Quantitative evaluation of transverse scalp sections[*]
Avaliação quantitativa em cortes histológicos transversais do couro cabeludo[*]

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Abstract: BACKGROUND - Punch-biopsy is a great tool for the diagnosis of scalp disorders, however experience is required for the dermatopathologist. Recognizing the standard structures found in normal scalp is very helpful in this aspect.
OBJECTIVE - The goal of this study was to determine quantitative data considering normal structures on the scalp of white adults.
METHODS - Twenty necropsies were followed by eighty 4mm-punch biopsies of clinically normal scalp. Samples were evaluated by transverse sections.
RESULTS - Scalp fragments from white adults, age 21 to 78, demonstrated (medians): 16.5 (12.0-23.0) total follicles; 8.0 (6.0-9.0) follicular units; 15.0 (10.0-20.0) terminal follicles; 1.0 (0.0-2.0) vellus follicle; and 1.0 (0.0-2.5) fibrous tract. Considering the hair cycle phase: 92.2% were in anagen; 1.6% in catagen and 6.2% in telogen.
CONCLUSION - Comparing the results, male samples demonstrated fewer follicular units, total and telogen follicles, and higher number of total follicles and fibrous tracts on frontal and vertex area.
Keywords: Alopecia; Dermatology; Hair follicle; Histology; Scalp

Resumo: FUNDAMENTOS – *A biópsia por punch é de grande auxílio no diagnóstico das doenças do couro cabeludo quando avaliada por patologista experiente. O conhecimento das estruturas encontradas no couro cabeludo normal facilita o diagnóstico histopatológico nessa área tão complexa da dermatopatologia.*
OBJETIVO – *Determinar dados quantitativos normais do couro cabeludo de adultos brancos.*
MÉTODOS – *Em 20 necrópsias, 80 fragmentos de couro cabeludo clinicamente normal foram obtidos por punch 4mm. As amostras foram avaliadas em cortes transversais.*
RESULTADOS – *Em fragmentos obtidos de adultos brancos, com idade variando entre 21 e 78 anos, foi possível observar (medianas): 16,5 (12-23) folículos totais; oito (6-9) unidades foliculares; 15 (10-20) folículos terminais; um (0-2) folículo velo; um (0-2,5) trato fibroso; 92,2% de folículos anágenos; 1,6% de catágenos e 6,2% de telógenos.*
CONCLUSÃO – *Além dos resultados apresentados, observaram-se ainda nos indivíduos do sexo masculino: menor número de unidades foliculares, folículos totais e folículos telógenos; e ainda, nas regiões frontal e vértex, maior número de folículos e tratos fibrosos.*
Palavras-chave: Alopecia; Dermatologia; Couro cabeludo; Folículo piloso; Histologia

INTRODUCTION

Scalp biopsy is one of the best ancillary tests for the diagnosis of scalp disorders. In order to get reliable information, the pathologist should be highly experienced. Knowledge of normal scalp structures enables a more precise histopathological diagnosis in this complex area of dermatopathology.

Kligman, in 1959,[1] performed the first full histological study of the scalp of men and women. This vertical histological assessment served as the basis to understand the hair cycle phases. In 1984, Headington characterized the cross-sectional microscopic structure of the hair follicle and pointed out the advantage of using it to assess the scalp.[2]

The aim of this study was to analyze the structure of follicles, the proportion of anagen, catagen and telogen follicles in normal scalp fragments, as well as to compare these data between genders and biopsy sites.

MATERIAL AND METHODS

This study was carried out at the *Instituto de Medicina Legal de Curitiba* [Forensic Medicine Institute], after approval by the Research Ethics Committee of the *Hospital de Clínicas, Universidade Federal do Paraná.* Individual features of the cadavers, such as age, skin color, hair color (gray, blond, brown or black) and hair type (straight, wavy or curly) were recorded. In order to obtain greater homogeneity, only specimens from white subjects of either sex were assessed. Specimens from cadavers having hospital stay longer than 24 hours, clinical signs of chronic disease or malnutrition, skin diseases, scalp lesions or alopecia were discarded.

Four scalp fragments of 20 white cadavers were assessed, totaling 80 samples. Two groups were established: Group 1 – specimens from 10 males aged 21 to 78 years; Group 2 – specimens from 10 females aged from 20 to 78. In Group 1, eight had straight, one wavy and one curly hair. In Group 2, six had straight, three wavy and one curly hair.

