

Computerized Morphometry of Hair Follicles in Alopecia Areata

Manal Bosseila, Bassem Saad*

Abstract

B **ackground:** Transverse sectioning of scalp biopsies of patients with alopecia areata gives simultaneous overview of many hair follicles. Computerized image analysis provides accurate changes in parameters of the follicles.

Aim: To demonstrate hair density and possible changes in hair follicles that may occur during alopecia areata (AA) using image analysis.

Patients and Methods: Scalp biopsies (4mm) were obtained from 20 patients with AA; and 5 individuals with healthy scalp and subjected to morphometric analysis of hair follicle parameters in the horizontal sections of biopsies.

Results: This study showed a statistically significant decrease in hair follicle area, hair follicle perimeter, hair shaft diameter and hair shaft area in AA compared to normal scalp hair; and a significant increase in hair follicle irregularity. However, hair follicle diameter, outer and inner sheath thickness and hair roundness did not vary significantly.

Conclusion: Computerized image analysis is a feasible technique for the assessment of hair density and various hair diameters in alopecia areata.

Key Words: Alopecia areata, hair follicle parameters, image analysis, transverse sectioning.

Introduction

Alopecia areata is a common form of non-scarring alopecia, which appears equally in males and females of any age, although children and adolescents are more commonly affected. The disorder is usually characterized by limited alopecic patches on the scalp, but more severe forms may affect the entire scalp (alopecia totalis) or body (alopecia universalis). Among many factors, an autoimmune etiology has been implicated in AA. Autoimmune diseases, such as diabetes mellitus and vitiligo, are linked to alopecia areata⁽¹⁾.

The histologic features of alopecia areata vary with the stage of the lesion. Active patches

Dermatology Department, Faculty of Medicine, Cairo University;
*Histology Department, Faculty of Medicine, Al-Azhar University
(Assiut Branch); Egypt

demonstrate peribulbar lymphoid infiltrate, a reduction in the anagen/telogen ratio and miniaturized dystrophic hairs. In later stages, a reduction in the anagen/telogen ratio, miniaturized hairs, and many fibrous tracts, some with lymphoid cells or pigment incontinence are seen. Eosinophils are common in all stages of AA, both within the peribulbar infiltrate and within fibrous tracts⁽²⁾.

Transverse sectioning of scalp biopsies taken from patients with alopecia areata gives a simultaneous overview of many follicles. This technique requires sectioning at several levels of the skin, since follicles will be at different depths, depending on the type of hair and part of the cycle they are in, and the pathology may lie anywhere along the length of the follicle. However, once appropriate sectioning is done, all the follicles in a given horizontally sectioned scalp biopsies can be viewed simultaneously, giving a much greater amount of information than is available from similar number of longitudinal sections⁽³⁾. Thus, transverse sections of scalp biopsies in alopecia areata provide more diagnostic and suitable information for the quantitative and morphometric analysis of follicular structures than do longitudinal sections⁽⁴⁾.

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 hairs to be measured in an automated fashion⁽⁵⁾.

Aim of Study

In this study, image analysis was applied to obtain detailed morphological and quantitative parameters of hair follicles, using horizontally sectioned scalp biopsies from normal controls and AA patients.

Patients and Methods

Patients:

The study was conducted on 20 patients presenting with alopecia areata. Study cases were

selected from those attending the dermatology outpatient clinic at Kasr El-Aini Hospital. A full disease history was obtained, and the cases were examined for clinical type of AA.

The control group consisted of 5 individuals with no diseases of the scalp, 3 males and 2 females. Their ages ranged between 18 and 25 years.

Methods

Skin biopsy specimens were taken from the scalp. Four mm punch biopsies were obtained from 20 patients with alopecia and 5 healthy controls.

The selected area was cleaned with alcohol and a local anaesthetic (Xylocaine 2%) was injected subcutaneously. Then, a 4mm biopsy punch was used to obtain the specimen and was put in formalin solution.

Each specimen was embedded in paraffin, sectioned serially and horizontally by a microtome (5 μ m 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 x 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, using image analysis (Figure 1,2):

1. The number of hair follicles per unit area.
2. The diameter (μ m), perimeter (μ m), area (μ m²) and irregularity of hair follicles (unit).

