Evaluation of Elastic Fibers Pattern with Orcein Staining in Differential Diagnosis of Lichen Planopilaris and Discoid Lupus Erythematosus

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Abstract- Differential diagnosis of lichen planopilaris and discoid lupus erythematosus especially in late stages is a problem for clinicians and pathologists. Our aim was to find discriminator histopathologic findings that help us to achieve definite diagnosis without using immunofluorescence study. The histopathologic findings in 77 cases of lichen planopilaris were compared with those of 26 cases of discoid lupus erythematosus with Hematoxylin & Eosin and especially staining (Alcian blue *pH* 2.5, Periodic Acid Shiff, Orcein). Final histopathologic diagnosis was based on histologic findings, clinicopathological correlation, past medical history and immunofluorescence studies if were applied before. Then elastic fibers pattern in dermis and follicular sheath with orcein staining were described without having information about final diagnosis. New and subtle presentations of histologic changes were assessed. We compared all histopathologic finding for each staining method. Some histologic changes such as hypergranulosis, epidermal atrophy, mucin deposition, diffuse scar and some other patterns were not specific for any diagnosis. A setting of histopathologic findings and clinicopathological correlation were needed for accurate diagnosis. We had only one specimen for the vertical section, and we had no horizontal sections. Description of elastic fibers pattern in orcein staining may be helpful in achieving a specific diagnosis, but this is not completely reliable, and we had overlap features. Finally, immunofluorescence study may be recommended for suspicious cases.

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Keywords: Lichen planopilaris; Discoid lupus erythematosus; Elastic fibers; Orcein staining; Mucin deposition

Introduction

Scarring alopecia encompasses a heterogeneous group of disorders characterized by permanent area of reduced concentration or complete absence of hair follicle and replacement by fibrosis (1). Our pathologists have many problematic cases. Unfortunately, it seems that the incidence of lichen planopilaris has increased in recent years in our country, and we have decreased age of patients. Lichen planopilaris (LPP) and discoid lupus erythematosus (DLE) in early stages and active lesions can be differentiated easily histologically but in late stage lesions and scarring areas we have difficulties in diagnosis (2), especially if the immunofluorescence (IF) studies are not available.

Therefore, it is necessary to search for histopathologic findings in hematoxylin & eosin (H&E) and especial staining which can helpful in the differential diagnosis. We undertook a histopathologic study on a case series of LPP and DLE to reassess histopathologic criteria for diagnosis and delineate which changes are sufficiently reliable to allow definite diagnosis. Another purpose was to identify new and subtle histologic alterations in elastic fibers patterns with orcein staining which only in two old articles (3, 4) were studied and not repeated again.

Materials and Methods

This research project was derived from a

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dermatopathology fellowship thesis carried out at Skin Research Center (SRC) of Shahid Beheshti University of Medical sciences.

Histopathologic findings of 90 biopsy specimens of LPP and 35 specimens of DLE between July 2009 and July 2012 at Skin Research Center (SRC) of Shahid Beheshti University Medical of sciences were reviewed. We had only one piece of tissue in paraffin blocks, and our technicians were more familiar with vertical sections, so we preferred to perform vertical sections to facilitate the differential diagnosis between long-standing LPP and DLE

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Table 1. Histopathologic findings in H&E staining for evaluation of epithelial changes

Histopathologic findings	LPP[1] (n=77)	DLE[2] (n=26)
Horny layer view		
Normal	10(12.99%)	1(3.85%)
Hyperkerathosis[3]	67(87.01%)	25(96.15%)
Orthokeratosis	66(98.51%)	25(100.00%)
Parakerathosis	1(1.49%)	-
Follicular plug view		
Absent	22(28.57%)	4(15.38%)
Present	55(71.43%)	22(84.62%)
Cup shaped[4]	12(21.82%)	12(54.54%)
Wedge shaped[5]	42(76.36%)	7(31.82%)
Cup and wedge shaped	1(1.82%)	3(13.64%)
Granular layer view		
Epidermal hypergranulosis	26(33.77%)	6(23.08%)
Only Infundibular hypergranulosis	30(38.96%)	6(23.08%)
Normal granular layer	21(27.27%)	12(46.15%)
Epidermal hypogranulosis	-	2(7.69%)
Epidermal view		
Normal	39(50.65%)	16(61.54%)
Reticulated pattern[6]	31(40.26%)	1(3.85%)
Atrophy	6(7.79%)	9(34.62%)
Hypertrophy	1(1.30%)	DLE (n=26)
Histopathologic findings	LPP (n=77)	-
Epithelial changes in epidermis		
Absen	36(46.75%)	3(11.54%)
Vacuolar degeneration	35(45.45%)	22(84.62%)
Lichenoid reaction	6(7.79%)	1(3.85%)
Hair follicle		
Absent	8(10.39%)	2(7.69%)
Present	69(89.61%)	24(92.31%)
Epithelial changes of follicle		
Absent	11(15.94%)	2(8.33%)
Vacuolar degeneration	6(8.70%)	9(37.50%)
Lichenoid reaction	52(75.36%)	13(54.17%)

