

HAIR FOLLICLE STEM CELLS IN THE PATHOGENESIS OF PRIMARY CICATRICAL ALOPECIA

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ABSTRACT

Background: Primary cicatricial alopecia (PCA) is a scarring disease. Although the scarring and deformity may affect any part of the body, such changes have been reported to be most obvious on the face and scalp. The pathogenesis behind this scarring process is not well understood. Once lesions have scarred, recurrent disease tends to occur at the edge of the scarred lesions but not within them. The fact that inflammation in PCA generally involves the bulge area of the hair follicles raises the possibility that damage to the stem cells of the bulge region may be one process leading to the permanent loss of hair follicles.

Objective : The aim of this study was to investigate the role of the epithelial hair follicle stem cells which reside in the bulge region in the scarring process in PCA.

Methods : We studied the reactivity to Mouse monoclonal antibody, Keratin 15 Ab-1 (LHK15), which recognizes cytokeratin (CK 15) and preferentially immunostains epithelial hair follicle stem cells without staining the remaining hair follicle, on skin biopsies (scalp lesions) from 40 patients with PCA (8 lichen planopilaris, 10 discoid lupus erythematosus, 7 folliculitis decalvans, 5 keratosis follicularis spinulosa decalvans, 10 acne keloid). Reactivity to Mouse monoclonal anti CD8 antibody (cd8-sp16) in cases of prominent inflammatory infiltrate (16 cases) was studied. Ten normal scalp biopsy specimens served as controls. The correlation between the extent of the cytotoxic inflammatory cell infiltrate (CD8+) and the presence of stem cells was investigated. Results were analyzed semiquantitatively.

Results : The expression of CK15 in epithelial hair follicle stem cells was absent at scarred PCA, and in cases of severe inflammation, it was weak or absent. There was normal to moderate CK15 expression at the bulge region of hair follicles when surrounded by mild or moderate inflammatory infiltrate (CD8⁺).

Conclusion : The bulge region appears to be involved in these diseases as a part of a broader involvement of the hair follicles; it is secondarily affected by the surrounding inflammatory cell infiltrate. Expression of CK15 immunostain was diminished and was then absent, indicating either damage to stem cells or differentiation to help in the repair process. Damage to follicular stem cells may help to explain the irreversible alopecia and the scarring process which characterize these diseases.

Key words : cytokeratin 15, primary cicatricial alopecia, hair follicle, scar, stem cell, hair bulge.

INTRODUCTION

The dynamic hair follicle cycles between growth (anagen), regression (catagen), and resting (telogen) phases throughout life. Presumptive hair follicle stem cells located at the lower most portion of the permanent follicle, a thickened region of the outer root sheath, named bulge⁽¹⁻³⁾. Cells below this area degenerate during the catagen stage and regenerate at the onset of a new anagen⁽⁴⁾. The bulge region is a reservoir of stem cells that are responsible for the regeneration of the cycling portion of the hair follicle. Hair follicle bulge stem cells are bipotent, because they can generate the hair follicle and also the epidermis⁽⁵⁾. The bulge stem cells can differentiate into hair follicle matrix cells, sebaceous gland basal cells, and epidermal cells⁽⁶⁾. Although stem cells in the hair follicle bulge can migrate to the epidermis and contribute to wound repair after epidermal injury, they are unnecessary for epidermal survival⁽⁷⁾.

Cicatricial (scarring) alopecias are a group of hair loss disorders characterized by permanent damage of the hair follicle⁽⁸⁻¹⁰⁾. Cicatricial alopecias can be categorized as primary and secondary. In

