Re-appraisal of Keratinocytes' Role in Vitiligo Pathogenesis

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Abstract

Background: Vitiligo is a common pigmentary disorder. Studies on its pathogenesis extensively investigated melanocytes’ abnormalities and few studies searched for keratinocytes’ role in disease development. Liver X receptor-α (LXR-α) is a member of nuclear hormone receptors that acts as a transcription factor. Its target genes are the main regulators of melanocyte functions. Aim: The aim of this study is to investigate keratinocytes’ role in vitiligo pathogenesis through immunohistochemical expression of LXR-α in lesional, perilesional, and distant nonlesional vitiligo skin. Materials and Methods: This case–control study was carried out on 44 participants. These included 24 patients with vitiligo and 20 age- and sex-matched normal individuals as a control group. Biopsies, from cases, were taken from lesional, perilesional, and distant nonlesional areas. Evaluation was done using immunohistochemical technique. Results: Keratinocyte LXR-α expression was upregulated in the lesional and perilesional skin (follicular and interfollicular epidermis) compared with control skin (P<0.001 for all). There was significant association between higher histoscore (H-score) in lesional epidermis (P<0.001) and in hair follicle (P=0.001) and the presence of angiogenesis. There was significant association between higher H-score in lesional epidermis and suprabasal vacuolization (P=0.02). No significant association was found between H-score or expression percentage and clinical data of selected cases. Conclusion: LXR-α upregulation is associated with keratinocyte damage in vitiligo lesional skin that leads to decreased keratinocyte-derived mediators and growth factors supporting the growth and/or melanization of surrounding melanocytes. Therefore, melanocyte function and survival are affected.

Keywords: Immunohistochemistry, keratinocytes, liver X receptor-α, melanocytes, pathogenesis, vitiligo

Introduction

Vitiligo is an acquired pigmentary disorder characterized by cutaneous milky white lesions which may be accompanied by hair whitening. Majority of the patients develop the disease before the age of 20 years without racial, regional, or gender differences.

Several hypotheses were suggested to explain vitiligo pathogenesis depending on melanocytes destruction by autoimmune mechanisms, cytotoxic mechanisms, an intrinsic defect, oxidant-antioxidant mechanisms, and neural mechanisms.

Liver X receptor (LXR)-α and LXR-β are ligand-activated transcription factors which orchestrate macrophage function, lipid homeostasis, and inflammation. The inducible LXR-α is highly expressed in skin, liver, intestine, adipose tissue, macrophages, lung and kidney, whereas LXR-β is ubiquitously expressed.

The role of LXR-α in skin pigmentation was suggested based on its downstream target genes which regulate melanocyte functions.

In human epidermis, keratinocytes and melanocytes form functional unit. Growth factors and cytokines produced by keratinocytes affect melanocyte function and survival.
Based on the role of keratinocytes in the maintenance of melanocytes and the role of LXR-α in regulation of melanocyte function and skin pigmentation, the present study aimed to investigate the role of keratinocytes in vitiligo pathogenesis through the immunohistochemical expression of LXR-α in vitiligo skin biopsies.

Materials and Methods

Studied population

This case–control study was carried out on 44 participants. These included 24 patients with vitiligo and 20 age- and sex-matched normal individuals as control. The clinical diagnosis was based on the presence of well-demarcated, depigmented patches, confirmed by Wood’s lamp examination. Controls were selected from persons attending Plastic Surgery Department.

All studied patients were subjected to complete history taking, general and dermatological examination. Clinical data describing patients’ demographics (age and gender) as well as the clinical variables (distribution, disease duration, clinical type, and family history) were all documented. Vitiligo was clinically classified according to Taieb and Picardo. Selected cases were either newly diagnosed without history of previous treatment or old cases with completely depigmented lesions.

Assessment of disease activity was done according to vitiligo disease activity score.\(^7\)

Exclusion criteria

All participants with dermatologic diseases other than vitiligo were excluded. Patients with systemic and/or autoimmune disorders were also excluded.

Ethics

Written consent form approved by the Local Research Ethics Committee at Menoufiya Faculty of Medicine was obtained from every participant before the study initiation. This was in accordance with Helsinki Declaration in 1975 (revised in 2000).

