Clinical, Histopathological Characteristics and Immunohistochemical Findings in Lichen Planus Pigmentosus

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Abstract

Background: Lichen planus pigmentosus (LPP), a rare variant of lichen planus, is reported in various ethnic groups, more often from the Indian subcontinent and the Middle East.

Aims: Although the condition is encountered quite often by dermatologists of this region, the data on the clinical, pathological, and immunohistochemical (IHC) aspects of LPP are limited. This prospective study is aimed towards filling this lacuna.

Materials and Methods: Data were collected from thirty clinically diagnosed cases of LPP who presented to the dermatology outpatient department. Skin biopsy and blood investigations were conducted and the specimens were further analyzed for their histopathological features and IHC staining for CD4⁺, CD8⁺ T-lymphocyte subsets along with CD45RO (UCHL-1), and CD68. The results were statistically analyzed.

Results: The study showed a female preponderance (56.7%). Photoaggravation as a precipitating cause was seen in 40% of the individuals. The lesions with duration <4 months had a more intense inflammatory infiltrate on histology. CD4⁺ and CD8⁺ cells showed very good Pearsons correlation on statistical analysis. CD45 was seen in association with CD8⁺, and staining for CD68 to assess the macrophage density showed a close correlation with CD45RO.

Limitations: Small sample size.

Conclusion: LPP represents a misguided lesional immune response pattern. The intense inflammatory infiltrate seen in the early lesions necessitates prompt treatment to arrest progression which may prevent the chronic pigmentary phase of the disease.

Key Words: CD45RO (UCHL-1), CD4⁺, CD8⁺, CD68, immunohistochemistry, lichen planus pigmentosus

Introduction

Pigmentary disorders of the skin are common in non-Caucasian populations and account for the third most common dermatologic diagnosis in Afro-Americans, Afro-Caribbean, Africans, Hispanics, and Asians.² Furthermore, persons of color tend to have more intense pigmentation than others when pigmentation occurs.²

Lichen planus pigmentosus (LPP), a rare variant of lichen planus (LP), that is seen in middle-aged individuals with darker pigmented skin, was first reported in a series of Indian patients in 1974.³,⁴ Clinically characterized by the insidious onset of focal or diffuse gray-blue or dark brown macules on exposed areas, LPP is reported to have a female preponderance.⁵ Pigmentary disorders with similar clinical features have been reported in literature but it is still not fully understood as there is a lack of contemporary evidence of the published writings. Histopathological evaluation shows atrophy of the dermis with loss of rete pattern, focal basal cell vacuolization, and sparse dermal infiltrate,⁶ but little is known regarding the etiopathology of LPP. An autoimmune attack is generally accepted, as demonstrated by the inflammatory infiltrate of T-lymphocytes with varying populations of CD4⁺ and CD8⁺ cells,⁷ and the autoreactive cytotoxic T-lymphocytes are implicated as the effector cells, which cause degeneration and destruction of keratinocytes.⁸

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How to cite this article: Bhat RM, Mathanda TR, Jayaprakash CS, Dandakeri S. Clinical, histopathological characteristics and immunohistochemical findings in lichen planus pigmentosus. Indian J Dermatol 2017;62:612-7.

Received: March, 2017. Accepted: August, 2017.
This study presents the common clinical presenting features and histopathological and immunohistochemical (IHC) characteristics of LPP lesions.

Materials and Methods
The study was approved by the Institutional Ethics Committee and registered with the Clinical Trials Registry of India (CTRI/2013/03/003491). Thirty clinically diagnosed cases of LPP were included in this study after obtaining informed consent. Patients who presented with idiopathic, itchy/asymptomatic, and hyperpigmented/violaceous macules that were clinically diagnosed as LPP were included. A detailed history and clinical examination was carried out, and routine hematological and biochemical tests were done along with hepatitis C virus (HCV) spot and HBsAg card test. Patch test was conducted on suspected patients with the cosmetic series kit. An incisional biopsy was done, and sections for hematoxylin and eosin and IHC stain were then obtained from the formalin-fixed and paraffin-embedded tissue.