Cylindrical scalp fragments were obtained on the following regions: A) frontal, B) vertex, C) occipital and D) right temporal. One square centimeter of hair was trimmed from these regions, in order to determine the direction of hair growth.

Disposable punches measuring 4-mm in diameter were inserted parallel to the hair growth direction, cutting down to the subcutaneous tissue. The lower part was cut with curved scissors, so that the sample was cylindrical. Fragments were fixated in buffered 10% formaldehyde and the vials were labeled with the case number and the letter corresponding to the area.

Fragments were included in paraffin, its epidermal aspect facing the center. Six 5-μ fragments, 250μ apart, were obtained and the sections were stained with hematoxylin-eosin (Figure 1). The deepest dermal slice was used to study the follicular structures. Only the structures that could be fully observed in the slices were assessed.

The follicles were classified as vellus (the diameter of the hair canal smaller than the inner root sheath) and terminal (the diameter of the hair canal larger than the inner root sheath). Terminal follicles were classified as anagen, catagen or telogen. Anagen follicles in the deep dermis were recognized when fully developed inner and outer root sheaths were identified, with no signs of apoptosis in the outer root sheath (Figure 2A). Follicles with thickening of the basal membrane or apoptotic cells were interpreted as catagen (Figure 2B). Follicles with central wrinkling of the hair canal (tricholemal keratinization) were considered as telogen (Figure 2C). These features were not clearly observed in some follicles. These follicles and others with oblique section were classified as undetermined.

Two other important structures were observed: telogen germinative units (TGU) and fibrous tracts. TGUs, the probable site of germinative cells, were identified by the presence of clusters of epidermal cells with peripheral palisade with no central keratinization in the deep dermis (Figure 2D). Fibrous tracts remaining in lower region when follicles are in the catagen phase were characterized as epithelial cells amidst thickened and concentric collagen fibers with increased vascularization in deep dermis and hypodermis (Figure 2E).

The Excel® spreadsheet was used to input data. Nonparametric tests were used for univariated analysis of the data. Medians and interquartile range (25 and 75 percentiles) are presented. The continuous
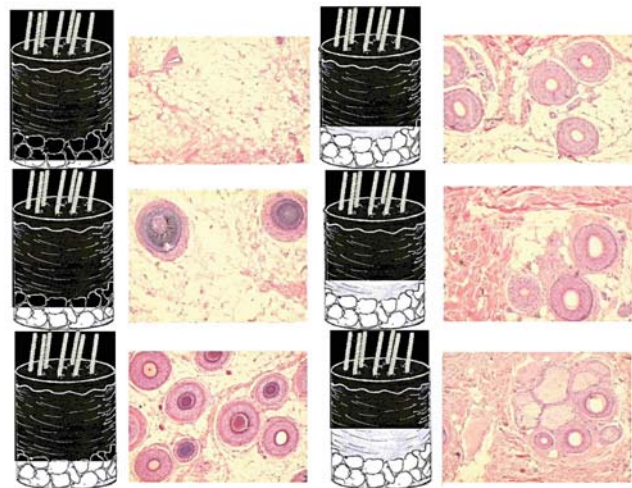

FIGURE 1: Cross-section levels correlated to histological aspects in photomicrographs (HE, 25X)
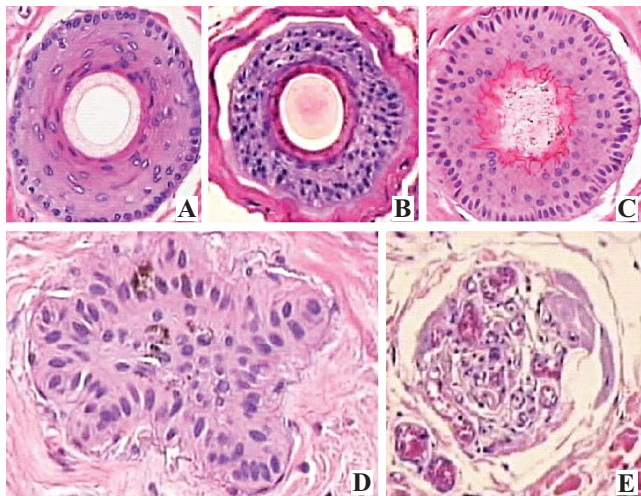
FIGURE 2: Histological aspect in transverse section of follicles: A) terminal anagen with inner and outer root sheath, B) terminal catagen with thickening of the basal membrane, and C) terminal telogen with wrinkling of the inner root sheath. Other structures observed in transverse sections: D) telogen germinative unit, and E) fibrous tract (HE, 100X)