primary cicatricial alopecias, the hair follicle is the specific target of an inflammatory process. In contrast, secondary cicatricial alopecia involve hair follicle damage coincidentally as part of a more generalized destructive proceeding within the skin (e.g. thermal burn, infection, trauma, or skin cancer)⁽¹¹⁾. Scarring and nonscarring alopecias are different in the pattern of skin inflammation. In scarring alopecias, the inflammatory infiltrate is mainly focused on the permanent, noncycling region of the hair follicle, which contains the bulge stem cells, and this leads to irreversible hair loss. However, in nonscarring alopecias, which are reversible hair loss, the location of the inflammatory cell attack is around the bulb and the transient cycling region⁽¹²⁾. Permanent bulge stem cell destruction may play a crucial pathogenetic role in PCA⁽¹³⁻¹⁵⁾. The bulge stem cell marker cytokeratin 15 (CK15) is absent in PCA, with moderate to heavy inflammation, suggesting that hair follicle stem cells in the bulge region are damaged by inflammatory events⁽¹⁶⁾. Markers of proliferating stem cells are down-regulated in the bulge of lichen planopilaris (LPP) hair follicles compared with

uninvolved hair follicles, also supporting the concept that bulge stem cell deletion is an important mechanism for hair loss, at least in LPP, the prototype of cicatricial alopecias⁽¹⁷⁾. In discoid lupus erythematosus, another type of cicatricial alopecia, CK15 expression is weak or absent in the bulge area, with severe inflammation further confirming that bulge stem cell destruction leads to permanent loss of hair follicles in this disease⁽¹⁸⁾.

Cytokeratin 15 is a type I keratin without a defined type II partner. A monoclonal antibody C8/144B raised against the last 17 amino acids of the CK15 polypeptide showing that CK15 is expressed primarily in stem cells of the follicular bulge region⁽¹⁹⁾.

PATIENTS AND METHODS

Patients: This study has been conducted on forty patients with PCA and ten normal control selected from the outpatient clinics of the Dermatology Department, Zagazig University Hospitals during the period from January 2013 to December 2017. They were 28 males and 12 females with their ages ranging from 20 to 72 years with a mean of 34.5 ± 9.32 . The diagnosis of PCA was clinically established by trichoscopy and histopathological examination.

Methods:

*Immunohistochemical Study:

Four mm punch biopsy specimens were obtained from each patient from the edge of the lesion. The specimens were fixed in 10% neutral buffered formalin and processed for paraffin embedding. Serial 4 μ m sections were obtained from paraffin blocks and stained with the following:

- Haematoxylin and Eosin (Hx.& E.) stain for routine histopathological diagnosis⁽²⁰⁾.

- Immunohistochemical staining using the Streptavidin – Biotin immunoperoxidase technique (Strept A-B staining method) for the detection of:

a-CK15 expression using CK15 Ab-1 mouse monoclonal antibody

b- CD8 expression using mouse monoclonal AB.

Principle of the procedure:

The procedure is based on the application of the primary antibody specific for the antigen to be localized. Biotin-labeled secondary antibody binds to the primary antibody and then peroxidase conjugated with streptavidin is added. Streptavidin binds strongly and irreversibly to biotin of the secondary antibody. So, the biotinylated secondary antibody (linking antibody) links between the

primary antibody on one hand and the streptavidin peroxidase complex on the other hand.

The peroxidase enzyme is visualized by adding hydrogen peroxide and chromogen. Hydrogen peroxide is reduced and chromogen become oxidized yielding a colored reaction product. When 3, 3-diaminobenzidine tetrachloride is used as chromogen, the reaction product is brown⁽²¹⁾.

REAGENTS

- Mouse monoclonal antibody, Keratin 15 Ab-1 (LHK15; 1: 250; NeoMarkers, Fremont, CA, U.S.A.). Cat. # MS-198 (Purified antibody with BSA and Azide)⁽²²⁾.

- Mouse monoclonal anti CD8 antibody CD8 (CD8 antibody monoclonal (cd8-sp16)⁽²³⁾.

Evaluation of Staining results:

* CK15 positive staining was noted by ascertaining CK15 expression in cytoplasm. CD8 expression was noted when uniform /circumferential staining expression.

* **Criteria for grading CK15 stained sections were:**

- Negative (-):** if <5 % positive cells.
- Weakly positive (+):** if 5% - 25% positive cells.
- Moderately positive (++):** if >25-50% positive cells.
- Strongly positive (+++):** if >50% positive cells⁽²⁴⁾

***Criteria for grading CD8 stained sections were :**

- Negative (-):** if <5 % positive cells.
- Weakly positive (+):** if .< 10 % positive cells.
- Moderately positive (++):** if 11-49 % positive cells.
- Strongly positive (+++):** if >50% positive cells⁽¹⁶⁾.