The following histological features were evaluated by two independent observers for, orthokeratosis/hyperkeratosis; epidermal thinning; “v” (wedge) hypergranulosis/normal; acanthosis; epidermal or dermal Civatte bodies (apoptotic cells); basal cell vacuolization; melanophages; pigment incontinence: conspicuous/nonconspicuous; inflammatory infiltrate: mild/remarkable; inflammatory infiltrate level: dermoepidermal junction (DEJ)/lower reticular dermis; quality of inflammatory infiltrate: lymphocytic only/plasma cells and histiocytes among inflammatory cells; and signs of regression: present/absent.

The data were stratified into two categories (present or absent), and the discordant results were reviewed for consensus achievement.

Immunohistochemical staining
For anti-CD4, CD8, CD45R0 (UCHL-1) and CD68, the cell count was performed using five observed fields. Briefly, four 1µm-thick sections were dewaxed and rehydrated in graded ethanol. The primary antibodies used were CD4 (IS 649; Dako) RTU, CD8 (IS623; Dako) RTU, CD45R0 (AM113-5M; Biogenex) RTU, and CD68 (IS 613, Dako) RTU.

For all antibodies, a steamer was used for epitope retrieval with either citrate buffer or Tris-EDTA buffer. The Advance polymer (K 5007; Dako) was used as a reaction amplifier. Visualization of the antibody complex was achieved using 3,3-diaminobenzidine tetrahydrochloride (K 5007; Dako) according to the manufacturer’s instructions. Sections were counterstained with Harris’ hematoxylin.

Appropriate negative and positive controls were included in each assay. The expression of these markers was calculated as the percentage of positivity in relation to the total amount of nuclei counted per section.

Statistical methods
Statistical analysis was done using independent t-test and Pearson correlation to test the correlation between variables. In the analysis, P < 0.05 was considered statistically significant.

Results
The study revealed a female preponderance with 17 (56.70%) females and 13 (43.30%) males with the disease occurring mostly around the third to fourth decade of life (38 ± 15 years). Females showed an earlier age of onset (35.94 ± 15.86) compared to males (41.00 ± 14.16). The duration at presentation ranged from 1.5 months to 6 years (15.9 ± 21.1) with no associated family history of similar lesions in any individuals. An aggravating cause was noted in 16 (53.30%) patients [Table 1].

Associated systemic illness was noted in 8 (26.70%) patients, which included a single case each of hypertension, diabetes mellitus, hypothyroidism, malaria, epilepsy, and cerebrovascular accident. One patient gave a history of being treated for chikungunya fever before the onset of lesions. Patch testing with the cosmetic series kit on individuals with a history of cosmetic use gave no positive result.

Clinically, all 30 (100%) individuals presented with macules and 2 (6.70%) also had additional papular and plaque-like lesions. Koebnerization of the lesions were noted in 1 (3.30%). Itching was an associated symptom in 15 (50%) patients [Table 2]. The lesions initially appeared as small, ill-defined oval to round macules and progressed to become confluent involving large areas [Figure 1a and b].

On histological examination [Figure 2a and b], with the exception of thinning of the epidermis and abundance of melanophages, the features were indistinguishable from LP [Table 3]. The inflammatory infiltrate was noted at the DEJ in early lesions and at the upper reticular dermis in late lesions. The intensity of inflammation was

| Precipitating causes seen in lichen planus pigmentosus | Percentage |
|-------------------------------------------------------|------------|
| Precipitating factors (53.30%)                         |            |
| Dental amalgam                                        | 1 (3.30%)  |
| Friction                                              | 4 (13.30%) |
| Hair dye                                              | 2 (6.70%)  |
| Psychological stress                                  | 1 (3.30%)  |
| Cosmetics                                             | 1 (3.30%)  |
| Photoaggravation                                      | 12 (40.40%)|
higher in early lesions and showed an inverse relation with the duration of disease.

