Interleukin-36α Expression in Vitiligo Skin Lesions and Its Association with Disease Pattern, Activity, and Severity

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Abstract

Background: Vitiligo is a pigmentary disorder defined by the presence of well-circumscribed milky-white macules on the skin and mucous membranes caused by the loss of functional melanocytes in the affected areas. There are various theories for vitiligo’s origin, including neurological, auto cytotoxic, genetic, and immunological theories. Because the cytokine IL-36 is involved in the development and pathophysiology of autoimmune disorders such as psoriasis, rheumatoid arthritis, and SLE, it’s probable that IL-36 plays a role in the progression of vitiligo. Objectives: The purpose of this study was to look at the tissue expression of IL-36 in vitiligo lesions and non-lesional skin. Patients and Methods: There were 41 vitiligo patients and 5 healthy controls in this cross-sectional analytic study. Each patient’s history was collected, and all patients were examined by a dermatologist to determine the pattern, activity, and severity of vitiligo. Biopsies were extracted from lesional and nonlesional skin of all patients, and immunohistochemistry staining for IL-36 expression was performed. Results: In vitiligo patients, IL-36 expression was elevated in both lesional and nonlesional skin, primarily in the inflammatory infiltrate in the dermis. In terms of age of onset, sex, disease duration, course, activity severity, and pattern, there is no significant variation in IL-36 expression. Conclusion: Our findings suggested that IL 36 may have a role in the etiology of vitiligo, which could shed light on the disease's pathogenesis.

Keywords: IL-36α, interleukin 36α, interleukin 36, vitiligo, disease severity

Introduction

Vitiligo is a multifactorial, progressive skin and mucous membrane depigmenting illness characterized clinically by well-defined white patches caused by the selective death of functioning melanocytes. Vitiligo has a significant impact on people’s lives. Many patients experience distress, embarrassment, shame, anxiety, dysthymia, and social isolation as a result of their treatment. Vitiligo patients' self-esteem suffers, which can lead to depression and suicide attempts. Because of its cosmetic implications, vitiligo carries a societal stigma. Depending on many theories, the cause of vitiligo is an overlap between the occurrence of many different factors, including oxidative stress, neurogenic mechanisms, infections, mutations, deficiency of melanocyte growth factors, and defective melanocyte migration in patients who have a genetic susceptibility. The link of vitiligo with autoimmune disorders such as Addison's disease, diabetes mellitus, and alopecia areata, as well as the fact

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that autoantibodies and autoreactive T lymphocytes against melanocyte antigens are seen in many vitiligo patients, support an autoimmune etiology\(^5\). Dendritic cells are activated by innate immune responses and present melanocyte-specific antigens to T cells. In addition, innate immune cells' interleukin (IL)-8 and interleukin (IL)-6 cytokines serve as the initial signal for T cells to find stressed melanocytes, resulting in targeted cell killing by CD8+ cytotoxic T cells\(^6\). Interleukin (IL)-36 is a member of the interleukin (IL1) superfamily, which includes the excruciating cytokines interleukin 36α, interleukin 36 β, and interleukin 36γ, as well as an inhibitor, interleukin 36Ra, formerly known as IL1F5\(^7,8\). Because it may promote expression of itself, interleukin (IL)-36 possesses an autocrine/paracrine loop. Both interleukin (IL)-17 and tumor necrosis factor (TNF) can stimulate the expression of interleukin (IL)-36, and agonists in keratinocytes when they are synergized by interleukin (IL)-22. Moreover, multiple studies show that epidermal growth factor (EGF) signaling regulates the expression of interleukins (IL)-36 in the skin\(^9\). Much research, including psoriasis, have been done to support the critical function of interleukin 36 in many skin illnesses, especially autoimmune ones. Psoriasis patients had higher levels of interleukin (IL)-36 expression\(^10\). By activating keratinocyte cells, increasing macrophage C-C motif chemokine ligand (CCL3, CCL4, CCL5, and CCL22), T cell chemoattractants, and assisting in the differentiation of Th 0 cells into Th1 cells, interleukin (IL)-36 cytokines actively propagate skin inflammation and immunological responses\(^11\). Interleukin (IL)-36 cytokines are a set of inflammatory mediators that begin nonspecific immune responses, and an interface between nonspecific immunity & acquired immunity. Also, having important functions in tissue inflammation and tissue damage. Both innate and acquired immunity play important role in the pathogenesis of vitiligo. So, this study will be conducted to assess the possible involvement of interleukin (IL)-36α in vitiligo pathogenesis.

**Patients and Methods**

**Patients**

This cross-sectional study included 41 vitiligo sufferers, the patients were diagnosed to have vitiligo clinically by woods light and a biopsy was taken and 5 healthy controls. The patients and controls were taken from a dermatological outpatient clinic. Biopsies were taken from lesional skin and non-lesional clinically apparent normal skin in patients with vitiligo. This research was carried out in accordance with the Helsinki Declaration 2013 criteria and STROBE items. The Institutional Review Board and research ethics committee of Suez Canal University's college of medicine gave their approval. Healthy controls with no history of autoimmune disease were enrolled and were age and sex-matched to our cases. All participants signed a written informed consent form. Patients with vitiligo of any age or sex were included. Patients who had had any treatment in the previous 8 weeks or who had a history of any other autoimmune condition were excluded. To establish the pattern, activity, and severity of vitiligo, all patients were examined.

