INTRODUCTION

Conventionally, histopathologists have relied entirely on vertical sections (VSs) of most tissues, except nerve, skeletal muscle and blood vessel, for diagnostic purposes. Although VS are satisfactory for the study of dermatologic diseases, their utility in the evaluation of alopecia is questionable. The hair follicle lies obliquely in relation to the epidermis. As a result of this, the follicle is often missed, cut tangentially, or incomplete. In 1984, Headington described and illustrated the morphology of hair follicles in transverse sections (TSs) of the scalp. He indicated that the morphometric analysis of TSs would provide information that could be used to study hair follicles in health, disease, and assess response to therapy. Subsequent workers have shown that horizontal scalp sections are the preferred method for evaluating all forms of hair loss.

The histologic diagnosis of alopecia can be challenging. To maximize the diagnostic yield, multiple biopsies may be necessary. Elston have also demonstrated that combined vertical and horizontal sections increase the diagnostic yield.

When only a single biopsy specimen is submitted, the pathologist is faced with a choice. Either VS or TS may be...
performed. A consensus from a working group of alopecia experts stated that combining VS and TS are optimal, but suggested that when a single biopsy is submitted it should always be sectioned transversely. VSs may be superior for some forms of alopecia (especially scarring alopecia) and TSs may be superior for other forms (especially nonscarring alopecia).

The purpose of this study is to assess the histological changes in the various types of alopecia and to evaluate the impact of combining vertical and horizontal sections in a single 4 mm scalp biopsy specimen by a new tissue processing technique.

MATERIALS AND METHODS

Scalp biopsies were received in the Histopathology Department from 100 patients with alopecia, from July 2006 to July 2016. Except for 2 biopsies that were 3 mm in diameter, the rest were 4 mm punch biopsies, taken from sites where the disease was active, yet not too advanced. This was usually at the periphery of the lesion. They were sent in 10% formalin and processed in an automatic tissue processor before paraffin embedding. Nine VS were taken and mounted on 3 glass slides [Figure 1] and stained with H and E, Verhoeff van Gieson (VVG) or Alcian blue/periodic acid-Schiff (AB/PAS) as required. The block was then melted and the tissue reembedded with the subcutaneous tissue facing downward and the epidermal aspect toward the technician to obtain horizontal sections [Figure 2]. On a microtome (LEICA RM22255) with a disposable blade until the tissue was exhausted and every 25th section was mounted on glass slides and each slide had 4 levels, for example, 25, 50, 75, and 100 and stained with H and E. Unmounted sections were saved in the refrigerator between filter paper sheets until reporting was completed [Figure 3]. The steps are depicted in line diagram [Figure 4].

RESULTS

One hundred cases were included in the study. The distribution of these cases is depicted in pie diagram [Figure 5]. The group of nonscarring alopecia consisted of alopecia areata [Figure 6] 22 cases, androgenetic alopecia 11 cases, trichotillomania 8 cases, and 2 cases each of tinea capitis and psoriatic alopecia.

Among the 44 cases of primary scarring alopecia, there were 19 cases of lichen planopilaris (LPP) [Figure 7] and 8 cases of discoid lupus erythematosus (DLE) [Figure 8]. 2 cases showed overlap features of LPP and DLE. 6 cases of end-stage scarring alopecia, 3 cases of folliculitis decalvans, 2 cases each of acne keloidalis, keratosis follicularis spinulosa decalvans, and fibrosing alopecia in a pattern distribution were diagnosed.

There were 11 cases of secondary scarring alopecia and consisted of 3 cases of scleroderma, 2 cases of lichen sclerosis et atrophicus and 1 case of atypical necrobiosis lipoidica. The unusual causes of secondary scarring alopecia included 2 cases each of nevus sebaceous, Vitamin D associated alopecia and 1 case of pemphigus vulgaris.

The diagnosis of nonscarring alopecia (n = 45) could be made exclusively on TS and VS in 44 (98%) cases and 33 cases (73.3%), respectively. When combining the TS and VS, the diagnosis was possible in all the cases (100%). The availability of more number of hair follicles, better visualization of the follicular variation, miniaturization and ratio of anagen and telogen hair follicles were possible only in the TS.

Of the 55 patients with scarring alopecia (both primary and secondary), 39 (70.9%) and 30 (54.5%) patients were diagnosed solely by TS and VS, respectively. The study of both the sections was necessary to arrive at a diagnosis in 53 out of 55 patients. The remaining two patients showed overlap features of LPP and DLE which underscores the importance of immunofluorescence study. TSs were superior in cases of lupus erythematosus, LPP with focal follicular involvement, fibrosing alopecia in a pattern distribution, Vitamin D associated alopecia, and keratosis follicularis spinulosa decalvans. Stelae and blank spots were more prominent in TSs. Interface changes and lupus panniculitis were better demonstrated in VSs. The histological types of primary scarring, secondary scarring including unusual causes and non scarring alopecia are listed in tables 1, 2 and 3 respectively.

