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# Utility of horizontal and vertical sections of scalp biopsies in various forms of primary alopecias

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## Abstract:

**BACKGROUND:** This study was performed to demonstrate the usefulness of horizontal and vertical sections of scalp biopsies in diagnosing various forms of primary alopecias and to highlight the importance of error-free grossing.

**MATERIALS AND METHODS:** A retrospective analytical review of 228 scalp biopsies was done, noting down the diagnostic histopathological features evident in horizontal and vertical sections of each cases. The idealness of the sections, especially horizontal section, was also analyzed.

**RESULTS:** Out of the 228 cases, 44 scalp biopsies were classified histologically as cicatricial alopecias and the remaining 162 as noncicatricial alopecia. 22 cases were inconclusive owing to erroneous grossing. We found horizontal sections to be more useful in cases of noncicatricial alopecias, whereas vertical sections proved superior in cicatricial alopecias.

**CONCLUSION:** Combining both horizontally and vertically sectioned scalp biopsies maximizes the diagnostic yield. When a single biopsy is submitted, the choice between horizontal and vertical section should depend on clinical diagnosis/suspicion.

## Key words:

Cicatricial alopecia, horizontal section, noncicatricial alopecia, vertical section

## Introduction

The microscopic evaluation of scalp biopsies forms an integral tool for the diagnosis of both cicatricial and noncicatricial alopecias.<sup>[1]</sup> However, rendering a histological diagnosis may, sometimes, prove daunting, especially if the grossing technique is improper. Substantial debate exists regarding the optimal sectioning technique of scalp biopsies. Both horizontal and vertical sections have their own potential advantages and disadvantages. However, when a single biopsy is received, the pathologist has to decide whether to section the specimen vertically or horizontally. Lack of clinical details further adds to the dilemma.

## Aims

In an effort to reconcile the conflicting views regarding horizontal versus vertical sectioning, this study was undertaken to illustrate the utility of vertically and horizontally sectioned scalp biopsies in various forms of primary alopecias and to analyze which is superior. We also intend to highlight the importance of precise grossing of scalp biopsies.

## Materials and Methods

A retrospective analytical study of scalp biopsies obtained from 228 patients attending Dermatology outpatient clinic attached to Bangalore medical college and research institute with the complaint of hair loss, from July 2011 to June 2014 was conducted. Two 4-mm punch biopsies, from appropriate sites, were obtained in each case

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for diagnostic purposes. These biopsies were received in 10% buffered formalin and were routinely processed. Grossing was carried out just before embedding. One of the biopsies was vertically sectioned, whereas the other biopsy was horizontally sectioned, approximately 1–1.5 mm below the epidermal surface. The grossed tissues were then embedded in paraffin with their cut surface down. About 3–4  $\mu$  thick microtomed sections were stained with hematoxylin and eosin (H and E). Both cicatricial and noncicatricial alopecias cases were included in the study. Cases, where only a single punch biopsy was received, were excluded.

All the H and E slides available, including the serial sections, were retrieved from the archives and jointly reviewed by two pathologists, along with the accompanying pathology request forms. Demographic data, clinical details, and provisional clinical diagnoses were also collected. The diagnostic histomorphologic features that were evident in horizontal and vertical sections of each case were noted down in detail. The utility of each section was analyzed qualitatively. The idealness of the sections, especially the horizontal sections, was also critically assessed.

## Results/Observation

Out of the 228 biopsies analyzed, cases of noncicatricial alopecia ( $n = 162$ ) outnumbered the cases of cicatricial alopecia ( $n = 44$ ). Among the nonscarring ones, alopecia areata (AA) topped the list. Table 1 depicts the distribution profile of the cases. In Table 2, we have mentioned the utility of vertical and horizontal sections in arriving at a histological diagnosis as experienced by us during the study. We also assessed the idealness of the horizontal sections in each case. Of the 228 cases, 156 cases (68.4%) had ideally cut horizontal sections displaying the pilosebaceous units [Figure 8a]. 28 cases (12.3%) were dissected at the level of subcutis, and the remaining 44 cases (19.3%) were obliquely sectioned [Figure 8b and

c]. We could not arrive at a final diagnosis in 22 (9.6%) cases owing to grossing errors.