Biopsies

From each patient, three 5-mm punch biopsies were obtained, using 2% lignocaine local anesthesia, from lesonal skin, from perilesional skin which is 1–5 mm peripheral-to-marginal area\(^8\) and from distant nonlesional skin. Biopsies from cases (lesional biopsies) and controls were site-matched. All specimens were fixed in 10% neutral-buffered formalin and subjected to routine tissue processing.

Sections were cut from paraffin-embedded blocks and stained with hematoxylin and eosin stain at Pathology Department, Faculty of Medicine, Menoufiya University for histopathological evaluation of epidermal thickness, pigment incontinence, basal and suprabasal vacuolization, dermal inflammation, hair follicle pigmentation, and angiogenesis.

Immunohistochemical staining

Four-micrometer-thick sections were cut from the paraffin-embedded blocks followed by deparaffinization and rehydration in xylene and graded series of alcohol, respectively. Antigen retrieval was performed by boiling in 10 ml citrate buffer (pH 6.0) for 20 min, followed by cooling at room temperature. After cooling, the slides were incubated overnight at room temperature with Rabbit polyclonal Anti-LXR-α antibody raised against LXR-α antigen. It was received as 0.1 ml concentrated (ab106464) (Abcam Inc., Cambridge, USA).

Detection of immunoreactivity was carried out using the ultravision detection system, ready-to-use anti-polyvalent horseradish peroxidase/diaminobenzidine (LabVision). Finally, the reaction was visualized by an appropriate substrate/chromogen (diaminobenzidine) reagent. Counter stain was carried out using Mayer’s hematoxylin.

Interpretation of immunostaining

A brown nucleocytoplasmic or cytoplasmic stain was considered positive in lesonal, perilesional, distant nonlesional, and control specimens.\(^9\)

The following items were evaluated in lesional, perilesional, distant nonlesional, and control biopsies:

a. Interfollicular and follicular epidermis were assessed for the following:

1. Expression - Positive or negative
2. Expression percentage – The percentage of the positive cells were assessed at ×200 magnification field\(^10\)
3. Histoscore (H-score) was calculated to all positive specimens according to the following equation
   \[ H\text{-score} = 1 \times \% \text{ of mildly stained cells} + 2 \times \% \text{ of moderately stained cells} + 3 \times \% \text{ of strongly stained cells} ; \]
4. Distribution was categorized as either
   - Patchy – Irregular or not uniform, or
   - Diffuse – Uniform.
5. Thickness pattern was categorized as either
   - Partial thickness staining, or
   - Whole thickness staining.

b. Dermis (adnexa, endothelial cells, and inflammatory cells) was assessed for:

   - Expression – Positive or negative.

Statistical analysis

Results were collected, tabulated, and statistically analyzed using an IBM personal computer and the statistical package SPSS version 11 (SPSS Inc., Chicago, IL, USA). Different statistical tests were carried out depending upon the type of data for comparison of different variables among the groups. \(P<0.05\) was considered statistically significant.
Results

Clinical characteristics of selected cases
The clinical characteristics of selected cases are summarized in Table 1.

Hematoxylin and Eosin findings in lesional skin
Epidermis was atrophic in 11 cases (45.8%). Pigmentary incontinence was present only in one case (4.2%). Basal vacuolization was present in 12 cases (50%). Suprabasal vacuolization was present in 15 cases (62.5%). The dermis showed inflammatory infiltrate in 19 cases (79.2%). Angiogenesis was present in 6 cases (25%).

Immunohistochemical expression of liver X receptor-α in studied groups

Control skin
In epidermis, all sections showed positive LXR-α expression with patchy distribution in 19 (95%) and diffuse distribution in one section (5%), and with partial thickness staining in 14 and whole thickness staining in six sections (30%).

Lesional skin
All cases showed positive LXR-α expression in epidermis with patchy distribution in 16 (66.7%) and diffuse distribution in 8 cases (33.3%), and with partial thickness staining in 12 (50%) and whole thickness staining in 12 cases (50%).

All cases showed positive dermal LXR-α expression. In follicular epidermis, LXR-α expression was positive in all cases, with patchy distribution in 22 (91.7%) and diffuse distribution in 2 cases (8.3%), and with partial thickness staining in 19 (79.2%) and whole thickness staining in 5 cases (20.8%). Detailed demonstration of LXR-α expression in control skin is shown in Table 2 and Figure 1.

