Morphometry of normal scalp hair follicles*

*Morfometria de folículos pilosos do couro cabeludo normal**

Fabiane Mulinari-Brenner[1]
Fernanda Manfron Batista Rosas[2]
José Fillus Neto[3]
Luiz Fernando Bleggi Torres[4]

Abstract: BACKGROUND - Histological description of normal human hair follicle has scarce literature. Recognizing normal scalp follicle and its variability throughout the scalp is fundamental to study scalp disorders.
OBJECTIVES - To evaluate normal scalp samples in Caucasians considering cross-section area, maximum and minimum diameters of terminal anagen follicles.
METHODS - Twenty necropsies of Caucasian individuals (10M, 10F) were followed by a 4mm punch biopsy of 4 scalp areas. Transverse sections were evaluated by light microscopy. Terminal anagen follicle cross-section area, maximum and minimum diameters were determined by computer image analysis. Results were compared between sexes and scalp areas.
RESULTS - Follicular area and maximum and minimum diameters are presented.
CONCLUSION - No statistically significant differences were observed in follicular maximum diameter and area in several scalp regions. However, the morphometric evaluation of terminal anagen follicles demonstrated greater dimensions within the male group.
Keywords: Alopecia; Hair; Hair follicle; Histology

Resumo: *FUNDAMENTOS - A caracterização histológica do folículo piloso normal em humanos é escassa na literatura, considerando que o reconhecimento da arquitetura folicular normal e suas variações nas diversas áreas do couro cabeludo é fundamental para o estudo das doenças do couro cabeludo.*
OBJETIVOS - Analisar fragmentos do couro cabeludo normal em indivíduos de raça branca quanto à área, os diâmetros máximo e mínimo dos folículos anágenos terminais.
MÉTODOS – Vinte necrópsias de indivíduos de raça branca, com idade variando de 20 a 78 anos, foram acompanhadas da coleta de biópsias por punch de 4mm de diâmetro em quatro áreas do couro cabeludo. Os casos foram divididos conforme o sexo em dois grupos, com 10 casos cada. A área e os diâmetros máximo e mínimo dos folículos anágenos terminais foram determinados por análise computadorizada das imagens de cortes transversais da microscopia óptica. Os resultados obtidos foram comparados entre os sexos e os locais de coleta.
RESULTADOS - Os resultados numéricos das áreas e diâmetro máximo e mínimo são expostos e comparados.
CONCLUSÃO - Não houve diferença estatisticamente significativa entre o diâmetro máximo e a área folicular nas diversas regiões do couro cabeludo; entretanto, foram evidenciadas dimensões de folículos anágenos terminais maiores no sexo masculino do que no feminino.
Palavras-chave: Alopecia; Cabelo; Folículo piloso; Histologia

Received on March 18, 2005.
Approved by the Consultive Council and accepted for publication on January 06, 2006.
* Work done at Universidade Federal do Paraná, Department of Internal Medicine, Service of Dermatology (PR), Brazil.
Conflict of interests: None

1 Dermatologist, MSc in Internal Medicine, Assistant Professor of Dermatology, Universidade Federal do Paraná (PR), Brazil.
2 Medical student, Universidade Federal do Paraná (PR), Brazil.
3 Pathologist, MSc in Pathology, Universidade Federal do Paraná, Professor of Basic Pathology, Universidade Federal do Paraná (PR), Brazil.
4 Pathologist, Ph.D. in Pathology from the University of London, England, Full Professor of Pathology, Universidade Federal do Paraná e da Pontifícia Universidade Católica do Paraná (PR), Brazil.

INTRODUCTION

Headington's description of transverse scalp sections a little more than 20 years ago brought a new drive to the morphometric study of the scalp.[1] Data collected from his study contributed to the comparative analysis and early diagnosis of hair loss. Few studies have evaluated information obtained with transverse sections in normal scalp after Headington's report. The findings and normal characteristics of the scalp have to be identified to characterize the changes occurring in dermatoses that affect this region, and especially in alopecia.

MATERIALS AND METHODS

Cylindrical scalp biopsy specimens were collected from the mid-frontal, vertex, occipital and right temporal regions. An approximately 1-cm² area of hair was trimmed in these regions, the hair shafts were left 3-5mm long, thus allowing the direction of hair growth to be viewed.

