HISTOPATHOLOGIC FINDINGS OF ALOPECIA AREATA IN VIETNAMESE PATIENTS

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SUMMARY

Introduction: Alopecia areata is considered as an autoimmune disease affecting hair follicles causing hair loss. Histopathological examination is the most valuable test for the diagnosis of disease. In addition to typical signs of diagnostic value such as lymphocytes densely gathered around the hair bulb into a "swarm of bee" and the ratio of hair follicle types on the specimen, there are a number of studies which have shown that the presence of eosinophils around the hair follicle, the features of follicular necrosis also play a role in the diagnosis and prognosis of alopecia areata.

Objectives: To describe histopathological characteristics and to analyze the relationship between those features with some clinical features of alopecia areata in Vietnamese patients.

Methods: We recruited 35 patients with alopecia areata diagnosed on clinical findings, dermoscopy and consent for histopathology. In each patient, we biopsied 2 pieces of scalp with a 4mm punch in the marked area. One piece was dissected in the horizontal section and the other piece was sectioned vertically.

Results: Average number of hair follicles was 20.1 ± 3.5, average number of hair follicular units was 9.3 ± 1.8. The percentage of specimens in the acute, subacute, and chronic stages was 48.6%, 28.6%, and 22.9%, respectively. There were no specimens in the recovery stage. The mean duration of lesions in the acute stage was 1.8 ± 1.0 months, the subacute and chronic stages were 4.7 ± 1.1 and 23.25 ± 26.3, respectively. In the acute stage slides, 100% of the slides had peribulbar anagen hair lymphocytic infiltration. The number of specimens showing eosinophils accounted for 22.8%. Vacuolization and necrosis accounted for 14.3% of the total specimens.

Conclusion: Diagnosis of alopecia areata is made best on histopathologic examination, the histopathological stage discribes more than peribulbar lymphocytic infiltration, such as: percentages of multiple types of hair follicles, variation of histopathologic traits through disease stages.

Keywords: *Alopecia areata, histopathology, punch biopsy, scalp biopsy.*

1. INTRODUCTION

Alopecia areata is a common condition in dermatological practice. The disease is characterized by spreading clusters of smooth, circular or oval hair loss and is often asymptomatic for no local or systemic accompanying symptoms. The disease has few effects on physical health but greatly affects mental health due to aesthetic problems.

Many studies have shown that there are a number of factors related to the generation and development of alopecia areata, such as genetic factors, infections, autoimmune, psychological rauma and other systemic diseases.

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Among them, the hypothesis of the etiology of autoimmune alopecia areata is supported by the evidence showing the concentration of T lymphocytes around the hair follicles on pathohistologic work-up. Histopathological examination is the most valuable test for the diagnosis of disease.

However, on histopathology, in addition to typical signs with diagnostic value such as lymphocytes densely gathered around the hair bulb into a "swarm of bee", there have been a number of studies in the world described some other characteristics. Among them, there may be eosinophils and some other cells around the hair follicles, signs of vacuolization and necrosis of hair follicle matrix cells. In addition, histopathological features also vary with disease stages; as the disease progresses through the acute to chronic stages, the infiltration degree of inflammatory cells and the proportion of hair follicle types such as anagen and terminal follicle decreased, accompanied by an increase in the number of catagen and telogen hair follicles and follicular streamers.

In Vietnam, there are no studies describing the histopathological characteristics of alopecia areata. Therefore, we caried out this study to discribe histopathologic features of alopecia areata and analyze those features in relationship with some clinical traits.

2. SUBJECTS AND METHODS

2.1. Study design

We conducted a case series study on 35 alopecia areata patients.

2.1.1. Selection criteria

- Alopecia areata patients diagnosed on clinical grounds such as: one or more clusters of alopecia areata, smooth, circular or oval, spontaneously occurring and without other local or systemic symptoms and on dermoscopy such as: exclamation mark hair, yellow dot, no follicular fibrosis.

- Age 16 and above for ethic requirement.

- Patients must consent for histopathology study.

2.1.2. Exclusion criteria

- Having medical conditions or medications that increase bleeding risks such as blood clotting disoders, abnormal platelet count, NSAIDs...

- History of allergy to local anesthetics.

- The condition of alopecia areata is improving with treatment or showing signs of recovery on dermoscopy for avoiding noise.

2.1.3. Materials

- 4mm punch biopsy.