[1] Lichen planopilaris
[2] Discoid lupus erythematosus
[3] For example, horny layer view in 67 (87.01%) of 77cases LPP was Hyperkerathosis. of 67 cases which had hyperkeratosis, 66 cases (98.51%) were orthokeratosis and one case (1.49%) had focal parakeratosis
[4] Cup shaped means O shaped view of follicular plug
[5] Wedge shaped means V shaped view of follicular plug
[6] Reticulated patterns was due to confluence of slightly elongated rete ridges

Table 2. Histopathologic findings in H&E staining in dermis

Histopathologic findings	LPP (n=77)	DLE (n=26)
Dermal inflammation		
Absent	-	-
Present	77(100.00%)	26(100.00%)
Mild	19(24.67%)	6(23.08%)
Moderate	56(72.73%)	20(76.92%)
Severe	2(2.60%)	-
Inflammation site		
Perivascular	77(100.00%)	26(100.00%)
Superficial	77(100.00%)	26(100.00%)
Deep	56(72.73%)	25(96.15%)
Perifollicullar	68(88.31%)	20(76.92%)
Upper segment(upper bulge)	31(45.59%)	-
Lower segment(below bulge)	3(4.41%)	2(10.00%)
Upper and lower segment	34(50.00%)	18(90.00%)
Perieccrine	7(9.09%)	17(65.38%)
Interstitial	3(3.90%)	1(3.85%)
Subcutaneous involvement	3(3.90%)	6(23.08%)
Inflammation type		
Lymphocyte and histiocyte	77(100.00%)	26(100.00%)
Neutrophil	3(3.90%)	6(23.08%)
Plasma cell	6(7.79%)	6(23.08%)
Eosinophil	3(3.90%)	1(3.85%)
Histopathologic findings	LPP (n=77)	DLE (n=26)
Mast cell	29(37.66%)	7(26.92%)
Mild[1]	16(55.17%)	6(85.71%)
Moderate	8(27.59%)	1(14.29%)
Severe	5(17.24%)	-
Telangiectasia		
Absent	62(80.52%)	3(11.54%)
Present	15(19.48%)	23(88.46%)
RBC Extravasation		
Absent	76(98.70%)	21(80.77%)
Present	1(1.30%)	5(19.23%)
Sebaceous glands		
Normal	2(2.60%)	4(15.38%)
Decreased	31(40.26%)	14(53.85%)
Absent	44(57.14%)	8(30.77%)
Arrector pili muscle		
Normal	17(22.08%)	15(57.69%)
Decreased	60(77.92%)	11(42.31%)
Fibrous tract		
Absent	9(11.69%)	14(53.85%)
Present	68(88.31%)	12(46.15%)

[1] Giemsa staining was applied for better observation of mast cells in cases we suspected more than average 2 mast cells in H&E stain on one high power field (HPF). We determined these values: Mild: <5 mast cells, Moderate 5-10mast cells, Severe>10 mast cells in each HPF. Moderate 5-10 mast cells, Severe>10 mast cells in each HPF

[2] Red Bloods Cell

Inclusion criteria were as follow: pathologic diagnosis of DLE or LPP in recorded pathology reports, adequate depth and diameter of tissue specimen in paraffin blocks and presence of scarring areas in primary sections to evaluate elastic fibers with orcein staining.

Exclusion criteria were as follow: no definite diagnosis of DLE or LPP, inadequate depth and diameter of specimen and absence of scar in primary sections (inflammatory lesions).