The specimens were collected using disposable punches (Miltex™, Japan), inserted parallel to the direction of hair growth, and the incision was performed down to the subcutaneous fat. The lower portion of the material was sectioned with curved scissors, providing the sample with a cylindrical appearance. The sections were fixed in 10% buffered formaldehyde solution.

The study was conducted at the *Instituto de Medicina Legal de Curitiba* [Institute of Legal Medicine of Curitiba] after approval of the Medical Research Ethics Committee of the Hospital de Clínicas da Universidade Federal do Paraná. The scalp specimens were collected from autopsies performed at the Legal Medicine Service in cases of violent death, and at the Pathological Anatomy Service in cases of natural death, both of the *Instituto Médico Legal do Paraná* [Institute of Legal Medicine of Paraná]. Four scalp specimens were collected from 37 autopsies, in a total of 148 pieces.

To obtain greater homogeneity of the material evaluated, specimens from Caucasian corpses of both sexes were selected for the study. Black and mixed-race individuals (eight autopsies), and those who died of chronic diseases (four autopsies) that could have altered the normal structure of the scalp were excluded. Specimens from corpses of patients who had been hospitalized for more than three days (five autopsies), with clinical signs of chronic disease or malnutrition, dermatoses, scalp lesions or evident androgenetic alopecia were discarded.

At the end, four scalp specimens from 20 Caucasian corpses, in a total of 80 pieces, were studied. The cases were divided into two groups: Group 1 with 10 males with age ranging from 21 to 78 years (median of 39) and Group 2 with 10 females with age ranging from 20 to 70 years (median of 53). Hair shaft characteristics of the individuals of each group were observed. In Group 1 the shafts were straight in eight cases and curly or wavy in two cases; regarding color, they were grey in four cases, brown in four, black in one and blond in one case. In Group 2 the shafts were straight in four cases and curly or wavy in six cases; regarding color, they were grey in six cases, black in three, brown in one and blond in one case.

Histological sections of the cylindrical piece were performed in series from the hypodermis to the epidermis (Figure 1) and mounted on a glass slide sequentially. To standardize the study, the terminal follicles of the superficial dermis (at the follicular isthmus) were first examined. These dermal sections corresponded to the fourth or fifth section mounted on the glass slide. At this level the follicles were characterized as anagen, catagen and telogen, according to Headington's initial description evaluating only the anagen follicles. To analyze the keratogenous zone of the follicles, hypodermic sections with a larger number of follicles – usually the second or third histological section – were also studied. The characterization of the follicles is more difficult in this region, and they were divided into anagen and others. These "others" included follicles with a modified structure because of full keratinization of the inner root sheath (usually telogen), or because of the thickening of the follicular basal membrane (usually catagen), or due to some technical artifacts.

Morphometry of area and maximal and minimal diameters at the isthmus (deep dermis) and at the keratogenous zone (hypodermis) were per-

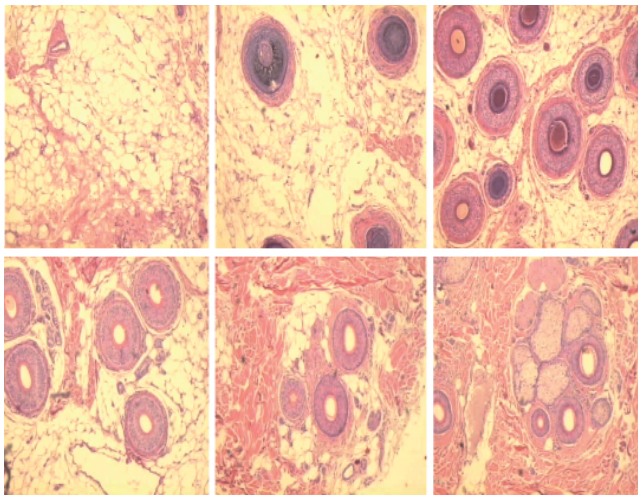

Figure 1: Correlation between histological aspect and sequence and depth of histological sections (HE) from hypodermis to dermis

formed. A digital imaging system composed of an Olympus™ microscope attached to a CCD-Iris, model DXC-107A color videocamera (Sony™, Japan) that transmits images to a microcomputer was used. The Optimas™ 6.2 (Media Cybernetics™, MD, USA) software was used to convert the images into a binary format for assessment in a Windows™ environment (Microsoft™, WA, USA). This software determines areas and maximal and minimum diameters of delimited regions.