STATISTICAL ANALYSIS

The collected data were computerized and statistically analyzed using SPSS program (Statistical Package for Social Science) version 18.0.

Results :

*Clinical types and causes of cicatricial alopecia:

The group enrolled in this study (40 patients and 10 controls) was clinically examined. Results of clinical examination of the forty patients proved the following diagnoses as shown in table 1:

- Lichen plano pilaris : 8 cases(20%)
- Keratosis follicularis spinulosa decalvans : 5 cases (12.5%)
- Folliculitis decalvans : 7 cases (17.5%)
- Acne Keloid : 10 cases (25%)
- Discoid lupus erythematosus : 10 cases (25%)

*Trichoscopy results :

Clinically diagnosed cases were examined by trichoscopy. Result of trichoscopy were 8 out of 10 DLE cases, 6 out of 8 LPP, in 5 out of 7 FD, while non specific features in KFSD and AK are observed. Results of trichoscopy were correlated with the clinical diagnosis and results show statistical significant difference ($P<0.05$) as shown in the table 2.

The trichoscopy features of the studied lesions was as follows:

-Discoid lupus erythematosus :

Yellow dots, large in size, yellow brownish color, thin and radial arborizing vessels are observed to emerge from these dots, (“red spider in yellow dots” appearance) as shown in fig 1.



Fig1: Discoid lupus erythematosus

-Lichen plano pilaris :

Intense perifollicular scaling, scales migrate along the hair shafts and form tubular structures that cover the proximal portions of the emerging hair shafts and elongated linear blood vessels in concentric arrangement. At the center of the lesion whitish areas lacking follicular openings“fibrotic white dots” are seen as shown in fig 2.



Fig2: Lichen plano pilaris

-Folliculitis decalvans :

Hair tufts are surrounded by a band of yellowish scales at the center of the lesion while at the active margin there are follicular pustules and yellow discharge as shown in fig 3.



Fig 3:Folliculitis decalvans

Histopathology results :

- Discoid lupus erythematosus :

Active scalp DLE is characterized by vacuolar interface alteration of the follicular epithelium, with a scattering of dyskeratotic keratinocytes. Dilated follicular plugs were noted in the form of aggregates of dense, hyperkeratotic stratum corneum in follicular infundibula. A variably dense periadnexal and interstitial lymphocytic infiltrate was noted. Perifollicular inflammation affects the upper

follicle. Perivascular inflammation is superficial and deep. Sebaceous glands are absent. Concentric perifollicular fibrosis was noted in most of the specimens examined. There were foci of follicular epithelial remnants and naked hair shafts surrounded by reticular fibrosis and an inflammatory infiltrate as shown in fig 4.

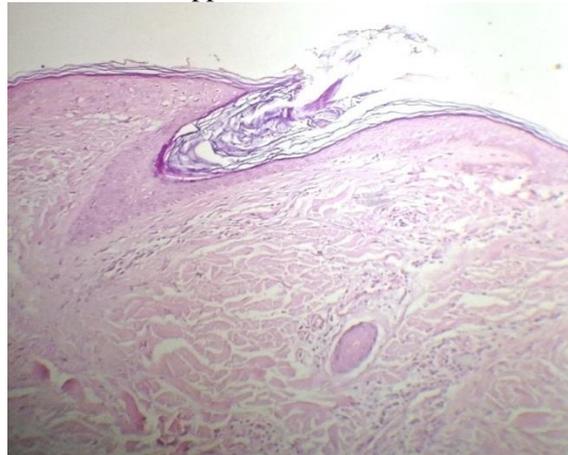


Fig 4 : Discoid lupus erythematosus with keratotic plug

-Lichen Planopilaris:

Biopsy of clinically active edge reveals the diagnostic features of lichenoid interface alteration, the upper follicle, and infundibulum in particular, is surrounded by a variably dense bandlike array of lymphocytes that often obscures the follicular epithelial-dermal junction. Infundibular hyperkeratosis with underlying hypergranulosis are seen. A lichenoid interface change was evident yet

with less prominent hydropic degeneration of the basal layer. Some dyskeratotic keratinocytes were detected. The follicular infundibulum revealed vacuolar basal change along with a dense, diffuse lymphocytic infiltrate hugging the hair follicles. A perifollicular concentric lamellar fibrosis was also noted. Sebaceous glands are absent as shown in fig 5.

