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Main Subject [Dermatology]

Original Article

Histochemical Study of Melanocytes and Langerhans Cells in Trichrome Non-Segmental Vitiligo

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Article information

Received: 15-08-2025

Professionally accepted: 01-10-2025

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Citation: Khalifa NKA, Ezz-El-Din SME, Salama OAM. Histochemical Study of Melanocytes and Langerhans Cells in Trichrome Non-Segmental Vitiligo. IJMA 2025; XX-XX [Article in Press].

Abstract

Background: Vitiligo is a frequent acquired depigmenting disorder that causes significant psychological stress.

Aim: To study the histopathological changes in the trichrome skin area together with other signs of the disease in the vitiligo skin and the adjacent perilesional skin.

Patients and methods: This descriptive study has been performed on thirty cases suffering from vitiligo disease of any duration, with special emphasis on those having the trichrome sign. We used CD1a for identification of Langerhans cells, Melan A for identification of melanocytes, and H&E for changes occurring in vitiligo. Cases were recruited from the dermatology outpatient clinic at Al-Hussein University Hospital, Al-Azhar University, Cairo, from September 2021 till September 2022.

Results: The number of melanocyte cells in the vitiligo skin area ranged from 0 to 1 cell/field, representing around 1/3rd the number of cells in the trichrome area and almost 1/9th the cell count in the adjacent perilesional skin. The number of Langerhans cells/field in the trichrome area (11-18 cells) is almost double the count in the adjacent perilesional skin (6-10 cells) and around triple the count in the vitiligo area (2-5 cells).

Conclusion: This study found a significant increase in CD1A Langerhans cells in vitiligo-affected areas and trichrome areas, with counts nearly double in trichrome compared to perilesional skin and approximately triple in vitiligo areas. Additionally, the melanocyte count in the trichrome area was notably decreased relative to adjacent perilesional skin.

Keywords: Melanocytes; Langerhans Cells; Trichrome Non-Segmental Vitiligo

INTRODUCTION

Vitiligo is a prevalent acquired depigmenting disorder that causes significant psychological stress. It is distinguished by the absence of pigmentary cells (melanocyte cells) from the epidermis that leads to white patches on the skin [1].

The precise cause of vitiligo is still unrevealed, but there are various theories to clarify its pathogenesis. Sometimes vitiligo has an association with autoimmune illnesses such as diabetes mellitus, autoimmune thyroid disease, pernicious anemia, and rheumatoid arthritis. [2].

Vitiligo presents clinically with white spots on the body distributed symmetrically and more clearly in individuals with dark skin. The lesions are distinguished by well-demarcated milky white macules of different shapes; the borders are well defined, with different sizes ranging from a few millimeters to different sizes covering big areas on the skin [3].

Initial lesions predominantly occur on the hands, forearms, legs, feet—especially in pressure areas—and the face, with a preference for periocular or perioral distribution [4].

Vitiligo presents various clinical forms, including quadrichrome, trichrome, pentachrome, and inflammatory types [5].

Trichrome vitiligo refers to lesions characterized by a tan zone situated among normal and completely depigmented skin. The importance of trichrome remains unclear; however, it is regarded as a transitional pigmentary state that may last for months to years with minimal alteration [6].

It has been suggested that Langerhans cells may contribute to the pathogenesis of vitiligo and so could act as a possible therapeutic target for novel treatments. Nonetheless, their involvement in the pathogenesis of vitiligo is still ambiguous, since published studies present conflicting findings concerning LCs in vitiligo [4-6].

The study aimed at studying the histopathological changes in the trichrome skin area together with other signs of the disease in the vitiligo skin and the adjacent perilesional skin.

PATIENTS AND METHODS

This descriptive study has been performed on thirty cases suffering from vitiligo disease of any duration; we choose patients with the trichrome sign. We used CD1a for identification of Langerhans cells, Melan A for identification of melanocytes, and H&E for changes occurring in vitiligo. Patients were recruited from the dermatology outpatient clinic at Al-Hussein University Hospital, Al-Azhar University, Cairo, from September 2021 until September 2022.