- Thin slicer: Shandong Fines 325 Rotary Microtome, 0.5 - 60 µm thickness.

- H&E dyes: Hematoxylin Harris and Eosin.

2.2. Study procedures

2.2.1. Punch biopsy of the scalp

We performed cutting 2 pieces of scalp with a 4mm punch in the marked area as follow.

Firtly, we created local anesthetic at biopsy site, choose punch size 4mm. Next, we punched a hole with 20^o direction to the scalp surface, along the axis of the hair follicle. Then we lift the specimen with surgical forceps and used scissors to cut at the bottom of the tissue, put it into the fixative solution. Next, we control bleeding, closed the skin with 1 - 2 stitches of nylon 4.0 or 5.0.

We cut the second piece similarly to the first piece.

2.2.2. Specimen sectioning and dyeing

In oder to get readable slides in vertical and horizontal manners, we processed samples as following steps.

The first biopsy piece was transversely cut at 1-1.5mm from the skin surface to create 2 parts of the specimen and the second biopsy piece was cut in half lengthwise. All these specimens were then placed with the cutting planes facing directly to the bottom of the paraffin mold then gently poured paraffin into the mold. Next, we sectioned the mass into multiple slides by the microtome and sticked the cut pieces on the slide.

Finally, we stained the slides with H&E and read the slides.

2.3. Variables

- Quantitative variables: duration of the specimen, number of hair follicle types, hair follicular unit types.

- Qualitative variables: pigmented cast, vacuolization - necrosis, lymphocytic and eosinophilic infiltration.

2.4. Data analysis

- Data were processed by SPSS 20.0 program.

- Quantitative data were expressed as $\bar{x} \pm SD$.

- Qualitative data were expressed as percentages.

- Comparative test:

+ For qualitative variables comparing between groups using Chi-square comparison test, if the number of theoretical expectation cells < 5 is greater than 20%, then use Fisher's exact test.

+ For quantitative variables compare values by T-test between two independent samples and One-Way ANOVA between multiple means.

+ The comparisons were statistically significant with $p < 0.05$.

3. RESULTS

3.1. Percentage of specimens by histopathological stage (%) (n = 35)

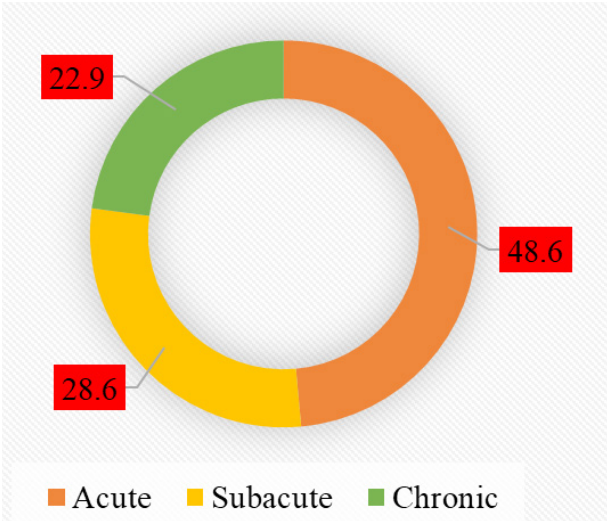

Chart 1. Percentage of histopathological stages (%) (n = 35)

Of the 35 investigated specimens, 17 specimens were in acute stage, accounting for 48.6%. The number of specimens in the subacute and chronic stages accounted for 28.6% and 22.9%, respectively (10 and 8 specimens, respectively). There are no specimens at the recovery stage (Chart 1).

Table 1. Average duration of the stages (months) (n = 35)

Stages	n	Average duration (X ± SD)
Acute	17	1.82 ± 1.0
Subacute	10	4.7 ± 1.1
Chronic	8	23.3 ± 26.3
General	35	7.5 ± 14.8

The mean duration of all specimens was 7.5 ± 14.8 months. The duration of the acute, subacute, and chronic stages gradually increased with 1.82 months, 4.7 months, and 23.3 months, respectively. The deviation of the mean time is quite large, indicating that this value is not representative (Table 1).

