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ORIGINAL PAPER

Altered levels of lymphocyte enhancer-binding factor-1 modulates the pigmentation in acral and non-acral lesions of non-segmental vitiligo patients: a follow-up-based study in North India

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Abstract

Background Lymphocyte enhancer-binding factor-1 (LEF1) is responsible for melanocyte proliferation, migration and differentiation and its downregulation may result in depigmentation in vitiligo. Narrowband UVB (NB-UVB) phototherapy is known to enhance melanocyte migration from hair follicles to lesional epidermis; hence, it may have a role in the upregulation of LEF1.

Objectives We intended to assess the expression of LEF1 both before and after NB-UVB therapy and correlate it with the extent of re-pigmentation.

Materials and methods In this prospective cohort study, 30 patients of unstable non-segmental vitiligo were administered NB-UVB phototherapy for 24 weeks. Skin biopsies were obtained from acral and non-acral sites in all patients, both prior to initiation and after completion of phototherapy and LEF1 expression was measured.

Results Amongst the 16 patients who completed the study, at 24 weeks, all patients achieved > 50% re-pigmentation. However, > 75% re-pigmentation was achieved in only 11.1% of acral patches, whereas it was achieved in a significantly higher number of non-acral patches (66.6%) ($p = 0.05$). A significant increase was observed in the mean fluorescent intensity of the LEF1 gene in both acral as well as non-acral areas at 24 weeks as compared to baseline ($p = 0.0078$), However, no difference was observed between acral and non-acral lesions in the LEF1 expression at 24 weeks or the change in LEF1 expression from baseline.

Conclusion LEF1 expression modulates the re-pigmentation of vitiligo lesions after treatment with NBUVB phototherapy.

Keywords Non-segmental vitiligo · LEF1 · NB-UVB phototherapy · Melanocyte · Wnt/β-catenin pathway

Introduction

Vitiligo is a common acquired disorder of depigmentation [1]. It is a multifactorial disorder that occurs due to a complex interaction of immunological, environmental, and genetic factors leading to the destruction of melanocytes. The Wnt/β catenin signaling pathway has been found to have a pivotal role in the differentiation of melanoblasts into performing melanocytes. Melanocytes in the skin express the lymphocyte enhancer-binding factor-1 (LEF1) and alterations in the Wnt/β-catenin pathway hampers the expression of LEF1 in vitiligo [2]. LEF1 stimulates the transcription of the microphthalmia-associated transcription factor (MITF) gene and also induces its own promoter, thereby facilitating the increased expression of MITF, which is also downregulated in vitiliginous skin. The activity of LEF1 promoter was also observed to be reduced in resident stem cells after oxidative stress, which might also have an impact on vitiligo pathogenesis [2, 3].

The treatment of vitiligo is often challenging Narrow band UVB therapy (NB-UVB) stimulates melanocyte proliferation, migration, and differentiation in hair follicles and lesional epidermis. Since in vitiligo, impaired signaling of the Wnt pathway impairs re-pigmentation, NB-UVB

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appears to be a promising modality to reverse the impaired pathway and stimulate re-pigmentation [4].

Therefore, in this study, we intended to evaluate the expression and activity of LEF1 both before and after NB-UVB therapy and its correlation with the extent of re-pigmentation.

Materials and methods

Study design and patients

This was a prospective study where the cohort was recruited from the patients attending the outpatient pigmentary clinic of the dermatology department of Post Graduate Institute of Medical Education and Research, Chandigarh. Institute Ethics Committee approvals (Intramural) were obtained before initiation of the study and all research was performed in accordance with relevant guidelines/regulations. Thirty patients aged 12–60 years with a clinical diagnosis of unstable, non-segmental vitiligo with a body surface area of 10–50% and having both acral and non-acral lesions were included in the study. Unstable disease was defined as the occurrence of new lesion(s), progression of existing lesion(s), or Koebnerisation with a VIDA score of 4 + . Patients with segmental vitiligo and universal vitiligo, pregnant and lactating mothers, any contraindication to NBUVB therapy like xeroderma pigmentosa, systemic lupus erythematosus and other photo-induced or photo-aggravated dermatoses, patients who have received any systemic treatment in the past 4 weeks and patients with unrealistic expectations were excluded from the study. After obtaining informed consent from the included patients, baseline characteristics, history, and clinical examination findings of the patient were noted. Disease activity and extent were evaluated by Vitiligo disease activity score (VIDA) and Vitiligo Area Severity Index (VASI), respectively. Lesions distal to the elbows and knees were considered acral lesions. Lesional VASI was calculated separately for acral and non-acral lesions.