Inclusion criteria: Age: from 18 to 45 years; gender: both sexes; confirmed cases of stable non-segmental vitiligo and having lesions for any duration.

Exclusion criteria: any associated skin disease, any associated systemic morbid disease (diabetes mellitus, dyslipidemia, thyroid disease, hypertension, liver disease, kidney disease), the use of any topical or systemic therapy within the last 3 months before entering into the study, unstable vitiligo, and pregnant and breastfeeding females.

Methods: Patients were subjected to the following: history taking, examination, and investigations.

A punch biopsy (4 mm) in size was taken from every patient. The procedure was done under local anesthesia using a 10.0% lidocaine solution and a 20-gauge needle to minimize pain during the procedure. Complete aseptic precautions were followed during and after taking the biopsy to prevent the skin infection. Betadine antiseptic solution 10.0% was used to sterilize the skin before and after the biopsy, sterilized cotton gauze was used for wound dressing, and no stitches were taken at the site of the biopsy. In all cases we aimed to include part of the skin from the adjacent perilesional, the vitiligo skin, and the trichrome skin area to compare the data in every area. The biopsy was immersed and preserved in 10.0% formalin solution till being sent for processing and staining. The sections have been stained utilizing hematoxylin-eosin, then we conducted immunohistochemical staining with CD1A for Langerhans cells & Melan A for melanocytes.

CD1A for Langerhans cells: The immunohistochemistry (IHC) method has been conducted utilizing a monoclonal mouse antibody from Biogenex Life Sciences Private Limited & Dako Cytomation, Denmark. Tissue sections were deparaffinized using xylene and subsequently hydrated in graduated concentrations of ethyl alcohol. Antigen retrieval has been performed. The tissue sections were treated utilizing a blocking agent called 'power block reagent' (Biogenix) to stop the natural activity of peroxidase. The sections underwent further incubation with the 1ry antibody anti-CD1a, followed by incubation with the secondary antibody poly-horseradish peroxidase (poly-HRP). Positive control sections comprised human skin tissue specimens that were subjected to the same treatment as the test groups. Negative control sections, which comprised one healthy tissue and one sick tissue, have been treated the same way as the test groups, except the primary antibody was left out and replaced with phosphate-buffered saline and a non-immune antibody (normal rabbit serum) at the same concentration.

Melan A STAINING for melanocytes: Immunostaining has been conducted via the avidin-biotin complex methodology. Four-micrometer-thick sections were deparaffinized in xylene and rehydrated using graded ethanol washes. Endogenous peroxidase activity has been inhibited by a ten-minute wash utilizing 3.0% hydrogen peroxide in methanol. Antigen retrieval has been conducted by microwave treatment in citrate buffer at pH 6.0. Non-specific binding was inhibited by a thirty-minute wash utilizing 1.0% bovine serum albumin (BSA) in Tris-buffered saline (TBS, pH 7.6). Sections have been treated overnight with a rabbit antibody specific to melan-A (NCL-L-Melan-A, diluted 1:25) for immunohistochemistry. After incubation with the 1ry antibody at four degrees Celsius, slices were treated with a biotinylated goat anti-rabbit secondary antibody (diluted 1:500; DAKO) for thirty minutes at room temperature. Sections were subsequently treated with a horseradish peroxidase-conjugated streptavidin-biotin complex (DAKO) for thirty minutes at room temperature. Immunostaining has been visualized with the 3-amino-9-ethylcarbazole (AEC) chromogen (DAKO). Sections were counterstained utilizing hematoxylin and subsequently covered with a slip. The negative control consisted of excluding the 1ry antibody and applying non-immune serum (0.1% BSA in TBS). Skin melanoma acted as a positive control for the two antibodies [8].

