INTRODUCTION

Alopecia is a common disease and can be divided into scarring and nonscarring. It includes primary and secondary types. In the primary type, hair follicles are the first target of destruction. While the etiology and pathogenesis of primary scarring alopecia are unclear, it is documented that the follicular units are substituted by fibrous tract, thereby causing permanent hair loss and scarring. Primary scarring alopecia is classified on the basis of the predominant cell type of inflammation. Four subgroups of scarring alopecia are composed of lymphocytic, neutrophilic, mixed, and nonspecific. Lichen planopilaris (LPP) and discoid lupus erythematosus (DLE) are in the lymphocyte-dominant subgroup. This is an open access journal, and articles are distributed under the terms of the Creative Commons Attribution-NonCommercial-ShareAlike 4.0 License, which allows others to remix, tweak, and build upon the work non-commercially, as long as appropriate credit is given and the new creations are licensed under the identical terms.

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The diagnosis of primary scarring alopecia is a challenge for pathologists and clinicians. Hematoxylin and eosin (H and E) staining with clinical correlation is routinely used for the classification of scarring alopecia. However, the definite diagnosis is often difficult due to the overlap of clinical and histologic features. As a result, an adjunctive diagnostic technique is needed to help achieve a more accurate diagnosis.

Recently, some studies have shown the presence of plasmacytoid dendritic cells (PDCs) in the cutaneous lupus erythematosus by CD123 immunostaining. These dendritic cells are not seen in normal epidermis, dermis, and follicular epithelium.[7-10] CD123 is an interleukin-3 receptor alpha chain on the PDC surface.[8,11,12] PDCs seem to have a significant role in the pathogenesis of lupus erythematosus due to the fact that they can produce large amounts of type I interferon. PDCs are present in the perivascular, periadnexal, and dermoepidermal junction infiltration and mostly form clusters of cells.[8,9,13]

This study aims to apply CD123 on scalp biopsies with proven DLE and LPP diagnosis and typical histologic features to evaluate the role of this marker in the differentiation of DLE from LPP.

Furthermore, the patterns of elastic fiber loss in elastic staining are often used to differentiate DLE from LPP, especially in challenging cases.[8,9] Therefore, elastic staining is done, and the results for DLE and LPP are compared.

**PATIENTS AND METHODS**

**Study design**

Pathology archives of Faghihi Hospital Laboratory, affiliated to Shiraz University of Medical Sciences, were searched for the clinical diagnosis of LPP and DLE with alopecia from 2015 to 2018. The study protocol was approved by the Institutional Review Board of Shiraz University of Medical Sciences and the Ethics Committee of Shiraz University of Medical Sciences (Ethics code: IR.sums. med.rec. 1395.s179). All H and E slides were reviewed by two dermatopathologists who blind to clinical diagnosis to confirm the diagnosis. In cases with disagreement, they jointly reviewed the slides to reach the final diagnosis. All LPP cases had to meet criteria including a typical clinical presentation, perifollicular lamellar fibroplasia at the level of the infundibulum or superficial isthmus with a perifollicular lymphocytic infiltrate at the same level, eccentric atrophy and squamatization of the outer root sheath, perifollicular clefting, and hypergranulosis of the infundibulum.[8,9] The inclusion criteria for DLE were a typical clinical presentation, follicular plugging, the presence of interfollicular epidermal interface dermatitis, thickened basement membrane, and the presence of a lymphocytic infiltrate in both the superficial and deep dermis and the subcutis.[8,13] All the cases had two 4-mm punch biopsies (horizontal and vertical sections). Both isolated CD123-positive PDCs and CD123 clusters (defined as at least five clustered PDCs) were examined within the dermis.[8,9] Cases with one punch biopsy and all the cases with unequivocal diagnoses or those with few mononuclear cell infiltrations were excluded.

**Techniques of immunohistochemical staining**

Immunohistochemistry (IHC) staining for CD123 antigens was done manually on 5-μm sections, obtained from formalin-fixed paraffin-embedded blocks, using the avidin–biotin–peroxidase complex method. Unstained tissue sections were collected on poly-L-lysine slides for IHC staining. Unstained slides were deparaffinized in xylene (30 min) and gradually rehydrated by placing them in distilled water (2 min) and then in a mixture of distilled water and H₂O₂, and were washed in phosphate-buffered saline (PBS) for 5 min.