Perilesional skin
All cases showed positive LXR-α expression in epidermis with patchy distribution in 10 (41.7%) and diffuse distribution in 14 cases (58.3%).

All cases showed positive dermal LXR-α expression. In follicular epidermis, LXR-α expression was positive in all cases, with patchy distribution in 14 (58.3%) and diffuse distribution in 10 cases (41.7%), and with partial thickness staining in 5 (20.8%) and whole thickness staining in 9 cases (37.5%). Detailed demonstration of LXR-α expression in perilesional skin is shown in Table 2 and Figure 3.

Distant nonlesional skin
All cases showed positive epidermal LXR-α expression with patchy distribution in 23 cases (95.8%) and diffuse distribution in one case (4.2%), and with partial thickness in 21 cases (87.5%) and whole thickness in 3 cases (12.5%).

Dermal expression was positive in 14 cases (58.3%). In follicular epidermis, LXR-α expression was positive in all cases with patchy distribution and with partial thickness in 19 cases (79.2%) and whole thickness in 5 cases (20.8%). Detailed demonstration of LXR-α expression in distant nonlesional skin is shown in Table 2 and Figure 4.

Table 1: Clinical data of the studied cases

| Variable                  | The studied cases (n=24) |
|---------------------------|--------------------------|
| Age Mean±SD               | 22.63±9.68               |
| Range                     | 12-53                    |
| Duration (in months) Mean±SD | 4.50±2.06              |
| Range                     | 2-19                     |
| Activity (in months) Mean±SD | 5.63±3.33              |
| Range                     | 1-12                     |
| Sex, n (%) Male           | 15 (62.5)                |
| Female                    | 9 (37.5)                 |
| Family history, n (%)     |                          |
| Positive                  | 3 (12.5)                 |
| Negative                  | 21 (87.5)                |
| Distribution, n (%) Focal | 2 (8.3)                  |
| Acrofacial                | 8 (33.3)                 |
| Segmental                 | 4 (16.7)                 |
| Generalized               | 10 (41.7)                |
| Clinical type, n (%) NSV  | 20 (83.3)                |
| Segmental                 | 4 (16.7)                 |
| VIDA score, n (%) Score +1 | 5 (20.8)                 |
| Score +2                  | 6 (25.0)                 |
| Score +3                  | 12 (50.0)                |
| Score +4                  | 1 (4.2)                  |

SD: Standard deviation, NSV: Nonsegmental vitiligo, VIDA: Vitiligo disease activity
Table 2: Liver X receptor-α expression in lesional, perilesional, distant nonlesional, and control skin

| The studied groups       | Lesional (n=24) | Perilesional (n=24) | Healthy skin (n=20) | Distant nonlesional (n=24) | P     |
|--------------------------|-----------------|---------------------|---------------------|----------------------------|-------|
| **Epidermis**            |                 |                     |                     |                            |       |
| Expression, n (%)        |                 |                     |                     |                            |       |
| Positive                 | 24 (100)        | 24 (100)            | 20 (100)            | 24 (100)                   | P1=NA |
| Negative                 | 0               | 0                   | 0                   | 0                          |       |
| **Distribution, n (%)**  |                 |                     |                     |                            |       |
| Patchy                   | 16 (66.7)       | 10 (41.7)           | 19 (95.0)           | 23 (95.8)                  | P1=0.08|
| Diffuse                  | 8 (33.3)        | 14 (58.3)           | 1 (5.0)             | 1 (4.2)                    | P2=0.03|
| **Thickness, n (%)**     |                 |                     |                     |                            |       |
| Partial                  | 12 (50)         | 7 (29.2)            | 14 (70)             | 21 (87.5)                  | P1=0.14|
| Whole                    | 12 (50)         | 17 (70.8)           | 6 (30)              | 3 (12.5)                   | P2=0.18|
| **Percentage**           | 68.5±27.56      | 85.8±20.19          | 33.5±19.27          | 35.4±21.87                 | P1=0.03|
| Mean±SD                  | 20-100          | 40-100              | 0-100               | 0-100                      | P2<0.001|
| Range                    |                 |                     |                     |                            |       |
| **H-score**              | 97.9±69.66      | 142.0±73.66         | 33.5±19.27          | 58.6±79.78                 | P1=0.009|
| Mean±SD                  | 30-300          | 50-300              | 0-100               | 0-100                      | P2<0.001|
| Range                    |                 |                     |                     |                            |       |
| **Dermis**               |                 |                     |                     |                            |       |
| Expression, n (%)        |                 |                     |                     |                            |       |
| Positive                 | 24 (100)        | 24 (100)            | 14 (70.0)           | 14 (58.3)                  | P1=NA |
| Negative                 | 0               | 0                   | 6 (30.0)            | 10 (41.7)                  |       |
| **Inflammatory cells, n (%)** |              |                     |                     |                            |       |
| Negative                 | 0               | 14 (58.3)           | 20 (100)            | 22 (91.7)                  | P1=0.66|
| Positive                 | 24 (100)        | 10 (41.7)           | 0                   | 2 (8.3)                    | P2<0.001|
| **Endothelium, n (%)**   |                 |                     |                     |                            |       |
| Positive                 | 12 (50)         | 10 (41.7)           | 12 (60)             | 14 (58.3)                  | P1=0.56|
| Negative                 | 12 (50)         | 14 (58.3)           | 8 (40)              | 10 (41.7)                  | P2=0.51|
| **Sweat glands, n (%)**  |                 |                     |                     |                            |       |
| Positive                 | 6 (25)          | 6 (25)              | 10 (50)             | 11 (54.8)                  | P1=1.0 |
| Negative                 | 18 (75)         | 18 (75)             | 10 (50)             | 13 (54.2)                  | P2<0.09|