DISCUSSION

Histopathologic examination is routinely done for the diagnosis of many dermatologic diseases. Although hair loss is distressing and not infrequent, scalp biopsies form a minor component of specimens received in the histopathology laboratory. For decades, the microscopic examination of hairs that have been pulled, plucked, or spontaneously shed have been used for the evaluation of hair loss. This is useful in diseases of the hair shaft and to an extent in nonscarring alopecia, but of limited value in
most cases of alopecia. Attempts to study the histological changes in alopecia, by conventional VS, were disappointing.

Figure 1: (a) Paraffin block for vertical sections (b) 4 slides, 3 vertical sections on each. Thickness of section = 4 µ

Figure 2: (a) Melting the paraffin block (b and c) Tissue reembedded with the subcutaneous tissue facing downward and the epidermal aspect toward the technician

Take 3 sections/slide X 3 slides
(First one slide with H and E, the other 2 slides can be used for special stains)
Melt the block
Re-embed the tissue with subcutaneous facing downwards (cutting surface) and the epidermal aspect towards the technician
Cut serial sections until the tissue is exhausted
Mount every 25th section in a glass slide, 4 sections/slide
(eg. first slide contains 1st, 25th, 50th & 75th sections, second slide contains 26th, 51st, 76th & 100th sections... etc.)
Stained with H & E. For a 4 mm punch biopsy 4 to 5 slides are expected
Save un-mounted (in-between) sections in a refrigerator (4°C) between the filter paper. These sections can be used for further examination with other H & E or special stains when required.

The unused sections can be saved for research purposes

Figure 3: (a) Serial 4 µ sections were cut until the tissue was exhausted. (b and c) Unmounted sections were saved in the refrigerator between filter paper sheets until reporting was completed

Figure 4: Line diagram representing the embedding and sectioning steps for vertical sections and transverse sections, scalp biopsy

Figure 5: Pie diagram depicting histological types of alopecia

Figure 6: Alopecia areata – (a) peribulbar inflammation, vertical sections (H and E, ×10). (b) – nanogen hair follicle, transverse sections (H and E, ×40)
as no useful information was forthcoming and no clinical correlates could be established. Both dermatologists and pathologists groped for a diagnostic approach to the problem of alopecia.

In 1984, Headington established the morphology of hair follicles in TSs of the scalp. Subsequent workers have shown that horizontal scalp sections are the preferred method for evaluating all forms of hair loss.\[^{5-8}\]

The main advantage of VS is that the dermoepidermal junction, papillary dermis, and subcutis are better demonstrated. Interface dermatitis, lupus panniculitis, miniaturized hairs, and trichomalacia are more obvious.

The advantages of TSs, in addition to the higher yield of follicles are the rapid, easy, and accurate assessment of follicular density, follicle and shaft diameters, anagen, telogen, terminal, and vellus hairs. Since each sections have their own advantages, many authors\[^{2,3}\] have clearly demonstrated that the increased diagnostic utility of combining the vertical and horizontal sections.

Clinicians usually perform two biopsies; one for vertical and the other one for transverse sectioning. Due to difficulty in convincing the patient for two biopsies and to limit the morbidity, the clinicians started doing a single biopsy. In that situation, the drawbacks are the need to use a different protocol for specimen preparation and familiarity with the transverse microanatomy of the scalp.

There are several ways to slice the single scalp biopsy specimen for TSs.\[^{9-11}\] However, the novel HoVert technique is an effective method in diagnosing alopecia by combining the advantages of both VS and TS in a single biopsy sample.\[^{12}\]

When the study commenced, we were unfamiliar with the transverse microanatomy of the scalp in health and disease. Since we are accustomed to VS, we thought it would be better to have a few VS, in all biopsies for orientation. This was found to be particularly useful in the detection of interface dermatitis in primary scarring alopecia and for

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Table 1: Histological types of primary scarring alopecia

| Histological diagnosis                              | Number of cases (n=44) |
|-----------------------------------------------------|------------------------|
| Lichen planopilaris                                  | 19                     |
| Discoid lupus erythematosus                          | 8                      |
| End stage scarring alopecia                          | 6                      |
| Folliculitis decalvans                               | 3                      |
| Keratosis follicularis spinulosa decalvans           | 2                      |
| Acne keloidalis                                      | 2                      |
| Overlap features of LPP + DLE                        | 2                      |
| Fibrosing alopecia in a pattern distribution         | 2                      |

