A Comparison of Vertical and Transverse Sections in the Histological Diagnosis of Alopecia Areata Scalp Biopsy Specimens

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ABSTRACT

Context: Both vertical and transverse sections are used for the diagnosis of alopecia areata. However when a single biopsy is submitted the pathologist has to decide which type is better.

Aims: To compare the diagnostic histological features in vertical and transverse sections in alopecia areata scalp biopsy specimens. Settings and Design: Tertiary Care Hospital. Comparative Study. Materials and Methods: A total of 30 patients were enrolled in the study. Two four mm punch biopsy were taken. One was used to take vertical sections and the other for transverse section and histological features of alopecia areata noted in both.

Statistical Analysis Used: Chi-square test, percentage. Results: Diagnosis of alopecia areata could be made in 30 (100%) cases in transverse sections and 28 cases (93.3%) in vertical sections. The number of hair follicles available for evaluation was more in the transverse section. Nanogen follicles and miniaturization of follicles were better visualized in the transverse sections. However the catagen and telogen follicles were noted in both vertical and transverse sections but the number and the ratio of anagen and telogen hair follicles could be better assessed in the transverse sections. Presence of peribulbar lymphocytic infiltrate, eosinophils and pigment casts were noted in both transverse and vertical sections.

Conclusions: Transverse sections provide a better assessment of the histological features of alopecia areata than vertical sections and thus should be preferred.

Key words: Alopecia areata, histopathology, horizontal section, vertical section

INTRODUCTION

Alopecia areata (AA) is an unpredictable, usually patchy, nonscarring hair loss condition.[1] AA equally affects males and females at all ages and 60% of patients before the age of 20 years.[2,3]

The histologic changes vary depending on the stage of the disease and most pathologists have limited experience with their interpretation as classic AA is rarely biopsied.[4] AA progresses through acute, subacute, and chronic stages.[5] In the acute stage, there is inflammatory infiltrate around the terminal hair bulb, whereas in the chronic stage, there is a reversal of the terminal-vellus ratio.[3] AA may lack the characteristic peribulbar infiltrate, especially in the late stage of the disease, the other features such as melanin in fibrous tract remnant, eosinophils in fibrous tracts or around follicular papillae, pigment casts within follicular epithelium, presence of catagen/telogen follicles, miniaturized follicles, presence of small dystrophic follicles (nanogen) may be used in the diagnosis.[6] The follicular Swiss cheese pattern is an additional diagnostic clue.[7]

Both vertical and transverse sections are used in the histological interpretation of alopecia areata biopsy specimens. The combination of two may be optimal, but the pathologist is frequently only provided with a single specimen. The trend in recent years has been toward transverse sections, but there is a paucity of studies directly comparing the two methods.[8] Vertical sections

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demonstrate the full thickness of the skin from stratum corneum to deep subcutaneous fat in every section. However, they demonstrate only a few hairs in every section. Any histological study requiring accurate vellus hair counts and terminal vellus hair ratio requires the use of transverse sections. Quantitative assessment of hair follicle morphology is particularly valuable for diagnosis and disease of hair disorder including alopecia. However, they may fail to demonstrate the changes at dermoepidermal junction or the deep subcutaneous fat. The relative size of follicles, proportion of follicles reduced in diameter, and variation in diameter are more likely to be important in the evaluation of nonscarring alopecia. Although these features could be assessed in serial vertical sections, they are more easily assessed in single transverse section. Thus, we conducted this study with the aim of comparing the two types of sections in the histological diagnosis of AA.

**MATERIALS AND METHODS**

The study was conducted in the Department of Pathology and Department of Dermatology of our Institute. A total of thirty consenting adult patients attending the outpatient Department of Dermatology diagnosed clinically by one or two dermatologists as AA were included in the study.

Clinical features such as age, sex, type of AA, duration of the disease, and the local examination were recorded. Two 4 mm punch scalp biopsies were taken from same/similar lesions. One of the biopsies was used for taking the vertical sections. Other biopsy was cut into two slices horizontally, approximately 1–2 mm below the dermoepidermal junction. Both the cut surfaces were inked. Sections were embedded with inked surface down, and sections were taken and stained with hematoxylin and eosin. Histological features of alopecia were noted in both the vertical and transverse sections. Four sections each of the vertical and transverse sections were studied by a junior and senior dermatopathologists.

Following histological features were noted in both the sections:

1. Presence of miniaturized follicles
2. Presence of nanogen follicles
3. Eosinophils in fibrous tracts or around follicular papillae
4. Lymphocytes in fibrous tracts remnants of follicles
5. Presence of peribulbar lymphocytic infiltrate
6. Melanin in fibrous tract remnants
7. Presence of pigment casts within follicular spaces
8. Presence of catagen/telogen follicles.

**Statistical analysis**

The sample size was thirty which was chosen arbitrarily. For documenting the descriptive histological features in AA, frequency and percentages were calculated. For comparing the outcomes obtained from the two types of sections, Chi-square test was used, and $P < 0.05$ was considered statistically significant.

Informed consent from the patient and clearance from the Institutional Ethics Committee was obtained.

**OBSERVATIONS AND RESULTS**

The demographic profile of the patients was studied including the age, gender, location of the lesion, and the clinical characteristics. Out of the thirty cases, two cases were already diagnosed and had presented with the second episode, and 28 cases were diagnosed for the first time clinically by the dermatologist and confirmed histologically on transverse and vertical sections.

Age of all the thirty cases included in the study ranged from 5 to 30 years with a mean ± standard deviation (SD) of 19.23 ± 7.55 years. There were 16 (53.3%) females and 14 (46.6%) males with a female: male ratio of 1.14. In all the thirty cases, the lesions were located on the scalp.