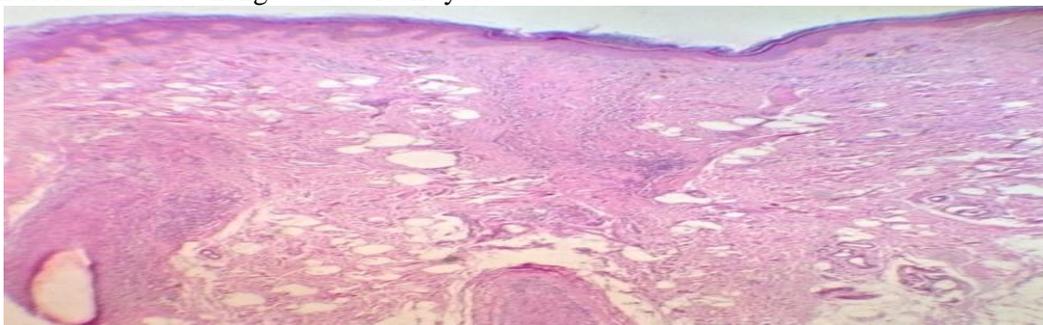


Fig 5 : old lesion with perifollicular fibrosis and lichenoid reaction

- Keratosis follicularis spinulosa decalvans :

Superficial intrafollicular and perifollicular edema and neutrophils are seen. There is compact hyperkeratosis and hypergranulosis of the upper follicular epithelium, follicular destruction, concentric perifollicular and horizontal adventitial

lamellar fibrosis (Fig 5). Some of the hair follicles are noted showing destruction of hair shafts and heavy infiltration by PNLs both surrounding and within the follicular epithelium.

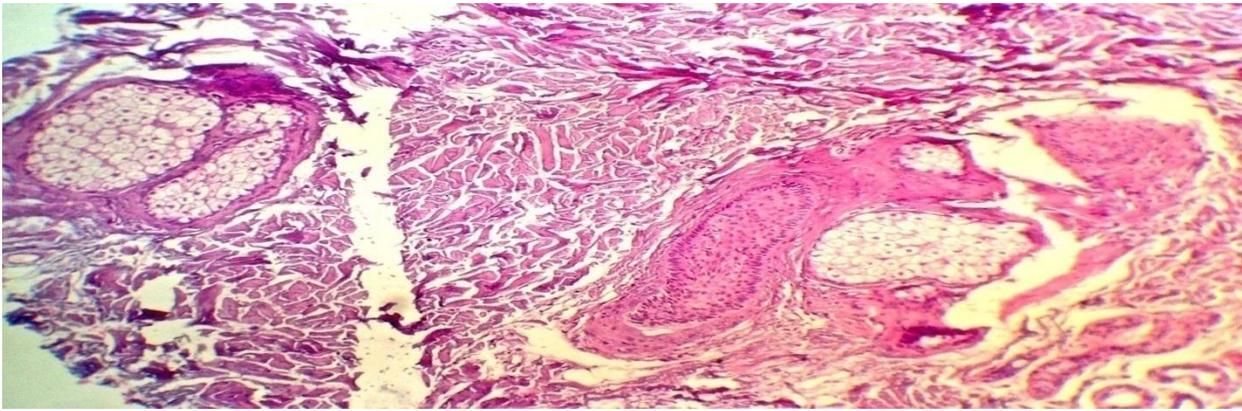


Fig 6

: Keratosis follicularis spinulosa decalvans, perifollicular fibrosis

-Folliculitis decalvans :

Many sections of comedonal follicular dilatation were noted. Neutrophils were seen both intra- as well as perifollicular. There are other sections

showing destruction of follicular epithelium with many PNL and foci of perifollicular lamellar fibroplasia as shown in fig 7 .