## Discussion

Several studies have been conducted to address the problem of horizontal versus vertical sectioning of scalp biopsies, and it is a well-known fact that both the techniques have their inherent pros and cons. Du *et al.*<sup>[2]</sup> meta-analyzed the published literature in this context and found that no significant difference exists between horizontal and vertical sectioning techniques in the diagnosis of alopecia and suggested future well-designed studies in this area. Elston *et al.*,<sup>[3]</sup> Sinclair *et al.*,<sup>[4]</sup> and Stefanato<sup>[5]</sup> concluded that combining both horizontal and vertical sections maximizes the diagnostic yield when compared to either section alone. Our findings also ascertain the same, that is, if two biopsies are available, the best way of processing is to section one of the biopsies horizontally and the other vertically.

A 2001 consensus meeting<sup>[6]</sup> acknowledged the merits of combined horizontal and vertical sections in various forms of alopecia and added that horizontal sectioning is to be preferred if a single biopsy is received. We suggest, whenever a single scalp biopsy is submitted, the decision should be based on the clinical diagnosis. We found horizontal sections to be more useful in cases of noncicatricial alopecias, whereas vertical sections conferred a diagnostic advantage in cicatricial alopecias. This is in concordance with the study by Elston *et al.*<sup>[7]</sup> Özcan *et al.*<sup>[8]</sup> opined that it is preferable to have horizontal sections in cases of noncicatricial alopecia, vertical sections in cases of suspected lichen planopilaris (LPP) and either type of sections in cicatricial alopecia when LPP is clinically ruled-out.

We observed that the vertically sectioned scalp biopsies provide the benefit of viewing full thickness of skin and complete hair profile, from root to top. Interface changes can be well made out. Subcutaneous pathology, if any, is not missed. The degree of peribulbar inflammation and fibrosis can be better assessed. Grossing is much easier as compared to horizontal sections. The major drawback of vertical sections is that the follicular counts and ratio cannot be assessed. Furthermore, vertical sections show only 10%–20% of the total number of follicles and the probability of sampling error is quite high. Serial sectioning may be required to find the 'diagnostic follicle' which adds to the laboratory expenses and increases the turnover time. Bathish *et al.*<sup>[9]</sup> found that serial vertical sectioning of scalp biopsies increases the diagnostic yield substantially, in noncicatricial as well as cicatricial alopecias. Tandon *et al.*<sup>[10]</sup> studied 27 cases of LPP and found vertical sections to provide sufficient histological clues.

**Table 1: Distribution of cases of primary alopecias**

Diagnosis	Number of cases (%)	Male:female ratio	Median age (years)
Noncicatricial alopecias ( $n=162$ )			
Alopecia areata	97 (59.9)	0.7:1	32
Telogen effluvium	25 (15.4)	1:4	40
Androgenetic alopecia	33 (20.4)	0.4:1	36
Trichotillomania	7 (4.3)	0.17:1	26
Cicatricial alopecias ( $n=44$ )			
Lichen planopilaris	21 (47.8)	0.3:1	41
Discoid lupus erythematosus	18 (40.9)	0.4:1	38
Pseudopelade of Brocq	4 (9.1)	0.33:1	58
Folliculitis decalvans	1 (2.2)	1 male	22
Inconclusive ( $n=22$ )			

**Table 2: Diagnostic utility of vertically and horizontally sectioned scalp biopsies in noncicatricial and cicatricial alopecia**