The duration of AA in the thirty patients ranged from days to years with a mean ± SD duration of 119.67 ± 264.65 days, the minimum duration being 5 days, and maximum being 4 years.

Scalp biopsies were taken from the area of the lesion and were processed. Both transverse and vertical sections were taken, and the histological features were studied in both the sections [Figures 1 and 2]. In the transverse sections, 100% of the cases were diagnosed as AA in the scalp biopsy, and in vertical sections, the diagnosis of AA was made in 93.3% of the cases. The mean number of hair follicles in transverse sections was more in transverse than in vertical sections ($P = 0.00$).

All the histological features included in the study showed a relatively higher frequency of positivity in transverse sections as compared to the vertical sections, and the features such as miniaturization and nanogen follicles were noted mostly in the transverse sections. The other features were comparable in both the transverse and vertical sections [Table 1].
DISCUSSION

Most of the patients were below 20 years of age. There was slight female predominance with female:male ratio of 1.14:1 which was consistent with Chaitra et al. As a classic AA is rarely biopsied, the pathologists have a limited experience in interpretation of biopsies of AA when clear-cut diagnostic features such as peribulbar lymphocytic infiltrate are not present.

The role of a pathologist is vital when dealing with atypical presentations, such as patients progressing to scarring, use of topical medications that alter the picture, in trying to provide prognostic information.

Some other studies have also compared the histological features in the transverse and vertical sections both. However, the number of cases where the direct comparison was done in both transverse and vertical sections were fewer than our study. Özcan et al. concluded that transverse sections were better in the assessment of noncicatricial alopecia and vertical sections were preferable in cicatricial alopecia. All of their thirty cases of noncicatricial alopecias could be diagnosed on transverse sections and 25/30 cases on vertical sections.

We found that out of our thirty cases, diagnosis of AA on transverse sections was made in all the cases and in vertical sections 28/30 cases could be diagnosed. Peckham et al. compared 15 cases of AA in transverse and vertical sections, and the diagnosis of AA was made in all 15 cases in vertical sections, whereas 14/15 cases were diagnosed in transverse sections. However, the one case which was not diagnosed showed evidence of follicular miniaturization and melanin in fibrous tracts which was not considered as a diagnostic characteristic of AA, but it strongly pointed toward AA. In a study by Chaitra et al. in Indian patients, all the twenty cases were diagnosed on vertical sections alone, but only five cases were available for direct comparison in transverse sections in which they found that either the findings were same or noncontributory.
Elston et al. directly compared the vertical and transverse sections including 102 diagnosed cases of both scarring and nonscarring alopecias and they found that out of the twenty cases which were discordant with the original diagnosis in both sections, three cases were of AA, and all the three cases were diagnosed only on vertical sections and transverse sections did not demonstrate the diagnostic features adequately.\textsuperscript{[6]} Serial sectioning of the vertical sections in noncicatricial alopecias increased the diagnostic yield; thus, overcoming the disadvantage that few follicles are visible in a section. Thus, when vertical sections are used, serial sectioning is required. However, the number of sections were very high 30–116 (mean 53) which is not practical.\textsuperscript{[9]}

The number of follicles in each case in both the sections and the mean number of follicles in transverse sections (14.10) was much higher than those in vertical sections (6.57), and the difference in the number of hair follicles was statistically significant with a $P < 0.001$. Thus, the quantitative analysis of the hair follicles could be done in a better way in transverse sections as suggested by the previous studies.\textsuperscript{[5,8]}

In our study, we observed that miniaturization, nanogen follicles, and eosinophils were the features which were assessed better in the transverse sections as compared to the vertical sections, and the difference was statistically significant with a $P < 0.05$. Peribulbar lymphocytes in fibrous tracts were the features seen most consistently in our patients apart from the catagen and telogen hair follicles. These features were noted in both the transverse and vertical sections.

Peckham et al. also reported a higher number of cases showing miniaturization in transverse sections than vertical sections, though there was a significant number of cases in vertical sections as well showing miniaturization of hair follicles.\textsuperscript{[6]}

Nanogen follicles are minute and cycle rapidly showing mixed features of anagen, catagen, and telogen.\textsuperscript{[6]} The presence of nanogen follicles was studied only by Peckham et al. and they found only 4/109 cases showed the nanogen follicles. However, out of the 109 cases studied by them, only 15 had transverse sections available.\textsuperscript{[6]}

Peckham et al. and Müller et al. studied the presence of eosinophils in the scalp biopsies in both transverse and vertical sections.\textsuperscript{[6,7]} The number of cases positive for eosinophils was very few in both transverse and vertical sections by Müller et al.\textsuperscript{[7]} Peckham et al. reported a higher percentage of cases positive for eosinophils in vertical than transverse sections.

Catagen/telogen follicles were found to be elevated in 88\% of those cases with marginally higher number of cases positive in vertical than transverse sections where there was absence of inflammatory infiltrate.\textsuperscript{[6]} Whiting also found that in the absence of inflammatory infiltrate the other most consistent finding is the presence of telogen and catagen hair and the reversal of anagen/telogen ratio.\textsuperscript{[3]} Our finding with increased percentage of telogen hair more in transverse sections than vertical sections was consistent with findings of Müller et al.\textsuperscript{[7]}

Peckham et al. found that the presence of melanin in fibrous tracts and pigment casts were comparable in both the sections; however, pigment casts were present in the fewer number of cases and also had a higher frequency in vertical sections as opposed to our findings where we found a higher frequency in total number of cases and also higher in transverse sections.\textsuperscript{[6]}

CONCLUSION

Thus, we found that when a single biopsy is submitted transverse sections are more useful in assessing the histological features of AA.

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Conflicts of interest

There are no conflicts of interest.

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