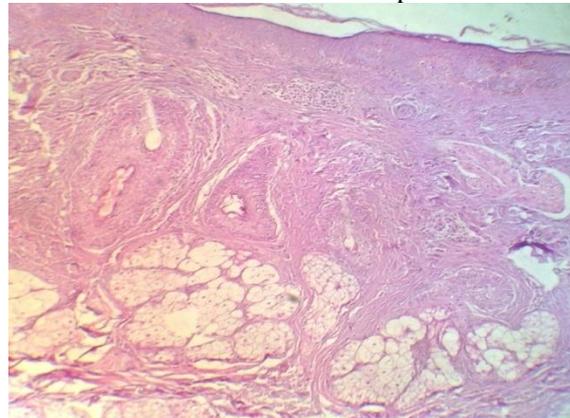


Fig 7 : Folliculitis Decalvans, perifollicular fibrosis

- Acne Keloid :

Sections examined revealed the presence of perifollicular reticular fibrosis along with thick collagen fibers having a keloid-like appearance. Plasma cells and neutrophils are detected. There are foci ranging from focal to complete follicular

destruction. Sebaceous glands are absent. Areas of chronic inflammation with numerous plasma cells and significant dermal fibrosis are a predominant feature as shown in fig 8.



Fig 8 : Acne Keloid perifollicular fibrosis and keloid like appearance of dermis, absent sebaceous glands

*** Immunohistochemistry results :**

- Results of CK 15immunohistochemical staining:

All CK 15 positive cells were located in the bulge region as shown in fig 9-11 .The staining reaction was strongly positive in the control group (10/10, 100%). Only 4 cases out of the total number of patients examined (n= 40) (10%) revealed a strongly positive reaction. A moderately to weakly positive reaction was observed in a total number of 13 cases. The reaction was however, negative in 23 cases (57.5%) with highly significance difference results (p<0.0001) as is shown in table (3) and fig 12. CK 15 reaction in various lesions of PCA shows no statistical difference as shown in table(4) .

- Evaluation of CD8 immunostaining reaction:

This was carried out on selected cases. Criteria of selection : Histologically detected prominent dermal lymphocytic infiltrate regardless of the nature of the lesion .It was found that 8 (50%)cases positive as shown in figures 13,14 and 8 cases (50%) negative as shown in fig 15. All -ve cases of Cd 8 were +ve in CK 15 while 75% of the +ve cases of Cd 8 were -ve in CK 15 and 25 % of them were +ve for both which are significant difference result (p<0.05). 16 cases are evaluated for CD8⁺ revealed positivity in 4 DLE, 2 LPP, 2 AK cases as showing in table 5,6,7 .

Table (1): Demographic data of the two studied groups:

This table shows that there were no statistical significance differences between cases and control in age or sex distribution.

Variable	Cases group (n=40)		Control group (n=10)		Test	P
					T	
Age (years)						
Mean ± SD	34.50 ± 9.32		35.5 ± 11.99			0.78
Range	20 – 62		21 – 62		0.29	N.S
Sex	No	%	No	%	χ^2	P
Male	28	70	7	70		1
Female	12	30	3	10	0	N.S

Table(2): Diagnosis results of the cases by both clinical and trichoscopy:

Diagnosis	Clinical (n=40)	Trichoscopy (n=19)	χ^2	P
Discoid lupus erythematosus	10	8	9.58	0.04*
Lichen planopilaris	8	6		
Keratosis follicularis spinulosa decalvans	5	-		
Folliculitis decalvans	7	5		
Acne Keloid	10	-		

Table (3): CK 15 results in the 2 studied groups:

Ck15	Cases (n=40)		Control (n=10)		χ^2	P
	No	%	No	%		
-ve	23	57.5	0	0	32.14	0.000**
Weakly +ve	8	20	0	0		
Moderately+ +ve	5	12.5	0	0		
Strongly++ +ve	4	10	10	100		

Table (4): CK 15 results in different types of lesions:

This table shows no statistical significance differences between different types of lesions in CK 15 results.

