INTRODUCTION

Alopecia areata is a common autoimmune cause of non-scarring alopecia presenting in either gender and all races. Prevalence in the general population is 0.1-0.2%. The lifetime risk of developing alopecia areata is estimated to be 1.7% and the frequency of positive family history for alopecia areata in affected patients is 10-20%. Prevalence of alopecia areata in Pakistani population has not been estimated; however, in Asians, it has been estimated to be 3.78% of dermatologic patients.

Diagnosis of alopecia areata might be a problem at times. During the past two decades, the use of transverse sections in the evaluation of scalp biopsy specimens has led to a better understanding of the histopathologic changes in both cicatricial and non-cicatricial alopecia. The technique was first introduced by John T Headington. Using this technique, numerous follicles can easily be seen in one tissue profile allowing evaluation of follicular density, follicular unit morphology, and follicular growth dynamics, i.e., anagen: telogen ratio. Many investigators have also used transverse sections to assess the therapeutic response to treatment. To further improve diagnostic yield, Elston et al. proposed combining two punch biopsy specimens in the same block, one cut vertically and the other horizontally. It is cost-effective and also improves the diagnostic yield.

Histopathological evaluation of alopecia areata has not been done in the local population and only a few studies have been carried out in Asian patients suggesting a different disease response. The purpose of this study was to correlate the histopathological findings with clinical stage of disease in terms of diagnostic accuracy and prognosis in Pakistani population.

PATIENTS AND METHODS

A cross-sectional study was carried out at the Dermatology Department of Combined Military Hospital,
Kharian Cantt, Pakistan from January 2002 to December 2004. Written informed consent was taken from all the participating patients or their parents. The medical ethics and scientific committee of the hospital approved the study.

Patients with a clinical diagnosis of alopecia areata who were willing for scalp biopsy and who had not taken any active treatment for their disease for four weeks prior to enrollment were included in the study. Patients known to have any known systemic illness or immunosuppression, patients taking any cytotoxic or immunosuppressive medication were excluded.

Fifty consecutive patients with alopecia areata fulfilling the inclusion criteria were included in the study. Detailed history regarding age of onset, duration of illness and associated symptoms was taken. Physical examination included evaluation of disease extent, presence of exclamation mark hairs and associated nail changes. First criteria of determining the clinical stage of disease was the extent of hair loss which was evaluated by dividing the patients into three groups. Group I comprised of < 25% scalp involvement; Group II comprised of 25 -75% scalp involvement and Group III more than 75% scalp involvement including alopecia totalis and alopecia universalis. Second criteria for determining the clinical stage was the duration of disease recorded as 0-6 months, > 6-12 months, >1-5 years and > 5 years.

A 4 mm biopsy punch was inserted into the scalp parallel to the direction of hair at a slight angle. Care was taken not to distort the specimen. The biopsy was extended well into subcutis so that the bulbs of terminal follicles, which lie in the superficial subcutis, were included in the specimen. Fixation was carried out in 10% neutral buffered formaldehyde solution for 24 hours. Specimen was then cut transversely 1 mm above the junction of dermis and subcutis under magnification and the cut surfaces marked with eosin. Both halves were embedded in the same paraffin block with the cut surface facing downwards. Multiple sections 5 micrometers thick were cut to study follicular anatomy at various levels from epidermis to subcutis. Staining was done with haematoxylin and eosin.

Cross-sectional area of 4 mm punch is 12.57 mm²; a round figure of 13 was taken to calculate the density of follicular units and follicles. Representative horizontal sections from lower and upper dermis were examined. All terminal anagen, catagen, and telogen hairs and vellus hairs (which included miniaturized hairs) were counted, as were follicular units and follicular stellae. Anagen and telogen percentages and terminal-vellus ratios were calculated from these follicular counts. In addition, inflammation and fibrosis around lower and upper follicles were rated as nil, mild, moderate, or dense. Follicular counts and inflammation ratings were then correlated with duration and extent of disease (Figure 1). Diagnostic criteria used for histological analysis of specimen have been described elsewhere.12 SPSS version 12 was used to manage and analyze the data. Frequencies and percentages were obtained for the variables where applicable. Mean and standard deviation were calculated for continuous variables. Descriptive data with percentages were reported. Chi-square test was used to test the statistical significance of relationship of histopathological findings and the clinical stage of disease.