Immunohistochemistry Protocol: The immunohistochemistry protocol for CD1a staining of Langerhans cells involved the use of a mouse monoclonal primary antibody (Clone O10, Cat# M3571, Dako) diluted 1:50 in PBS and incubated for sixty minutes at room temperature. Antigen retrieval has been conducted via heat-induced epitope retrieval

(HIER) in 10 mM citrate buffer (pH 6.0) at ninety-five degrees Celsius for a duration of twenty minutes, succeeded by a twenty-minute cooling period. Blocking was carried out using Power Block (Biogenex) for 10 minutes, followed by an additional blocking step with 5.0% normal goat serum for thirty minutes to reduce nonspecific binding. The secondary antibody used was a poly-HRP-conjugated anti-mouse IgG, incubated for 30 minutes at room temperature. Human skin tissue known to contain Langerhans cells served as the positive control, while the negative control involved the omission of the 1ry antibody and substitution with PBS.

The immunohistochemistry protocol for Melan- A staining of melanocytes utilized a mouse monoclonal primary antibody (Clone A103, Cat# NCL-L-Melan-A, Leica Biosystems) diluted 1:25 in 1.0% BSA/TBS and incubated overnight at four degrees Celsius. Antigen retrieval has been carried out utilizing microwave treatment in citrate buffer (pH 6.0) at 800 W for three cycles of 5 minutes each. Blocking was done with 1.0% BSA in TBS for 30 minutes, followed by an additional 30-minute incubation with 5.0% normal goat serum to minimize nonspecific binding. A biotinylated goat anti-mouse IgG secondary antibody (DAKO, Cat# E0433), diluted 1:500, was applied for thirty minutes at room temperature. Detection was achieved using streptavidin-HRP (DAKO) for thirty minutes at room temperature, and 3-amino-9-ethylcarbazole has been used as a chromogen. Skin melanoma tissue served as the positive control, while the negative control involved substituting the 1ry antibody with non-immune serum.

Ethical considerations: Approval from the Department of Dermatology and Venereology, Faculty of Medicine, Al-Azhar University for Boys was obtained. Cases are identified by their names in the data collection sheet, which is kept in privacy by the investigator. Informed written consent was obtained from all cases that were participating in this research. The protocol was declared for ethical and research approval by the local ethics committee of the dermatology and venereology department at Al-Azhar University.

RESULTS

Table 1 illustrates that the mean age of the studied group was 31.5 ± 6.75; there were seven men (23.3%) and twenty-three women (76.7%).

Table 2 illustrates that the mean melanocyte cell count was 1.67 ± 1.94, with a range from 0 to 9. The mean Langerhans cell count was 7.76 ± 4.09, ranging from 2 to 18.

Table 3 illustrates that the number of Langerhans cells/field in the trichrome area (11-18 cells) is almost double the count in the adjacent perilesional skin (6-10 cells) and around triple the count in the vitiligo area (2-5 cells).

Table 4 shows that the number of melanocyte cells in the vitiligo skin area ranged from 0 to 1 cell/field, representing around 1/3rd the number of cells in the trichrome area and almost 1/9th the cell count in the adjacent perilesional skin.

Table [1]: Distribution of demographic data in the studied group.

Variable		Studied group N=30	
		Mean	SD
Age		31.5	6.75
Sex	Male	7	23.3
	Female	23	76.7

Table [2]: Distribution of Melanocyte cell counts and Langerhans cell counts in the three studied groups.

Variable	Studied group N=30		
	Mean	SD	Range
Melanocyte cell counts	1.67	1.94	(0-9)
Langerhans cell counts	7.76	4.09	(2-18)

Table [3]: Categorizing the Langerhans cell count/fields according to the area of skin involved.

Category of the cell	Area of skin	Langerhans cells Count/field	Total number of fields
Category A	Vitiligo skin area	(2 – 5)	20 (19.4%)
Category B	Trichrome skin area	(11- 18)	50 (48.5%)
Category C	Perilesional adjacent skin area	(6 – 10)	33 (32.03%)
Total count of Langerhans cells in fields		796	103 (100.0%)

Table [4]: Categorizing the melanocyte cell count/fields according to the area of skin involved.