Type of Lesion	CK 15								χ^2	P
	-		+		++		+++			
	(n=23)		(n=8)		(n=5)		(n=4)			
	N	%	N	%	N	%	N	%		
Lichen planopilaris	6	26.1	1	12.5	1	20	0	0	10.64	0.58 N.S
Discoid lupus erythematosus	5	21.7	3	37.5	2	40	0	0		
Keratosis follicularis spinulosa decalvans	2	8.7	2	25	1	20	0	0		
Folliculitis decalvans	4	17.4	1	12.5	0	0	2	50		
Acne Keloid	6	26.1	1	12.5	1	20	2	50		

Table (5): CD 8⁺ results in the studied cases:

CD8 ⁺	Cases (n=8)	
	No	%
-ve	8	50
+ve	8	50

Table (6): Relation between CK 15 results and CD8⁺ results:

This tables shows that there were statistical significant differences between –ve cases in CK 15 and +ve cases in Cd 8 results.

Ck15	CD 8 ⁺				χ^2	P
	-ve (n=4)		+ve (n=4)			
	No	%	No	%		
-ve	0	0	6	75	4.80	0.03*
+ve	8	100	2	25		

Table (7): CD 8⁺ results in different types of lesions:

This table shows no statistical significance differences between different types of lesions in CD 8⁺ results.

Type of Lesion	CD 8 ⁺				χ^2	P
	-ve (n=4)		+ve (n=4)			
	N	%	N	%		
Lichen planopilaris	4	50	2	25	1.33	0.51 N.S
Discoid lupus erythematosus	4	50	4	50		
Acne Keloid	0	0	2	25		

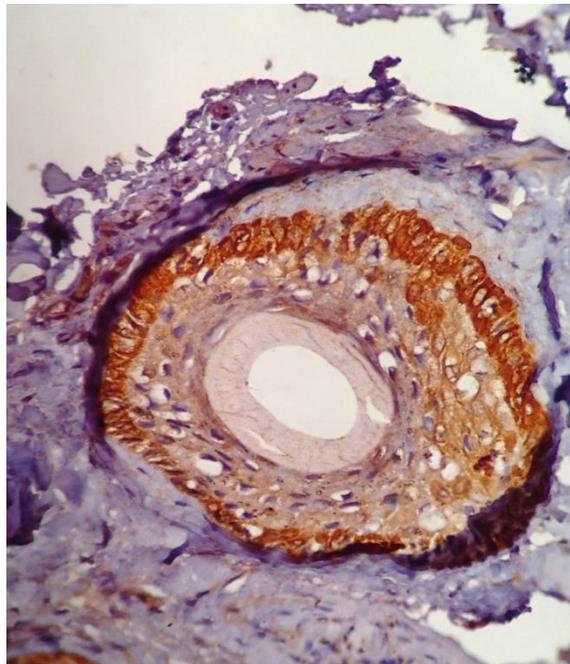


Fig 9 : Folliculitis Decalvans strongly positive CK 15.