Skin biopsy and immunohistochemistry

Skin biopsies were obtained using a 2 mm punch biopsy from target lesions chosen at both acral and non-acral sites. At baseline, biopsies were obtained from thirty recruited patients and were repeated at the end of the study period (24 weeks) from patients who completed the study. LEF1 expression was examined in paraffin-embedded skin tissue sections with immunohistochemistry (IHC) as described previously [5]. The expression of LEF1 was quantified as mean fluorescent intensity (MFI).

Narrow-band UVB phototherapy

The study patients were treated with whole-body NBUVB phototherapy unit, administered as per the standard protocol. Phototherapy operations were based on local public health recommendations, and in consultation with the institution's infection control unit (in view of the COVID-19 pandemic). NBUVB was administered for at least 24 weeks (72 sessions) in all patients. Patients who failed to develop any signs of re-pigmentation by the end of 16 weeks (48 sessions) were considered primary non-responders and phototherapy was discontinued.

Follow-up and patient evaluation

The patients were followed-up on day 8 and weeks 4, 8, 12, 16, 20, and 24 after starting NBUVB. At each follow-up visit, blinded physicians (DP, MKS, and VK) assessed the percentage of re-pigmentation by whole-body clinical photography using a 20.2 MP camera in the same settings with respect to patient positioning, background, lighting, and camera settings along with lesional VASI, VIDA, pattern of re-pigmentation (perifollicular, marginal, diffuse and combined) and colour matching ('somewhat lighter than', 'same as' or 'somewhat darker' than normal skin). Re-pigmentation of 50% or more was considered to be satisfactory in this study.

Assessment of treatment compliance

Patients were considered compliant with phototherapy if they had attended at least 90% of their scheduled phototherapy visits.

Statistical analysis

All the collective data were entered into a spreadsheet (Microsoft Excel 2016); subsequently these data were clean and coded for statistical analysis. Due to a small sample size, the results were reported with a 95% confidence interval rather than the p value approach wherever applicable. The statistical analysis was carried out using IBM SPSS Statistics for Windows 27.0 (IBM, Chicago, Illinois, United States). The descriptive data have been presented as mean ± SD, median, interquartile range, frequencies, and percentages depending upon the normality of the data. Normal quantitative data, after assessment by Kolmogorov–Smirnov test, were analyzed using Student's *t* test, and skewed and ordinal data were analyzed using Wilcoxon signed rank test. Categorical data were compared using Chi-square test. Fischer exact test was used for categorical data in

which more than 20% of the cells in the table had a value of less than five. Friedman test has been used to assess the difference in the repeated values of variables across multiple points of time. *P* value < 0.05 has been considered significant in all tests.

Results

Thirty patients with the diagnosis of non-segmental vitiligo were included in this study. However, due to the unprecedented COVID-19 pandemic, only 16 patients completed the 24 weeks study period. The baseline demographic details and patient characteristics of the patients that completed follow-up have been summarised in Table 1. The mean age of the patients was 36.66 ± 10.64 years, with a male: female ratio of 1:3. The mean total duration of illness was 10.55 ± 4.53 years. All patients had lesions on both acral and non-acral areas. The mean BSA as measured by Wallace's rule of nines was 27.2 ± 15.5%. Overall, the mean VASI was 24.3 ± 14.0, whereas the mean lesional VASI of acral areas was 8.97 ± 4.54 and that of non-acral areas was 15.34 ± 10.38. All patients at baseline had a VIDA of 4 + .