![Figure 1: Photomicrograph showing histopathological features of alopecia areata (early disease, H&E stain, 40x).](image)

**RESULTS**

A total of 50 patients were evaluated. Age range was 3- 52 years (mean 23.2, SD +12.5). Gender distribution was 72% males and 28% females. Age of onset of alopecia areata ranged from 2-50 years (mean 21.8 years, SD + 12.4). Duration of disease ranged from 2 months to 12 years (mean 1.5 years, SD ± 2.3). Thirty patients were in group I, whereas, 17 and 3 patients were in group II and group III respectively. A total of 23 patients had less than 6 months duration of disease while 9 had less than 1 year duration, 13 had 1-5 years duration, and 5 had more than 5 years duration of disease. In 31 patients (62%), a definite diagnosis of alopecia areata could be established and in another 10 patients (20%), a probable diagnosis of alopecia areata could be made. In 9 patients (18%), biopsies were not diagnostic.

The total follicular units decreased with the increasing duration of disease (p< 0.01). Density of follicular units per mm² decreased with duration of disease (p=0.01). The total number and density of vellus hair follicles was not found to be statistically significant when correlated with the duration of disease (p=0.62). The total number and density of anagen hair follicles was also not found statistically significant when correlated with the duration of disease (p=0.09). On the other hand, total number and density of catagen hair follicles, telogen hair follicle...
counts, and follicular stellae were found to be highly significant when correlated with increasing duration of disease ($p=0.0001$).

Amount of cellular infiltrate was found to be statistically significant when correlated with increasing duration of disease ($p=0.0001$). Cellular infiltrate was classified as sparse (n=29), mild (n=9), moderate (n=10), and dense (n=3). In 7 (14%) patients, eosinophils were also seen among the infiltrating cells. Cellular infiltrate was seen to correspond with the degree of inversion of anagen/telogen ratio.

Fibrosis increased with increasing duration of disease ($p=0.0001$). It was classified as absent (n=39), perifollicular (n=11), and mild (n=1). Moderate and severe fibrosis was not seen in any patient.

Table I shows correlation of disease duration with identified histological features. Follicular counts, density, cellular infiltrate, fibrosis, anagen telogen ratio, and vellus to terminal hair ratio were correlated with the duration of disease and found to be statistically significant.

<table>
<thead>
<tr>
<th>Duration of disease</th>
<th>0-6 months (n=30)</th>
<th>&gt;6m-1 year (n=17)</th>
<th>&gt;1-5 year (n=3)</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mean follicular units / 13mm$^2$ biopsy (range)</td>
<td>10.1 (7-14)</td>
<td>8 (7-12)</td>
<td>7.3 (5-9)</td>
<td>0.01</td>
</tr>
<tr>
<td>Terminal follicular density/mm$^2$</td>
<td>1.65</td>
<td>1.55</td>
<td>1.0</td>
<td>0.007</td>
</tr>
<tr>
<td>Cellular infiltrate (%)</td>
<td>27 (90%)</td>
<td>12 (70.5%)</td>
<td>2 (66.6%)</td>
<td>0.0001</td>
</tr>
<tr>
<td>Inversion of anagen: telogen ratio (%)</td>
<td>22 (73.3%)</td>
<td>8 (47%)</td>
<td>1 (33.3%)</td>
<td>0.001</td>
</tr>
<tr>
<td>Altered vellus to terminal hair ratio (%)</td>
<td>10 (33.3%)</td>
<td>11 (64.7%)</td>
<td>3 (100%)</td>
<td>0.0001</td>
</tr>
<tr>
<td>Perifollicular fibrosis (%)</td>
<td>4 (13.3%)</td>
<td>6 (35.2%)</td>
<td>2 (66.6%)</td>
<td>0.0001</td>
</tr>
</tbody>
</table>

**DISCUSSION**

Transverse sections of human scalp biopsy specimens can provide both qualitative and quantitative information about follicular histopathology not readily available in vertically sectioned specimens. Using a technique maintaining all sections in the same anatomic orientation (deep to superficial) in all tissue pieces on microscopic slides allows transverse sections for evaluation of alopecia to be processed in a more cost-effective manner. This compares favourably with previously published techniques in providing diagnostic information.9