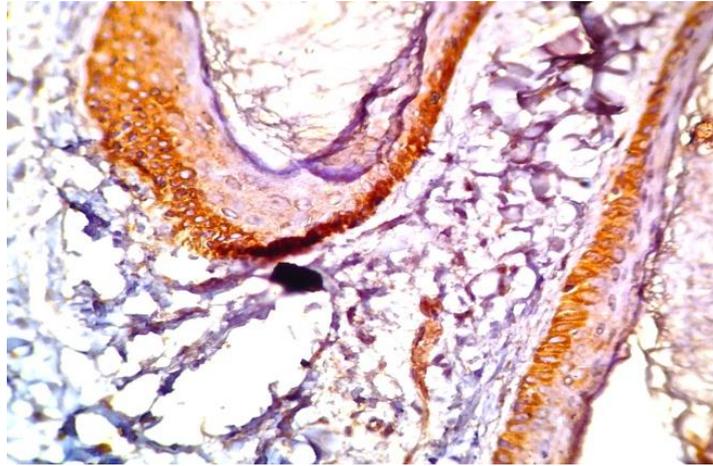


Fig 10 : Discoid lupus erythematosus. High power view of CK15 +ve

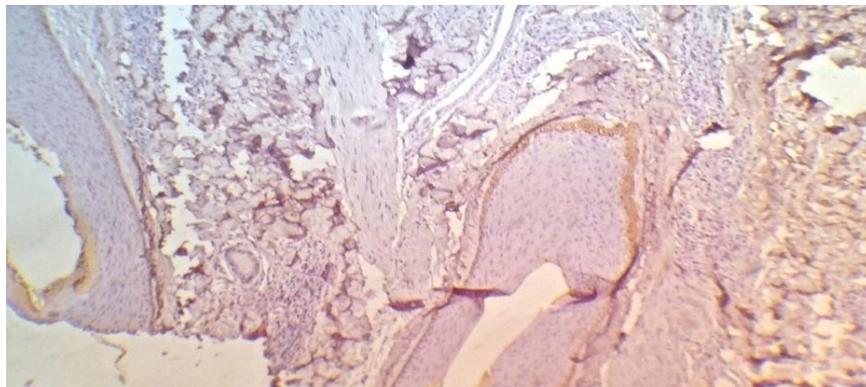


Fig 11 : Lichen Planopilaris,CK15, Mod- mild+ve

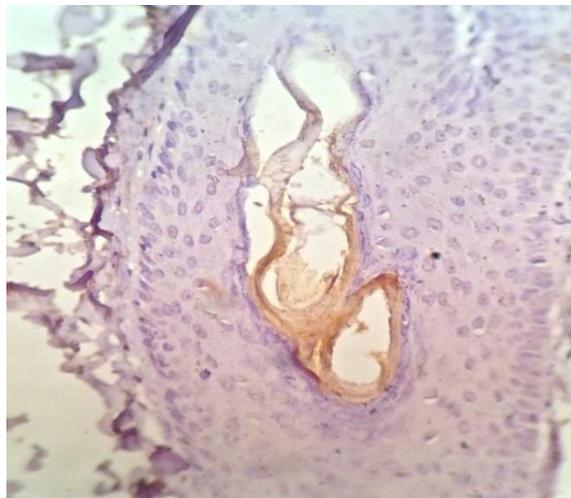


Fig 12 : Negative CK15

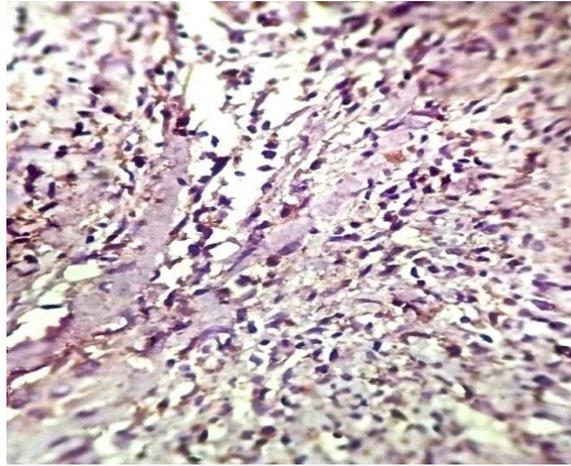


Fig 13 : Discoid lupus erythematosus, CD8, surrounding destroyed hair follicle

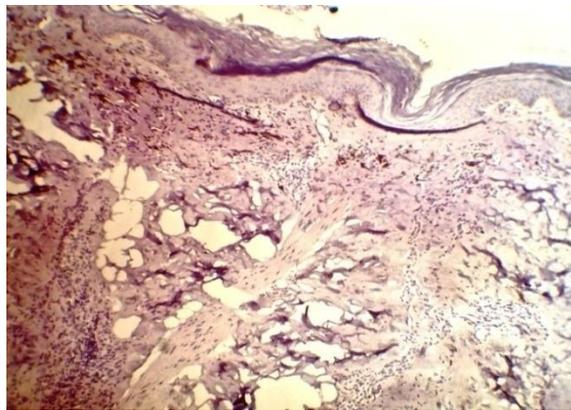


Fig 14 : CD8 positive. : Lichen Planopilaris around fibrotic hair follicle

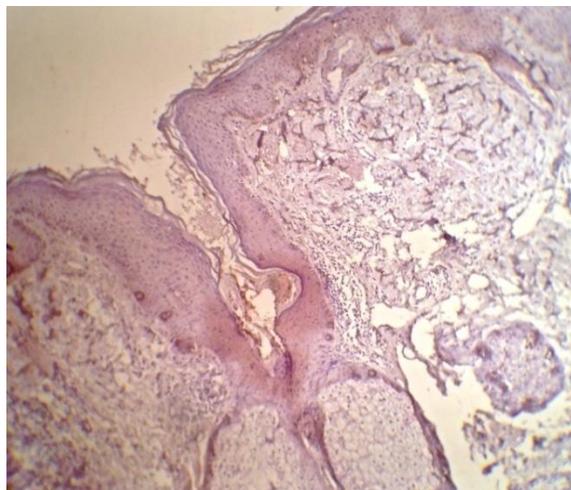


Fig 15 : CD8 negative

DISCUSSION

The origin and pathogenesis of primary cicatricial alopecia remain largely unclear, there are several theories to explain the etiopathogenesis of cicatricial alopecia. So far, there is still little supporting evidence for any one of these hypothesis and each hypothesis is not necessarily exclusive of another. It is even possible that a different hypothesis will eventually be linked to different cicatricial alopecia presentations⁽²⁵⁾.

These theories include autoimmune-mediated (through inappropriate presentation of self-antigen to the adaptive immune system) and immune privilege breakdown mechanisms, the danger versus non-danger hypothesis, bulge stem cell destruction, the hair follicle epithelial-mesenchymal communication inhibition hypothesis, sebaceous gland dysfunction, and the genetic mutation of keratin hypothesis⁽²⁶⁾.

In this study we focused on the bulge stem cell destruction examined by CK 15 expression. Bulge stem cell was negative in 23 cases (57.5%), but strongly positive in 4 cases (10%). It was negative in LPP (26.1%), DLE (21.7%), KFSD (8.7%), FD (17.4%), AK (26.1%). These findings raise the possibility that destruction of the hair follicle bulge cells may represent a primary event that leads to cicatricial alopecia.