Effect of NB-UVB phototherapy on re-pigmentation of lesions in acral and non-acral sites

At the end of the study period of 24 weeks, both disease stability and re-pigmentation were achieved (Table 2). While all patients had active disease at baseline (100%), only 43.8% of patients had active disease at 24 weeks ($p = 0.001$). The median VIDA at 24 weeks was 2 + . All 16 patients had achieved > 50% of re-pigmentation. However, > 75% re-pigmentation was achieved in only 11.1% of acral patches, whereas it was achieved in a

Table 2 Disease parameters and LEF1 expression at 24 weeks

Parameters	Acral lesion	Non-acral lesion	*p* value
Lesional VASI	3.53 ± 1.69	3.66 ± 3.27	1.000[1]
Change in VASI	− 5.44	− 11.68	**0.004**[1]
LEF1 expression in MFI	8.59 ± 1.94	8.74 ± 1.81	0.742[1]
Change in LEF1 expression as compared to baseline	3.58 ± 2.16	+ 3.74 ± 3.16	0.839[1]

Bold indicates statistical significance ($p < 0.05$)

Abbreviations: *LEF1* Lymphocyte enhancer-binding factor-1, *VASI* Vitiligo Area Severity Index, *MFI* Mean fluorescent intensity

[1]Unpaired *t* test

Table 1 Summary of baseline demographic and disease characteristics

	Parameters	*N* = 16
1.	Age (years) [mean ± SD]	36.66 ± 10.64
2.	Gender	
	Male	4 (25%)
	Female	12 (75%)
3.	Mean total duration of illness (years) [mean ± SD]	10.55 ± 4.53
4.	Leukotrichia present	5 (31.2%)
5.	Mucosal involvement	5 (31.2%)
6.	Lesions on face	9 (56.3%)
7.	History of topical treatment	16 (100%)
8.	Topical steroids	16 (100%)
9.	Topical treatment: tacrolimus	16 (100%)
10.	Topical treatment: calcipotriol	7 (43.8%)
11.	History of systemic treatment	4 (25%)
12.	History of phototherapy in the past	4 (25%)
13.	History of indigenous treatment	9 (56.3%)
14.	History of surgical management	0 (0%)
15.	Body surface area (mean ± SD)	27.2 ± 15.5%
16.	Total VASI (mean ± SD)	24.3 ± 14.0
17.	Lesional VASI—acral (mean ± SD)	8.97 ± 4.54
18.	Lesional VASI—non-acral (mean ± SD)	15.34 ± 10.38
19.	Baseline LEF1 expression—acral (mean ± SD) [MFI]	4.33 ± 1.55
20.	Baseline LEF1 expression—non-acral (mean ± SD) [MFI]	3.89 ± 1.86

SD Standard deviation, *VASI* Vitiligo Area Severity Index, *LEF1* Lymphocyte enhancer-binding factor-1, *MFI* Mean fluorescence intensity

significantly higher number of non-acral patches (66.6%) ($p = 0.05$). Two patients achieved > 90% re-pigmentation in the non-acral patches (Fig. 1). On assessment of VASI at 24 weeks, the total VASI significantly decreased from 24.31 at baseline to 7.19 ($p \leq 0.001$) with a reduction of 70.42% after NB-UVB phototherapy. The mean lesional VASI of acral areas significantly reduced from 8.97 at baseline to 3.53 (− 57.2%) at 24 weeks (Friedman Test: $\chi^2 = 51.6$, $p \leq 0.001$). The mean lesional VASI of non-acral areas also significantly reduced from a maximum of 15.34 at baseline to 3.66 (− 77.4%) at 24 weeks (Friedman Test: $\chi^2 = 52.6$, $p \leq 0.001$). The rate of re-pigmentation was not significantly different between acral and non-acral lesions (Friedman Test: $\chi^2 = 1.5$, $p = 0.221$). Though there was no significant difference in the absolute values of VASI both at baseline and at every follow-up visit between the acral and non-acral patches, the percentage change in VASI as compared to baseline was significantly higher in the non-acral lesions as compared to the acral lesions across all time points (post hoc pairwise tests for Friedman test performed using Nemenyi test). At 24 weeks, all patients showed a combination of both perifollicular pigmentation and peripheral migration of pigment in both acral and non-acral areas. With respect to colour match, 77.8% of lesions of acral group and 66.7% of lesions of the non-acral group achieved a good colour match as the surrounding skin ($p = 1.000$).

