# Horizontal and Vertical Sections in Alopecia: Study of Fifty Cases with their Clinicopathological Correlation

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Abstract: <u>Objective</u>: To compare the histopathological findings in horizontal and vertical sections in cases of alopecia with their clinicopathological correlation. <u>Materials and methods</u>: A study of 50 patients was conducted to study the histopathological findings in horizontal and vertical sections in cases of alopecia along with their clinicopathological correlation. <u>Results</u>: In our study, major causes of alopecia were lichen planopilaris (22%), discoid lupus erythematosus (16%), alopecia areata (16%), Pseudopelade of Broq (14%), other causes included folliculitis, psoriasis scalp, nevus sebaceous, spongiotic psoriasiform dermatitis, trichotillomania, post inflammatory dermatoses, lupus profundus, acne keloides, lichen simplex chronicus and solar keratosis. In our study, the histopathological features of alopecia were perifollicular lymphocytic infiltrate in 35% patients, perifollicular fibrosis in 32% patients, hyperkeratosis in 20% patients, necrotic keratinocytes in 18% and basal cell vacuolization in 16% patients. Pigment casts were seen in 2% of cases. <u>Conclusion:</u> Lichen planopilaris, Discoid lupus erythematosus were diagnosed better in vertical sections. Alopecia areata was diagnosed better on horizontal sections. Combined vertical and horizontal sections increased the chances of reaching a correct diagnosis.

Keywords: Alopecia, Discoid lupus erythematosus, Lichen planopilaris

#### 1. Introduction

Alopecia refers to loss of hair. They are usually classified as Non cicatricial and cicatricial, depending on their relative propensity to culminate in permanent hair loss. Scarring alopecia is further classified as primary or secondary. In primary scarring alopecia, the hair follicle is the prime target of destruction as opposed to secondary scarring alopecia in which follicles are lost as a result of collateral damage.

Clinical examination indicates whether the alopecia is noncicatricial (follicular ostia preserved) or cicatricial (ostia are lost). Some alopecia begin as non-scarring and progress to scarring in later stages, underscoring the fact that there is considerable overlap between the two.

Histopathologic examination of scalp biopsies remains the single most important and potent tool in clarifying the etiology.

A punch biopsy or incisional biopsy extending into fat to include terminal hair bulbs is necessary for proper evaluation of alopecia. At least a 4-mm punch should be used in order to obtain a sufficient number of hairs for study.<sup>1</sup>

The manner of sectioning a punch biopsy is a matter of debate. In vertical sectioning, the cylinder of tissue is bisected longitudinally which is a usual way to section any skin biopsy. In horizontal or transverse sectioning, the skin cylinder is bread loafed.<sup>2</sup>

Horizontal sections enable visualization of all the follicles in a specimen while vertical sections demonstrate only 10-15% of them. Transversely sectioned scalp biopsies allow for rapid evaluation of hair density and follicular units. The disadvantage of transverse sections is inability to properly evaluate the epidermis.<sup>3</sup>

The Ho-Vert technique yields vertical sections as well as horizontal sections that maximizes the histopathological information obtained.<sup>4</sup>

Histopathological diagnosis of scalp biopsies is still a challenging area in dermatopathology. The present study aimed to study both horizontal and vertical sections in cases of alopecia and their clinicopathological correlation.

#### 2. Materials and Methods

This study was conducted in Department of Pathology, Government Medical College, Amritsar after approval from Institutional Thesis and Ethical Committee. 50 cases were included after taking informed consent of the patient. From each patient presenting with clinical diagnosis of alopecia in Department of skin and VD of our institute, a 4mm punch biopsy of scalp was taken. History and clinical examination of each patient were recorded. Each scalp biopsy was cut both horizontally and vertically. Sections were cut and stained with Hematoxylin and Eosin. Histopathological features in both types of sections were recorded in each case.

#### 3. Results

The cases showed maximum incidence in age group of  $\geq$ 50 years comprising 16 (32%) of cases followed by age groups 21-30yrs (22%). The youngest patient in the present study was 9 yrs old while the eldest being 71 yrs old.

