Original Research Article

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Correlation between clinical, histopathological and direct immunoflourescence findings in cases of cicatricial alopecias

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ABSTRACT

Background: Cicatricial (scarring) alopecias form a group of disorders in which permanent hair loss results from replacement of follicles by fibrosis or hyalinized collagen. The aim was to find the correlation between clinical, histopathological and direct immunofluorescence findings in cicatricial alopecias.

Methods: A total of 20 cases were included in our study. Two 4mm punch biopsies were taken for haematoxylin and eosin (H & E) and DIF respectively. Vertical and horizontal section were cut and stained with haematoxylin and eosin.

Results: After histopathological examination 8 (40%) were confirmed as LPP, 6 (30%) as DLE, 2 (10%) as folliculitis decalvans and 1 (5%) each of acne keloidalis, trichotillomania, CTCL and morphea. Out of 8 confirmed cases of LPP 4 (50%) were positive for direct immunofluorescence and in 6 confirmed cases of DLE 4 (67%) were positive for immunofluorescence.

Conclusions: There was no statistical significance regarding the correlation between clinical, histopathological and immunofluorescence findings.

Keywords: Cicatricial alopecias, Histopathological examination, Immunofluorescence

INTRODUCTION

The term alopecia is derived from a Greek word "Alopex" meaning "fox mange" (baldness).¹ Alopecia of the scalp may be scarring (cicatricial) or non-scarring (non-cicatricial). Some cases of non-cicatricial alopecia in due course may become cicatricial and this is referred to as biphasic pattern of hair loss.² Permanent hair loss in cicatricial alopecia is due to predominant infiltration of inflammatory cells around the bulge region that contains the follicular stem cell reservoir followed by replacement of hair follicle by fibrosis or hyalinized collagen.

Cicatricial alopecia can also be classified as primary and secondary. Both primary and secondary involves the destruction of the hair follicle. Diseases included in the primary directly target the hair follicles and the surrounding tissue may be normal during early stages of the disease activity. However in secondary cicatricial alopecia the hair follicle and the bulge are innocent bystanders.

Cicatricial alopecia is also classified according to a consensus-issued classification scheme based on the predominant cell type present: lymphocytic, neutrophilic, or mixed and hence histopathology has important role in the diagnostic evaluation.³

Adequate sample size and optimal site of biopsy is very important for histopathological interpretation. Atleast two 4 mm biopsy samples from active site is required out of which 1 sample was used for histopathological examination and 2^{nd} sample is used for direct immunofluorescence (DIF).³

METHODS

The study included 20 patients with scarring alopecia attending the out-patient department of PSG Hospitals, Coimbatore between February 2014 and February 2015. Pregnant and lactating women, patients with classical androgenetic alopecia and alopecia areata were excluded.

A detailed history with relevance to age, gender, occupation, onset and duration of disease, symptoms associated with the disease process were recorded. A detailed scalp examination was carried out in terms of morphology, site(s) and extent of involvement. General physical examination and cutaneous examination were done. Complete blood count (CBC), KOH mounts and antinuclear antibodies (ANA) testing were undertaken whenever necessary. Clinical photographs were taken after patient's consent.

Two 4 mm punch biopsy were taken for haematoxylin and eosin (H & E) and DIF respectively. The specimens obtained were fixed with formalin and phosphate buffer saline for DIF and transported to the lab immediately. Vertical and horizontal section were cut and stained with haematoxylin and eosin (H & E), Verhoeff-van-Gieson elastic stain, Alcian blue (AB), periodic acid-Schiff (PAS) were done in clinically suspected cases of discoid lupus erythematosus (DLE) and Lichen plano pilaris (LPP).

RESULTS

A total of twenty patients were studied. The age range of the patients was between 11-63 years with the mean age of 37 years. Of the twenty patients, 7 were men and 13 were women. Out of 20 cases 10 (50%) were clinically diagnosed as LPP as shown in Figure 1, 7 (35%) were diagnosed as DLE as shown in Figure 2, 2 (10%) as folliculitis decalvans and 1 (5%) as acne kelodalis as given in Table 1.



Figure 1: Cicatrial alopecia secondary to LPP.

Table 1: Clinical, histopathological and DIF findings.

S.no	Clinical dianosis	Histopathology	DIF
1	Folliculitis	Folliculitis	No
-	decalvans	decalvans	deposits
2	Acne keloidalis	Acne keloidalis	No
		LDD	deposits
3	LPP	LPP	LPP No
4	LPP	LPP	deposits
5	DLE	DLE	DLE
			No
6	DLE	DLE	deposits
7	LPP		No
· .	211	Trichotillomania	deposits
8	LPP	LPP	No
			deposits
9	DLE	DLE	DLE
10	DLE	DLE	DLE
11	LPP	LPP	LPP
12	DLE	DLE	No
			deposits
13	DLE	DLE	DLE
14	LPP	Morphea	No deposits
15	LPP	LPP	LPP
16	LPP	LPP	LPP
17	LPP	LPP	No
			deposits
18	DLE	CTCL	No
			deposits
19	LPP	LPP	No
			deposits
20	Folliculitis	Folliculitis	No
	decalvans	decalvans	deposits



Figure 2: Cicatrial alopecia secondary to DLE.

After histopathological examination 8 (40%) were confirmed as LPP, 6 (30%) as DLE, 2 (10%) as folliculitis decalvans and 1 (5%) each of acne keloidalis, trichotillomania, cutaneous T cell lymphoma (CTCL) and morphea as in Table 2. Out of 8 confirmed cases of LPP 4 (50%) were positive for direct immunofluorescence and in 6 confirmed cases of DLE 4 (67%) were positive for immunofluorescence.