**Assessment of disease pattern**

There are three types of vitiligo: generalized, localized, and universal. Vulgaris, acrofacial, and mixed forms are among the generalized types. Segmental and focal vitiligo are two types of localized vitiligo\(^12\).

**Assessment of disease activity**

The Vitiligo Disease Activity Score (VIDA) is a measure used to determine how active vitiligo is. It is influenced by one’s own assessment of the severity and duration\(^13\).
Assessment of disease severity
The Vitiligo Area Scoring Index (VASI) is a quantitative scale that has been validated. It was created to test different vitiligo therapies. The trunk, hands, feet, upper and lower limbs are the five components of the patient's body that are segregated in VASI. In the next study a sixth site, the head, and neck areas were added. It is calculated by the percentage of the participation of each area of the body vitiligo by using the volar aspect of the hand. The volar aspect of the patient was used to estimate and determine 1.0% of the total body surface area. Then an assessment of the pattern of pigmentation of the skin by clinical examination and reduce remaining pigmentation(14).

Assessment of IL-36α expression
In vitiligo skin lesion and nonlesional apparently normal skin by immunohistochemical staining (IHC): five mm. skin punch biopsies were taken from healthy controls, the center of the vitiligo lesions (group A), and from the clinically apparent normal skin of the vitiligo patients (group B) after intradermal injection of local anesthesia. Each skin biopsy sample was preserved in buffered formalin before being processed for paraffin slices). To study the pathological alterations, the sections were stained with hematoxylin and eosin. Immunohistochemical stain: sections were incubated with the primary antibody of IL-36α; IgG a polyclonal goat concentrate, diluted 5-15 μg/ML, stained overnight at 4 c in a humidity chamber. Followed by incubation with biotinylated antimouse IgG (Universal clinilabSAB2 kit), and streptavidine peroxidase was applied. Incubation steps were interspersed with three washings steps (phosphate buffered saline). The color reaction was developed using DAB substrate system containing diamino benzidine) Finally, sections were counterstained with mayers haematoxylen solution (clinilab) (R&D Systems). H score (15) was used depending on the percentage of stained cells at x 400 magnification (0: none, 1:<5%, 2:5-25%, 3: 25-75% and 4:>75%), and the intensity of staining (0: absent, 1: weak, 2: moderate, and 3: strong). The immunoreactive score was calculated by multiplying the two parameters with a total score 1-12. Positive expression was considered if the score ≥1 (1-4: mild,5-8: moderate, and 9-12: marked)

Statistical analysis
The findings were analyzed statistically using IBM Statistical Package for Social Science Software (SPSS), version 23 for Windows (SPSS Inc., Chicago, Illinois, USA). The mean, median, SD, and range were used to describe quantitative variables. Frequency and percentage were used to express categorical data. For multiple comparisons, we used the Kruskal-Wallis test, and for two-group comparisons, we used the Mann-Whitney test. P-values of less than 0.05 were considered significant.

Results
The participants in the study were 41 vitiligo sufferers and 5 healthy controls who were age and sex-matched. The patients' mean age was 34.71 ±16.89 years, with a range of 9 to 67 years, whereas the controls’ mean age was 36.40± 19.40 years. The percentage of male patients was 29.3%, and the percentage of female patients was 70.7 %, whereas the percentage of male healthy controls was 60.0 % and the percentage of female patients was 40.0 %. In terms of sex and age, between patients and controls, there is no discernible difference, as indicated in (table 1).
Table 1: Comparison between the two studied groups according to demographic data

<table>
<thead>
<tr>
<th></th>
<th>Cases (n = 41)</th>
<th>Control (n = 5)</th>
<th>p</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>No.</td>
<td>%</td>
<td>No.</td>
</tr>
<tr>
<td>Sex</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Male</td>
<td>12</td>
<td>29.3</td>
<td>3</td>
</tr>
<tr>
<td>Female</td>
<td>29</td>
<td>70.7</td>
<td>2</td>
</tr>
<tr>
<td>Age (years)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>≤ 40</td>
<td>27</td>
<td>65.9</td>
<td>3</td>
</tr>
<tr>
<td>&gt; 40</td>
<td>14</td>
<td>34.1</td>
<td>2</td>
</tr>
<tr>
<td>Min. – Max.</td>
<td>9.0 – 67.0</td>
<td>18.0 – 60.0</td>
<td></td>
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<tr>
<td>Mean ± SD.</td>
<td>34.71 ± 16.89</td>
<td>36.40 ± 19.40</td>
<td>0.836</td>
</tr>
</tbody>
</table>

FE: Fisher Exact test

The distribution of the examined sample in lesional skin according to IL-36 is expressed in (figure 1). The distribution of the examined sample in non-lesional skin according to IL-36 is expressed in (figure 2). The degree of inflammatory infiltration and IL-36 expression in vitiligo patients’ lesional and nonlesional skin was shown in (table 2), there is no significant difference between the lesional and nonlesional skin in the expression of interleukin 36α and according to tissue expression of IL36α in control cases showed negative expression of IL36α. Vitiligo patients had a mean age of onset of 27.44 ± 17.86 years. In 82.9 % of cases, the condition progressed, while only 17.1 % remained constant. According to the results, 46.3% of patients had a positive family history of vitiligo. According to the pattern of the disease, it was generalized in 78.1% of them; the mixed subtype was 29.3 while vulgaris and acrofacial were 26.8% and 22.0% respectively.