Table 2. Relationship between disease stage and lesion progression time (%)

	Stage (n,%)			Total
Progression time	**Acute**	**Subacute**	**Chronic**	
≤ 1 month	5 (100%)	0	0	5 (100%)
> 1 - 3 months	11 (84,6%)	1 (7.7%)	1 (7.7%)	13 (100%)
> 3 - 6 months	1 (10%)	9 (90%)	0	10 (100%)
> 6 months	0	0	7 (100%)	7 (100%)
p	< 0.05*			

**: Fisher's exact test*

The total number of specimens with disease duration of no more than 1 month is 5 and all are in the acute stage. Among the specimens with the disease duration from 1 to 3 months, the stage with the highest proportion is the acute stage with 11 specimens accounting for 84.6%, the subacute and chronic stages accounting for 7.7 % with 1 template for each stage. In the group from 3 to 6 months, 90% of the lesions were in the subacute stage, the remaining 10% were 1 specimen in the acute stage. In time group more than 6 months, all 7 specimens are in chronic stage (Table 2).

Table 3. Correlation between number of hair follicles and number of hair follicular units with the progression time (X±SD)

Progression time	n	Number of hair follicles	Number of hair follicular units	p
≤ 1 month	5	21.6 ± 2.9	9.4 ± 1.7	> 0.05†
> 1 - 3 months	13	18.9 ± 3.0	8.8 ± 1.6	
> 3 - 6 months	10	21.1 ± 4.7	9.6 ± 2.3	
> 6 months	7	20.0 ± 2.5	9.7 ± 1.5	
Total	35	20.1 ± 3.5	9.3 ± 1.8	

†: One-Way ANOVA test

On total specimens, the average number of hair follicles was 20.1 ± 3.5; the average number of hair follicular units was 9.3 ± 1.8. The specimens in the ≤ 1 month group had an average of 21.6 ± 2.9 follicles and 9.4 hair follicular units. The group >1-3 months had an average of 18.9 ± 3.0 follicles and 8.8 ± 1.6 hair follicular units, the group >3-6 months had an average of 21.1 ± 4.7 follicles and 9.6 ± 2.3 hair follicular units and the group >6 months had an average of 20.0 ± 2.5 follicles and 9.7 ± 1.5 units of hair folliculars. However, these differences were not statistically significant with p>0.05 (Table 3).

Table 4. Percentage of follicular types in different disease stages (%)

Follicle types	Acute	Subacute	Chronic
Anagen	51.8 ± 8.9	14.1 ± 6.5	3.1 ± 4.6
Vellus	27.4 ± 10.5	47.8 ± 8.8	49.2 ± 8.8
Nanogen	8.2 ± 6.3	14.8 ± 5.5	42.5 ± 9.0

In the acute stage, the percentage of anagen follicles accounted for an average of 51.8%, vellus follicles accounted for 27.4%, anagen follicles accounted for 8.2%. In subacute specimens, the average rate of anagen follicle accounted for 14.1%, vellus follicles accounted for 47.8%, nanogen follicles accounted for 14.8%. In the chronic stage group, anagen follicles accounted for an average of 3.1%, vellus follicles accounted for 49.2%, nanogen follicles accounted for 42.5% (Table 4).

Table 5. Percentage of lymphocytic infiltration around diffenrent hair follicular types in different stages (%)

	Acute	Subacte	Chronic
Anagen	100	40.0	0
Catagen	64.7	40.0	0
Nanogen	29.4	80.0	50.0
Follicular streamer	41.2	80.0	75.0

In the acute stage, 100% of the slides had lymphocytic infiltration around the anagen follicles, 64.7% of the slides had lymphatic infiltration around the catagen follicles, 29.4% of the slides had a peribulbar infiltration of the nanogene follicles and 41.2% had follicular streamer infiltrates. In the group of subacute stage specimens, the inflammatory infiltrate was mainly around the nanogene cysts and fibrovascular structures with the prevalence in 80% of the specimens, the lymphocytic infiltration around the catagen and anagen cysts was less common with 40% of the specimens. In the group of chronic stage specimens, there was no lymphocytic infiltration around the anagen and catagen follicles, but only in the nanogene follicles and fibrovascular structure with the rate of occurrence in 50% and 75% of the specimens at this stage (Table 5).

Table 6. Perifollicular eosinophils infiltration by histopathologic stages

Eosinophils infiltration	Acute n (%)	Subacute n (%)	Chronic n (%)	Overall n (%)
Present	7 (41.2)	1 (10)	1 (12.5)	9 (25.7)
Absent	10 (58.8)	9 (90)	7 (87.5)	26 (74.3)
Total	17 (100)	10 (100)	8 (100)	35 (100)

Out of the total of 35 specimens, there are 9 specimens showing eosinophil, accounting for 25.7%. Among the acute stage specimens, there are 7 specimens with peribulbar eosinophils infiltrate, accounting for 41.2%. Among the subacute specimens, there is 1 specimen out of 10 with the presence of eosinophils. The chronic stage slides do not have the presence of eosinophils (Table 6).