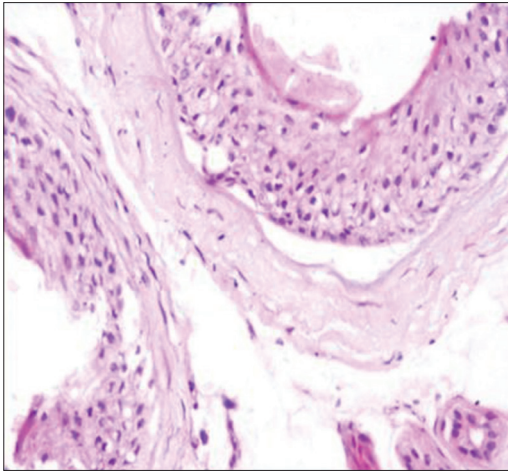
Diagnosis	Utility of vertical sections	Utility of horizontal sections	Remarks
Noncicatricial alopecias			
Alopecia areata (n=97)	Perifollicular fibrosis and mononuclear cell infiltrate could be well appreciated on both horizontal and vertical sections although vertical sections provided better picture of the degree of fibrosis and inflammation [Figure 1] The hallmark peribulbar lymphoid infiltrate was evident on vertical sections	Perifollicular fibrosis and mononuclear cell infiltrate [Figure 2] Derivation of follicular counts and ratios	Both sections were complimentary to each other
Telogen effluvium (n=25)	-	Increased number of telogen and terminal-to-vellus hair ratio could be demonstrated only on horizontal sections [Figure 3]	Vertical sections yielded minimal diagnostic information
Androgenetic alopecia (n=33)	-	Derivation of follicular counts and ratios such as increased number of vellus hair, terminal: Vellus follicle <4:1, decreased anagen-to-telogen ratio, decreased anagen-to-catagen ratio Variation in diameter of hair follicles due to miniaturization [Figure 4]	We could derive at a diagnostic conclusion based only on the findings noted in horizontal sections
Trichotillomania (n=7)	Melanin pigment cast, perifollicular/intrafollicular hemorrhage, dysmorphic, follicles, and folliculocentric hemosiderin was appreciated in both the sections [Figures 5 and 6]	Melanin pigment cast, perifollicular/intrafollicular hemorrhage and folliculocentric hemosiderin was appreciated in both the sections Additional information regarding follicular counts and ratio (increased catagen: Telogen)	Horizontal and vertical sections were equally informative
Cicatricial alopecias			
Lichen planopilaris (n=21) and discoid lupus erythematosus (n=18)	The diagnostic dermoepidermal junctional changes such as band-like lymphocytic infiltrate, basal layer vacuolar degeneration were easily discernable [Figure 7]		Vertical sections were advantageous
Pseudopelade of Brocq (n=4)	Prominent perifollicular fibrosis was visualized in the vertical sections Minimal perifollicular lymphocytic inflammation	Significant reduction in the number of hair follicles was noted on horizontal sections. One case had no hair follicles at all Minimal perifollicular lymphocytic inflammation	Both sections were complimentary to each other
Folliculitis decalvans (n=1)	The diagnostic perifollicular mixed inflammatory infiltrate was more evident in the vertical section as compared to horizontal section		Vertical sections proved advantageous

The potential utility of horizontal sections lies in deducing follicular counts, density and ratio. It allows visualization of all the hair follicles present in the biopsy core. Follicular growth dynamics and hair morphometry can be studied. Hence, we found horizontal sections to be crucial for diagnosis in cases of Telogen effluvium and androgenetic alopecia where follicular ratios are important. In cases of Discoid lupus erythematosus and LPP, we failed to assess the dermoepidermal junction on horizontal sections; and hence, it was of minimal diagnostic utility in these cases. Another disadvantage of horizontal sections is

