Dermoscopic Features of Nail Psoriasis: An Observational, Analytical Study

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Keywords

Dermoscopy · Nail psoriasis · Fuzzy lunula · Oil spot/salmon patch · Red lunula · Nail pitting

Abstract

Introduction: Nail involvement in psoriasis occurs in up to 30–50% of patients, and 5–10% may have isolated nail disease. Onychoscopy, a noninvasive tool, might obviate the need for nail biopsy, which is a diagnostic gold standard. **Objective:** The aim of this study was to evaluate onychoscopic features of nail unit in patients with nail psoriasis. **Methods:** Fifty-five patients with clinically diagnosed and histologically documented nail psoriasis were recruited. Onychoscopy was performed for each nail (excluding the 5th toenail). Clinically, 443/550 fingernails and 101/440 toenails were involved. The frequency distributions of various onychoscopic features was assessed and compared using the χ^2 test (p value <0.05 was considered significant). **Results:** With onychoscopy, additional 52 fingernails and 64 toenails showed psoriatic involvement. Pitting was the commonest finding in fingernails (60.5%) followed by subungual hyperkeratosis (SUH) (52.8%), onycholysis (40.8%), and dotted capillaries in hyponychium, proximal, and lateral nail folds (38.6 vs. 35.8

vs. 35.8%). In toenails, we observed SUH (85.1%), nail plate thickening (82.1%), onycholysis (77.2%), and dotted capillaries in hyponychium and nail folds (59.4 vs. 53.4 vs. 45.5%). Fuzzy lunula was a novel onychoscopic finding noted in 33.6% fingernails and 4.95% toenails ($p < 0.00001$). **Limitations:** Small sample size. **Conclusions:** Onychoscopy may aid in diagnosing nail lesions even before the clinical signs are apparent.

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Introduction

Psoriasis, a chronic immune-mediated, debilitating disease affects approximately 2–3% of population with prevalence ranging between 0.5 and 2.5% worldwide [1, 2]. Nail affliction occurs in about 30–50% of cases with lifetime incidence of 80–90%. Nail involvement is associated with extensive disease, longer disease duration, and psoriatic arthropathy (PsA) [3, 4]. Approximately, 5–10% cases tend to have isolated nail disease [5–7].

Nail psoriasis occurring with concomitant skin lesions is easy to diagnose. However, in patients with isolated nail involvement, there is a diagnostic dilemma due to its

close resemblance to other causes of dystrophic nails like onychomycosis, lichen planus, alopecia areata, pityriasis rubra pilaris, traumatic onycholysis, etc. [8]. Nail biopsy serves as the gold standard but proves to be diagnostic in only ~50% nail psoriasis cases [9]. Dermoscopy is an in vivo, noninvasive, and rapid method with well-established diagnostic utility in pigmented skin and nail disorders [10]. Dermoscopy may serve as the best tool to detect early nail involvement which might obviate the need for biopsy for diagnosis and follow-up of nail psoriasis.

There is paucity of studies evaluating the dermoscopic features of nail psoriasis [11–14]. In this study, we have attempted to evaluate and describe the utility of onychoscopy in nail psoriasis.

Methods

This prospective, observational, analytical study was conducted over a period of 18 months in the departments of Dermatology & STD and Pathology, Guru Teg Bahadur Hospital and University College of Medical Sciences, Delhi, India. The study protocol was approved by the Institutional Ethics Committee. Fifty-five patients with clinically diagnosed and histologically documented psoriasis (50 skin lesion, 3 nail bed, and 2 nail matrix biopsies) with nail lesions/isolated nail psoriasis were recruited after obtaining written informed consent. Patients with psoriatic erythroderma, reactive arthritis, psoriasiform dermatitis/drug reaction, and severe systemic disease were excluded. A detailed history was collected, and general physical and systemic examinations, including joint examination, were carried out. Eighteen nails (10 fingernails and 8 toenails, excluding the 5th toenail of both feet) and representative skin lesion were examined for clinical and dermoscopic findings, and photographic documentation was done. Dermoscopy of nail unit was carried out with USB dermatoscope (AM-7013MZTS [4S] Dino-Lite Premier), with mineral oil as an interface medium. The severity of cutaneous and nail involvement was assessed with Psoriasis Area and Severity Index (PASI) and Nail Psoriasis Severity Index (NAPSI), respectively.