Twelve cases of LPP group and 6 cases of DLE group were excluded because of inadequate depth in specimen or absence of scarring area, also one case in LPP group and 3 cases in DLE group which had incorrect diagnosis in overview were excluded. Finally 77 specimens in LPP group and 26 specimens in DLE group were reviewed by two pathologists. We used H&E staining for routine assessment, Periodic Acid Schiff (PAS) for evaluation of basement membrane thickening, Alcian blue *pH* 2.5 for evaluation of mucin and synthetic orcein staining to evaluate elastic fibers.

In the article, data are reported as number (percentage) for categorical variables. The Fisher exact test or Pearson $\chi 2$ tests were used to compare categorical variables between groups, wherever appropriate. All p-values were two-sided and tests were conducted at the p=0.05 level of significance. Data analysis was conducted using SPSS for Windows version 16.0.0 (SPSS Inc., Chicago, IL, USA).

Result

This study comprised 77 cases with LPP (55 women and 22 men), ranging in age from 17 to 74 years (mean age, 44.05±13.17 years) at the time of diagnosis. Also, 26 cases with DLE were recruited to this study (19 women and 7 men). In this group, the mean age at the time of diagnosis was 40.69±12.99 years (Range 15-64 years).

We only mentioned important findings in this section and referred to table for more information.

Histopathologic findings were summarized in tables designed for this purpose, as follow:

Histopathologic changes of epithelium and dermis in H&E staining of two groups are shown in tables 1 and 2, respectively. Table 3 demonstrates mucin deposition in Alcian blue staining of two groups. The patterns of elastic fibers in Orcein staining of two groups are presented in table 4.

Table 3. Histopathologic findings in Alcian blue staining

Histopathologic findings	LPP (n=77)	DLE (n=26)
Perifollicular mucinous fibroplasia		
Absent	33(42.86%)	26(100.00%)
Present	44(57.14%)	-
Above bulge area	22(50.00%)	-
Below bulge area	4(9.09%)	-
Above and Below bulge area	18(40.91%)	-
Dermal mucin		
Absent	51(66.23%)	5(19.23%)
Present	26(33.77%)	21(80.77%)
Mild	24(92.31%)	8(38.10%)
Moderate	2(7.69%)	5(23.80%)
Severe [1]	-	8(38.10%)

[1] We considered severe mucin deposition when prominent mucins were seen in deep dermis and perieccrine glands.

Table 4. Histopathologic findings in Orcein staining

Histopathologic findings	LPP (n=77)	DLE (n=26)	p-value
Dermal elastic fibers pattern			
A-Loss of elastic fibers of dermal papillae *without* wedge shaped scar view	3(3.90%)	13(50.00%)	<0.0001
B-Loss of elastic fibers of dermal papillae *with* wedge shaped scar view	60(77.92%)	2(7.69%)	<0.0001
C-Loss of elastic fibers of dermal papillae and upper reticular dermis (*band* shaped scar)	6(7.79%)	-	0.33
D-*Fragmentation* of elastic fibers in reticular dermis	52(67.53%)	5(19.23%)	<0.0001
E-*Partial loss* of elastic fibers in reticular dermis	13(16.88%)	21(80.77%)	<0.0001
F-*Increased* elastic fibers in reticular dermis [1]	-	-	-
***Associated* B and D patterns**	46(59.74%)	1(3.85%)	<0.0001
***Associated* B and E patterns**	8(10.39%)	1(3.85%)	0.44
***Associated* A and D patterns**	2(2.60%)	2(7.69%)	0.26
***Associated* A and E patterns**	1(1.30%)	11(42.31%)	<0.0001
Elastic sheath (coat) pattern around hair follicles and fibrous tract			
Normal pattern [2]	8(10.39%)	20(76.92%)	<0.0001
Destruction in upper part of elastic sheath but normality in lower part	61(79.22%)	5(19.23%)	<0.0001
Destruction in upper part of elastic sheath but increased in lower part with narrow fibrous tract	7(9.09%)	1(3.85%)	0.68
Destructions in upper part of elastic sheath but increased in lower part with wide fibrous tract	1(1.30%)	-	1.00

In these tables, we used North American Hair Research Society (NAHRS) criteria for pathologic evaluation of cicatricial alopecia (5), which were appreciable in vertical sections.

