MJMR, Vol. 35, No. 1, 2024, pages (257-263)

Open Access
ISSN:2682-4558

Research Article

Alopecia areata hair pull test and its correlation to histopathological findings.

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DOI: 10.21608/MJMR.2023.244358.1530

Abstract

Background: Alopecia areata (AA) is a common, non-scarring type of hair loss that affects 2% of the general population and has an unpredictable course. **Aim**: Histopathological comparison of perifollicular lymphocytic infiltrate in alopecia areata in both longitudinal and transverse sections and its relation to the hair pull test. **Methods**: The study was conducted on 18 AA patients attending the dermatology outpatient clinic, Minia University Hospitals from September 2022 to December 2022. A three-millimeter punch biopsy was taken from the edge of the lesion in each patient, processed routinely for longitudinal sections, stained with hematoxylin and eosin stain, then re-embedded, and transverse sectioning was performed. Perifollicular mononuclear inflammatory infiltrate was evaluated as either significant or not in each case and in each follicle. **Result**: Significant higher numbers of terminal hair follicles with a significant existence of perifollicular infiltrate were found in transverse sections when compared to longitudinal sections, and immune infiltrates were positively correlated to the positive hair pull test.

Key words (histopathology, pull test, transverse section, alopecia)

Introduction

Alopecia areata (AA) is an autoimmune disease with a fluctuating, relapsing course that can be permanent. It is a common kind of hair loss in humans after male and female-pattern alopecia. In AA, the clinical hair loss patterns are typically highly unique. The most typical pattern is a round or spotty bald lesion (patchy alopecia areata), typically on the scalp, which may proceed to total hair loss solely on the scalp (alopecia totalis) or total hair loss on the entire body (alopecia universalis). [(1)]

Numerous factors, including immunologic, genetic, viral, and psychological issues, have an impact on the etiopathogenesis of AA. There are also claims that trace elements can exacerbate or cause AA. [(1-2)]

Immune privilege (IP) at the hair follicle (HF) broke down, which resulted in aberrant immune responses against it, which is the main cause of AA. [(3)]

There is compelling evidence that CD8+ T cells are important in the emergence of alopecia areata. It was also noted that these cytotoxic T lymphocytes (CTL) had entered the hair follicles. The restoration of hair growth in certain alopecia areata patients following immunosuppressive medication and the decline in the number of cytotoxic T cells invading the restored hair follicles revealed that T cells were functionally significant in the development of AA.[(4)]

Follicular-related inflammatory infiltrates have been noticed in AA. An increase in catagen and telogen follicles, a peribulbar lymphocytic inflammatory infiltrate, and eosinophils in the stellae are all signs of early AA. Lymphocytes were found invading the hair matrix, and there

is also vacuolar damage, pigment incontinence, and matrix cell death. In terminal hair follicles, the inflammatory infiltration is very pronounced. T lymphocytes CD+4 and CD+8 make up this infiltrate. Langerhans cells, lower follicular keratinocytes, melanocytes, and dermal papillae can all show signs of degeneration. [5]

Hair pull test conducted at the periphery of the lesion is usually used as an indicator of disease activity. [6]

Aim of work: Evaluating perifollicular inflammatory infiltrate in transverse and longitudinal sections in alopecia areata and correlating that to the hair pull test outcome.

Patients and methods

1: Selection of cases

In this study, there were a total of 18 cases diagnosed as AA. Three cases were excluded due to insufficient data. The patients were collected from the dermatology Outpatient Clinic at Minia University Hospitals, where they **were picked randomly.**

2: Clinical data

- A complete medical history was obtained from each patient, including their personal history (age, sex, and occupation), present history (onset, course, and duration of the condition), past history (previous diseases or medications), and family history of AA.
- The cases underwent general and dermatological examinations. The diagnosis was made by clinical examination.
- Hair pull test was done for each case, and the results were recorded for further statistical analysis.

3: Ethical approval

The goal and design of the study were conveyed to each parent before they participated. By getting parents' informed consent prior to participant recruitment, which allowed participants the choice to withdraw from our research at any moment, we avoided using misleading techniques. Additionally, this study was approved by the ethics committee of the Minia University faculty of medicine. Approval number: 579-224.

4: Skin biopsy

After infiltrating the skin with local anesthesia consisting of two percent lidocaine (Debocaine, Sigma-Tec, Egypt), skin biopsy specimens were retrieved using 3 mm punches. Biopsies were obtained at the border of the lesions on each patient. The biopsies were processed for routine longitudinal sectioning after they were fixed in ten percent formalin. Transverse sectioning was done for each biopsy 1 mm above the dermal subcutaneous junction (Headington, 1984) [7], after re-embedding.