Table 7. Percenteage of disease stages in bulbar vacuolization - necrosis and pigmented cast features (n,(%))

Stage	Acute	Subacute	Chronic	Total
Vacuolization - necrosis	5 (100)	0	0	5 (100)
Pigmented cast	5 (45.5)	3 (27.25)	3 (27.25)	13 (100)

There are a total of 5 specimens with vacuolization - necrosis, 100% are in the acute stage. There are 13 specimens with pigmented cast encountered in the acute stage (45.5%) more than the other 2 stages with 27.25% per stage (Table 7).

4. DISCUSSION

This is the first study on the histopathological characteristics of alopecia areata in Vietnam. Besides, there are no studies describing the normal histological characteristics of the scalp in Vietnamese people. Therefore, we have consulted

the method of 4mm punch biopsy and compared with some histological features of normal human scalp in some Asian countries.

Primarily, it is understandable that the changes in histopathological stage are compatible with the time of progression. Our result suggests that maybe the 3-month mark is the milestone that has a high probability of the lesions turning from the acute phase to the subacute phase, the 6-month mark may turn the lesions to the chronic stage. However, studies with larger sample size will be needed for the values to be more representative and to dertermine at which duration of time the histopathological characteristics transform clearly to later stages'.

Among our total 35 biopsies, the mean number of hair follicles was 20.1 ± 3.5, the mean number of hair follicular units was 9.3 ± 1.8. In comparision to normal Asian subjects, our study showed compatible results, such as on Taiwanese scalp biopsy, samples found that the average number of hair follicles was 21.3 ± 4.8 and the average number of hair follicular units was 9.4 ± 1.9; in Thailand, the average number of hair follicles was 20.5 ± 5.2 and the average number of hair follicular units was 9.2 ± 1.6.

We did not find marked fibrosis distances between follicular units which usually indicates fibrosis transformation of follicles. A study with a sufficiently long follow-up period in years and biopsies from time to time may be needed to monitor follicular fibrosis in alopeia areata.

On histopathology, along the stages and duration of the lesion, the proportion of anagen follicles and terminal hair decreased, accompanied by an increase in the number of telogen and vellus follicles. This is also contributable to the making of diagnosis. Our results are consistent with Whiting's study in 2003 statistics, during the course of 1-12 months, the percentage of vellus follicles increased from 31.8% to 49.7%.

Besides, according to the histopathological progression from acute to chronic stage, we found lymphocyte and eosinophil infiltration around the hair follicle structures more in the acute stage and gradually decreased to the chronic stage. In our study, there were 9 specimens showing eosinophils, accounting for 25.7%, lower than Elston's study on 71 patients, the number of specimens with eosinophils accounted for 53.5%.

According to our study, the characteristic of vacuolization and necrosis of follicular matrix cells is only encountered in the acute phase when many inflammatory cells are concentrated and the inflammatory response is taking place strongly. Pigmented cast characteristics can be seen in all three stages, but the acute phase is more common. However, according to Peckham's study, 44% of the specimens had pigmented cast expression in the hair follicles, which is higher than our study.

5. CONCLUSION

Histopathology is the most valuable diagnostic tool for many dermatological diseases. Histopathological examination of the hair or scalp should be performed with a 4mm punch biopsy to produce biopsies of uniform size that can be compared with normal and pathological values from different studies. It is necessary to combine quantitative and qualitative features in the interpretation of scalp biopsy specimens in order to increase the accuracy of the diagnosis. On this study, we found the acute phase was predominant and usually lasts 3 months. The average number of hair follicles were 20.1 ± 3.5. The average number of hair follicles were 9.3 ± 1.8. The most common

type of follicular units were the ones with 2 follicles accounting for 36.8% of the total number of follicular units. Anagen follicles were more common in the acute stage, gradually decreased in the subacute and chronic stages. Vellus hair, telogen, and nanogen follicles increased in subacute and chronic. Eosinophils were mainly seen in the acute stage and rarely occured in the later stages. Vacuolization - necrosis only occured in the acute stage.

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