There were following findings in LPP group:

Hyperkerathosis (87.01%), follicular plugging (71.43%), epidermal hypergranulosis (33.77%), infundibular hypergranulosis (38.96%), reticulated pattern of epidermis (40.26%) due to the confluence of slightly elongated rete ridge , absent epithelial changes in epidermis (46.75%), lichenoid reaction in basal layer of follicular epithelium (75.36%), superficial (100.00%) and deep (72.73%) perivascular inflammation, perifollicular inflammation (88.31%), perieccrine inflammation (9.09%), mast cell infiltration (37.66%), interstitial and subcutaneous involvement which composed of mast cells (3.90%), present fibrous tract (88.31%), telangiectasia (19.48%), absent sebaceous glands (57.14%), perifollicular mucinous fibroplasia (57.14%), dermal mucin deposition (33.77%), loss of elastic fibers of dermal papillae with wedge shaped scar (77.92%) (Figure 1), fragmentation of elastic fibers in reticular dermis (67.53%) (Figure 2), association of these two patterns (59.74%), loss of elastic fibers of dermal papillae and upper reticular dermis (band shaped scar) (7.79%) (Figure 3), destruction in upper part of follicular elastic sheath but normality in lower part (79.22%).

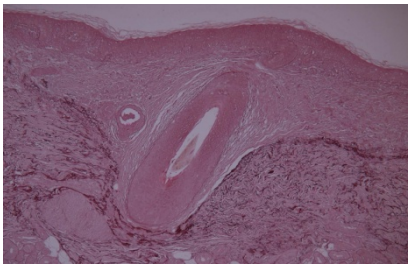

Figure 1. Lichen planopilaris, wedge shaped scar in orcein staining.x10

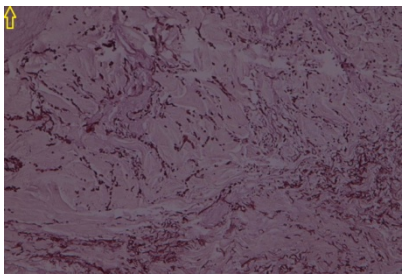

Figure 2. Lichen planopilaris, fragmentation of dermal elastic fibers in orcein staining.x40

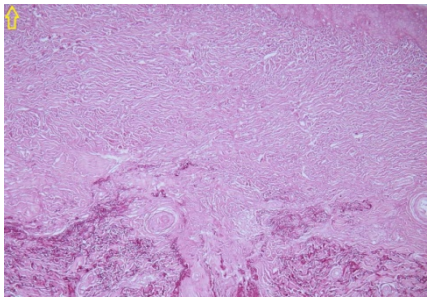

Figure 3. Lichen planopilaris, band shaped scar (Loss of elastic fibers in dermal papillae and upper reticular dermis) in orcein staining.x10

Fisher exact test showed a significant association between mast cells infiltration and band shaped scar (p=0.03). About one third of LPP cases with moderate to severe mast cells infiltration had band shaped scar, and there was not band shaped scar in LPP cases without significant mast cells infiltration.

There were following findings in DLE group:

Hyperkerathosis (96.15%), follicular plugging (84.62%), normal granular layer (46.15%), epidermal atrophy (34.62%), vacuolar degeneration of epidermis (84.62%), lichenoid reaction in basal layer of follicular epithelium (54.17%), superficial (100.00%) and deep perivascular inflammation (96.15%), perifollicular inflammation (76.92%), perieccrine inflammation (65.38%), telangiectasia (88.46%), neutrophil and plasma cell infiltration (23.08%), mast cells infiltration (26.92%), red blood cells (RBCs) extravasation (19.23%), decreased sebaceous glands (53.85%), absent fibrous tract (53.85%), thickening of basement membrane (76.92%), dermal mucin deposition (80.77%), loss of elastic fibers of dermal papillae without wedge shaped scar view (50.00%), partial loss of elastic fibers in reticular dermis (80.77%) (figure 4), association of these two patterns (42.31%), normal pattern of elastic sheath around hair follicle (76.92%).