5: Histological examination

H&E staining was done for both longitudinal and transverse sections. The presence of any detected perifollicular mono-nuclear inflammatory infiltrate in longitudinal sections for each case was evaluated as either significant or not. All hair follicles were examined individually for the detection of perifollicular inflammatory infiltrate in transverse sections as either significant or not. The sections were examined by three independent pathologists to confirm the findings.

6: Statistical analysis

IBM's SPSS version 25 was utilized for the data analysis process. Quantitative data that did not fit a normal distribution were presented as a median and interquartile range (IQR), while qualitative data were presented numerically and qualitatively. For non-parametric quantitative data, analyses were performed using the Mann-Whitney test comparing the two groups. The Chi-square and Fischer exact tests were used to compare categorical variables. A p-value less than 0.05 was considered significant.

Results

The study was conducted on 18 patients with AA attending the dermatology outpatient clinic, Minia University Hospital, Minia, Egypt. Three cases were excluded due to insufficient data or the loss of samples. Of these patients, 6 (40%) were males and 9 (60%) were females. Their age ranged from 5 to 40 years, with a mean± SD of 19.6±10.2. The duration of the disease ranged from 5 days to 36 months, with a mean±SD of 5.5±8.9. No family history of AA was present in any of the patients. As regard the

disease activity (with the hair pulling test), 8 (53.3%) were active and 7 (46.7%) were stable. One case gave a history of vitiligo on the same AA lesion **(Table 1)**.

The total number of terminal follicles found in longitudinal sections was 39, but in transverse sections the total number was 81, with a statistically significant difference (P = 0.010) **(Table 2)**.

Histopathologic examination of all cases in longitudinal sections revealed that 7 cases had a significant perifollicular mono-nuclear inflammatory infiltrate **(Fig. 1)**. On the other hand, H&E staining of all cases in cross sections revealed that 52 follicles (64.2%) had a significant mono-nuclear inflammatory infiltrate **(Figure 2–3)** and 29 follicles (35.8%) had an insignificant infiltrate. A case-by case study confirmed the presence of a significant infiltrate in 13 patients and an insignificant in 2 with a statistically significant difference (P = 0.020) **(Table 3)**.

The correlation between positivity of hair pull test and the existence of perifollicular inflammatory infiltrate in cross sections was statistically significant (P = 0.026) **(Table 4**).

Table (1): Demographic and clinical Data of all patients.

		Descriptive statistics N=15
Age	**Mean±SD** **IQR**	19.56±10.17 9.5-27
Sex	**Female** **Male**	9(60%) 6(40%)
Activity (hair pull test)	**Negative** **positive**	7(46.7%) 8(53.3%)
History of other autoimmune diseases	**Negative** **Positive**	14(93.3%) 1(6.7%)
Family history of autoimmune diseases	**Negative** **Positive**	15(100%) 0(0%)
Duration	**Mean±SD** **IQR**	5.54±8.9 1-4.8

Table (2): Mann Whitney test demonstrating the number of terminal follicles in both longitudinal and transverse section.

		Longitudinal	**Cross**	**P value**
		N=15	**N=15**	
Number of terminal hair follicle	**Median** **IQR**	2 (1-3)	5 (2-7)	0.010*

*** Significant level at P value < 0.05**

Table (3): Comparing perifollicular inflammatory infiltrate in both longitudinal and transverse section as regard chi square test.

		Longitudinal	**Cross**	**P value**
		N=15	**N=15**	
Perifollicular Inflammatory infiltrate	**No** **Yes**	8(53.3%) 7(46.7%)	2(13.3%) 13(86.7%)	0.020*

***significant level at P value < 0.05**

Table (4): Perifollicular inflammatory infiltrate in both longitudinal and transverse section of active cases as regard fisher exact test.

		Longitudinal	Cross	P value
		N=8	N=8	
Perifollicular infiltrate	**No**	5(62.5%)	0(0%)	0.026*
	Yes	3(37.5%)	8(100%)	