Morphometric data were obtained from a central field of the 2-mm^2 histological sections, corresponding to the field observed by the videocamera in the 40X magnification, and the measurements were calibrated in a micrometered slide. After delimiting the anagen follicles by means of a mouse click on the basal membrane region, data obtained were initially entered into the Excel version 7.0 software.

The variables were analyzed with their continuous quantitative values. The Mann-Whitney U test was applied to the independent variables in the comparison of follicle measurements in the different scalp sites between groups 1 and 2. Statistical significance was considered at alpha ≤ 0.05.

The Kruskal-Wallis test was used to compare each of the measurements obtained in the different scalp sites (frontal, parietal, occipital and vertex) in each group. Statistical significance was considered at alpha ≤ 0,05.

RESULTS

Terminal anagen follicles corresponded to 90.6% of the follicles in dermal sections. Measurements of 140 terminal anagen follicles at the isthmus were evaluated in Group 1, and of 126 in Group 2. Data were analyzed as a whole, in the groups separately and according to the scalp site in each group.

Total and group median values at the follicle isthmus can be observed in tables 1 and 2. A statistically significant difference was observed in all measurements of the groups, and measurements in Group 1 were larger than those in Group 2 ($p<0.05$). Also at the follicle isthmus, the measurements in the different areas for both groups are shown in Table 3, and no statistically significant differences were observed between them using Mann-Whitney U test.

The terminal anagen follicles corresponded to 90.3% of the follicles in hypodermic sections. Measurements of 78 anagen follicles at the keratogenous zone were analyzed in Group 1 and of 83 in Group 2. Data were analyzed as a whole, in the groups separately and according to the scalp site in each group.

TABLE 1: Area and maximal and minimum diameters of anagen follicles observed in the total of cases at the follicle isthmus

Measurements	Median (interquartile interval)
Area (μm^2)	55,486.2 (42,792.3-75,329.4)
Maximum diameter (μm)	288.3 (258.1-339.5)
Minimum diameter (μm)	258.1 (229-300.5)

Table 4 demonstrates medians obtained from all studied cases, while isolated groups are shown in table 5. The measurements in Group 1 were larger than those in Group 2, with a statistically significant difference ($p<0.05$).

The measurements in the different scalp sites in the groups are shown in table 6. In Group 2, the values of minimal diameters decreased progressively toward the frontal, occipital, vertex, and parietal scalp sites. The comparison between sites showed a statistically significant difference ($p<0.05$) using the Mann-Whitney U test in the median values in the four sites.

DISCUSSION

Scalp samples can be evaluated from the vertical or horizontal. In vertical or horizontal sections, only 10 to 15% of the follicles of the specimen can be observed.[1] Horizontal or transverse histological sections, which are currently more used, improved the quality of the histopathological study of hair follicles of the scalp.[1-3] These sections allow the global assessment of the piece providing quantitative specimen data such as follicle units (structure, number, density and phase of the growth cycle), number of follicles in each phase of the cycle, of telogen germinative units, of fiber tracts, and of vellus and terminal follicles. These data can be evaluated at different depths, from the superficial dermis to the subcutaneous fat.

The pilosebaceous structure and morphological variations resulting from the hair growth cycle may make the histological assessment difficult. The optimal site for routine observation of scalp hair follicles is a plane close to the entrance of the sebaceous duct into the follicle.[1] Sections at this level can be obtained by sectioning the piece 1mm above the junction between the dermis and the subcutaneous. Since this section is hard to obtain in practice, the deepest dermal section of the series was considered one of the bases for the histological analysis in this study. In addition to this level, measurements of the keratogenous zone of the follicle at the distal portion of the hair bulb were also evaluated. This region marks the cell transition for hair keratin and does not include the bulge area with its increased diameter.