Distribution of dermoscopic nail features according to each nail (18 nails) was determined using SPSS Statistics for Windows, version 20.0 (SPSS Inc., Chicago, IL, USA). Pearson correlation between the severity scores, NAPSI and PASI, was calculated. Difference in frequency distributions of clinical and dermoscopic findings was found using the χ^2 test of significance, and a p value <0.05 was considered significant.

Results

Fifty-five patients were recruited of whom 40 (72.7%) were men and 15 (27.3%) were women, with a male-to-female (M:F) ratio of 2.66:1. The mean age of the cases was 28.9 ± 14.2 years (range 10–70 years). Maximum number of patients ($28/55 = 51\%$) belonged to the age

group of 10–25 years. The reported disease duration ranged from 2 months to 18 years (mean = 5.29 ± 4.39 years). Age at disease onset varied from 3 to 64 years (mean = 23.7 ± 14.36 years). Out of 55 cases, 50 (90.90%) had concomitant skin lesions of varying morphology and 5 (9.1%) had isolated nail psoriasis. Chronic plaque psoriasis was the most common morphology noted in $32/55$ (58.18%) patients. Associated joint involvement was observed in $5/50$ cases with cutaneous lesions. However, none of the patients with isolated nail psoriasis had evidence of PsA. The mean PASI was 7.67 ± 7.87 (range 0–32.2). A total of 544 nails were found to be involved in 55 cases (average of 9.8 nails per patient). Out of these, 443 (81.43%) were fingernails and rest 101 (18.56%) were toenails. NAPSI scores for fingernail were higher than toenail (25.67 ± 15.57 vs. 6.36) with a mean total NAPSI score of 32.03 ± 21.20 . There was a weak positive correlation between the PASI score and NAPSI score (Pearson's correlation coefficient [r] = 0.442 and $p = 0.001$).

Clinical Features

Various nail changes suggestive of psoriasis were observed on clinical examination in $443/550$ fingernails and $101/440$ toenails. The most common finding among fingernails and toenails was pitting ($265; 59.81\%$) and crumbling ($84; 83.16\%$). This was followed by distal onycholysis (FN: $202; 45.5\%$ vs. TN: $78; 77.2\%$) and subungual hyperkeratosis (SUH) (FN: $181; 40.85\%$ vs. TN: $74; 73.26\%$) in both fingernails and toenails. The difference in frequency distribution of clinical features in fingernails and toenails was statistically significant ($p < 0.05$) for all features except Beau's lines and splinter haemorrhages.

Dermoscopic Features

Dermoscopy was performed on all the fingernails ($n = 550$) and toenails except 5th toenails ($n = 440$). Dermoscopic findings of all involved fingernails and toenails are summarized in Table 1. The nail matrix features observed in involved fingernails in the descending order were pitting, longitudinal ridging, fuzzy lunula, leukonychia, nail plate thickening and crumbling, and red lunula (Fig. 1a–d). Among involved toenails, nail plate thickening and crumbling was the most common finding (82.17%). The difference in frequency distribution of dermoscopic features in fingernails and toenails was statistically significant for all features except Beau's lines and onychomadesis. Fuzzy lunula (Fig. 2) was a novel dermoscopic nail matrix finding seen in 33.63% of involved fingernails and 4.95% of toenails. It appeared as irregular, wide white lunula.

Table 1. Dermoscopic features in nail psoriasis in fingernails ($n = 443$) and toenails ($n = 101$)