***significant level at P value < 0.05**

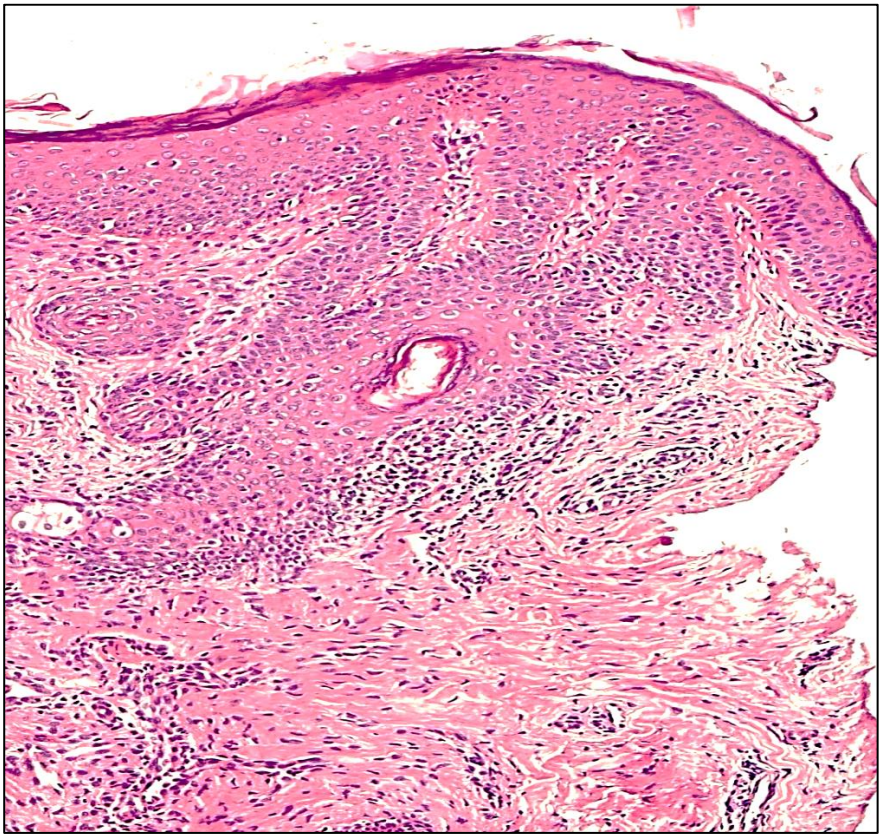

Figure (1): H&E staining of AA specimen showing significant inflammatory infiltrate around hair follicle in a longitudinal section (40x).

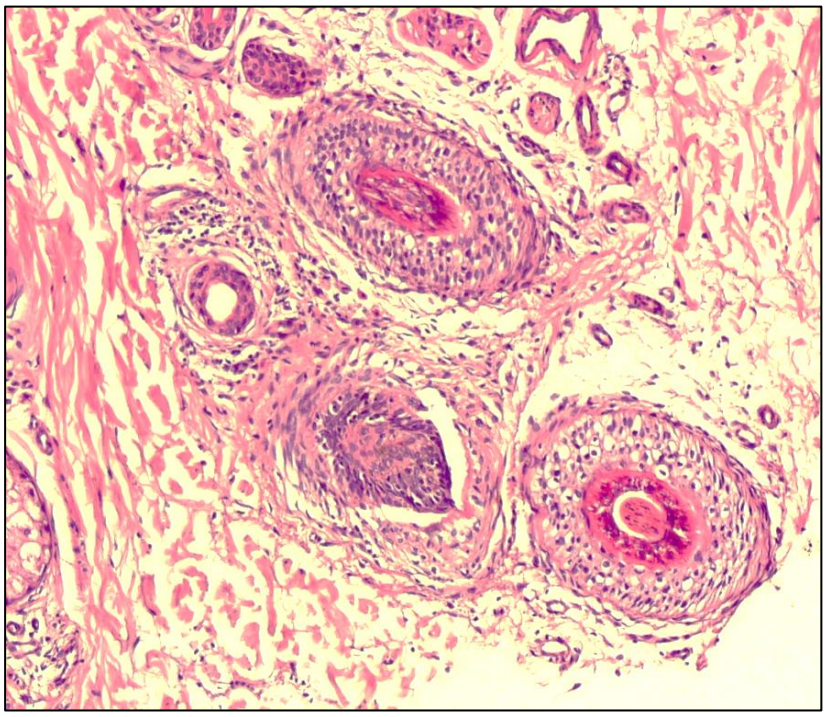

Figure (2): H&E staining of AA specimen showing significant perifollicular inflammatory infiltrates in cross section (40).