TABLE 2: Area and maximal and minimum diameters of anagen follicles at the follicle isthmus in Groups 1 and 2

	Group 1	Group 2	p value
Area (μm^2)	59,547.1 (45,388.7-78,790.6)	51,695.3 (42,236.6-66,698.9)	0.011
Maximum diameter (μm)	301.1 (267.8-352.3)	281.2 (252.4-323.4)	0.016
Minimum diameter (μm)	270 (233.5-311.4)	251.4 (223-279.7)	0.03

Scalp biopsy is a simple and slightly painful procedure. There is a current consensus that the specimen should be obtained using 4 to 6-mm punch incisions performed in parallel with the hair that emerge from the scalp.[1,2,4-6] Since it is fundamental that the specimens include the bulbs of the terminal anagen follicles located in the hypodermis, the piece should be removed by sectioning the inferior portion of the material with scissors, thus obtaining a cylindrical specimen.[3,5,7] Conical specimens show a reduced number of terminal anagen bulbs, which interferes with the assessment.

The scalp biopsy procedure and its histological evaluation were standardized in this study using a 4-mm punch in Caucasian adults from 21 to 78 years of age (median of 44 years) with similar groups between which the main difference was sex. The use of a disposable punch with a precise cut surface was essential for obtaining adequate material. Four-mm-diameter specimens are easy to obtain; smaller specimens are of little use for histological study; larger ones make the procedure more difficult.

Little information on follicle dimensions and their normal variations is found in the literature. The definition of these follicular morphometric parameters helps characterize follicle miniaturization espe-

TABLE 3: Area and maximal and minimum diameters of anagen follicles of the frontal, vertex, occipital and parietal regions at the follicle isthmus in Groups 1 and 2

Group 1	Frontal	Vertex	Occipital	Parietal	p value
Area (μm^2)	59,641.3 (53,706.2-81,128.1)	53,086.7 (42,140.6-75,303.8)	67,021.8 (45,587.5-82,537.5)	60,694.4 (45,065.1-78,303.1)	0.529
Maximum diameter (μm)	296.9 (272.6-348.5)	291.4 (247.6-335.1)	313.1 (272.7-353.3)	312.1 (264.4-371.3)	0.5
Minimum diameter (μm)	279 (256.4-317.9)	258.4 (229.2-306.5)	283.2 (229.7-313.7)	257.1 (231.8-307.2)	0.238
Group 2					
Area (μm^2)	53,049.8 (42,300-63,126.4)	49,725 (38,487.5-70,151.3)	58,328.1 (42,803.2-78,243.7)	48,948.4 (38,801.5-63,500.5)	0.309
Maximum diameter (μm)	281.2 (247.9-309.9)	275.4 (245.7-322.3)	290.6 (269.9-351.7)	265.8 (252-319.5)	0.27
Minimum diameter (μm)	251.4 (228.2-270.2)	241.5 (213.1-274.3)	268.1 (229.2-300.1)	247.2 (202.3-279.7)	0.474

Table 4: Area and maximum and minimum diameters of anagen follicles at the keratogenous zone observed in the total of cases

Measurements	Median (interquartile interval)
Area (μm^2)	49,869.6 (37,228.9-65.798.8)
Maximum diameter (μm)	274.9 (243-318.1)
Minimum diameter (μm)	244.5 (211.8-277.6)

cially in non-cicatricial alopecia. Based on normal values, a variety of studies on scalp may be developed by using a comparative analysis of transverse sections. This information may be used for the early detection of pathological changes, thus collaborating with the diagnosis of scalp disorders, as well as with therapeutic assessment.

The hair growth phase may only be identified in sections performed in the lower segment, in the portion that undergoes anatomical changes throughout the hair cycle. The anagen phase is the period between the final of the telogen (or exogen) phase and the beginning of the catagen phase. Although this definition is difficult from a biological standpoint, the histological characteristics are well identified.[8] The anagen follicles represent 75 to 100% of the follicles in a normal scalp specimen.[6,9] In the total sample of cases studied, approximately 90% of the follicles were characterized as anagen, showing that the samples were normal. Of these, 266 follicles were measured at the dermis and 161 at the hypodermis.