The IHC findings are related to the cells present in both DEJ and upper reticular dermis. A cell counting average of the five fields on IHC staining (cells per field) was considered. Higher number of cells staining for CD4 and marginally lower values of CD68 were associated with remarkable inflammation as expressed in histology [Figure 3a-d]. When compared with the duration of disease, those presenting with duration <4 months had a higher CD4 and CD8 count [Figure 4]. A good significant correlation with Pearson’s correlation of 0.760 and significance of <0.001 was found between CD4 and CD8. On comparing CD45 and CD68, a positive correlation of 0.414 and significance of 0.023 were noted. Furthermore, between CD4 and CD45, the significance was <0.001 with an excellent correlation of 0.961. A fair correlation was noted with CD68 and CD4 [Table 4]. On comparing these findings with the histopathological features, the intense inflammatory infiltrate seen in lesions of a short duration was associated with a higher CD4+ count.

Routine blood examination with liver function test showed no abnormality in any of the patients. No patients were found to be positive for HBsAg or HCV. Moreover, there was no positive reaction seen in any of the individuals who underwent patch testing with the standard cosmetic series. No immunofluorescence study was done.

Discussion
LPP, a variant of LP, first described by Bhutani et al.,[3] from India, is an inflammatory dermatoses involving mucocutaneous surfaces that can present with a variety of clinical manifestations.[9] The frequency of LPP varies on the basis of the population studied, with a particularly high rate of disease noted on the Indian subcontinent, most commonly affecting middle-aged people.[10] The mean age of our cases was 38 ± 15 years with slight female preponderance (male:female 1:1.30) and duration at presentation ranging from 1.5 months to 6 years.

Gold probably being the most common drug producing an LPP-like eruption,[11] several retrospective studies have reported associations between LPP and topical applications of mustard and amla oils.[3,4] These oils are used for body massage, hair dressing, cooking, and the preparation of local medicines in India. Mustard oil contains allyl

![Table 2: Clinical presentation of lichen planus pigmentosus](image)

**Table 2: Clinical presentation of lichen planus pigmentosus**

| Pattern of pigmentation | Shades of pigmentation | Areas of involvement (%) |
|-------------------------|------------------------|--------------------------|
| Diffuse                 | Bluish black           | Upper limbs 15 (50.00)   |
| Reticular              | Slate gray             | Lower limbs 8 (26.70)    |
| Blotchy                | Brownish black         | Trunk 6 (20.00)          |
| Perifollicular         | Dark brown             | Face 9 (6.70)            |
| -                      |                        | Periorbital 1 (3.30)     |
| -                      |                        | Mucosal 5 (16.70)        |
| -                      |                        | Nail 7 (23.30)           |

**Table 3: Histopathological features in lichen planus pigmentosus (n=30)**

| Histological features                        | LPP (%) |
|----------------------------------------------|---------|
| Orthokeratosis                               | 9 (70.00) |
| Epidermal thinning                           | 23 (76.70) |
| “v” hypergranulosis                          | 9 (30.00) |
| Acanthosis                                   | 6 (20.00) |
| Civatte bodies                               | 1 (3.30) |
| Basal cell vacuolization                      | 24 (80.00) |
| Melanophages                                 | 30 (100.00) |
| Pigment incontinence - conspicuous           | 26 (86.70) |
| Pigment incontinence - nonconspicuous        | 4 (13.30) |
| Inflammatory infiltrate - mild               | 23 (76.70) |
| Inflammatory infiltrate - remarkable         | 6 (20.00) |
| Inflammatory infiltrate at DEJ               | 11 (36.70) |
| Inflammatory infiltrate at perivascular/upper reticular dermis | 27 (90.00) |
| Lymphocytes                                  | 30 (100.00) |
| Plasma cells                                 | 28 (93.30) |
| Histioocytes                                 | 21 (70.00) |
| Signs of regression                          | 6 (20.00) |

DEJ: Dermoepidermal junction, LPP: Lichen planus pigmentosus

![Figure 1](image)

