mean	513.986 μm	496.186 μm
S.D.	169.66	115.35
P-value		>0.05 NS
Hair follicle area		
Mean	357128.433 μm^2	185668.68 mm^2
S.D.	141474.84	29518.03
P-value		<0.01 Sign. dec.
Hair follicle perimeter		
Mean	2067.683 μm	1620.59 μm
S.D.	357.65	164.685
P-value of *t*-test		<0.01 Sign. dec.
Outer sheath thickness		
Mean	102.88 μm	146.5 μm
S.D.	22.527	89.18
P-value		>0.05 NS
Inner sheath thickness		
Mean	49.29 μm	49.46 μm
S.D.	31.11	33.538
P-value		>0.05 NS
Hair follicle irregularity units		
Mean	0.1376	0.4
S.D.	0.05	0.03
P-value		<0.01 Sign. inc.
Hair shaft diameter		
Mean	178.756 μm	89.9 μm
S.D.	76.88	65.05
P-value		<0.01 Sign. dec.
Hair shaft area		
Mean	44061.61 μm^2	10718.14 μm^2
S.D.	16985.32	11421.61
P-value of *t*-test		<0.01 Sign. dec.
Hair shaft perimeter		
Mean	716.93 μm	1492.23 μm
S.D.	185.167	2499.014
P-value		>0.05 NS
Hair roundness units		
Mean	0.7666 units	0.5166 units
S.D.	0.22	0.37
P-value		>0.05 NS

S.D.: standard deviation; Sign. inc.: significant increase; Sign. dec.: Significant decrease; NS = Nonsignificant.

Using image analysis in a study done on 12 healthy subjects and 46 patients with AA a significant increase in irregularity of outer root sheath and significant decrease in diameter of hair shaft were measured; which is consistent with the present results. However, the thickness of inner root sheath (TIRS) and diameter of outer root sheath (DORS) of the AA group were significantly smaller in patients than in controls [3], whereas in the present study there was no significant difference between these two parameters in both groups.

If the insult in AA was sufficiently severe, keratinization of the hair shaft would have been impaired [4]. This may account for the significant decrease in hair shaft diameter and hair shaft area in our patients in relation to controls.

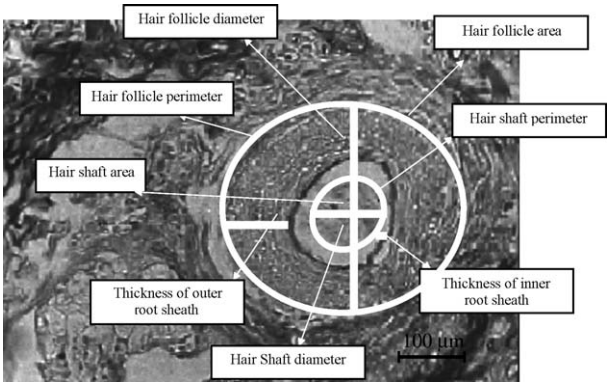

Fig. 1 A transverse section of a hair follicle showing the different morphometric measurements using an Image Analyzer (Mallory trichrome stain).

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