The anagen phase is characterized by the full development of follicles in active growth. In scalp follicles it lasts approximately six years.[4] We chose to study follicle measurements in this long-lasting predominant phase. The catagen and telogen phases are phases of follicle regression and represent five to 15% of the follicles in a normal sample.[6,9]

Lee et al. introduced the computer image analysis in measurement of hair follicles of scalp biopsies in androgenetic alopecia in 1995.[11] From then on, other authors have used the computerized morphometric study of the scalp.[10-12] The present morphometric study focused on terminal anagen follicles (90.6% of the follicles observed). The following median values were observed in deep dermal sections (follicle isthmus): 55486.2 (42792.3-75329.4) μm^2, for area, 288.3 (258.1-339.5) μm for maximum diameter, and 258.1 (229-300.5) μm for minimal follicle diameter. At the hypodermis close to the keratogenous zone the following median values were observed: 49869.6 (37228.9-65.798.8) μm^2, for area, 274.9 (243-318.1) μm for maximal diameter, and 244.5 (211.8-277.6) μm for minimum follicle diameter. All dimensions obtained were larger in males, with a statistically significant difference.

The measurements obtained in the present study included Costa's results, who studied terminal follicles of 12 male volunteers and obtained means of 75657.77 μm^2 for follicle area in the occipital region, and 59791.25 μm^2 in the parietal region, minimum mean diameters of 273.46 μm in the occipital region and 259.74 μm in the parietal region.[10] Costa's assessments evidenced statistically significant differences between regions, which was not corroborated by the present assessment with a larger sample size and including female individuals.

When the hypodermic sections were compared within the Group 2 (females), a statistically significant difference was observed between the minimum follicle diameters. Oval or elliptical follicles (associated with wavy or curly hair) have smaller minimum diameters than round follicles (associated with straight hair). The possibility of a relation with the type of hair shaft was considered. Analyzing the initial notes, six individuals in Group 2 (females) presented wavy or curly hair versus only two in Group 1 (males). Transverse sections of the follicle shaft of wavy hair are oval or reniform (negroid hair); little is known, however, about these follicle differences.[8] This assessment is complex and was not observed

Table 5: Area and maximum and minimum diameters of anagen follicles at the keratogenous zone in Groups 1 and 2

	Group 1	Group 2	p value
Area (μm^2)	55,326.5 (41,767.1-69,630.40)	46,059.3 (34,450.1-61,500)	0.009
Maximum diameter (μm)	286.2 (264.1-333.2)	267.65 (228.3-312.4)	0.010
Minimum diameter (μm)	260.6 (217-280.2)	229.0 (204.2-267.9)	0.011

TABLE 6: Area and maximum and minimum diameter of anagen follicles in the frontal, vertex, occipital and parietal regions at the keratogenous zone in Groups 1 and 2

Group 1	Frontal	Vertex	Occipital	Parietal	p value
Area (μm^2)	56,821.8 (47,145.2-71,105.4)	45,278.1 (38,711.7-62,981.2)	56,237.5 (41,422.8-68,879.6)	62,500.7 (51,539-70676)	0.186
Maximum diameter (μm)	287.7 (260.4-323.6)	274.3 (253.9-334.9)	313.1 (272.7-353.3)	312.1 (264.4-371.3)	0.407
Minimum diameter (μm)	268.1 (234.9-291.5)	237.1 (208.2-278.5)	241.8 (212.1-278.6)	271.5 (247-280.7)	0.186
Group 2					
Area (μm^2)	49,869.6 (40,959.5-66,284.2)	46,059.3 (33,543-68,296.4)	48,047.5 (34,980.4-56,524.9)	36,678.1 (29,973-42,408.5)	0.067
Maximum diameter (μm)	274.4 (243.3-304.7)	285.4 (228.3-357.6)	270.6 (227.7-313.4)	239.8 (217.7-262.6)	0.206
Minimum diameter (μm)	251.2 (219-276.3)	225.6 (199.4-288.8)	237.7 (206.7-259)	206.3 (181.5-228)	0.014

only in the measurements at the isthmus and at the keratogenous zone.