Feature	Fingernails, n (%)	Toenails, n (%)	p value*
<i>Nail matrix dermoscopic features</i>			
Pitting	268 (60.49)	6 (5.94)	<0.00001
LR	254 (57.33)	23 (22.77)	<0.00001
Fuzzy lunula	149 (33.63)	5 (4.95)	<0.00001
Leukonychia	117 (26.41)	12 (11.88)	0.001948
Nail plate thickening and crumbling	101 (22.79)	83 (82.17)	<0.00001
Red lunula	57 (12.86)	4 (3.96)	0.01047
Lamellar splitting	53 (11.96)	40 (39.60)	<0.00001
Beau's lines	29 (6.54)	6 (5.94)	0.822851
Dystrophy	26 (5.86)	48 (47.52)	<0.00001
Onychomadesis	5 (1.12)	3 (2.97)	0.165286
<i>Nail bed dermoscopic features</i>			
SUH	234 (52.82)	86 (85.14)	<0.00001
Dilated capillaries	222 (50.11)	11 (10.89)	<0.00001
Onycholysis	181 (40.8)	78 (77.2)	0.005123
Splinter haemorrhage	180 (40.63)	32 (31.68)	0.096081
Scales	67 (15.12)	35 (34.65)	<0.00001
Oil spot	45 (10.15)	3 (2.97)	0.021549
Brown discoloration	41 (9.25)	21 (20.79)	0.000993
White streaks	22 (4.96)	2 (1.98)	0.18728.
<i>Hyponychium dermoscopic features</i>			
Dotted capillaries	171 (38.6)	60 (59.40)	0.000135
Scales	72 (16.25)	64 (63.36)	<0.00001
Dilated capillaries	13 (2.93)	1 (0.99)	0.265423
Pustules	5 (1.12)	1 (0.99)	0.904224
<i>PNF dermoscopic features</i>			
Scaling	208 (46.95)	90 (89.10)	<0.00001
Dotted capillaries	159 (35.89)	54 (53.46)	0.001094
Dilated capillaries	26 (5.86)	1 (0.99)	0.041616
Pustules	2 (0.45)	1 (0.99)	0.509497
<i>LNF dermoscopic features</i>			
Scales	241 (54.40)	87 (86.13)	<0.00001
Dotted capillaries	159 (35.89)	46 (45.54)	0.070837
Hyperkeratosis	33 (7.44)	35 (34.65)	<0.00001
Dilated capillaries	14 (3.16)	0	
Pustules	3 (0.67)	6 (5.94)	0.000182

PNF, proximal nail fold; LNF, lateral nail fold. SUH, subungual hyperkeratosis; LR, longitudinal ridging.
 * p values of <0.05, shown in bold, are statistically significant.

The most common nail bed dermoscopic finding (Fig. 3a–d) in both fingernails and toenails was SUH. This was followed by dilated nail bed capillaries (50.11%), distal onycholysis (40.8%) in fingernails; and onycholysis (77.2%), scaling (34.65%), and splinter haemorrhages (32.68%) in toenails. Oil spots/salmon patches were observed in 10.1% of involved fingernails and 2.9% of toenails. This difference in frequency distribution was statis-

tically significant for all features except splinter haemorrhages and white streaks. Dermoscopic examination of nail folds in involved nails revealed presence of dotted capillaries in hyponychium (fingernails: 38.6% vs. toenails: 59.40%), proximal nail fold (PNF) (fingernails: 35.89% vs. toenails: 53.46%), and lateral nail fold (LNF) (fingernails: 35.89% vs. toenails: 45.54%) (Fig. 4a–d). Dilated capillaries could be appreciated in 2.93% of hypo-

Fig. 1. Nail psoriasis nail matrix dermoscopic features: coarse pitting (**a**), leukonychia (**b**), red lunula (black arrow) and dotted capillaries in PNF (red arrow) (**c**) (170 × polarized mode), and Beau's lines (**d**). PNF, proximal nail fold.

Fig. 2. Nail psoriasis novel nail matrix dermoscopic feature: fuzzy lunula (polarized, 70×).

nychium, 5.86% of PNF, and 3.16% of LNF in fingernails and 0.99% of hyponychium and PNF in toenails. The statistical significance in frequency distribution was noted for dotted capillaries and dilated capillaries in hyponychium.

Dermoscopy of clinically uninvolved fingernails ($n = 107$) and toenails ($n = 339$) revealed findings suggestive of psoriasis in 52 fingernails and 64 toenails. These findings were appreciated in the form of red lunula (fingernails: 16.82% vs. toenails: 2.94%), fuzzy lunula

(fingernails: 8.41% vs. toenails: 1.76%), dilated nail bed capillaries (fingernails: 28.03% vs. toenails: 2.35%), and hyponychial dotted capillaries (fingernails: 34.57% vs. toenails: 5.6%). So, features of nail psoriasis were evident in additional 52 (48.59%) uninvolved fingernails and 64 (18.87%) uninvolved toenails on dermoscopy.