All the slides were boiled in Tris buffer (PH = 9.0) for 50 min and then were cooled and put in PBS (5 min). The slides were blocked with 10% goat serum and incubated (20 min) at room temperature in a humidified chamber for 1 h.

In the subsequent stage, an anti-human CD123 mouse monoclonal antibody (Master diagnostic CD123 antibody, 1:250) (7G3) was used. After washing for 20 min in PBS and putting them in a wet chamber, one drop of horseradish peroxidase polymer was applied on sections (30 min) at room temperature, and they were washed in PBS for 10 min. After adding 3,3’-diaminobenzidine chromogen, they were washed again in PBS for 5 min. Next, the slides were counterstained with hematoxylin, rinsed in running water for some minutes, dehydrated in graded ethanol solutions, cleared with xylene, and mounted.

**Immunohistochemical scoring**

The sections, stained by IHC, were examined alongside H- and E-stained slides to identify the precise locations of the lesions. CD123 was considered positive when it showed cytoplasmic and/or membranous staining. CD123

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was classified as negative when it was completely unstained or showed <10% and positive when >10% and >20%.[8]

The presence of PDC clusters with 10–20 cells or >20 cells were also considered. Moreover, the pattern and location of the CD123-positive cell infiltrate (perivascular, perifollicular, intrafollicular, follicular junction, perieccrine, or mainly interstitial) were mentioned.

Elastic staining

Elastic staining (Verhoeff’s stain method) was done on vertical sections of all cases, and then, fibrous tract formation and pattern of elastic fiber loss in the papillary dermis, wedge-shaped loss, or diffuse loss, were considered.

Statistical analysis

The results were presented as mean ± standard deviation for quantitative variables and were summarized by frequency (percentage) for categorical variables. To analyze the data, the Chi-square test was used; moreover, the sensitivity and specificity of CD123 were calculated based on a diagnostic test for 2-by-2 tables. For the statistical analysis, the SPSS software for Windows version 21.0 (IBM Corp., 2012, Armonk, NY, USA) was used. P < 0.05 was considered statistically significant.

RESULTS

The study was conducted on 43 patients with clinical diagnosis of LPP (23) and DLE (20) confirmed by histopathology. [Figure 1a and b]. The results of the presence and pattern of distribution of PDC and sensitivity and specificity are shown in Tables 1 and 2. Infiltration of PDC was seen in 90% of DLE cases. PDCs were >10% of infiltration in 18/20 cases of DLE and 15/23 of LPP [Figure 2a]. In 17/20 (85%) DLE cases, an infiltration of more than 20% of inflammatory cells was seen [Figure 2b]. These results were statistically significant (P = 0.0048). The presence of more than 10% PDC cells in inflammatory cells had 90% sensitivity and 34.7% specificity, whereas the presence of more than 20% PDC in inflammatory cells had 85% sensitivity and 91.3% specificity for DLE diagnosis. The presence of clusters of PDC was more specific. PDC clusters more than 20 cells had 100% specificity for DLE [Table 1 and Figure 2c]. There were no clusters with more than 20 cells in LPP. Perieccrine infiltration of PDC cells was seen only in DLE. Perivascular infiltration of PDC was seen in both DLE and LPP. Intrafollicular and follicular junction infiltrations of PDC were found mainly in DLE. However, these results were not statistically significant (P = 0.378).

The presence of the fibrous tract and pattern of loss of elastic fibers in the papillary dermis in elastic staining are shown in Table 3. The fibrous tract was seen in most cases of LPP and DLE. The pattern of elastic fiber loss in the papillary dermis was different in LPP and DLE [Figure 3a and b]. The wedge-shaped loss was the dominant pattern in LPP, and the diffuse loss of elastic fibers was the dominant pattern in DLE (P = 0.006).