**Figure 1:** (a) Lichen planus pigmentosus involving the face and neck. (b) Hyperpigmented coalescent macules over the back

![Figure 2](image)

**Figure 2:** (a) Histopathology showing atrophy of the epidermis, melanin incontinence, and moderate fibrosis in dermis (H and E, ×10). (b) Basal cell layer degeneration, melanin incontinence, and presence of Civatte bodies (H and E, ×40)
thiocyanate, which is a potential photosensitizer and a potential pathogenetic agent in LPP.\textsuperscript{[4]}

Although no definite etiological factors have been identified, precipitating causes in our study included friction at the site of lesion, presence of dental amalgam, psychological stress, use of hair dye and cosmetics and chikungunya fever. Photoaggravation of lesions was associated with 40% of the cases. There has been a reported association of LPP with acrokeratosis of Bazex,\textsuperscript{[12]} but no systemic illness has been implicated to be associated with LPP.

The macular hyperpigmentation chiefly involves the upper limbs, face, and neck. While it can be more widespread and varies from slate gray to brownish black, it is mostly diffuse, but reticular, blotchy, and perifollicular forms are also seen.\textsuperscript{[4]} Two cases of LPP presenting with a linear pattern over the extremities have been reported where authors suggest that the linearity of the lesions is probably related to Blaschko’s lines, implying that predisposition to LPP might be determined during embryogenesis.\textsuperscript{[13]} The mucous membranes, palms, and soles are usually not involved, but involvement of mucous membranes has been observed.\textsuperscript{[14]} Our study revealed pruritus as an associated symptom in 50% of the cases with oral mucosal involvement in 16.70% of the cases and nail changes in the form of hyperpigmentation and longitudinal ridging in 7 (23.30%).

Except for a few subtle epidermal changes on histopathological evaluation, the features of LPP are indistinguishable from LP suggesting that LPP probably represents a lichenoid reaction to an unknown agent or stimulus and that a histopathological similarity exists between LPP and LP.\textsuperscript{[3-5]} The inflammatory infiltrate was more intense at the DEJ in the early lesions with their concentration higher at the upper reticular dermis.

**Table 4: Correlation between markers: Pearsons correlation**

|                         | CD4\textsuperscript{+} present | CD8\textsuperscript{+} present | CD45RO       |
|-------------------------|---------------------------------|---------------------------------|--------------|
| **CD8\textsuperscript{+} present** |                                |                                 |              |
| Pearson correlation     | 0.760                           |                                 |              |
| Significant (two-tailed)| <0.001                          |                                 |              |
| n                       | 30                              |                                 |              |
| **CD45RO**              |                                 |                                 |              |
| Pearson correlation     | 0.961                           |                                 |              |
| Significant (two-tailed)| <0.001                          | <0.001                          |              |
| n                       | 30                              | 30                              |              |
| **CD68**                |                                 |                                 |              |
| Pearson correlation     | 0.407                           | 0.303                           | 0.413        |
| Significant (two-tailed)| 0.026                           | 0.104                           | 0.023        |
| n                       | 30                              | 30                              |              |

**Look at the Pearsons correlation value if it is**

| 0.1         | 0.3         | 0.5         | 0.7         | 0.9         |
|-------------|-------------|-------------|-------------|-------------|
| Poor correlation | Fair correlation | Good correlation | Very good correlation | Excellent correlation |

**Figure 3:** (a) Immunohistochemical staining showing CD4\textsuperscript{+} T-cell subsets (×40; Dewinter select microscope). (b) CD8\textsuperscript{+} T-cell subsets (Dewinter select microscope, ×40). (c) CD45RO\textsuperscript{+} (UCHL-1) (Dewinter select microscope, +40). (d) CD68 (Dewinter select microscope, +40).