quantitative values of the variables were studied. Mann Whitney U test was used to compare follicle dimensions in different scalp areas between Groups 1 and 2. Significance was established at alpha ≤ 0.05. The Kruskal-Wallis test was used to compare each of the independent measurements in the different scalp areas (frontal, parietal, occipital, and vertex) within each group. Significance was established at alpha ≤ 0.05.

RESULTS

Results were obtained from 20 cases with median age of 45 (31.5–66.7) years. Median age in Group 1 was 39 (27.5– 65) years and in Group 2 was 53 (34.5– 69) years. Using the Mann-Whitney U test on this variable, the p value was 0.384. Hence, age was not statistically significant different between the two groups.

The median results of the quantitative analysis obtained from deep dermis of the 80 samples are depicted in tables 1 to 5. All results were analyzed, by each group and by collection site in each group.

DISCUSSION

Scalp biopsy is an easy procedure, associated with little pain. The current recommendation is to obtain the material with a 4-6-mm punch placed parallel to the hair emerging from the scalp.[2-6] Samples must include the terminal anagen follicle bulbs; therefore, the punch must reach and include the subcutaneous tissue.[2,7] Special care should be given to rotating and excising fragments, as well as the lower cut with scissors to obtain a cylindrical sample that is suitable

for assessment.[3,4,8] Conical samples contain a reduced number of terminal anagen follicle bulbs, which interferes with the assessment.

Scalp fragments can be both analyzed in the vertical and the horizontal planes. In vertical (longitudinal) sections only 10 to 15% of sample follicles are seen, and therefore several serial sections are needed for an appropriate assessment.[2] Horizontal or cross sections currently employed improve the quality of the histological study of scalp hair follicle.[2,5,8-15] Transverse sections enable the overall assessment of fragments, yielding quantitative information about the sample. They also enable observing the follicular structure, hair cycle phases and other structures, such as telogen germinative units and fibrous tracts. Such findings can be assessed at different depths, from the superficial dermis to the subcutaneous tissue.

The pilosebaceous structure and the morphological variation due to different hair cycles may hinder the histological assessment. The best area for routine assessment of the scalp hair follicles is at the plane of the sebaceous duct entry.[2] Such samples may be obtained by cutting the fragment one millimeter above the dermis-subcutaneous junction, according to Sperling.[7,12] Despite descriptions in the literature of ideal sections, it is difficult to attain them in practice, which justifies the deep dermis section as performed in this study.

Scalp biopsy and histological assessment were standardized, using a 4-mm diameter punch in white adults with median age of 45 (31.5 – 66.7) years in similar groups with clinically normal subjects, differing mainly in sex. The use of disposable punch with sharp cutting edge was essential to obtain suitable samples. Four-millimeter samples are easy to obtain and of notice is the fact that smaller samples are unsuitable for histological analysis because they do include enough structures.

The follicular units are the fundamental scalp structure. The histological analysis should be performed in the superficial or deep dermis where these follicles have clear limits. Terminal follicles and vellus hairs are well identified as well as the different hair cycle phases in the deep dermis and thus it is the preferred level for studying. Eight (6-9) follicles were observed in each sample; this number was significantly lower in males. Early cross-sectional studies demonstrated 12 a 14 follicular units per sample.[3,7,14] This difference may be explained by the different populations assessed and depth of the section. In one of these studies, the depth is not described and in another the superficial dermis was assessed. In a Brazilian study of occipital scalp biopsies of women the mean number of follicular units observed was

TABLE 1: Median of structures observed in the total number of cases (absolute numbers)

Structures	Median (interquartile range)
Total follicles	16.5 (12-23)
Follicular units	8 (6-9)
Terminal follicles	15 (10-20.5)
Vellus follicles	1 (0-2)
Anagen follicles	14 (9-19)
Catagen follicles	0 (0-0)
Telogen follicles	0 (0-1)
Undetermined follicles	0 (0-0)
Telogen germinative units	0 (0-1)
Fibrous tracts	1 (0-2.5)