DISCUSSION

PDCs play a critical role in the inflammatory process of autoimmune and immune-allergic dermatoses. Some studies showed that PDCs are seen in skin biopsies of systemic lupus erythematosus, DLE, and Jessner’s lymphocytic but not in normal skin.[8,9,11,16,17] The presence of PDCs in the cutaneous lupus erythematosus indicates the role of these cells in the pathogenesis of the disease.[8] In practice, the diagnosis

| Diagnosis | >10% infiltration, n (%) | >20% infiltration, n (%) | Cluster with 10-20 cells, n (%) | Cluster with >20 cells, n (%) |
|-----------|--------------------------|--------------------------|---------------------------------|-----------------------------|
| LPP       | 15 (65)                  | 2 (8)                    | 2 (8)                           | 0                           |
| DLE       | 18 (90)                  | 17 (85)                  | 15 (75)                         | 6                           |
| Sensitivity for diagnosis DLE | 90 (68.3-57.2) | 85 (62.1-96.7) | 75 (50.8-91.3) | 30 (11.9-54.3) |
| Specificity for diagnosis DLE | 34.7 (16.7-57.2) | 91.3 (71.9-98.9) | 91.3 (71.9-98.9) | 100 (85.1-100) |

DLE – Discoid lupus erythematosus; LPP – Lichen planopilaris

| Table 2: Distribution of CD123-positive cells at vertical sections |
|---------------------------------------------------------------|
| Perieccrine, n (%) | Intrafollicular, n (%) | Perifollicular, n (%) | Follicular junction, n (%) | Interstitial, n (%) | Perivascular, n (%) | P |
|---------------------|------------------------|-----------------------|---------------------------|-------------------|-------------------|---|
| LPP (23)            | 0(0)                   | 4(3)                  | 9(39)                     | 2(8.6)            | 1(4.3)            | 14(60)   |
| DLE (20)            | 2(10)                  | 6(33)                 | 17(85)                    | 11(55)            | 2(10)             | 19(95)   |

DLE – Discoid lupus erythematosus; LPP – Lichen planopilaris

| Table 1: The percentage of plasmacytoid dendritic cells, size of clusters, sensitivity, and specificity |
|----------------------------------------------------------------------------------------------------------------|
| Diagnosis | >10% infiltration, n (%) | >20% infiltration, n (%) | Cluster with 10-20 cells, n (%) | Cluster with >20 cells, n (%) |
|-----------|--------------------------|--------------------------|---------------------------------|-----------------------------|
| LPP       | 15 (65)                  | 2 (8)                    | 2 (8)                           | 0                           |
| DLE       | 18 (90)                  | 17 (85)                  | 15 (75)                         | 6                           |
| Sensitivity for diagnosis DLE | 90 (68.3-57.2) | 85 (62.1-96.7) | 75 (50.8-91.3) | 30 (11.9-54.3) |
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DLE – Discoid lupus erythematosus; LPP – Lichen planopilaris

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we apply this IHC marker to differentiate DLE from LPP, being the most frequent clinical and pathological differential diagnosis.

In this study, we found that an infiltration of PDCs was predominantly seen in DLE versus LPP. Moreover, we found that PDCs’ clusters, particularly those of 20 or more cells, were specific to DLE. The presence of PDC clusters and an infiltration of >20% were found to exclude LPP. The findings of this study are consistent with those showing the effectiveness of CD123 IHC in the diagnosis of cutaneous lupus erythematosus. Fening et al.[8] showed that PDC had a higher percentage of infiltration in DLE versus LPP and central centrifugal cicatricial alopecia, and clusters more than 20 cells were mostly seen in cases of DLE. Kolivras and Thompson[9] also revealed that clusters of CD123-positive PDCs are a reliable morphologic sign to distinguish DLE from LPP. Sleiman R.[18] found similar results that PDCs with cutoff >10% of mononuclear infiltrating cells and presence of clusters more than 10 cells are in favor of DLE.