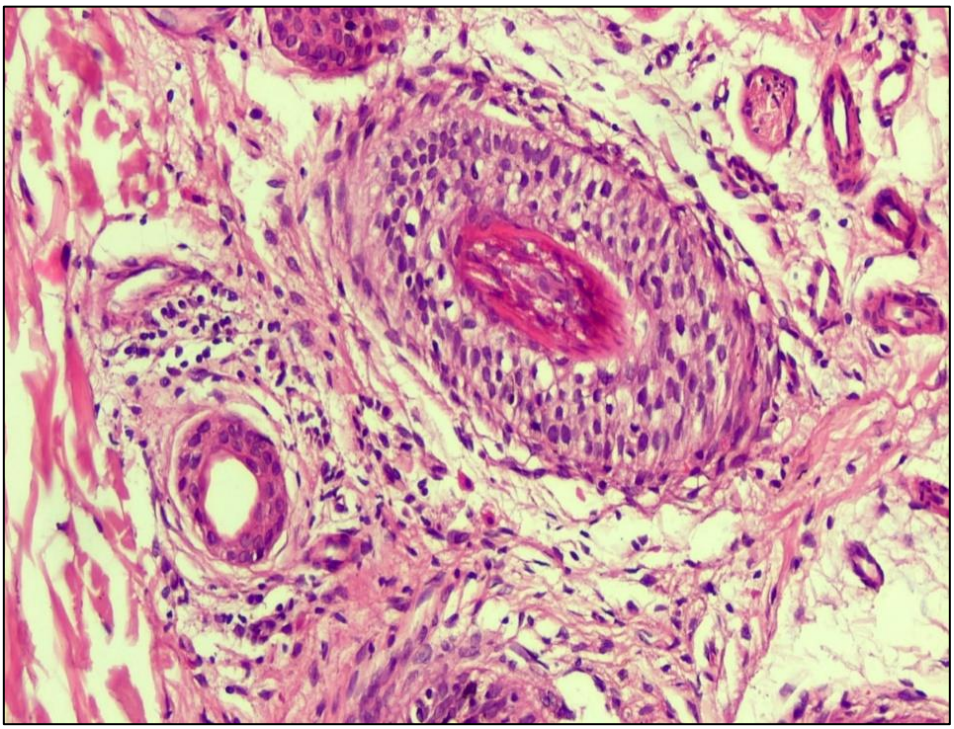

Figure (3): H&E staining of AA specimen showing significant perifollicular inflammatory infiltrates in cross section (40x).

Discussion

Patients of all ages can experience alopecia areata, which is a frequent type of non-scarring alopecia; however, children and young adults are more likely to experience it. AA appears to be an autoimmune process that targets follicular melanocytes or keratinocytes and has a somewhat equal sex preference. [8–9]

The most prominent histologic characteristic feature of AA is the peribulbar lymphocytes infiltrate "swarm of bees." Other findings could be required to make the diagnosis because this trait might not be present in persistent instances. Eosinophils, melanin, and lymphocytes have all been documented to be present in fibrous tract remanent. Alopecia areata has also been associated with an increased number of catagen/telogen follicles, pigment casts inside the hair canal, and shrunken follicles; however, these reports are not specific. [10]

The histological analysis of alopecia areata biopsies employs both vertical and transverse sections. Although two together could be the best option, the pathologist typically only receives one specimen. Transverse sections have been used more frequently; however, there are few studies that explicitly compare the two approaches. [11]

Although vertical sections usually visualize the skin from the stratum corneum to the deep subcutaneous fat, they only show a few hairs in each segment. [12]

When evaluating non-scarring alopecia, the relative size of follicles, the percentage of follicles with reduced diameter, and variation in diameter are more likely to be significant factors. Although these characteristics could be evaluated in a series of vertical sections, a single transverse section makes the evaluation process simpler. [13]

Thus, we conducted this study with the aim of comparing the two types of sections in the histological diagnosis of AA and comparing the hair pull test as a method of activity indication to the existence of perifollicular inflammatory infiltrate.

In our study, perifollicular lymphocytic infiltrate was significant in 8 cases in longitudinal sections and 13 cases in transverse sections. This was in agreement with Singh et al., 2016 [11] who noted many histopathologic diagnostic features of AA e.g., perifollicular inflammatory infiltrate clearly exists in transverse sections. Özcan et al., 2011 [13] also noted that both the transverse and the vertical sections showed the diagnostic features of non-cicatricial alopecia, such as peribulbar lymphocytic infiltration and follicular lymphocytic exocytosis of alopecia areata.

Chaitra et al., 2010 [14] noted that the extent and location of perifollicular inflammation could not be assessed in the transverse sections.

As regards the total number of terminal hair follicles, it was more in transverse sections than in longitudinal sections. This is in agreement with Singh et al., 2016 [11], who mentioned that the number of follicles in each case in both sections and the mean number of follicles in transverse sections was much higher than those in vertical sections.

In our research, out of 15 cases, eight were active with the hair pull test, and all of them had significant infiltrates in cross sections. There was a statistically significant correlation between the positivity of the hair pull test and the existence of inflammatory infiltrates in cross sections ($P = 0.026$).

On the other hand, 7 cases were negative with the hair pull test; 4 of them had significant perifollicular infiltrate in cross sections. Thus, we assume that negativity of the hair pull test does not mean absence of perifollicular infiltrate, which agrees with Cua et al., 2021, [15] who reported a case of diffuse alopecia areata with a negative hair pull test and perifollicular inflammatory infiltrate.

Conclusion:

Transverse sectioning is better for the demonstration and evaluation of hair follicles and their related immune-inflammatory infiltrate in alopecia areata patients, and a positive hair pull test is a good sign for the

disease activity that significantly correlates to the inflammatory infiltrate and eventually to the immune attack. Yet, a negative hair pull test cannot deny disease activity or the existence of the immune attack on AA follicles. Bigger sample size may be recommended to further confirm the findings.

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