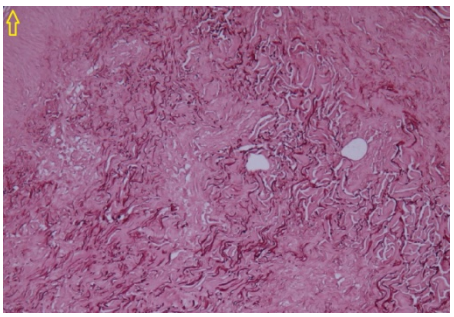

Figure 4. Discoid lupus erythematosus, partial elimination of elastic fibers in mid dermis, in orcein staining.x20

In DLE cases with mucin deposition (n=21), severe (massive and deep) mucin deposition was observed in 4 out of 14 cases with perieccrine infiltration and in 4 out of 7 cases without perieccrine infiltration. No significant association was observed between the amount of mucin deposition in DLE cases and absence of perieccrine inflammation (p=0.34).

In orcein staining, loss of elastic fibers of dermal papillae with wedge shaped scar view, fragmentation of elastic fibers in mid dermis and association of these two patterns were in favor of LPP diagnosis (all P<0.0001); on the other hand, loss of elastic fibers of dermal papillae without wedge shaped scar view, partial loss of elastic fibers in reticular dermis and association of these two patterns were in favor of DLE diagnosis (all P<0.0001, Table 4). In the evaluation of elastic sheath of follicles, destruction in the upper part of elastic sheath and normality in lower part demonstrated LPP but normal elastic sheath was indicator of DLE diagnosis (P<0.0001, Table 4). Comparison of other parameters in orcein staining was not statistically significant between two groups (Table 4).

Discussion

This study describes histopathologic findings of 103 cases with scarring alopecia which had the definite diagnosis of DLE or LPP.Histopathlogic criteria, clinical history or previous IF study results were in favor of these diagnosis. In these series, LPP and DLE affected mainly adult women. Mean age in each group in our research was about 6-8 years lower than other studies (6, 7), and this showed either lower age at presentation of the diseases or earlier referral of patients to dermatologist.

Both LPP and DLE had orthokerathosis in most cases, but focal parakeratosis was seen only in one case of LPP group but parakeratosis was more common in earlier reports in DLE and LPP (2, 8). Follicular plugging was seen in most cases of LPP and DLE. Cup shaped view of the follicular plug was more prominent in DLE group and wedge shaped type was more prevalent in LPP group similar to previous study (9). Cup shaped follicular plug may be due to abundant laminated keratin in dilated infundibulum in the DLE series (10), but this view was not specific for any diagnosis, and we observed two views simultaneously in one lesion. Interestingly, epidermal and infundibular hypergranulosis was seen in DLE group, in addition to LPP series and, there was hypogranulosis only in a minority of DLE cases.

Reticulated pattern of epidermis was observed in 40% of LPP and 3.8% of DLE cases. This pattern was in favor of LPP in the previous report (1).

There was epidermal atrophy in 7.7% of LPP cases which was lower than 34.6% of cases in DLE group. Absent epithelial changes in the epidermis were more common than vacuolar degeneration of basal layer and lichenoid reaction in LPP cases similar to previous study (6). The most common epithelial change of epidermis in DLE group was vacuolar degeneration, such as another study (11). In follicular epithelium, lichenoid reaction in LPP and DLE cases was more common than normal and vacuolar degeneration pattern.

Perivascular infiltration around deep vessels of reticular dermis was observed in 96% of DLE cases and 72% of LPP cases. Also, higher rate of deep perivascular infiltration in LPP cases was seen in another study (6) but this is in contrast with no deep vessel involvement in LPP in other research (1).

Inflammatory infiltration around lower segment of the hair follicle in LPP without upper segment involvement was very low (4% of cases) as in previous similar study (6). Upper and lower segment involvement of the hair follicle in DLE was more common than LPP (90% vs. 50%).

Eccrine gland involvement in LPP cases was present in 9% of cases only in one direction in the vicinity of follicular inflammation, but there was not total involvement of eccrine glands such as DLE. Thus this view should not be mistaken with DLE.

Interstitial and subcutaneous infiltration in LPP cases was present but mostly composed of mast cells and as we know mast cells have a probable role in the pathogenesis of lichen planus (12, 13). Neutrophils and plasma cells were more common in DLE group, but one case of LPP group with the history of Crohn's disease had many neutrophils with hyper segmented nuclei and many extravasated RBCs. Two cases of LPP group had neutrophils because of folliculitis.