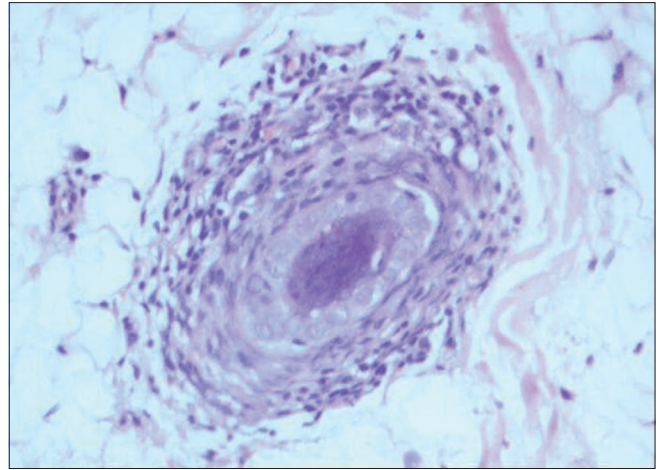
that the grossing is cumbersome and demands some degree of expertise and experience.

We studied 97 cases of AA and noted horizontal and vertical sections to be complementary to each other. If a single biopsy is submitted, then we prefer horizontal sectioning of the specimen. Singh *et al.*<sup>[11]</sup> could diagnose 100% cases of AA by horizontal sections alone and 93.3% cases by vertical sections alone. They found horizontal sections to be superior as it provided a better assessment of the histological features of AA. Jameel *et al.*<sup>[12]</sup> also found horizontally sectioned scalp biopsies

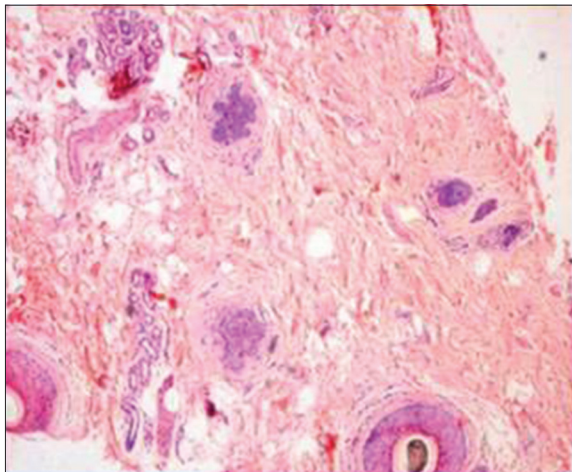




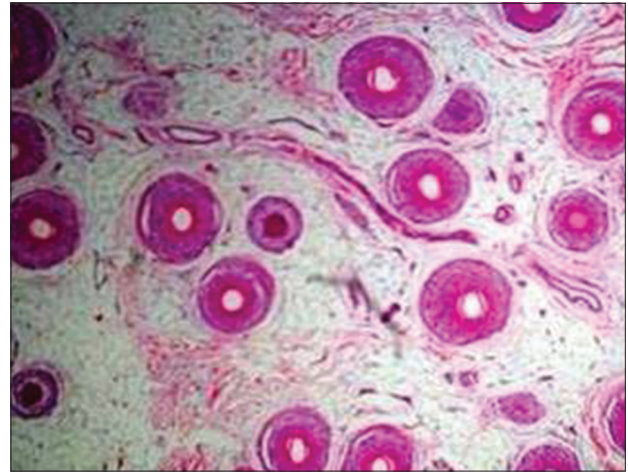
**Figure 1:** Perifollicular fibrosis seen on vertical section in a case of alopecia areata (H and E, ×400)



**Figure 2:** Perifollicular mononuclear cell infiltrate seen on horizontal section in a case of alopecia areata (H and E, ×400)