Category of the cell	Area of skin	Melanocyte cells Cell count / field	Total number of fields

Category A	Vitiligo area	(0 – 1)	40 (53.3%)
Category B	Trichrome area	(2 – 3)	24 (32.0%)
Category C	Normal adjacent area	(4 – 9)	11 (14.7%)
Total count of melanocyte cells in fields		126	75 (100.0%)

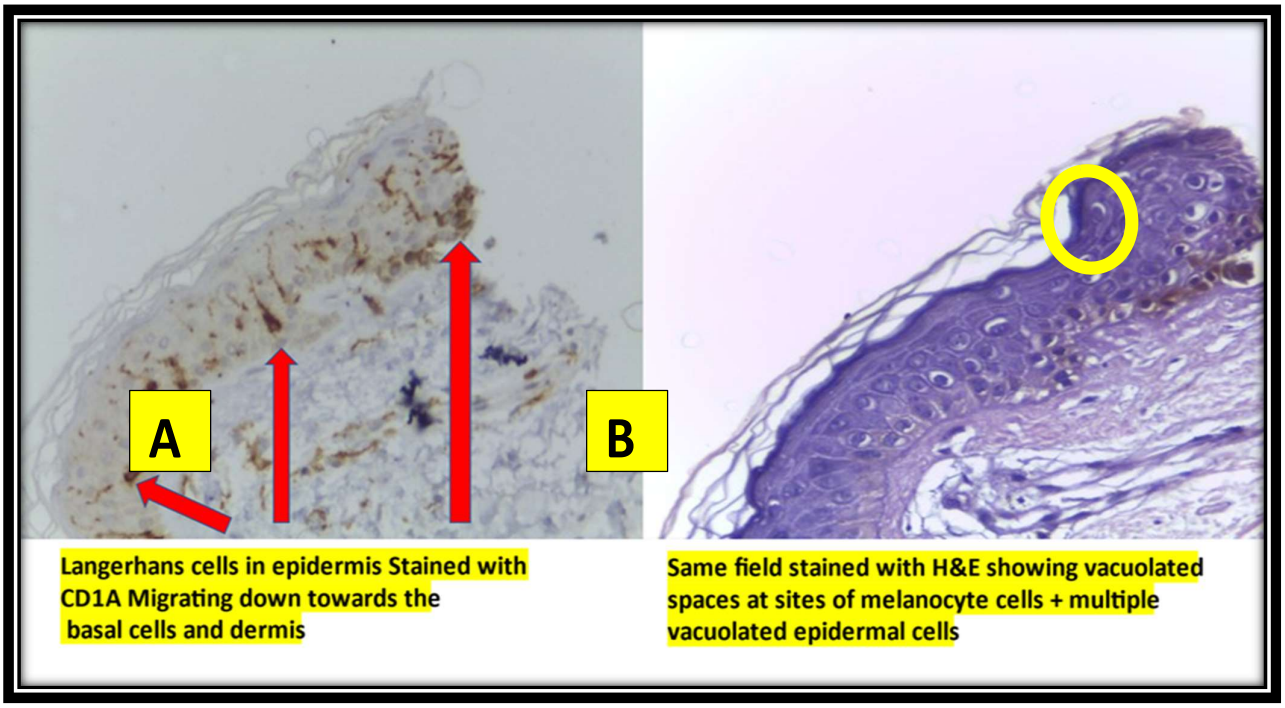

Figure 1: CD1A stain and H&E stain for the same field; CD1A shows scanty Langerhans cells in the epidermis migrating down towards the basal cells and dermis. H&E stain shows vacuolated spaces at sites of melanocyte cells and multiple vacuolated epidermal cells. This is a vitiligo skin area. X400.