Discussion

The mean age of 55 study subjects was 28.9 ± 14.2 years (range: 10–70 years) which was lower than those in other Indian studies, Yadav and Khopkar [11] (38.36 years), Wanniang et al. [14] (45.02 years), and Daulatabad et al. [15] (36.3 years), as well as studies reported from the West by van der Velden et al. [16] (48 years), Brazzelli et al. [17] (52.53 years), Marina et al. [18] (51.89 years), and Hashimoto et al. [19] (47.5 years). This could be attributed to an absence of lower age limit in our inclusion criteria and higher awareness with regards to personal grooming and cosmesis among all strata. We also observed male preponderance with M:F = 2.66:1 which was similar as recorded by Yadav and Khopkar [11] (2.83:1), Wanniang et al. [14] (3.1:1), Daulatabad et al. [15] (2.1:1), Brazzelli et al. [17] (2.6:1), van der Velden et al. [16] (1.13:1), and Hashimoto et al. [19] (5:1).

Fig. 3. Nail psoriasis nail bed dermoscopic features: dilated nail bed capillaries with a surrounding white halo (polarized 170×) (a), compact SUH (b), distal onycholysis with erythematous border (red arrow) (polarized 70×) (c), and oil spot/salmon patch (polarized 70×) (d). SUH, subungual hyperkeratosis.

Fig. 4. Nail psoriasis nail folds dermoscopic features: dotted capillaries in hyponychium (a), dotted capillaries in PNF (b), dotted capillaries in LNF (c), and dilated capillaries in LNF (polarized, 170×) (d). PNF, proximal nail fold; LNF, lateral nail fold.

We found pitting to be the most common clinical feature in fingernails followed by onycholysis and SUH. In toenails, crumbling (83.16%) was the commonest followed by onycholysis and SUH. This finding was in concordance with earlier studies [14, 15]. Red lunula, an infrequent nail finding in psoriasis, was noticed only in 1

fingernail (0.22%; 1.8% cases). Mean PASI score in our cohort was 7.67 ± 7.87 (0–32.2), which was less as compared to previous studies by Wanniang et al. [14] (19.8), Brazzelli et al. [17] (11.26), Daulatabad et al. [15] (14.4), and Schons et al. [20] (11.1). Mean NAPSI score found in our study was 32.03 (fingernail NAPSI: 25.67; toenail

NAPSI: 6.36). NAPSI was comparative in patients with concomitant arthritis and scores were higher in fingernails than toenails. Mean NAPSI reported in other studies was 11.93 (Sharada and Thomas) [21] and 83.16 (Daulatabad et al.) [15]. Schons et al. [20] reported NAPSI as 18.3 in patients with PsA and 6.2 in patients without PsA. Daulatabad et al. [15] reported slightly higher mean NAPSI scores for toenails (42.61) than fingernails (40.29). Wanniang et al. [14] reported mean clinical NAPSI score and mean dermoscopic NAPSI score as 23.82 and 26.68, respectively.

We found a weak positive correlation between the PASI score and NAPSI score (Pearson's correlation coefficient [r] = 0.442 and p = 0.001). Rich et al. [22] reported no correlation between PASI and NAPSI, while Patsatsi et al. [23] detected significant correlation. However, Rich et al. [22] used target NAPSI (0–8), contrary to Patsatsi et al. [23] who assessed total NAPSI (0–160). In a study by Daulatabad et al. [15], PASI showed weak positive but statistically insignificant correlation with NAPSI (r = 0.26; p = 0.12), demonstrating worsening of nail involvement with increased severity of skin disease. Hallaji et al. [24] reported significant correlation between total NAPSI and PASI in patients with plaque psoriasis. Van der Velden et al. [16] reported no significant correlation between NAPSI and PASI, whereas Kyriakou et al. [25] reported significant correlation (r = 0.515; p = 0.029). Wanniang et al. [14] reported significant positive correlation between PASI and dermoscopic NAPSI scores (r = 0.34; p = 0.013).