**Figure 4:** Correlation between duration and immunohistochemical markers.
Various studies have shown that the subpopulation of inflammatory cells in lichenoid dermatitis, i.e., both subtypes of T-lymphocytes (CD4+ and CD8+) are present and act synergistically but probably by different mechanisms.11-13 This study showed a positive correlation between CD4+ and CD8+ T-cells. They together contribute to activation of signals required for the phosphorylation of key substrates taking part in the immunology of LPP. The cases presenting with duration of <4 months had a more remarkable inflammatory infiltrate, and this was supported by a higher number of cells staining for CD4+ and CD8+ T-cells. Significant accumulation of CD4+ and CD8+ subsets have been seen both in the epidermis and dermis of LP lesions, with gradual accumulation of CD8+ T-lymphocytes within the epithelium with the progression of disease.16 The autoreactive cytotoxic CD8+ T-lymphocytes are the effector cells which cause degeneration and destruction of keratinocytes; therefore, important in the pathogenesis of LP.8,10 Once within the epithelium, CD8+ lymphocytes secrete granzyme B, a serine protease around keratinocytes, triggering nuclear injury.20 A few studies have reported a significant correlation between CD4+ cells and the other variables. However, a study by Devadas et al. showed that granzyme B plays a role in cell death by CD4+ Th2-type lymphocytes and mediates the in vivo regulation of Th-cell responses.21 Study by Lage et al. comparing LP with lichenoid drug eruption did not find a significant positive correlation between the number of T-(UCHL-1) and CD8+ cells,17 but we found that the circulating “memory” subset (CD45RO+/UCHL-1) of T-helper cells (CD4+) showed a positive correlation with CD8+ cells [Table 3].

Macrophages are responsible for digestion and presentation of antigens to T-lymphocytes. As they phagocyte antigens and undergo maturation, they activate T-cells which cause clonal proliferation of cells, initiating a lichenoid drug reaction. Staining for CD68 assessed macrophage density at 11 ± 7 cells per field. The CD4 set has been divided into two functional subsets by the expression of different isoforms of the leukocyte common antigen (CD45). The T-cells lose the high molecular isoform CD45RA and synthesize the 180 kDa CD45R0+ molecule on in vitro stimulation. These two subsets are implicated to represent the resting T-cells and cells that have recently encountered antigen. Antigenically primed CD4+ cells remain CD45R0 positive as denoted by the corresponding excellent positive Pearson’s correlation.22

Because of its varied morphology simulating several pigmentary disorders, there are a number of closely related or possibly even the same pigmentary disorders that have been described under the different entities worldwide. Although limited by the small sample size, the study attempts to identify and quantify certain specific functional subsets of T-lymphocytes. Most studies so far have reported a predominance of CD8-positive cells over CD4+ T-lymphocytes. Our study showed an early predominance of both CD4+ and CD8+ T-cells that seem to play an important role in disease pathogenesis following antigen priming. These findings are similar to the previous observations in LP that the inflammation represents a common lesional immune response pattern which might have developed from an evolutionary perspective for the normal immune functions such as antiviral or antitumor activity and environmental insults, but which is misguided in autoimmune diseases.23 The histopathology of LPP is, in general, subtle with sparse inflammation. This requires a thorough evaluation of the biopsy, and the diagnosis of LPP should be based on a clinicopathological correlation. The recognition of increased CD4+ inflammatory cells at the early phase of the disease indicates the need for a prompt, targeted treatment to arrest the autoimmune course of the disease and prevent persistence of the inflammation. Thus, we presume that early interference may help in preventing the chronic persistent pigmentation of LPP, which is a concern both for the patients and the treating physicians.

**Conclusion**

LPP challenges dermatologists with a diagnostic and therapeutic challenge, and conducting a large-scale study to formulate a diagnostic criterion can perhaps attempt to solve dilemma.

**Financial support and sponsorship**

IADVL-L'Oreal hair and skin research grant.

**Conflicts of interest**

There are no conflicts of interest.

What is new?

We report a significant correlation between CD4+ cells and the other variables of the disease. This study highlights the need for focusing on early control of the inflammatory reaction and the formation of a precise diagnosis and management guideline.

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