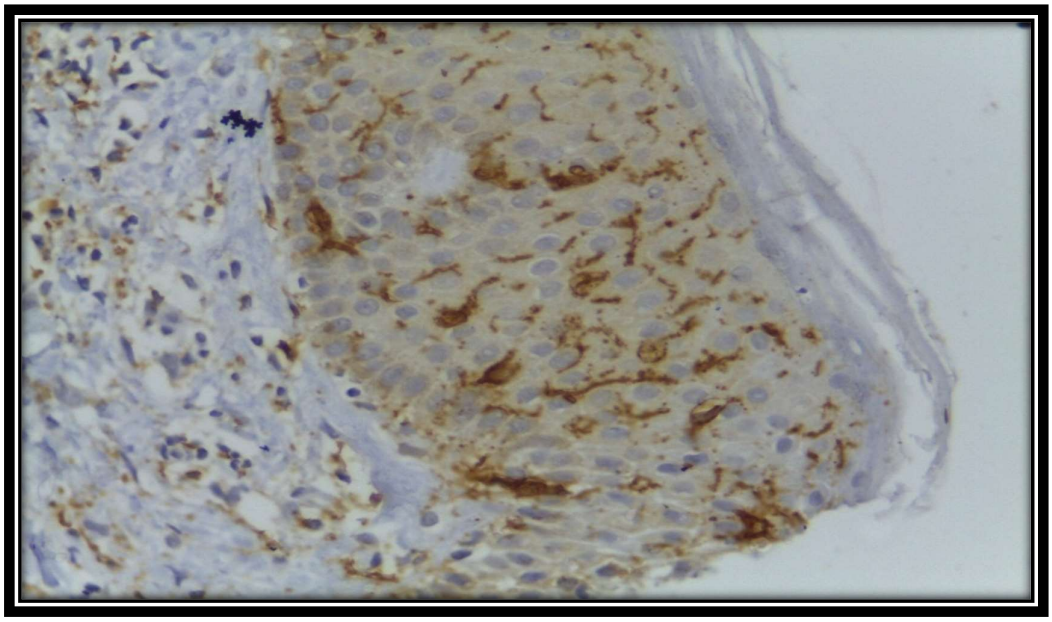

Figure 2: CD1a stain in the trichrome vitiligo area shows abundant Langerhans cells, abundant inflammatory cells in the upper dermis, abundant melanophores in the upper dermis, and thin epidermis. Magnification x 400.