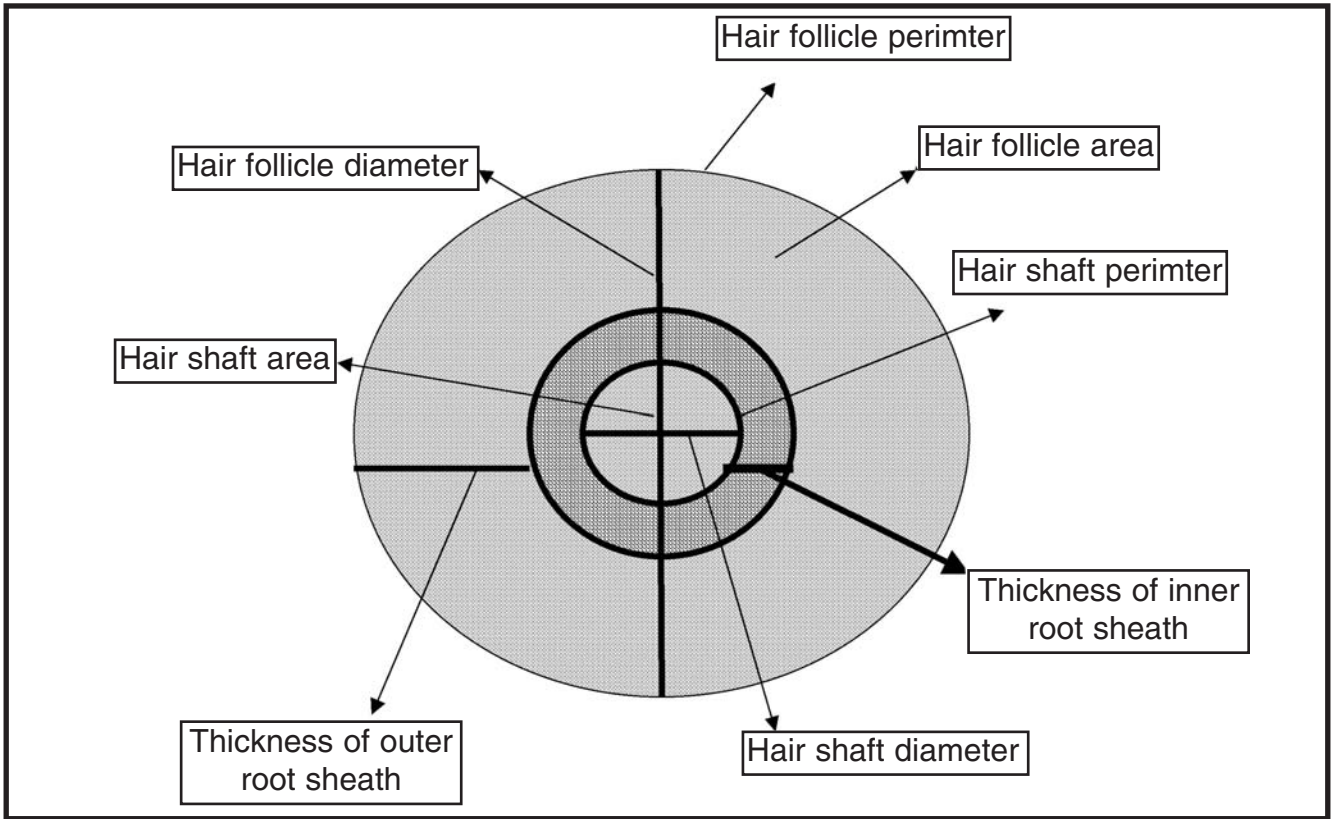


Fig .1. A diagram of the different morphometric measurements of a hair follicle.

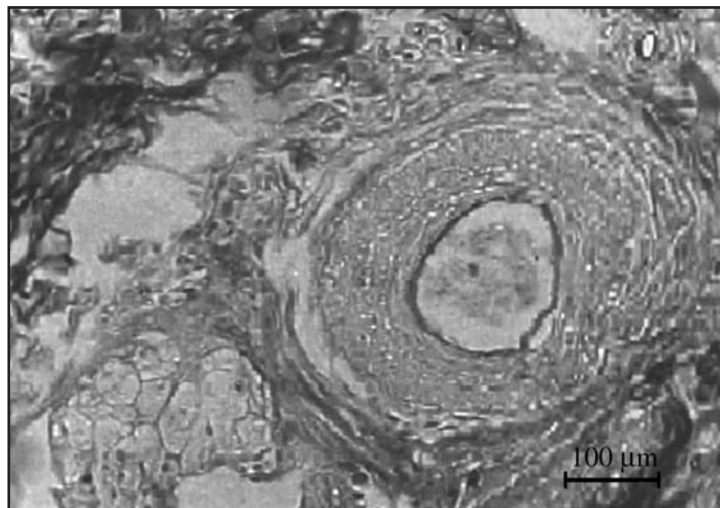


Fig .2. A transverse section of a hair follicle showing the different morphometric measurements using an image analyzer (Mallory trichrome stain).