In our study, perivascular and perifollicular PDC infiltrations were seen in both DLE and LPP, and perieccrine localization was only seen in DLE. Intrafollicular and follicular junction PDC infiltration was mostly seen in DLE. However, these findings were not statistically significant. Unlike our study, Kolivras and Thompson reported that infiltration of PDCs beneath the perifollicular epidermis and beneath the interfollicular epidermis had a similar specificity but less sensitivity for the diagnosis of DLE.[8] Furthermore, Fening et al.[8] found that PDCs seen in DLE are more likely in perivascular, perifollicular, and perieccrine location as well as along the follicular junction. However, distribution of PDCs in LPP was in the form of single cells at interstitial location. Ko et al. also reported PDCs at the dermoepidermal junction and clusters of these cells at perivascular and periadnexal location.[19]

Miyashita et al. evaluated the relationship between the percentage of PDCs in the dermis and adipose tissue and response to therapy in lupus erythematosus profundus. The patients with higher percentages of PDCs had a better response to therapy.[14] Thus, the evaluation of PDCs can be both diagnostic and prognostic for response to treatment.[14] The most important features which could be effective in the diagnosis of cutaneous lupus include the presence of a high percentage of PDCs, clustering of these cells, and finally, distribution of PDCs in the periaxial and the dermoeipidermal junction.[8,16,19] In scalp DLE, PDCs cells are present at the follicular epithelium and the follicular junction.[8,19] Our study showed the usefulness of CD123 staining to detect PDCs in scarring alopecia and of lymphocytic alopecia is a challenge for clinicians and pathologists. In cases with severe inflammation, it is difficult to arrive at a correct diagnosis. Therefore,
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In summary, CD123 along with elastic staining and histopathologic features could be suggested for the diagnosis of challenging cases of scarring alopecia with clinical differential diagnosis of LPP and DLE.

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Conflicts of interest

There are no conflicts of interest.

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Table 3: Fibrous tract formation and pattern of elastic fiber loss at papillary dermis lichen planopilaris and discoid lupus erythematosus

|                | Fibrous tract in H and E (%) | Fibrous tract at elastic stain (%) | Wedge-shaped loss (%) | Diffuse loss | P     |
|----------------|-------------------------------|-----------------------------------|-----------------------|-------------|-------|
| LPP            | 20/23 (86)                   | 23 (100)                          | 15 (65)               | 5 (21.7)    | 0.006 |
| DLE            | 15/20 (75)                   | 18 (90)                           | 4 (20)                | 12 (60)     |       |

DLE – Discoid lupus erythematosus; LPP – Lichen planopilaris; H and E – Hematoxylin and eosin

differentiate DLE from other alopecia with lymphocytic infiltration, especially LPP. However, no significant difference was found in distribution patterns of PDCs between LPP and DLE.

Elastic staining is commonly used to detect fibrous tract in order to differentiate scarring alopecia from nonscarring alopecia. In nonscarring alopecia, elastic staining tends to highlight an intact elastic network consisting of smooth, delicate, and thin elastic fibers around the streamers. However, in follicular scars of scarring alopecia, elastic staining shows increased elastic fiber attenuation and loss and also elastic fiber thickening, clumping, and recoil.[14]

In H and E sections, recognition of fibrous tract is sometimes difficult, particularly in a background of hyalinized dermis. Elastic staining shows the extent of scarring as well as the preserved fibrous tract remnants.[20] In DLE, perifollicular scarring is seen with the destruction of the perifollicular elastic sheath, which involves the whole length of follicle. The elastic fibers of the dermis between follicular units vanish, so in the final stage DLE, it is absent through the dermis. Fibrous tract remnants are not visible, as both the elastic sheath and the normal dermal elastic tissue network have been destroyed by the inflammatory process. Scarred lesions of LPP generally demonstrate wedge-shaped scars involving the upper third of the fibrous tract, destroying the upper portion of the elastic sheath. The remainder of the fibrous tract is clearly visible, outlined by its elastic sheath.[20]

In our study, elastic staining did not improve the detection of fibrous tract. However, the wedge-shaped pattern was the dominant feature in LPP and diffuse loss of elastic fibers at the papillary dermis in DLE (P = 0.006), which is similar to some other studies.[14,20,21] Therefore, elastic staining might prove useful for the diagnosis of scarring alopecia, helping to differentiate LPP from DLE.

The limitation of our study was exclusion of cases with few inflammatory cells. Therefore, we cannot decide whether CD123 is helpful in differentiation between LPP and DLE scarring alopecia in a specimen with few inflammatory cells.
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