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 Table 1: Showing Age Wise Distribution

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Age group	No. of cases	Percentage (%)		
0-10 yrs	1	2		
11-20 yrs	8	16		
21-30 yrs	11	22		
31-40 yrs	8	16		
41-50 yrs	6	12		
>50 yrs	16	32		
Total	50	100		

Males outnumbered females (54% and 46% respectively) with male to female ratio of 1.17:1.

Table 2: Showing Sex Distribution			
Sex	No. Of cases	Percentage (%)	
Male	27	54	
Female	23	46	
Total	50	100	

30% of cases were from rural area while 70% from urban area.

**Table 3:** Showing Rural/Urban Distribution

Status	No. of cases	Percentage (%)
Rural	15	30
Urban	35	70
Total	50	100

Maximum numbers of patients presented with clinical diagnosis of Lichen planopilaris (LPP). This was followed by Psuedopelade, alopecia areata and Discoid lupus erythematosus (DLE). Other diagnoses were as shown in table below.

Table 4: Showing Clinical Diagnosis for the Scalp Lesions

Clinical diagnosis	No. of cases	Percentage (%)
LPP	13	26
Psuedopelade	9	18
Alopecia areata	8	16
DLE	8	16
Psoriasis scalp	2	4
Post inflammatory dermatoses	2	4
Nevus sebaceous	2	4
Trichotillomania	1	2
Spongiotic psoriasiform dermatitis	1	2
Prurigo nodularis	1	2
Acne keloides	1	2
Lupus panniculitis	1	2
Actinic keratosis	1	2
Total	50	100

92% of patients presented with multiple patches of hair loss, 4% with single patch and 4% with diffuse hair loss. Of the 50 scalp biopsies taken, on histopathology 11cases were typified as LPP, 8 each as Alopecia areata and DLE. Other diagnoses were as shown in table below. 
 Table 5: Showing spectrum of histologic diagnosis for the scalp biopsies

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Histological diagnosis	No. of cases	Percentage
		(%)
LPP	11	22
Alopecia areata	8	16
DLE	8	16
Psuedopelade	7	14
Psoriasis scalp	3	6
Folliculitis	3	6
Spongiotic psoriasiform dermatitis	2	4
Nevus sebaceous	2	4
Trichotillomania	1	2
Post inflammatory dermatoses	1	2
Lupus profundus	1	2
Acne keloides	1	2
Lichen simplex chronicus	1	2
Solar keratosis	1	2
Total	50	100

In our study, the histopathological findings in case of LPP were orthohyperkeratosis, mild acanthosis, follicular plugging, lymphoplasmacytic infiltrate at the dermo-epidermal junction and around hair infundibula, pigment incontinence and necrotic keratinocytes.



Figure 1: Diffuse hair loss in LPP



Figure 2: Lymphoplasmacytic infiltrate around hair infundibulum in LPP (H&E, 40X)

The histopathological findings in most cases of DLE were orthohyperkeratosis, keratoticplugging, focal thinning of epidermis, necrotic keratinocytes, thickening of basement membrane, basal vacuolization and squamatization.



Figure 3: Basal vacuolization and basement membrane thickening in DLE (H&E,100X)

Cases of alopecia areata on histopathology in vertical section revealed normal looking epidermis and mild superficial perivascular lymphocytic infiltrate. Transverse sections revealed peribulbar lymphocytic infiltrate.



**Figure 4:** Minimal peribulbar lymphocytic infiltrate in alopecia areata (H&E, Horizontal section,400X)

Histopathology in cases of Psuedopelade revealed orthohyperkeratosis, mild acanthosis, absence of hair follicles, fibrous tracts, mild perivascular lymphocytic infiltrate and pigment incontinence.



Figure 5: Multiple patches of hair loss in psuedopelade



Figure 6: Absence of hair follicle and formation of fibrous tracts in Psuedopelade(H&E,100X)

Few hair follicles in catagen phase and melanin cast inside the follicle were noticed in case of trichotillomania.