TABLE 2: Percentage of terminal follicles, vellus, anagen, catagen, telogen and undetermined follicles in the total number of cases and in each group

	Total	Group 1	Group 2
		%	
Terminal follicles	91	92.2	90.1
Vellus follicles	9	7.8	9.9
Terminal anagen follicles	90.6	94.5	87.4
Terminal catagen follicles	1.6	1.3	1.8
Terminal telogen follicles	6	2.8	8.7
Undetermined follicles	1.8	1.4	2.1

nine, with no clear information about the depth of the section.[13]

Two to four follicles clusters formed the follicular units. Some cases had follicular units with up to five terminal follicles. Units formed by less than two follicles usually display one or two fibrous tracts. This finding was in accordance with the classic description: a group of two to four terminal hairs and associated with one or two vellus hair.[2,3]

In each slice, 16.5 (12–23) follicles were identified, with no statistical difference between sexes. In males, the number of total follicles was higher in the vertex and frontal regions. The correlation of the finding and scalp areas has never been reported. Earlier studies demonstrated 20 to 40 follicles per section.[2,14] In a study by Steiner (1998), 35 follicles, in average, were found in each histological section of the occipital scalp.[13] The depth of the slice directly influences the number of follicles observed, since the more superficial sections display higher number of vellus follicles. In samples derived from males, the highest number of follicles observed was 27. In only three slices derived from females more than 30 follicles were observed. In a study by Sperling, 1996, an

excess of vellus follicles were found in the superficial dermis compared to the deep dermis, what could explain the discrepancies.[7]

In both groups assessed, over 90% of follicles observed were terminal (92.0% in Group 1 and 90.1% in Group 2), which is compatible with normal scalp. Vellus follicle bulbs are located in the dermis, many of which in are in its superficial region. Deep dermal slices do not encompass most of these follicles. The terminal: vellus ratio (T/V) was 15:1, not significantly different between groups or scalp regions. T/V ratio greater than 2:1 is considered normal.[16] Whiting, in 1996, and Steiner, in 1988, found a T/V ratio of 7:1 in rather superficial slices.[7,13]

Hair growth cycle causes continuous follicular changes. Histological assessment of hair growth cycles is done by the observation of the follicular structure changes. The establishment of criteria of hair growth cycle characterization in cross-sectional slices was essential for this assessment. The deep dermis, below the sebaceous duct, was the area chosen for study, since in there follicles with unharmed inner root sheaths are found, which allows for improved identification of the cycle phases.

In the dermis, 1.6% follicles were catagen, 6% telogen and 1.8% undetermined. Similar findings were reported, with 80 to 90% anagen, 1 to 2% cata-

TABLE 3: Median of structures observed in groups 1 and 2 (absolute numbers)

	Group 1	Group 2	p value
Total follicles	15.5 (10-20.7)	18 (12.2-24)	0.069
Follicular units	7 (6-8)	9 (7-9)	0.042
Terminal follicles	14 (9-18)	16 (12-22)	0.070
Vellus follicles	1 (0-2)	1 (0-2)	0.354
Anagen follicles	13 (8-16.5)	14 (9-20)	0.444
Catagen follicles	0 (0-0)	0 (0-0)	0.696
Telogen follicles	0 (0-1)	0 (0-3)	0.030
Undetermined follicles	0 (0-0)	0 (0-0)	0.417
Telogen germinative units	0 (0-0)	0 (0-1)	0.032
Fibrous tracts	1 (0-4)	1 (0-2)	0.139

TABLE 4: Median of structures observed in the frontal, vertex, occipital and parietal regions in Group 1 (absolute numbers)