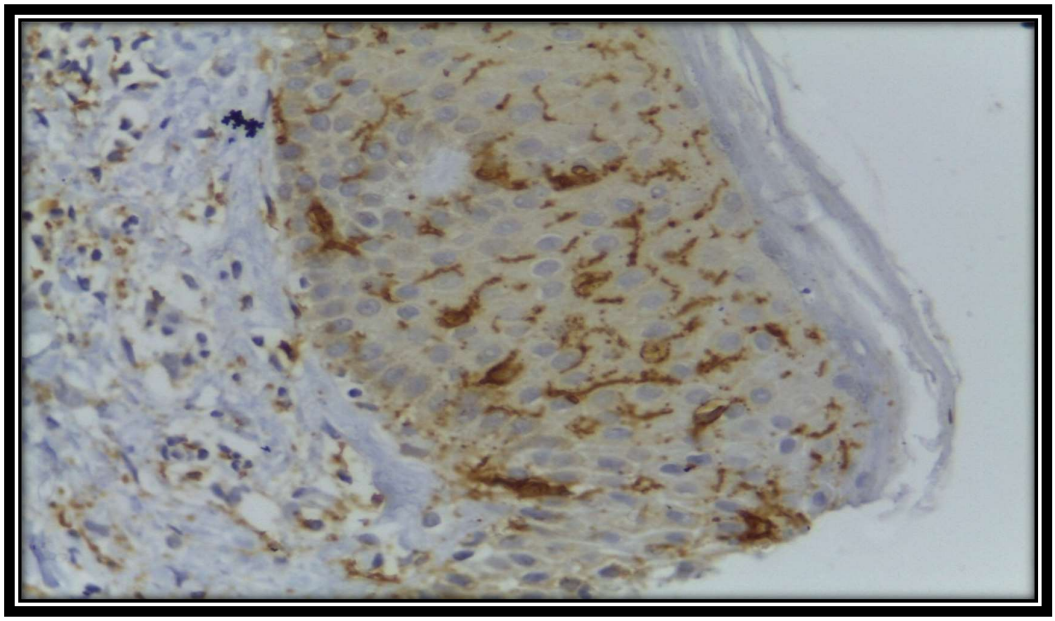

Figure 3: CD1A stain; shows Langerhans cells in different shapes and sizes, spheroidal cells inside red rings, oval-shaped cells inside yellow rings directed towards the basal cell layer, and multi-shaped cells inside blue rings. X400.

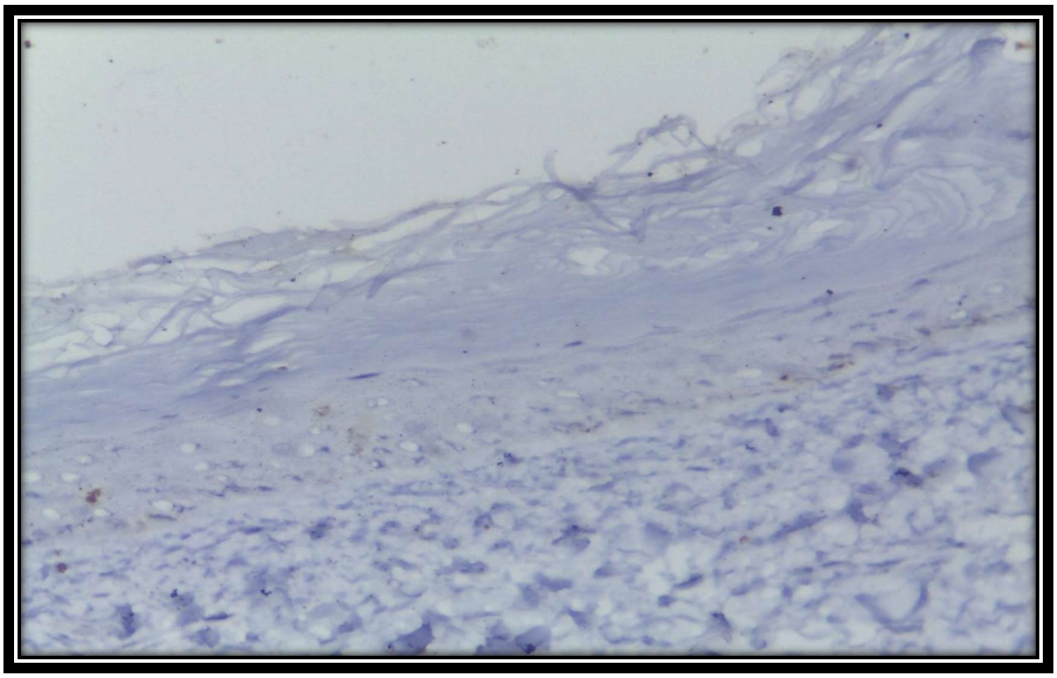

Figure 4: Melan A stain in the vitiligo area of skin shows an absence of melanocyte cells, few vacuolated epidermal cells, and an absence of inflammatory cells and melanophores in the upper dermis. X400.

Figure 5*:* Milan A-stained section in a trichrome skin area showing faint melanin dust in the basal cell layer with few melanocyte cells. X400.

DISCUSSION

Trichrome vitiligo refers to lesions characterized by a tan zone situated among normal and completely depigmented skin. The importance of trichrome remains unclear; however, it is regarded as a transitional pigmentary condition that may last for months to years with minimal alteration [9].

In Egypt, no statistical studies were done for the disease incidence in the whole country, but, in one study done by **El-Khateeb *et al.***, [10] they found that it affects around 0.06% of the population between 6 and 12 years.

In our study, the CD1a immunohistochemical marker has been used to illustrate the rise in epidermal Langerhans cells in cases of vitiligo with the trichrome sign. As for melanocyte cell expression, we used the Melan A immunohistochemical marker to observe changes in these cells in the biopsies examined.