**Figure 3:** Horizontal section showing increased number of telogen in a case of telogen effluvium (H and E, ×100)



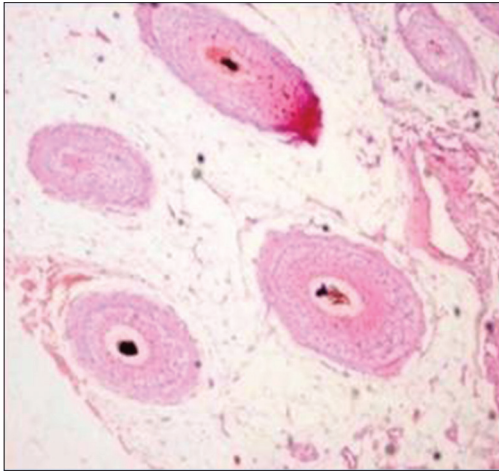
**Figure 4:** Miniaturized hair follicles seen on horizontal section in a case of androgenetic alopecia (H and E, ×100)

to be beneficial in diagnosing AA as well as predicting the disease stage.

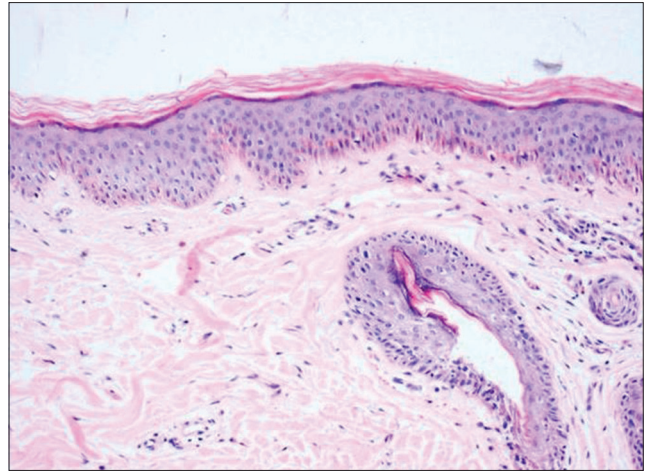
In the present study, we assessed the idealness of the horizontal sections in all the cases. Out of 228 cases, 72 (31.6%) cases were inappropriately grossed, that is, either obliquely sectioned (44 cases) or cut at the level of subcutis (28 cases). Serial sectioning had to be done in these 28 erroneously grossed cases to reach the pilosebaceous apparatus for microscopic evaluation, whereas the 44 obliquely sectioned ones had to be reoriented, reblocked, and then resectioned. These factors led to tissue loss, increased the turnover time and decreased the cost-effectiveness. A conclusive diagnosis was not possible in 22 (9.6%) cases due to improper grossing. Hence, we lay emphasis on efficient grossing techniques of the scalp biopsies, especially the horizontal ones.

Headington<sup>[13]</sup> demonstrated that “the best area to bisect a punch biopsy for the horizontal section is 1 mm above the

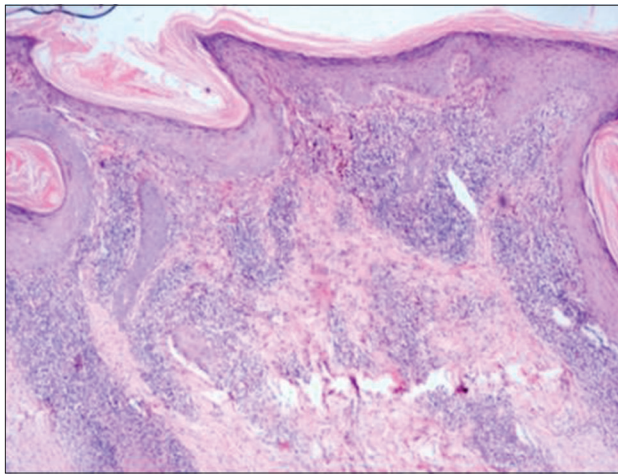
dermal-subcutaneous junction.” In our practice, we find it difficult to pinpoint the dermal-subcutaneous junction in a processed specimen. It is equally difficult to make a clean cut at the aforementioned area in an unprocessed biopsy sample. Frishberg *et al.*<sup>[14]</sup> advocated the idea of slicing the biopsy specimen into 3 or 4 horizontal sections, and embedding all the slices with cut surface down in the same cassette. We feel that this technique demands proper embedding and recuts will lead to critical tissue loss. Another interesting technique, “the Tyler technique,” involves first vertically sectioning the entire specimen into two halves, followed by horizontal sectioning of one of the halves.<sup>[15]</sup> The demerit of this method is that it limits the evaluation of hair density and ratio as only half of the actual biopsy is studied microscopically. In the “St. John’s Protocol,” two 4-mm punch biopsies of the scalp are procured. In suspected cases of cicatricial alopecias, one biopsy is bisected horizontally and the other vertically whereas in cases of noncicatricial alopecias, both the biopsies are transected horizontally 1 mm below the