	Frontal	Vertex	Occipital	Parietal	p value
Total follicles	18.5 (13.2-25)	22.5 (14.2-26)	12 (9-15.2)	13 (9-16.7)	0.029
Follicular units	8 (7-9)	8 (6-10)	7 (5.7-8)	6 (5.7-7.2)	0.086
Terminal follicles	16 (9.5-20.5)	24 (13-24.5)	11 (8.75-14.2)	13 (8-16.5)	0.107
Vellus follicles	2 (1-3)	1 (0-2)	0 (0-1.2)	1 (0-2)	0.100
Anagen follicles	16 (8.5-20)	22 (12-23.5)	10.5 (8-13.2)	13(8-14.5)	0.098
Catagen follicles	0 (0-0)	0 (0-1)	0 (0-0)	0 (0-0)	0.494
Telogen follicles	0 (0-1)	0 (0-1.5)	0 (0-1)	0 (0-0.2)	0.712
Undetermined follicles	0 (0-0)	0 (0-0.5)	0 (0-0.2)	0 (0-0)	0.864
Telogen germinative units	0 (0-0)	0 (0-1)	0 (0-0)	0 (0-0.2)	0.240
Fibrous tracts	3 (1-6)	3 (1.5-5.5)	1 (0-1.2)	1 (0-1.7)	0.030

gen and about 10%, telogen.[8] In the present study, women had significantly higher telogen follicles than men, unlike earlier trichogram results displaying higher number of telogen follicles in men.[16] In addition, telogen germinative units were significantly higher in women. Little is known about the function and importance of such structures that apparently encompass follicular germinative cells.[17-19] In clinical practice women complain more often than men of telogen effluvium. Common belief was that women were more likely to notice shedding, however some physiological mechanisms to explain a shorter cycle cannot be ruled out. Additional studies with larger sample sizes are necessary to clarify this finding.

Fibrous tracts were found in at least one scalp region of all cases. Such structures are usually found in the hypodermis and represent either latent follicles or old follicle remnants. In males, these structures were preferably seen in the frontal and vertex regions and, in some cases, more than five fibrous tracts were observed. Curiously, total follicles were also predominantly found in these regions in the same group. Fibrous tracts are undistinguishable from collapsed follicles found in alopecia areata and from those below the miniaturized follicles of androgenic alopecia. The vertex is the region more commonly affected by androgenic alopecia nonetheless all samples were obtained from subjects with normal hair density. This finding could be due to greater susceptibility to alopecia or to greater follicular activity in this region, which could explain the preference for hair rarefaction in this area. It is important to stress that fibrous tracts are found in normal scalps and its single finding in histopathological specimens cannot be assumed as diagnostic of alopecia of any sort. Whiting (1993) found in average 1.8 fibrous tract per superficial dermis section and 21 fibrous tracts in a sample of normal male vertex scalp as well.[14]

CONCLUSION

In normal scalp fragments of white adults aged 21 to 78 years, obtained with a 4-mm punch, 16.5 (12–23) total follicles were observed: 15 (10-20) terminal follicles and one (0-2) vellus follicle in 8 follicular units. At least one (0-2.5) fibrous tract was found in each slice. Regarding the hair cycle phases, 92% were anagen, 1.6% catagen and 6.2% telogen. Men had smaller number of follicular units, total follicles and telogen follicles than women; in men, total follicles, fibrous tracts were found in higher number in the frontal and vellus regions than in the other regions. ❑

TABLE 5: Median of structures observed in the frontal, vertex, occipital and parietal regions in Group 2 (absolute numbers)

	Frontal	Vertex	Occipital	Parietal	p value
Total follicles	20.5 (17.7-24.7)	19.5 (15.7-26.2)	17.5 (12.7-20.7)	12.5 (11.5-20.5)	0.200
Follicular units	9 (6-10)	9 (7.7-9.7)	8 (7-9.5)	6.5 (4-9.2)	0.241
Terminal follicles	18 (14.7-23.2)	18.5 (13-26)	16 (11-20.5)	12 (10-17)	0.264
Vellus follicles	2 (0-4)	1.5 (1-2.2)	1 (0-2)	1 (0-2)	0.500
Anagen follicles	16.5 (12.7-20)	16.5 (8.5-22.7)	15 (9.5-19.5)	10 (8.2-14.7)	0.341
Catagen follicles	0 (0-1.2)	0 (0-0.2)	0 (0-0.5)	0 (0-0)	0.344
Telogen follicles	0.5 (0-3.2)	1 (0-3)	0 (0-3)	0 (0-2)	0.579
Undetermined follicles	0 (0-0)	0 (0-0)	0 (0-0)	1 (0-1.2)	0.018
Telogen germinative units	0 (0-1)	0 (0-1)	1 (0-1.5)	0 (0-1)	0.813
Fibrous tracts	2 (0-2.5)	1 (0.7-2.5)	1 (0-2)	1 (0-1)	0.401

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