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

Statistical method: Microsoft Office Excel 2003 was used for data management and analysis. Frequency and percent were calculated for qualitative values, while mean + standard deviation were 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.

Results

I. Results of the clinical data:

Twenty patients were enrolled in the study, with a mean age of 24 years, and a disease duration varying from 1 week to 5 years. Clinical data of the 20 investigated patients with alopecia areata are represented in table (1).

II. Results of the total hair count in control and alopecia areata groups using image analysis:

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

III. Results of the morphometric measurements of hair follicles using image analysis (Table 2):

This study showed a statistically significant decrease in hair follicle area, hair follicle perimeter, hair shaft diameter and hair shaft area in AA compared to normal scalp hair; and a significant increase in hair follicle irregularity. However, hair follicle diameter, outer and inner sheath thickness, hair shaft perimeter and hair roundness did not vary significantly between the 2 groups.

Discussion

Alopecia areata is essentially a hair cycling problem: There is characteristic anagen arrest or anagen inhibition, which terminates hair growth

in a short time. In the chronic stage, decreased terminal and increased miniaturized hairs are found with variable inflammation⁽⁶⁾.

A major challenge in the histopathological examination of scalp biopsies is to perform an adequate evaluation of all the hair follicles present in tissue. The technique of sectioning a scalp punch biopsy transversely has been proposed to demonstrate the entire complement of hair follicles within a biopsy specimen thereby enabling more accurate diagnosis⁽⁷⁾. Demonstration of cross-sectional surfaces of the tissue guarantees that parts of the follicular structures are not omitted, which is a prerequisite to precise quantitative measurements⁽⁸⁾.

As regards quantitative measurements, the density of terminal hairs in a 4-mm punch biopsy was 15.44 in AA patients and 24.56 in the control group. This significant decrease in the number of terminal hairs in patients with AA as compared to the control was consistent with the findings of Whiting, 1995⁽⁴⁾. His study done on 287 AA patients and 22 controls revealed 14 and 35 terminal hairs in 4-mm punch biopsies taken from AA patients and controls, respectively. This finding was present in 67% of the horizontal sections and only in 49% of the vertical sections. It is to be noted that the hair count in our controls was lower than that demonstrated in the previous study (24 and 35, respectively). This is thought to be due to racial variations between white population and african population. Similarly, evaluating the normal hair density in the scalp of African Americans revealed a count of 22.4 hairs/4mm punch biopsy, thus denoting further the possibility of racial variation in scalp hair density⁽⁹⁾.

In contrast, Kim et al., 1999⁽¹⁰⁾ showed in their study a little difference between the number of terminal hairs in both AA and control populations. The average number was 3.0/mm² surface area in AA patients as compared to 3.3/mm² surface area in controls. Similarly, pilar units were present in normal numbers in transverse sections of biopsies taken from alopecia totalis, even in longstanding cases⁽¹¹⁾.

Image analysis with transverse sections is a simple technique and much faster to perform than linear measurements and can therefore be used to

quantify rapidly the severity and extent of AA. It also may represent a method of quantifying the effective uses of hair growth promoters in clinical trials⁽¹⁰⁾.

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. There was no significant difference between the hair follicle diameter, outer root sheath thickness, inner root sheath thickness, hair shaft perimeter and roundness in AA and control groups.

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⁽¹⁰⁾, whereas in the present study there was no significant difference between these 2 parameters in both groups.

Using a light microscope, hair follicles in AA were found generally smaller than normal follicles, and this was more pronounced in longstanding disease. It was mentioned that if the insult in AA was sufficiently severe, keratinization of the hair shaft would be impaired⁽¹¹⁾. This may account for the significant decrease in hair shaft diameter and hair shaft area in patients in relation to controls.

When comparing hair follicle parameters using computer image analysis in androgenetic alopecia with normal scalp biopsies, a significant decrease in hair shaft diameter, outer root sheath thickness and hair follicle diameter was noted; whereas inner root sheath thickness and hair density did not vary significantly⁽⁵⁾.

Very few studies in literature compared the various parameters of hair follicles in non-scarring alopecias with the same parameters in normal hairs^(5,8,10). It is needed to perform more studies on common diseases inducing hair fall, to be able to detect consistent or characteristic changes

occurring in them, thus aiding in diagnosing doubtful cases.

Conclusions

Transverse sections of scalp biopsy specimens in cases of alopecia areata can provide more information than do longitudinal sections, as regards follicular count and structures.

Computerized image analysis is a feasible technique for the assessment of hair density and/or hair diameters in alopecia areata.