Contd...
Comparison between liver X receptor-α expression in studied groups

Lesional versus control skin
Higher expression percentage, higher epidermal H-score, and positive expression in dermal inflammatory cells (P<0.001 for all) were significantly associated with lesional skin compared with control skin [Table 2].

Regarding hair follicle expression, higher expression percentage and higher H-score (P<0.001 for both) were significantly associated with lesional compared with control skin [Table 2].

Lesional versus perilesional skin
Higher epidermal expression percentage (P=0.03) and higher epidermal H-score (P=0.009) were significantly associated with perilesional compared with lesional skin [Table 2].

Regarding hair follicle expression, higher expression percentage (P=0.003), higher H-score (P<0.001), and positive expression in dermal inflammatory cells (P<0.001) were significantly associated with perilesional compared with lesional skin.

Table 2: Contd...

| The studied groups                                                                 | Lesional (n=24) | Perilesional (n=24) | Healthy skin (n=20) | Distant nonlesional (n=24) | P     |
|-----------------------------------------------------------------------------------|-----------------|---------------------|---------------------|---------------------------|-------|
| Sebaceous glands, n (%)                                                           |                 |                     |                     |                           |       |
| Positive                                                                          | 11 (45.8)       | 13 (54.2)           | 14 (70)             | 13 (54.2)                 | P1=0.56 |
| Negative                                                                          | 13 (54.2)       | 11 (45.8)           | 6 (30)              | 11 (45.8)                 | P2=0.11 |
|                                                                                   |                 |                     |                     |                           | P3=0.28|
|                                                                                   |                 |                     |                     |                           | P4=0.56|
| Hair follicle                                                                     |                 |                     |                     |                           |       |
| Expression, n (%)                                                                |                 |                     |                     |                           |       |
| Positive                                                                          | 24 (100)        | 24 (100)            | 20 (100)            | 24 (100)                  | P1=NA  |
| Negative                                                                          | 0               | 0                   | 0                   | 0                         | P2=NA  |
|                                                                                   |                 |                     |                     |                           | P3=NA  |
|                                                                                   |                 |                     |                     |                           | P4=NA  |
| Distribution, n (%)                                                              |                 |                     |                     |                           |       |
| Patchy                                                                           | 22 (91.7)       | 14 (58.3)           | 19 (95.0)           | 24 (100)                  | P1=0.008|
| Diffuse                                                                          | 2 (8.3)         | 10 (41.7)           | 1 (5.0)             | 0                         | P2=1.0 |
|                                                                                   |                 |                     |                     |                           | P3=0.005|
|                                                                                   |                 |                     |                     |                           | P4=0.49|
| Thickness, n (%)                                                                 |                 |                     |                     |                           |       |
| Partial                                                                          | 19 (79.2)       | 5 (20.8)            | 13 (65)             | 19 (79.2)                 | P1=0.001|
| Whole                                                                            | 5 (20.8)        | 19 (79.2)           | 7 (35)              | 5 (20.8)                  | P2=0.29|
|                                                                                   |                 |                     |                     |                           | P3=0.003|
|                                                                                   |                 |                     |                     |                           | P4=1.0 |
| Percentage                                                                       |                 |                     |                     |                           |       |
| Mean±SD                                                                          | 58.75±26.26     | 79.79±20.56         | 31.5±21.83          | 31.25±22.13               | P1=0.003|
| Range                                                                            | 30-100          | 45-100              | 0-100               | 0-80                      | P2<0.001|
|                                                                                   |                 |                     |                     |                           | P3<0.001|
|                                                                                   |                 |                     |                     |                           | P4<0.001|
| H-score                                                                          |                 |                     |                     |                           |       |
| Mean±SD                                                                          | 61.25±29.08     | 117.92±52.75        | 28.0±19.89          | 25.0±17.94                | P1<0.001|
| Range                                                                            | 30-120          | 60-210              | 0-100               | 0-80                      | P2<0.001|
|                                                                                   |                 |                     |                     |                           | P3<0.001|
|                                                                                   |                 |                     |                     |                           | P4<0.001|