Table 2: Histopathological diagnosis.

Skin disorder	No of cases	percentage
LPP	8	40
DLE	6	30
Folliculitis decalvans	2	10
Acne keloidalis	1	5
Trichotillomania	1	5
CTCL	1	5
Morphea	1	5

Histopathological feature of lymphocyte-mediated lichenoid band–like interface dermatitis involving the follicle and interfollicular epidermis was present in all 8 patients of LPP. In addition they had basal cell vacuolization and pigmentary incontinence. Both infundibular and isthmic portion of hair folloicle was affected .There was no appendageal involvement in these cases as shown in Figure 3.

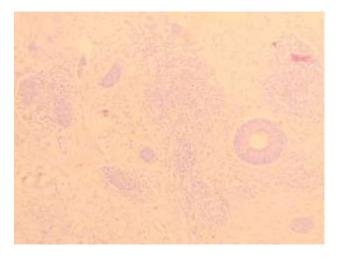


Figure 3: Periadenexal infiltrate in DLE (H & E 40X).

Out of 8 clinically suspected DLE cases, seven of them showed interface dermatitis with basal cell vacuolization and lymphoplasmocytic infiltrate around the appendages and hair follicles. Special staining with Alcian blue periodic acid Schiff stain showed mild increase in dermal mucin in reticular dermis and loss of elastic fibre with Verhoeff-van-Gieson elastic stain.

Direct immunofluorescence finding in LPP cases was mostly IgM surrounding the upper portion of hair follicle and fibrinogen deposits in basement membrane zone. DLE cases showed granular or linear pattern of immunoreactivity to IgG, IgM, C3 along the dermal epidermal junction and follicular epithelial dermal junction.

Statistical analyses was done using Chi square test. The correlation between clinical, histopathological and immunofluorescence findings for LPP and DLE was not statistically significant as given in Table 3.

Table 3: Correlation between LPP and DLE.

Skin disorder	Clinical diagnosis	Histopathology	DIF
LPP	10	8	4
DLE	7	6	4

DISCUSSION

Scarring alopecia or cicatricial alopecia results from follicular damage which is sufficient to cause the destruction and replacement of pilosebaceous structures by scar tissue. Primary scarring alopecias represent a group of disorders that primarily affect the hair follicles as compared to secondary scarring alopecias, which affect the dermis and secondarily cause follicular destruction.⁴ Cicatricial alopecias that mainly involve lymphocytic inflammation include discoid lupus erythematosus, lichen planopilaris, frontal fibrosing alopecia, central centrifugal alopecia, and pseudopelade (Brocq). Cicatricial alopecias that are due to predominantly neutrophilic inflammation include folliculitis decalvans, tufted folliculitis, and dissecting cellulitis of the scalp. Acne keloidalis is a cicatricial alopecia with a mixed inflammatory infiltrate.

Deep punch biopsies are required to confirm the diagnosis. Both transverse and horizontal sections are required. Transverse sections allow visualization of as many follicular units as possible and at multiple levels; vertical sections are useful to visualize the entire follicle.⁵

The classical histological findings of LPP are lichenoid band–like infiltrate with interface changes involving the infundibulum, isthmus and variably the interfollicular epidermis. Cytoid bodies, basal vacuolization, pigment incontinence, and Max Joseph spaces along the follicular epithelium are also seen.⁶

The classical findings of DLE include interface dermatitis with basal vacuolization, dyskeratotic or apoptotic keratinocytes, epidermal atrophy or hyperplasia, follicular plugging and an inflammatory infiltrate composed predominantly of lymphocytes with admixed plasma cells distributed around the superficial and deeper dermal vasculature as well as adnexal structures.⁷

The distinguishing features to differentiate LPP from DLE include superficial and deep perivascular and periadnexal inflammation that may span length of follicle, mucin deposition and thickened periodic acid–

Schiff-positive basement membrane zone in cases of DLE. In LPP the infiltrate is confined to the superficial dermis, there is no eccrine involvement, dermal mucin or thickened basement zone.⁸ These features helped us to differentiate LPP from DLE histopathologically.

Direct immunofluorescence shows shaggy fibrinogen deposition and IgM and C3 deposits along basement membrane zone in LPP.⁹ The findings are usually non-specific in LPP and may provide an additional help in establishing the diagnosis. Previous DIF studies in LP patients have shown positive results in 37–97% of cases. In our study DIF was positive in 50% of cases.¹⁰

In DLE direct immunofluorescence shows a granular or linear pattern of immunoreactivity to immunoglobulins IgG, IgM and complement C3 along the dermal– epidermal junction and the follicular epithelial dermal junction. Direct immunofluorescence is positive in 63-100% cases of DLE.¹¹ In our study DIF was positive in 67% cases of DLE. A negative result does not exclude the possibility of DLE. A positive result can help to distinguish difficult cases of DLE from LPP.

The clinical correlation between the clinical, histopathological and direct immunofluorescence was not statistically significant in our study. This may be due to the small sample size. A study was undertaken by Thakur al to study the clinical, trichoscopic, and et histopathological characteristics of cicatricial alopecias of the scalp and to find out the concordance between trichoscopic and histopathological diagnosis.¹² They concluded that there was 89% concordance between trichoscopic and histopathological diagnosis in their study.

We conclude that histopathological is the gold standard investigation in diagnosing cicatricial alopecias. DIF can be used as additional aid in diagnosing the condition especially in cases of DLE and to some extent in LPP. Studies need to be conducted with larger sample size to find a positive concordance between the clinical, histopathological and immunofluorescence findings in cicatricial alopecias.

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