Dermoscopic evaluation in 55 cases revealed that pitting was the most common nail matrix finding present in 268 (60.49%) fingernails and was seen only in 5.9% toenails which was in concordance with a study by Yadav and Khopkar [11], Yorulmaz Artuz [12] (58.2%), and Wanniang et al. [14] (84%). Longitudinal ridging could be appreciated in 57.3% of fingernails and 22.7% toenails in our study. The frequency of this dermoscopic finding has not been studied in the past. Dermoscopy revealed leukonychia in 26.4% of fingernails and 11.8% of toenails, which was comparable to naked eye examination. Nail plate thickening and crumbling was noted in approximately 22% of fingernails and 82% of toenails. In a previous study by Yorulmaz and Artuz [12], leukonychia and nail plate crumbling were reported in 6 and 17.9% of cases, respectively, and Wanniang et al. [14] reported these findings in 22 and 16% cases, respectively. Red spot in lunula is a very infrequent finding. However, dermoscopy increased the detection of this finding from 0.22 to 12.86% in fingernails and from nil to 3.96% in toenails in clini-

cally involved nails. Lunular red spots were described only in 1.5% cases by Yorulmaz and Artuz [12] and 8% cases by Wanniang et al. [14]. On further examination of rest 446 clinically uninvolved nails, this finding was evident in 18/107 (16.82%) fingernails and 10/339 (2.94%) toenails. Fuzzy lunula was a novel finding that appeared as irregular wide white lunula and has not been described previously in the literature. In clinically involved nails, it was reported in 33.63% fingernails and 4.95% of toenails. Among clinically unaffected nails, we could elicit this finding in 8.41% fingernails and 1.76% toenails.

Among nail bed features, both finger and toenails revealed SUH as the most common feature (52.8% fingernails and 85.14% toenails; increased from 40 to 73%, respectively, on naked eye examination). Salomon et al. [26] reported that toenails more commonly present with this finding; Yorulmaz and Artuz [12] reported this in 9% cases (both fingernails and toenails combined), whereas Wanniang et al. [14] reported in 46% cases. Dilated nail bed capillaries were observed in approximately 50% of fingernails and 10.8% of toenails and were visualized as bright red to dusky coloured dilated vessels arranged parallel over the onychodermal band of the nail plate and surrounded by a prominent halo. These dilated vessels are appreciated well on dermoscopic examination [27]. Additional 28.03% fingernails and 2.35% toenails showed this finding on examination of clinically uninvolved nails. Yadav and Khopkar [11] reported this finding in 19.5% of cases. Distal onycholysis that appears as opaque nail proximally bordered by characteristic erythematous band is considered an important dermoscopic finding in nail psoriasis, especially in patients presenting with isolated onycholysis [28]. In our study, this feature was noted in 40.8% of fingernails and 77.2% of toenails, and 5% patients with confirmed nail psoriasis failed to reveal this finding. Erythematous band was reported in 21.7, 55.2, and 54% cases by Yadav and Khopkar [11], Yorulmaz and Artuz [12], and Wanniang et al. [14], respectively. Splinter haemorrhages, a nonspecific finding, were visible as longitudinal purple (new onset) to black streaks (long-standing). Dermoscopy increased their detection from 20 to 40% in fingernails and from 21 to 31% in toenails in our study. Yadav and Khopkar [11], Yorulmaz and Artuz [12], Hashimoto et al. [19], and Wanniang et al. [14] reported this finding in 10.86%, 73.1%, 4/6 cases, and 62%, respectively. We found oil spot/salmon patch in 10% fingernails and 2.9% toenails as compared to 4.34, 22.4, and 44% cases in a study by Yadav and Khopkar [11], Yorulmaz and Artuz [12], and Wanniang et al. [14], respectively. Di Chiacchio et al.

Table 2. Comparative nail psoriasis dermoscopic features between our study and other studies

Dermoscopic features	Present study, <i>n</i> = 55 (%)	Yadav and Khopkar [11], <i>n</i> = 46 (%)	Yorulmaz and Artuz [12], <i>n</i> = 67 (%)	Wanniang et al. [14], <i>n</i> = 50 (%)
<i>Nail matrix dermoscopic features</i>				
Pitting	42 (76.36)	18 (39.13)	39 (58.2)	42 (84)
Red lunula	10 (18.18)	–	1 (1.5)	4 (8)
Fuzzy white lunula	17 (30.90)	–	–	–
Leukonychia	16 (29.09)	–	4 (6)	11 (22)
Nail plate thickening	19 (34.54)	–	12 (17.9)	8 (16)
<i>Nail bed dermoscopic features</i>				
Onycholysis	37 (67.27)	10 (21.7)	37 (55.2)	27 (54)
Oil spot	9 (16.36)	2 (4.34)	15 (22.4)	22 (44)
Dilated capillaries	33 (60)	9 (19.5)	–	–
Splinter haemorrhage	24 (43.63)	5 (10.86)	49 (73.1)	31 (62)
<i>Hyponychium dermoscopic features</i>				
Dotted capillaries	21 (38.18)	–	24 (35.8)	5 (10)
<i>PNF dermoscopic features</i>				
Dotted capillaries	19 (34.54)	–	–	–
PNF, proximal nail fold.				