Immunohistochemical assessment of LEF1 expression

Expression of LEF1 in both acral and non-acral areas was assessed by IHC at baseline and 24 weeks post-phototherapy (Table 2). In acral lesions, the MFI of LEF1 gene was significantly increased from 4.33 ± 1.55 at the baseline to 8.59 ± 1.94 MFI ($p = 0.0078$) at 24 weeks (Fig. 2). Similarly, the MFI of LEF1 measured at non-acral lesions also revealed significant augmentation from 3.89 ± 1.86 MFI at baseline to 8.74 ± 1.81 MFI ($p = 0.0078$) at 24 weeks. There was no statistical difference in the absolute MFI value of LEF1 or the change in LEF1 value as compared to the baseline, between the acral and non-acral group at 24 weeks. However, the change in LEF1 expression with both the groups as compared with baseline expression was significant as specified above (Fig. 2). On further bivariate analysis, no significant correlation was found between the change in VASI and the change in LEF1 expression in both acral (Spearman coefficient = − 0.07, $p = 0.864$) and non-acral areas (Spearman coefficient = 0.47, $p = 0.213$). No significant correlation was found between the change in LEF1 expression and the baseline patient and disease characteristics. However, a significant negative correlation was observed between the change in LEF1 expression in non-acral areas at the end of 24 weeks and the baseline LEF1 expression of non-acral areas (Spearman coefficient = − 0.84; $p = 0.004$). Though a similar negative correlation was observed in acral areas as well, it did not achieve statistical significance (Spearman coefficient = − 0.65; $p = 0.06$).

Adverse effects

Amongst the 16 study patients, 14 experienced one or the other adverse effects due to phototherapy. Thirteen patients (81.3%) showed lesional erythema at some point during phototherapy. Blistering was experienced by five patients (31.3%) which easily subsided with a short course of oral steroids. Five patients (31.3%) complained of pruritus, which was managed with standard doses of anti-histamines and emollients. Six patients (37.5%) experienced diffuse hair-fall after starting phototherapy which resolved by the end of the study period. One patient (6.25%) had a reactivation of herpes labialis.

Discussion

Vitiligo is a common acquired disorder of hypopigmentation which presents as milky white macules and/or patches over the skin. Over the years, various hypotheses and theories namely autoimmune theory, neural theory, autocytotoxic theory, biochemical theory, and melanocytorrhagy have been put forward to further elucidate the etiopathogenesis

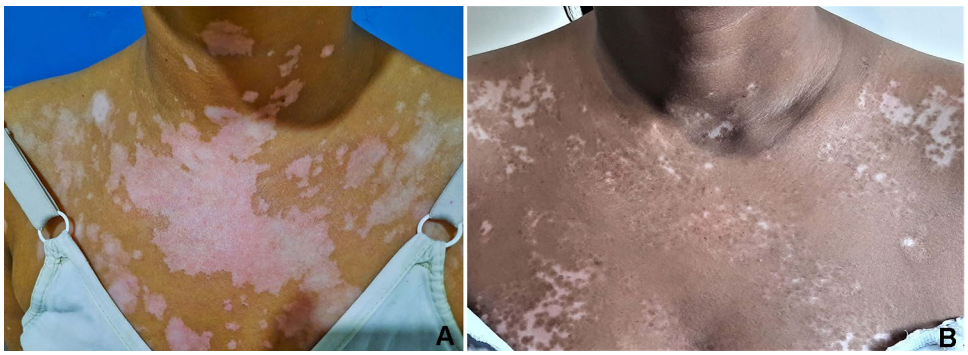

Fig. 1 **a** Depigmented patches of vitiligo present over the chest at baseline. **b** > 90% repigmentation at 24 weeks after NB-UVB therapy