Five cases of LPP had abundant and large mast cells (figure 5), but in clinical evaluation, these patients did not have any symptoms of systemic mastocytosis, unlike the previous case report about association of mastocytosis and scarring alopecia (14), for this reason we did not measure the level of urinary N-methyl histamine because this test was not specific for mastocytosis and may be positive in any mast cell degranulation phenomenon such as severe systemic allergic reaction (15). These patients had only history of atopy, asthma and scalp pruritus in contact with some materials (for example formalin).

Decreased size of sebaceous glands (atrophy) was

more prevalent in DLE, but loss of sebaceous glands was more significant in LPP cases which these morphologic features may be associated with the pathogenesis of them.

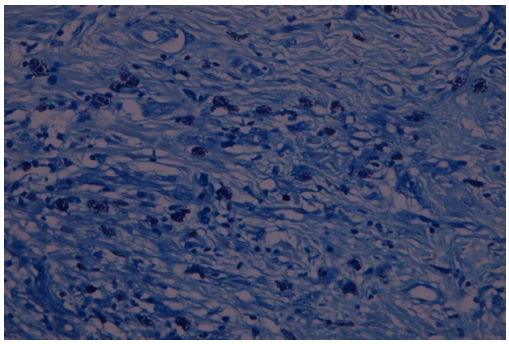

Figure 5. Lichen planopilaris, severe mast cells infiltration in dermis in toluidine blue staining.x40

Arrector pili muscles were not disappeared completely in any cases, but their numbers were decreased. Fibrous tract formation was more prominent in LPP cases because fibrosis of interfollicular dermis in DLE cases resulted in indistinct fibrous tract (3).

Basement membrane thickening was detected in 76.9% of DLE cases such as another research (11). Mucinous fibroplasia was detected in 57% of LPP cases which was more common than the previous report (6). 9% of these cases had mucinous fibroplasia only below bulge area which was not recorded in previous studies.

Dermal mucin was found in 33.77% of LPP cases; thus this finding was not specific for DLE diagnosis.

In orcein staining wedge shaped scar in papillary dermis and around upper follicle was seen in 77.9% of LPP cases which in 59.7% of LPP cases this view was in keeping with fragmentation of elastic fibers in reticular dermis. We do not know if fragmentation of elastic fibers was initiation of scar formation or not, so we considered this feature separately.

In previous studies wedge, shaped scar was seen in 60% and 80% of LPP cases respectively (3,8). Lichenoid reaction in upper dermis resulted in this pattern of scar (1).

Eighty percent of DLE cases had partial elimination (loss) of elastic fibers in mid dermis which resulted from previous patchy infiltration in this region. In 42.3% of DLE cases, this view was associated with papillary dermal fibrosis without wedge shaped scarring. In prior researches, interfollicular scar was found in 70% of DLE cases (3).

DLE cases had wedge shaped scar only in 7.69% of cases, on the other hand, partial elimination of elastic fibers in mid dermis was seen only in 16.8% of LPP cases, then overlap scar patterns were present in DLE and LPP groups. Previous study reported the diffuse scar which involved interfollicular dermis in 22% (6) and association of perifollicular with mid dermal fibrosis in 50% of LPP cases (8).

Band shaped scar in upper dermis in LPP group may be due to fibrogenic roles of mast cells. Mast cells release IL-4, which may stimulate fibroblast activities (16).

Normal histologic view of elastic fibers around the hair follicle is characterized by loose arrangement of elastic fibers in infundibular portion and well organized elastic coat in isthmus portion with more aggregation in connection of arrector pili muscle (bulge) and sparse elastic fibers on lower segment .This view is quantitatively variable between different people (4), for this reason sometimes judgment was difficult for us.

Seventy-six percent of cases in DLE group had normal elastic sheath around the hair follicle because of lower rate of wedge shaped scar in DLE cases. In DLE cases with papillary dermal fibrosis, we observed that dermal papillae far from the hair follicle had fibrosis, but dermal papillae adjacent to hair follicle had normal elastic fibers.

Increased elastic fibers around lower segment of fibrous tract are not necessarily the indicator of idiopathic pseudopelade (4); because in 10% of LPP cases and 3.8% of DLE cases, lower segment of fibrous tract had increased elastic fibers.

In conclusion; in the differential diagnosis of DLE and LPP, we need a setting of histopathologic findings with routine and especial staining, information about overlap features of them in histopathology and clinicopathological correlations; especially if study is not available, however, negative IF results does not exclude DLE diagnosis (2).

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