This study showed diagnostic changes of alopecia areata in 62% of study population and suggestive changes in another 20%. To-date, the largest series of transverse scalp biopsies in alopecia areata were published by Whiting. He found diagnostic changes in 84% of cases on transverse sections in his first published series.13 Later, he reported diagnostic features in 67% of 287 patients.14 He concluded and we agree that the diagnostic sensitivity of transverse section scalp biopsy depends upon how strictly the diagnostic criteria are applied.15

Patients in this series did not equally represent the groups in terms of duration and extent of disease. Majority of the patients were in early and limited disease. Disease duration of more than 5 years was represented by only 3 patients in group II and 2 patients in group III. Such a small number of patients cannot provide a significant inference to be drawn from the results. However, being a pilot project and first of its kind in Pakistani literature, this should enable further research in this direction.

The results of this study show that the mean follicular count can be regarded as the best indicator of disease activity. It falls with increasing duration as well as extent of the disease. The present counts were much less than Whiting who showed a mean follicular count of 30 in study group compared to 40 in normal controls. Moreover, in his study, follicular counts did not correlate with the severity of disease.15 One reason for the higher follicle counts in Whiting study (30) compared to the present (24.8 in <6 month) could be the genetic difference in hair follicle density as Whiting’s study was done in western population. Asians have been shown to have lesser hair density compared to western population.11 We could not find any study in our population analyzing the normal hair follicle counts. However, Fanti et al. have not found any significant correlation between follicle count and duration of the disease.16

Perifolbul inflammatory infiltrate is known to be a diagnostic marker of alopecia areata.17 There was a high percentage of inflammatory infiltrate in patients with early disease but it reduced as the disease progressed. Although the number of patients is too small to deduce significant results in long-standing disease, it appears that diagnosis of alopecia areata in late disease cannot rely solely on the extent of inflammatory infiltrate. Eosinophils have been shown to be a diagnostic marker in the absence of other features.18 Authors report presence of eosinophils in about 50% of biopsies even in the absence of perifolbul infiltrate. Eosinophils were visible in only 7% of the presently reported cases. Presence of eosinophils should not be considered a mandatory diagnostic marker. Further research is needed in this direction to validate the claim.

Anagen-telogen inversion and vellus terminal hair ratio are additional markers of disease severity and duration.
The present results suggest inversion of anagen telogen ratio in early disease, which reverses in long-standing cases, whereas, vellus to terminal hair ratio reverses in long-standing disease. These findings are consistent with Whiting’s series. These inverted ratios can help in prognosis of disease but as a diagnostic marker their utility is questionable as they may be found in other patterns of non-scarring alopecias.

It can be deduced from this study that the features of early disease include peribulbar lymphocytic infiltrate, and reversal of anagen/telogen ratio. These features are associated with better prognosis. On the other hand, reduced follicular count and density, altered vellus to terminal hair ratio and perifollicular fibrosis are features associated with prolonged and severe disease and therefore, carry a worse prognosis. Whiting has shown that follicular count is not obviously affected by the type, percentage of hair loss or total duration of disease. However, in this study, 2 patients with long-standing alopecia totalis/universalis had reduced follicular counts. The number of patients in group III in this study is too small to be of significance, larger studies should be done in this regard to further confirm the results.

Recently, the validity of transverse section in the diagnosis of scalp alopecias has been questioned on the basis of lack of quantitative approach to the diagnosis. Transverse section scalp biopsy is a good indicator of disease progress and severity when all the histological features are calculated and analyzed in correlation with the clinical stage of disease. This can help narrow down the differential diagnosis and in making better prognostic assessment in a particular patient. On the other hand, results should be carefully analyzed as the diagnostic accuracy of transverse section biopsy depends upon the subjective evaluation of follicular morphology in qualitative as well as quantitative terms. Recently, computer-based morphometry and three-dimensional (3D) image reconstruction software has been used successfully to evaluate scalp biopsies from non-cicatricial alopecias, which will take histological diagnosis of scalp alopecias one step further.

CONCLUSION

Histopathological changes in alopecia areata, as assessed with transverse section scalp biopsy, correlate with clinical stage of the disease.

REFERENCES