SD: Standard deviation, NA: Not applicable, P1: Lesional versus perilesional, P2: Lesional versus control, P3: Perilesional versus control, P4: Lesional versus distant nonlesional skin

Figure 1: Normal skin showing (a) diffuse liver X receptor-α expression in epidermis (red arrow) and hair follicle (blue arrow), (b) patchy staining of epidermis (red arrow) and hair follicle (blue arrow)
Bakry, et al.: LXR-α in vitiligo

diffuse distribution (P=0.008), and whole thickness staining (P<0.001) were significantly associated with lesional compared with distant nonlesional skin [Table 2].

Lesional skin versus distant nonlesional skin

Higher epidermal intensity (P=0.008), higher expression percentage (P<0.001), higher epidermal H-score (P=0.001), positive dermal expression (P<0.001), diffuse epidermal distribution (P=0.02), and epidermal whole thickness staining (P=0.005) were significantly associated with lesional skin compared with distant nonlesional skin [Table 2].

Regarding hair follicle expression, higher expression percentage (P<0.001) and higher H-score (P<0.001) were significantly associated with lesional skin compared with distant nonlesional skin [Table 2].

Perilesional versus control skin

Higher epidermal expression percentage, higher epidermal H-score, diffuse distribution, and positive inflammatory cell expression (P<0.001 for all) were significantly associated with perilesional compared with control skin [Table 2].

Regarding hair follicle expression, higher expression percentage and higher H-score (P<0.001 for both) were significantly associated with perilesional skin compared with control skin [Table 2].

Relationship between Histoscore and expression percentage in lesional skin and clinical data of studied cases

There was no significant association between H-score or expression percentage in lesional epidermis and hair follicle and clinical data of selected cases [data not shown in Tables].

Relationship between Histoscore and expression percentage in lesional skin and histopathological data

There was significant association between higher H-score in lesional epidermis (P<0.001) and in hair follicle (P=0.001) and the presence of dermal angiogenesis [Table 3].

There was significant association between higher H-score in lesional epidermis and suprabasal vacuolization (P=0.02) [Table 3].

There was significant association between higher LXR-α percentage in lesional epidermis (P=0.009) and hair follicle (P=0.002) and presence of dermal angiogenesis [Table 4].

Discussion

Despite being a common dermatologic disease, vitiligo pathogenesis is not yet well established. Several hypotheses have been suggested but none of them completely explains all aspects of the disease. Vitiligo is a multifactorial disease with genetic and nongenetic factors working in concert. [6]

The importance of LXR-α in vitiligo development comes from the known functional link between epidermal keratinocytes and melanocytes. Keratinocyte-derived cytokines and growth factors affect melanocyte function and survival. [5]

LXRs are transcription factors that regulate genes involved in immunity, inflammation, and lipid biosynthesis. Cutaneous LXRs are involved in regulation of keratinocyte, melanocyte, and sebocyte functions. [4]
In the present work, all control sections showed positive epidermal LXR-α expression. This was in agreement with Alestas et al.\textsuperscript{[12]}