The diagnostic value of this computer-assisted morphometric analysis needs to be validated by more studies on patients with non-scarring alopecias.

Table (1): Summary of clinical data of patients, n=20.

Age	12 - 40 years Mean = 23.6
Sex	Males 16, 80% Females 4, 20%
Disease duration	1 week - 8 years
Disease activity	Active: 16, 80% Stable: 4, 20%
Family history of AA	Positive in 1 patient, 5%
Relation of AA to stress	Positive: 9 patients, 45% Negative: 11 patients, 55%
Clinical type of alopecia	Single patch: 6 patients, 30% Multiple patches: 5 patients, 25% Diffuse pattern: 4 patients, 20% Reticular pattern: 4 patients, 20% Alopecia universalis: 1 patient, 50%

Table (2): 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 (μm)	Mean SD p-valua of t-test Significance	513.986 μm 169.66	496.186 μm 115.35 >0.05 NS
Hair follicle area (μm^2)	Mean SD p-valua of t-test Significance	357128.433 μm^2 141474.84	185668.68 mm^2 29518.03 <0.01 Sign. Dec
Hair follicle Perimeter (μm)	Mean SD p-valua of t-test Significance	2067.683 μm 357.65	1620.59 μm 164.685 <0.01 Sign. Dec
Outer sheath thickness (μm)	Mean SD p-valua of t-test Significance	102.88 μm 22.527	146.5 μm 89.18 >0.05 NS
Inner sheath thickness (μm)	Mean SD p-valua of t-test Significance	49.29 μm 31.11	49.46 μm 33.538 >0.05 NS
Hair follicle irregularity units	Mean SD p-valua of t-test Significance	0.1376 units 0.05	0.4 units 0.03 <0.01 Sign. Inc.
Hair shaft diameter (μm)	Mean SD p-valua of t-test Significance	178.756 μm 76.88	89.9 μm 65.05 <0.01 Sign. Dec
Hair follicle area (μm^2)	Mean SD p-valua of t-test Significance	44.61.61 μm^2 16985.32	10718.14 μm^2 11421.61 <0.01 Sign. Dec.
Hair follicle Perimeter (μm)	Mean SD p-valua of t-test Significance	716.93 μm 185.167	1492.23 μm 2499.014 >0.05 NS
Hair roundness units	Mean SD p-valua of t-test Significance	0.7666 units 0.22	0.5166 units 0.37 >0.05 NS

SD = Standard Deviation
Sign. Dec = Significant Decrease

Sign. Dec = Significant increase
NS = Non significant

References

1. Papadopoulos AJ, Schwartz RA, Janniger CK. Alopecia areata. Pathogenesis, diagnosis, and therapy. *Am J Clin Dermatol*, 2000; 1(2): 101.
2. Elston DM, Bergfeld WM, Heibel M. Eosinophils in fibrous tracts and near hair bulbs: a helpful diagnostic feature of alopecia areata. *J Am Acad Dermatol*, 1997; 37: 101.
3. Olsen EA. Hair disorders. In: Freedberg IM, Eisen AZ, Wolff K, Austen KF, Goldsmith LA, Katz SI, Fitzpatrick TB (eds): *Fitzpatrick' Dermatology in General Medicine*; New York, McGraw-Hill, 1999, p 729.
4. Whiting DA. Histopathology of alopecia areata in horizontal sections of scalp biopsies. *J Invest Dermatol*, 1995; 104: 26S.
5. Lee MS, Kossard S, Wilkinson B, et al. Quantification of hair follicle parameters using computer image analysis: a comparison of androgenetic alopecia with normal scalp biopsies. *Australas J Dermatol*, 1995; 36(3): 143.
6. Whiting DA. Histopathologic features of alopecia areata. A new look. *Arch Dermatol*, 2003; 139: 1555.
7. Frishberg DP, Sperling LC, Guthrie VM. Transverse scalp sections: a proposed method for laboratory processing. *J Am Acad Dermatol*, 1996; 35: 220.
8. Shum DT, Lui H, Martinka M, et al. Computerized morphometry and three-dimensional image reconstruction in the evaluation of scalp biopsy from patients with non-cicatricial alopecias. *Br J Dermatol*, 2003; 148:272.
9. Sperling LC. Hair density in African Americans. *Arch Dermatol* 1999; 135: 656.
10. Kim IH, Jo HY, Cho CG, et al. Quantitative image analysis of hair follicles in alopecia areata. *Acta Derm Venereol (Stockh)* 1999; 79:214.
11. Messenger AG, Slater DN, Bleehen SS. Alopecia areata: alterations in the hair growth cycle and correlation with the follicular pathology. *Br J Dermatol*, 1986; 114: 337.