Several sites were assessed because some clinical and experimental situations suggest that follicles of different sites are not similar. Follicles differ as to the length, thickness, color, cross-sectional area, and sensitivity to hormones.[8] However, in the present study specimens from different sites were similar. Follicle characteristics seem to be established by the anagen papilla. Despite this fact, aging, systemic diseases, and some drugs may change the growth pattern. The embryonic origin of the scalp dermis may determine the follicle pattern: the vertex, face and anterior neck mesenchyma derives from the neural crest, whereas the temporal and occipital scalp mesenchyma derives from the cephalic or somitic mesoderm.[8]

The basis for the heterogeneity of the follicles may result from initial determinants in the follicle development expressed by a complex genetic pattern. Among the molecular mediators of follicle growth it is possible to find several hormone factors that could influence follicle differences between sexes. Some possible follicle influences in females include 17-β-estradiol blocking hair growth, the presence of estrogen receptors in the follicle papilla inducing the anagen phase, and the presence of prolactin receptors in the papilla, matrix and outer root sheath stimulating anagen and catagen phases.[8] The presence of androgen receptors in the follicle papilla, of aromatase in the outer root sheath, and of 5-β reductase type 1 (in sebaceous glands and hair follicle papilla) and type 2 (in the follicle epithelium, interfollicular dermal cells and outer root sheath)[8] may also be involved in this process. Further assessments with a higher number of specimens and biochemical studies are necessary for a better understanding of the findings of this study.

CONCLUSIONS

Measurements of transverse sections of anagen follicles at the isthmus and keratogenous zone of normal scalp samples obtained with a 4-mm punch from Caucasian adults with age ranging from 21 to 78 years (median of 44 years) were determined. These measurements provide normal parameters for further comparisons. The terminal anagen follicles presented larger cross-sectional dimensions at both levels in males; little is known, however, about the mechanisms that lead to this difference. The mini-

mum follicle diameter varied among the scalp sites in hypodermic sections in females with a statistical significance. Broader assessments are necessary for a better understanding of these data. ❑

ACKNOWLEDGEMENTS

To Dr. Lúcia de Noronha, Professor of Pathology at *Pontifícia Universidade Católica do Paraná*, for her orientation and help with computerized morphometry.

REFERENCES

1. Headington JT. Transverse microscopic anatomy of the human scalp. A basis for a morphometric approach to disorders of the hair follicle. Arch Dermatol. 1984;120:449-57.
2. Frishberg DP, Sperling LC, Guthrie VM. Transverse scalp sections: a proposed method for laboratory processing. J Am Acad Dermatol. 1996;35:220-2.
3. Templeton SF, Santa Cruz DJ, Solomon AR. Alopecia: histologic diagnosis by transverse sections. Semin Diagn Pathol. 1996;13:2-18.
4. Steiner D, Nicola CF, Bedin V, Landman G, Pinto CAL, Pasello RR. A importância da biópsia transversal para o diagnóstico da alopecia androgenética feminina. Anais do 53º Congresso Brasileiro de Dermatologia; 1998 Set 21; Belo Horizonte – Brasil.
5. Caserio RJ. Diagnostic techniques for hair disorders - Part II: microscopic examination of hair bulbs, tips, and casts. Cutis. 1987; 40:321-2.
6. Whiting DA, Howsden FL. Normal hair. In: Color Atlas of Differencial Diagnosis of Hair Loss. New Jersey: Canfield Publishing; 1996. p. 2-17.
7. Sperling LC. Evaluation of hair loss. Curr Probl Dermatol. 1996; 8: 97-136.
8. Stenn KS, Paus R. Controls of hair follicle cycling. Physiol Rev. 2001;81:449-94.
9. Bergfeld WF, Mulinari-Brenner F. Shedding: a common cause of hair loss. Cleve Clin J Med. 2001;68:256-61.
10. Costa IMC. Alopecia Androgenética: Contribuição ao estudo morfométrico e de imuno-histoquímica em folículos pilosos. [tese]. São Paulo: Escola Paulista de Medicina; 1998.
11. Lee MS, Kossard S, Wilkinson B, Doyle JA. Quantification of hair follicle parameters using computer image analysis: a comparison of androgenetic alopecia with normal scalp biopsies. Australas J Dermatol. 1995; 36:143-7.
12. Shum DT, Lui H, Martinka M, Bernardo O, Shapiro J. Computerized morphometry and tree-dimensional image reconstruction in the evaluation of scalp biopsy from patients with non-cicatricial alopecias. Br J Dermatol. 2003; 148:272-8.

MAILING ADDRESS:
Fabiane Mulinari Brenner
Rua da Paz,195 conj. 423
80160-060 - Curitiba - PR
Tel./fax: (41) 264-3755
E-mail: fmbrenner@uol.com.br