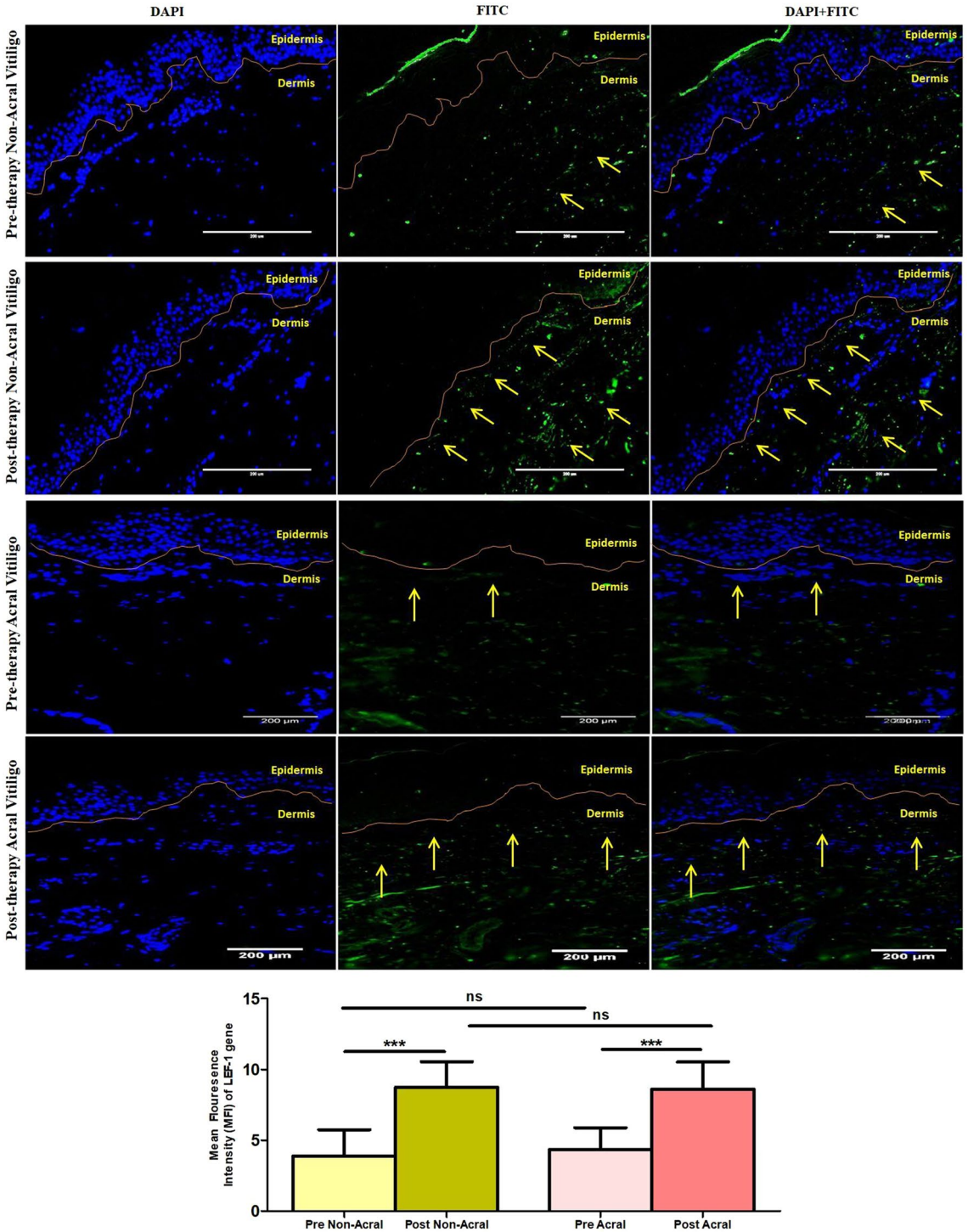

Fig. 2 Immunohistochemistry (IHC) images showing the expression of LEF1 expression in pre and post therapy non-acral/acral vitiligo skin. Panel (from left to right) showing nucleus staining by DAPI (Blue), LEF-1 (FITC; Green), and Merged (DAPI + FITC) respectively in skin tissue sections (original magnification at X200). Graph representing the mean fluorescence intensity (MFI) of LEF-1 gene. MFI was measured by ImageJ software. Statistical significance is shown by $*p < 0.05$, $**p < 0.01$, $***p < 0.001$

of the disease. The convergence pathway which involves an interplay of all the above-described pathways is now more widely accepted [1, 6].