The histopathological findings in our study were in congruence with the existing literature, **Wang *et al.***, [11]. **Elsherif *et al.***, [12]. **Yadav *et al.***, [13].

As regards the distribution of melanocyte cell counts in the studied group, it was found that the range of melanocyte cell counts in the three different pathological areas—perilesional adjacent skin, trichrome area, and the vitiligo area—the mean value was 1.67 ± 1.94 SD.

The number of Langerhans cells/field in the trichrome area (11-18 cells) is almost double the count in the adjacent perilesional skin (6-10 cells) and around triple the count in the vitiligo area (2-5 cells).

As regards the average value of melanocytes between the trichrome area and adjacent skin around it. There was a statistically significant rise in the incidence of the average value of melanocytes (2–3) in the trichrome area, which was 63.3%, while the rise in the frequency of the average value of melanocytes (4–9) in the adjacent skin was around 66.7.0% with a p-value of p=0.004.

Their results were contradictory to other investigations of vitiligo, **Hann SK**, ***et al.*** [14], **Le Poole IC**, ***et al***, [15]. The report of the absence of melanocytes in the depigmented patches was confirmed through electron microscopy or immunohistochemical staining.

These values denote that the melanocyte cell count in the trichrome area is markedly reduced compared to that of the adjacent perilesional skin. Although the latter exhibits active inflammatory reactions during the destruction process of melanocytes, that reaction in the trichrome area was markedly advanced in killing the melanocytes and representing the area of the acme immunological reactions in killing the melanocyte cells. These figures, from our point of view, are correct with our pathological findings in this work and are statistically significant.

As regards the average value of Langerhans cells between the vitiligo area and the normal skin around it. We found a statistically significant rise in the incidence of the average value of Langerhans cells

(2–5) in the vitiligo area, which was 63.3%, while the increase in the frequency of the average value of Langerhans cells

In our study we noticed scanty Langerhans cells in the vitiliginous skin areas, which agrees with the work of **Kao CH**, & **Yu HS**, [16].

Their findings, including inflammation of the dermis and epidermis in trichrome skin and perilesional normal skin, together with alterations in melanocytes, indicate that cell-mediated immunity may play a role in the pathogenesis of trichrome vitiligo. **Kao CH, Yu HS**. [16]

In one study, a rise in, as well as activation of, Langerhans cells from progressive non-segmental vitiligo **Itoi *et al***., [17].

In our work the number of melanocyte cells in the vitiligo skin area ranged from 0 to 1 cell/field, representing around 1/3rd the number of cells in the trichrome area and almost 1/9th the cell counts in the adjacent perilesional skin. The count and morphology of Langerhans cells were not prominent compared with the trichrome and adjacent skin.

In a study done by Shoeib and co-workers, they stated a decrease in Langerhans cells percentage in skin impacted by vitiligo **Shoeib *et al***., [18].

This result agrees with our findings regarding the population of Langerhans cells in the vitiliginous area of the skin.

A new study indicates that epidermal Langerhans cells in lesional skin from progressive vitiligo are significantly raised in number and demonstrate structural morphological changes compared to Langerhans cells from stable vitiligo or healthy skin. **Yang *et al***., [19].

A study revealed a rise and activation of Langerhans cells in progressive non-segmental vitiligo **Itoi *et al***., [17].

Shoeib and colleagues conducted a study including twenty vitiligo cases and ten age- and sex-matched controls, revealing a decrease in the number of Langerhans cells in vitiligo-affected skin, with a statistically insignificant alteration in LCs.

% following NB-UVB therapy, **Shoeib *et al***., [18].

New research indicates that epidermal Langerhans cells in lesional skin from progressive vitiligo are significantly elevated in number and demonstrate morphological changes compared to Langerhans cells from stable vitiligo or healthy skin. Moreover, these Langerhans cells exhibit modified shape and heightened expression of S100B **Yang *et al***., [19].

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