[29] recently reported a novel finding: pseudo-pitting of the nail, wherein pits were observed only above the oil spot. Histopathology of nail bed in this case showed features suggestive of typical psoriasis with dilated capillaries in papillary dermis responsible for orange discoloration and parakeratosis with accumulation of neutrophils in the overlying nail plate possibly explaining the formation of pits. It was named so because of its similarity with normal pitting, but the location of defect is in the nail bed. Decreased frequency of oil spot in toenails compared to fingernails can be attributed to increase dystrophy and crumbling of toenails, which makes visualization of oil spots difficult even with dermoscopy.

Dermoscopy of hyponychium of clinically involved nails showed red-coloured regularly arranged dots in 38.6% fingernails and 59.4% of toenails and dilated, tortuous, irregularly arranged capillaries in 2.9% fingernails and 0.9% toenails. In addition, dermoscopy could pick up similar changes in clinically uninvolved finger and toenails (34.6 vs. 5.6%). This was reported in 35.8% cases by Yorulmaz and Artuz [12] and 10% of cases by Wanniang et al. [14]. Iorizzo et al. [13] reported this finding in hyponychium of all 30 nail psoriasis patients and suggested that capillary density was positively correlated with disease severity.

Bhushan et al. [30] reported diminution in capillary bed density at nail fold of patients with nail psoriasis, and

their findings were in line with the study by Zaric et al. [31, 32]. In our study, regularly arranged red dots in PNF were seen in 35.8% fingernails and 53.4% toenails; dilated capillaries in PNF were seen in 5.8 and 0.9% of fingernails and toenails, respectively. Regularly distributed red dots and dilated capillaries in LNF were seen in 35.8% fingernails versus 45.5% toenails and 3.16% fingernails versus none of the toenails, respectively, in our study. Further, dermoscopic examination of PNF and LNF of uninvolved nails did not reveal any finding suggestive of nail psoriasis.

Other findings studied were scaling (fingernails: 54.4% vs. toenails: 86.1%), hyperkeratosis (fingernails: 7.4% vs. toenails: 34.65%), and pustules (fingernails: 0.67% vs. toenails: 5.9%). These findings have not been described in previous studies. The difference in frequency distribution of different dermoscopic features in fingernails and toenails was found to be statistically significant in our study as shown in Table 1, for most of the features except Beau's lines, onychomadesis, splinter haemorrhages, white streaks, pustules in hyponychium, and PNF and hyponychial dilated capillaries. Though nail psoriasis is considered an independent risk factor for PsA, none of the patients of isolated nail psoriasis in our study had evidence of PsA. Table 2 shows comparative nail psoriasis dermoscopic features between our study and other studies.

Conclusion

Dermoscopy acts as an interface between clinical and histological examination and aids in the diagnosis of nail psoriasis even before clinical signs of nail involvement are apparent. The presence of dermoscopic features in clinically uninvolved nails can possibly serve as an early marker of disease activity. This study comprehensively describes the dermoscopic features of different parts of nail unit in patients of nail psoriasis. However, further controlled studies on a larger sample size are required to determine the specificity and sensitivity of each dermoscopic feature to standardize the diagnostic utility of each one.

Statement of Ethics

Institutional Ethics Committee – Human Research (IEC-HR), University College of Medical Sciences, University of Delhi, India, approved the research protocol, and the study was performed according to the World Medical Association Declaration of Helsinki. Written informed consent was obtained.

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Author Contributions

Dr. Ankita Chauhan contributed in patient recruitment, dermoscopic examination, gathering and analysis of data, and writing of manuscript. Dr. Archana Singal had main contributions in the concept, design, analysis, and improvisation of the manuscript. Dr. Chander Grover had main contributions in the concept, design, analysis, and improvisation of the manuscript. Dr. Sonal Sharma contributed in histopathological examination.

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