Generalized vitiligo often follows an unpredictable course, making the treatment very challenging, especially when larger body surface areas are involved. Multiple

modalities of management are used like topical and systemic drugs, phototherapy, and surgical techniques, either alone or in combination, to stabilize the disease and re-pigment the lesion. NBUVB facilitates melanocyte proliferation, migration, and differentiation in hair follicles and lesional skin and hence helps in both disease stabilization and re-pigmentation [7, 8]. In our study, disease activity showed significant stabilization at the end of the study period with NBUVB as compared to baseline. This finding was consistent with the findings of Bhatnagar et al. [9]. We also observed a 70.4% reduction in total VASI as compared to the baseline. This was higher than the improvement seen in other studies, which have reported an improvement of 40–50%. However, the extent of re-pigmentation was lesser in acral areas as compared to non-acral areas, which can be explained by multiple mechanisms like relatively low melanocyte density, minimal density of hair follicles (which are a melanocyte reservoir), and a greater chance of repeated friction or trauma, which can induce koebnerisation [10]. Overall, all patients were able to achieve the satisfactory outcome of > 50% re-pigmentation of lesions.

The Wnt/β catenin pathway plays an important role in cell proliferation, polarity, and homeostasis. Apart from carcinogenesis and embryogenesis, it plays a pivotal role in the differentiation of melanoblasts into melanocytes. LEF1 is a transcription factor that acts as a positive mediator for the downstream Wnt/β catenin pathway. Oxidative stress in vitiligo is one of the factors that results in attenuation of the Wnt pathway, which further results in decreased LEF1 expression downstream. It has been demonstrated that NBUVB phototherapy up-regulates the expression of Wnt7 in the epidermis and also facilitates translocation of β catenin into the melanocyte stem cell (McSC) and hence subsequently results in re-pigmentation of vitiligo lesions [11, 12]. Regazzetti et al. also showed that lesional vitiligo skin is characterized by downregulation in the expression of LEF1 and other downstream effectors like cadherin 2, cadherin 3, and interferon regulatory factor 4 [2]. In our study, we observed a 98.4% enhancement of LEF1 expression in acral lesions and 124.7% enhancement in non-acral lesions following NB-UVB phototherapy which signifies reactivation of the Wnt/β-catenin signaling pathway and the resultant re-pigmentation. The increase in expression, however, failed to show a significant correlation with the re-pigmentation of the lesions, which could be explained by a small sample size.

Limitation

The limitations of this study are the small sample size because of unavoidable circumstances of the COVID-19 pandemic as well as a short duration of follow-up.

Conclusion

LEF1 expression modulates the re-pigmentation of vitiligo lesions after treatment with NBUVB phototherapy. Further studies with different modalities of management need to be conducted with a larger sample size so that the correlation between the increase in LEF1 expression post-treatment and the extent of re-pigmentation can be further explored.

Author contributions DN, AD, AB, MSK, VK and DP were responsible for patient enrolment and management and follow-up. DN and NS were responsible for sample collection and processing. NS was responsible for laboratory techniques and interpretation of laboratory data. DN, AD, VK and DP were responsible for data assimilation, analysis and interpretation. DN, NS, AD and DP prepared the first draft of the manuscript and all authors were involved in critical revision of the manuscript. Figures were prepared by DN, NS and AD.

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Data availability statement Data shall be provided by the authors upon reasonable request.

Declarations

Conflict of interest The authors declare that they have no conflict of interest.

Ethical approval The study was approved by institute intramural ethics committee (INT/IEC/2020/SPL-543) and the study was performed in accordance with the ethical standards as laid down in the 1964 Declaration of Helsinki.

Consent to participate Written informed consent was taken from all the patients prior to enrolment in the study.

Consent to publish The authors affirm that human research participants provided informed consent for the publication of the images in Fig. 1a and b.

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