Figure 7: Distorted hair follicles in catagen phase along with melanin cast in Trichotillomania (H&E,100X)

A case diagnosed as lupus profundus revealed normal looking epidermis, superficial and deep perivascular and periadnexal infiltrate and dilated blood vessels.In case of and folliculitis, infundibular area interstitial lymphoplasmacytic infiltrate along with few neutrophils seen. In a case diagnosed as Acne keloides, histopathologic features were interface dermatitis, perivascular and perifollicular inflammatory infiltrate composed of lymphocytes, plasma cells, histiocytes and occasional eosinophils . Hair follicle destruction and fibrosis were also noticed. No fungus or demodex was seen. The histopathological findings in case of solar keratosis revealed chronic inflammatory infiltrate in dermis.

Clinicopathological concordance was seen in 82% cases.

#### 4. Discussion

In our study, maximum number of patients with alopecia were  $\geq$ 50 years (32%). Males outnumbered females with a ratio of 1.17:1. 92% patients had multiple patches of alopecia. In our study, the maximum number of cases of alopecia were due to LPP (22%) followed by DLE and

Alopecia areata (16% each) and Pseudopelade of Broq (14%). Less frequent causes were 6% each as folliculitis and psoriasis scalp, 4% each as nevus sebaceous and spongiotic psoriasiform dermatitis, 2% each as trichotillomania, post inflammatory dermatoses, lupus profundus, acne keloides, lichen simplex chronicus and solar keratosis.

Table 6: Most common cause of alopecia in different

studies			
Study	Year	Most common cause	%
Whiting et al <sup>7</sup>	2001	Psuedopelade of Broq	40.6
Tan et al <sup>8</sup>	2004	Discoid Lupus erythematosus	33.9
Puri N et al <sup>5</sup>	2013	Lichen Planopilaris	27.5
Thakur BK et al <sup>6</sup>	2015	Discoid Lupus erythematosus	41.6
Present study	2016	Lichen Planopilaris	22

The commonest histopathological feature of alopecia was perifollicular lymphocytic infiltrate seen in 35% patients, perifollicular fibrosis in 32% patients, hyperkeratosis in 20% patients, necrotic keratinocytes in 18% and basal cell vacuolization in 16% patients. Pigment casts were seen in 2% of cases.

In 2013, Puri N et al studied the finding of a bandlike fibrotic thickening of papillary dermis and fibrotic tracts at sites of destroyed follicles in old lesions while most early lesions of lichen planopilaris showed a focally dense band like perifollicular lymphocytic infiltrate at the level of infundibulum.<sup>5</sup>

In 2015, Thakur BK et al noticed in one case of LPP there was a lichenoid infiltrate around the infundibulo-isthmic segment of one follicle in addition to fibrous tracts replacing follicles.<sup>6</sup> In all the others, absence of inflammation, absence of sebaceous epithelium, and atrophy of the bulge area occurred. Fibrous tracts replacing the follicle, with or without colloid bodies, were observed. The basal membrane was not thickened on PAS-stained sections. In a study by Puri N et al in 2013, the histopathological findings in case of Discoid lupus erythematosus were a lymphocytic infiltrate mainly directed to the mid portion of the follicle and a normal anagen: telogen ratio.<sup>5</sup>

In a study by Thakur BK et al in 2015, characteristic histopathologic findings of the late phase of Discoid lupus erythematosus included hyperkeratosis, horn plugs, atrophy of the malpighian layer, slight vacuolar degeneration of the basal layer, and fibrous tract replacing the follicles.<sup>6</sup> A thickened basal membrane could be seen in 58.8% of the cases on PAS –stained sections.

# 5. Conclusion

Vertical sectioning of a scalp biopsy enables the dermal– epidermal junction to be better visualized as in LPP and DLE, although fewer follicles can be evaluated by this method. Horizontal sectioning enables the entire follicular unit to be assessed so that focal pathology can be identified as in alopecia areata. The use of combined vertical and horizontal sections in a single scalp biopsy can increase the chances to arrive at a definite diagnosis.

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