LPP – Lichen planopilaris, DLE – Discoid lupus erythematosus

Table 2: Histological types of secondary scarring and unusual causes of alopecia

| Histological diagnosis                          | Number of cases (n=11) |
|-------------------------------------------------|------------------------|
| Scleroderma                                      | 3                      |
| Lichen sclerosus et atrophicus                   | 2                      |
| Necrobiosis lipoidica                            | 1                      |
| Unusual causes                                   |                         |
| Nevus sebaceous                                  | 2                      |
| Vitamin D associated alopecia                    | 2                      |
| Pemphigus vulgaris                               | 1                      |

Table 3: Histological types of nonscarring alopecia

| Histological diagnosis                          | Number of cases (n=45) |
|-------------------------------------------------|------------------------|
| Alopecia areata                                  | 22                     |
| Androgenetic alopecia                            | 11                     |
| Trichotillomania                                 | 8                      |
| Tinea capitis                                    | 2                      |
| Psoriatic alopecia                               | 2                      |

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Figure 7: Lichen planopilaris – (a) Subepidermal wedge-shaped scar, vertical sections, (Verhoeff van Gieson, x10). (b) Lichenoid inflammation around hair follicle, transverse sections (H and E, x40)

Figure 8: Discoid lupus erythematosus – (a) Basement membrane thickening, vertical sections (Alcian blue/periodic acid-Schiff, x10) (b) Perieccrine inflammation, transverse sections (H&E, x40)
demonstrating the subepidermal wedge-shaped scars of LPP. Since we had 2 more unstained VS, special stains such as AB-PAS (mucin) and VVG (elastic) can also be done when required, apart from the H and E section.

The diagnosis of scarring alopecia was easily made on VS. However, the TS may provide an insight into the mechanism of hair loss. LPP was the most common variant of scarring alopecia. It was possible to distinguish it from DLE by the absence of inflammation in the deep dermis, diffuse deposition of dermal mucin, and extensive dermal scarring. However, in 2 cases, there was an overlap of microscopic features and this underscores the need for immunofluorescence studies in cases of scarring alopecia.

Unusual forms of secondary scarring alopecia—scleroderma, lichen sclerosus et atrophicus, and necrobiosis lipoidica were also studied.

The diagnosis of nonscarring alopecia requires TSs since they are related to abnormalities in the growth cycling of hair follicles. The features such as variation in size of the hair follicles with miniaturization and presence of nanogen follicles in case of alopecia areata were easily studied only on TSs. Alopecia areata was the most common cause of hair loss and was diagnosed on the presence of peribulbar inflammation and/or nanogen hair follicles.

The present technique allows us to analyze the epidermis and entire thickness of the dermis with subcutis on VSs. However, which is not possible in the previously described methods, where the biopsy is transected and used for vertical and TSs separately.

The main drawback of this method includes a need for a trained technical staff to obtain proper TS without having oblique sections.

**CONCLUSION**

This new tissue processing technique is useful in the diagnosis of all types of alopecia (both scarring and nonscarring) as all the aspects of the hair follicles including the size (terminal, vellus, and indeterminate), phase of the hair cycle (anagen, catagen, and telogen), bulbs, follicular units (evenly spaced/blank spots etc.), presence of inflammation (at the level of bulb, suprabulbar, isthmus, and infundibulum) are clearly demonstrated. In addition to that, this technique may also be useful in studying the epidermis, dermoepidermal junction, and subcutis. This technique also provides unmounted sections at all levels of the hair follicles which are saved in the refrigerator between filter paper sheets until reporting was completed.

Although it is cumbersome, a more complete and thorough examination is possible by combining vertical and transverse sectioning of the same punch biopsy specimen by this effective technique.

To summarize, this is a histological study of scalp biopsies, sectioned vertically and horizontally, in 100 cases of alopecia. The series is fairly representative of the various clinicopathological types of alopecia. Alopecia areata was the most common cause of alopecia, both types, put together. LPP was the most common type of scarring alopecia. TSs were particularly useful in the histology of nonscarring alopecia. Since most patients are reluctant/refuse to permit a second punch biopsy, a single 4 mm punch can be used to obtain diagnostically satisfactory vertical and horizontal sections by modifying the embedding procedure, thereby avoiding the need for the second biopsy.

**Declaration of patient consent**

The authors certify that they have obtained all appropriate patient consent forms. In the form the patients have given his their consent for their images and other clinical information to be reported in the journal. The patients understand that their names and initials will not be published and due efforts will be made to conceal their identity, but anonymity cannot be guaranteed.

**Financial support and sponsorship**

Nil.

**Conflicts of interest**

There are no conflicts of interest.

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