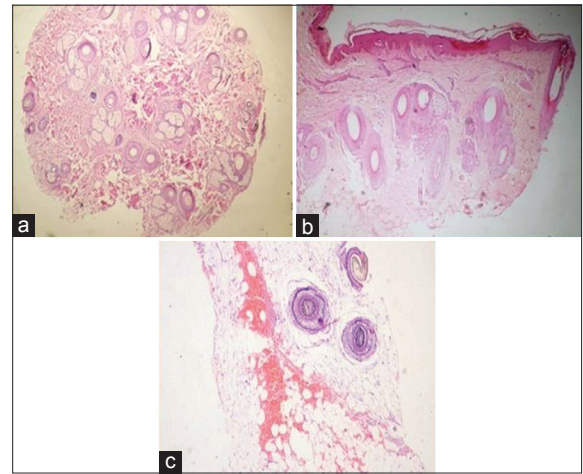
**Figure 5:** Melanin pigment cast noted on horizontal section in a case of trichotillomania (H and E, ×100)



**Figure 6:** Dysmorphic hair follicle noted on horizontal section in a case of Trichotillomania (H and E, ×100)



**Figure 7:** Characteristic features of discoid lupus erythematosus noted on vertical section (H and E, ×100)



**Figure 8:** (a) An ideal horizontal section displaying the pilosebaceous units (H and E, ×40). (b) An obliquely cut scalp biopsy (H and E, ×40). (c) A scalp biopsy sectioned at the level of subcutis (H and E, ×100)

epidermis.<sup>[3]</sup> After employing this very protocol, Taylor *et al.*<sup>[16]</sup> observed a significant increase in the diagnostic yield of scalp biopsies.

In our institution, we usually receive two scalp biopsies. One of the biopsies is bisected vertically, making sure that the cut is made parallel to the direction of hair growth. The other biopsy is cut horizontally, following the technique proposed by Solomon.<sup>[17]</sup> We dissect the scalp biopsies 1–1.5 mm below the epidermal surface after overnight processing, just before the embedding step. We find this procedure quite feasible, easy, and rewarding. Needless to say, special attention must be given to the final microtomy step to avoid tangential cuts and obtain a perfect horizontal section.

Recently, the new “Hovort technique”<sup>[18]</sup> is gaining popularity, wherein the scalp biopsy is transected approximately 1 mm below the epidermal surface to obtain an “epidermal disc.” This epidermal disc is subjected to vertical sectioning in

conventional fashion, whereas the remaining lower portion of the biopsy is sliced for horizontal sections. Kolivras and Thompson<sup>[19]</sup> employed the Hovort technique and found the procedure to improve and simplify the diagnostics. However, more studies need to be conducted to affirm the efficiency and reproducibility of this newer method.

## Conclusion

We found that the combination of two punch biopsies (one horizontally and other vertically sectioned) is ideal for microscopic diagnosis in cases of alopecia. Whenever a single biopsy is submitted, grossing technique should be dictated by the clinical diagnosis. Vertical sectioning is to be preferred in noncicatricial alopecias and horizontal sectioning to be preferred in cicatricial alopecias. Furthermore, utmost care should be taken to make grossing error-free to avoid serial sectioning and/or inconclusive diagnosis.



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## Conflicts of interest

There are no conflicts of interest.

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