LXR ligands stimulate keratinocyte differentiation by inducing the expression of genes involved in cornified envelope formation, namely, transglutaminase 1, involucrin, loricrin and filaggrin.\textsuperscript{[13]}

Epidermal homeostasis is critical for survival of an organism, and any change in skin barrier function through alterations in keratinocyte differentiation and/or lipid synthesis/transport may predispose individuals to cutaneous inflammation. To avoid severe consequences of epidermal barrier perturbations and to swiftly adjust epidermal homeostasis, the skin system appears to employ lipid-sensing nuclear receptors, namely, LXRs and peroxisome proliferator-activated receptors.\textsuperscript{[14]}

In the present work, all examined control sections showed positive hair follicle expression of LXR-α. This was in agreement with Russell et al.\textsuperscript{[15]} who noted marked LXR-α expression in cells adjacent to the dermal papillae of hair follicles. This may correlate with the site of hair follicle melanocytes, suggesting a contribution to hair follicle melanocyte activity. Dermal papillae also play a pivotal role in hair formation, growth, and cycling.\textsuperscript{[16]}

The positive LXR-α expression in the sebaceous glands, noted in the current work, went with Russell et al.\textsuperscript{[17]} who recorded that both LXR isotypes are expressed in sebocytes and LXR-α agonists stimulate lipogenesis and inhibit proliferation of sebocytes.

In the current study, endothelial expression of LXR-α was positive in 60% of examined control sections. This was previously noted by Morello et al.\textsuperscript{[18]} In addition, Yu et al.\textsuperscript{[19]} reported that LXR-α is expressed and functional in rat bone marrow-derived endothelial progenitor cells which play a pivotal role in endothelial regeneration, repair, and migration.\textsuperscript{[19]}

Many genes governed by LXR-α are related to the regulation of melanocyte functions.\textsuperscript{[20]} Moreover, LXR-α was upregulated in perilesional melanocytes of vitiligo skin compared with the normal unaffected regions, suggesting that LXR-α might have a role in the pathogenesis of vitiligo.\textsuperscript{[21,22]}

Chang et al. reported that microphthalmia-associated transcription factor (MITF) is a master transcription factor for melanogenesis. Activation of LXR-α inhibits the expression of melanogenic enzymes through the acceleration of extracellular signal-regulated kinase (ERK) - mediated MITF degradation, ultimately suppressing melanogenesis.\textsuperscript{[23]}

Previous studies had investigated keratinocytes participation in vitiligo pathogenesis by different
mechanisms.\textsuperscript{24-26} To the best of our knowledge, LXR-α expression was not investigated before in vitiliginous keratinocytes.

In the current work, keratinocytes showed positive expression of LXR-α in lesional, perilesional, and distant nonlesional skin sections in the epidermis and dermis and was upregulated in lesional skin compared with distant nonlesional and control skin.

Most studies on vitiligo focused on melanocyte defects. However, vitiligo is not a disease confined to melanocytes. Direct cell-to-cell contact between melanocytes and keratinocytes stimulates \textit{in vitro} proliferation of melanocytes. Growth factors produced by adjacent keratinocytes regulate proliferation and differentiation of melanocytes.\textsuperscript{27}

These factors include endothelin-1, stem cell factor, and granulocyte-monocyte colony-stimulating factor which stimulate melanogenesis and melanocyte proliferation.\textsuperscript{3} Keratinocytes can secrete additional cytokines, such as interleukin-6 and tumor necrosis factor-α, which function as paracrine inhibitors of melanocytes.\textsuperscript{28}

Altered levels of keratinocyte-derived mediators have been described in vitiligo epidermis, suggesting a role of keratinocytes in the pathogenesis of vitiligo. In addition, keratinocytes in vitiligo lesions have been reported to be more susceptible to apoptosis.\textsuperscript{29}

LXR-α activation and synthetic LXR-α-specific agonists decrease keratinocytes proliferation, increase cell death, and decrease epidermal thickness.\textsuperscript{13,15}

Therefore, LXR-α upregulation is associated with keratinocyte damage in vitiligo lesional skin which is suspected to adversely affect melanocyte function and survival. Further large-scaled investigation is required for firmer conclusion.