In cases of dense inflammatory infiltrate of cytotoxic cells (CD8⁺), CK15 expression was weak or absent. These findings of a decrease or absence of CK15⁺ cell staining at the bulge of hair follicles in lesions in which there was a severe inflammatory infiltrate of cytotoxic cells supports a contributory role for cytotoxic lymphocytes in the pathogenesis of the scarring process in these diseases. These changes in CK15 staining may indicate destruction of stem cells or possibly differentiation of stem cells to repair the damage caused by the surrounding cytotoxic cells. Similarly, comparing expression of SC markers in scarring versus non scarring alopecia, reported CK15 expression in bulge region in 53% (23/43) of cicatricial alopecia⁽²⁷⁾.

The bulge region appears to be involved in DLE (as demonstrated by using CK 15 as a marker of stem cells) as part of broader involvement of the hair follicles; it is secondarily affected by the surrounding inflammatory cell infiltrate. Expression of stem cells markers diminished and were then absent⁽¹⁴⁾.

Decrease and absence of CK 15 staining in (75%) 12/16 at late stage of LPP were demonstrated, while

in all cases studied showed a lichenoid lymphocytic infiltrate at the bulge region, the bulb area was spared⁽¹⁷⁾. Within inflammatory infiltrate, CD8⁺ T cells were increased in LPP cases studied, at late stage of disease absence of bulge stem cell in (57.1%) 20/35⁽¹⁶⁾. It is consistent with our study

The loss of CK15⁺ stem cells in most affected hair follicles in LPP was confirmed (40/55), with unaffected hair follicles retaining CK15⁺ stem cells demonstrated⁽²⁸⁾. It is similar to current study results.

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