The upregulation of LXR-α in hair follicles of lesional skin compared with control and distant nonlesional skin may raise a question; whether this will affect follicular melanocyte reservoir or not? As repigmentation of vitiliginous skin arises mostly from hair follicle units, wherever hair is available.\textsuperscript{30} Therefore, additional research is required to get an answer.

The significant association between higher H scores of LXR-α in vitiligo skin and the presence of angiogenesis in our study can be explained by the transcriptional regulation of the vascular endothelial growth factor (VEGF) by LXR-α.\textsuperscript{31}

LXR-α does not seem to be involved in basal VEGF expression but in response to inflammation and some other pathological conditions.\textsuperscript{19}

### Table 4: Relationship between liver X receptor-α expression percentage in lesional epidermis and histopathological data

| Variable                              | Interfollicular epidermis | Follicular epidermis |
|---------------------------------------|---------------------------|----------------------|
|                                       | LXR-α expression percentage | P          | LXR-α expression percentage | P            |
|                                       | Mean±SD                   | P          | Mean±SD                   | P            |
| **Epidermis**                         |                           |            |                           |              |
| Normal                                | 70.0±26.46                | 0.81       | 63.85±27.25                | 0.24         |
| Atrophic                              | 66.82±30.02               |            | 52.73±24.94                |              |
| **Pigmentary incontinence**           |                           |            |                           |              |
| Absent                                | 69.35±27.89               | 0.51       | 59.57±26.54                | 0.42         |
| Present                               | 50.0                      |            | 40.0                      |              |
| **Basal vacuolization**               |                           |            |                           |              |
| Absent                                | 61.67±25.52               | 0.19       | 57.50±27.01                | 0.75         |
| Present                               | 75.42±28.88               |            | 60.0±26.63                |              |
| **Suprabasal vacuolization**          |                           |            |                           |              |
| Absent                                | 47.22±17.87               | 0.004      | 45.56±8.82                 | 0.17         |
| Present                               | 81.33±24.46               |            | 66.67±30.16               |              |
| **Inflammation**                      |                           |            |                           |              |
| Absent                                | 54.0±23.02                | 0.19       | 40.0±10.0                  | 0.06         |
| Present                               | 72.37±27.91               |            | 63.68±27.12               |              |
| **Pigmented hair follicle**           |                           |            |                           |              |
| Negative                              | 68.54±27.56               | -          | 58.75±26.26                | -            |
| Positive                              | 100.0±0.0                 | 0.001      | 88.33±18.35                | 0.002        |
| Negative                              | 58.06±23.71               |            | 48.89±20.55               |              |

SD: Standard deviation, LXR: Liver X receptor
The present study showed that higher percent of expression of LXR-α in vitiligo skin was significantly associated with keratinocyte vacuolization. This observation might be due to the effect of LXR-α activation on keratinocytes which was previously detected by Köműves et al. who reported that activation of LXR-α induced growth arrest and apoptosis in keratinocytes.[32]

The present work demonstrated that LXR-α was upregulated in perilesional skin compared with lesional skin. Prignano et al. reported that keratinocytes from perilesional skin of vitiligo showed significant biochemical alterations, such as increased production of reactive oxygen species, lipoperoxidation, mitochondrial alterations, and increased apoptotic markers compared with lesional or healthy skin. This led to hypothesize that perilesional vitiligo skin may represent the substrate where melanocyte death is initiated, with a substantial role played by keratinocytes in the development of disease.[33]

Therefore, we can postulate that therapeutic options for vitiligo may need to be extended to pigmented perilesional skin which may help to prevent early events in “silent” vitiligo melanocytes and prevent the spread of the disease.

And now, a question arises: what is the precipitating factor that leads to LXR-α upregulation in lesional vitiliginous skin with all its subsequent events? Is it induced by oxidative stress? Or there are unknown controlling mechanisms? The answer requires more molecular investigations to be demystified. It is also worthy enough to study the interaction between LXR-α and other factors suggested to play roles in vitiligo pathogenesis as vitiligo is a multifactorial disease.

Conclusion
In summary, LXR-α is upregulated in vitiliginous skin keratinocytes with the highest expression in perilesional area. The cause of this upregulation is not clear and may lead to keratinocyte death that leads to decreased keratinocyte-derived mediators and growth factors supporting the growth and/or melanization of surrounding melanocytes.

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Nil.

Conflicts of interest
There are no conflicts of interest.

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