Table 2: Comparison between Lesional and Non lesional according to Infiltrate and IL36

<table>
<thead>
<tr>
<th>Infiltrate</th>
<th>IL36</th>
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<tbody>
<tr>
<td></td>
<td>Lesional</td>
</tr>
<tr>
<td></td>
<td>No.</td>
</tr>
<tr>
<td>Negative</td>
<td>0</td>
</tr>
<tr>
<td>Mild</td>
<td>27</td>
</tr>
<tr>
<td>Moderate</td>
<td>12</td>
</tr>
<tr>
<td>Marked</td>
<td>2</td>
</tr>
</tbody>
</table>

MC p 0.383 0.240

MC: Monte Carlo.

The minority of vitiligo patients had a localized pattern 19.5%. The mean activity of vitiligo by using VIDA score was 2.49 ± 1.53 with a range of -1.0 – 4.0. The mean severity of vitiligo by using VASI score was 2.43 ± 2.18 with a range of 0.01 - 7.65. There is no significant difference in IL-36α expression with respect to age of onset, sex, course, duration, pattern, activity, or severity of disease in lesional and nonlesional skin.

Discussion

In our study there is increased expression of IL-36α in vitiligo patients in lesional and
Interleukin 36α and vitiligo

Nonlesional skin expressed mainly in inflammatory infiltrate in the dermis and this may be explained that the skin of the patient with vitiligo whether lesional skin or clinically apparently normal is diseased and may change at any time to clinically vitiligous lesions. Expression of IL-36α was moderate in the lesional skin of 2 patients only (4.9%), while the expression of IL-36α was mild in the lesional skin of 29 patients (70.7%), and the nonlesional skin of 26 (63.4%) of the studied patients. There was no expression of IL-36α in (24.4%) of lesional skin, and in nonlesional skin was (36.6%). MDDCs produce IL-12, IL-1, IL-6, IL-23, and IL-18 in response to IL-36, and IL-36R is abundantly expressed on naive T-cells.

Figure 1: Distribution of studied sample according to IL-36α in lesional skin

Figure 2: Distribution of studied sample according to IL-36α in non-lesional skin

T-cell proliferation is stimulated by IL-36, which also causes the production of IL-2. Furthermore, IL-36 is engaged in the pathogenesis of vitiligo by inducing T cell proliferation and playing a key role in the transformation of Th0 cells into Th1 cells that produce IFN-γ. This is the first study that we are aware of that shows elevated IL-36 expression in vitiligo. Other autoimmune and inflammatory illnesses, such as psoriasis, generalized pustular psoriasis, SLE, and Sjögren’s syndrome, allergic contact dermatitis, rheumatoid arthritis, psoriatic arthritis, and osteoarthritis, have been linked to IL-36 in certain studies (19-21). Psoriatic skin lesions express IL-36Ra and IL-36, and there was a link between their expression and the expression of other cytokines such as IL-17, TNF-α, and IFN-γ, according to Blumberg et al., 2007, suggesting that psoriasis may have a positive gene expression loop. Also, transgenic mice that overexpress the IL-36 gene in basal keratinocytes have macroscopic and histological evidence that IL-36 cytokines play a role in regulating skin inflammation (22). According to Tortola et al., 2012, imiquimod, a TLR7 agonist that can create psoriasis-like lesions, is a good IL-36 inducer. Furthermore, imiquimod promotes skin thickening, IL-17 production, and neutrophil, macrophage, and T cell infiltration into the skin, all of which are almost totally dependent on IL-36R signaling. Indeed, animals without IL-36R were protected from imiquimod-induced skin inflammation, but mice lacking IL-36Ra had a more severe phenotype (23). According to Blumberg et al., 2010, engrafting immunodeficient mice with human psoriasis skin offered one of the most compelling lines of evidence for the activity of IL-36 as a cause of skin inflammation.
After therapy with an anti-human IL-36R-neutralizing mAb, the skin pathology of these mice was considerably improved\(^{(22)}\). According to Man et al., 2015, IL-36 has a role in the pathogenesis of SLE, with measurable levels of IL-36, IL-36R, and IL-36 in the plasma of both normal controls and SLE patients. All SLE patients had similar plasma IL-36R concentrations, however IL-36 and IL-36 levels were significantly greater in active SLE patients than in normal controls (3.6 0.2 vs. 2.0 0.2 ng/mL and 1.2 0.1 vs. 0.7 0.1 ng/mL, respectively, both \(p<0.05\))\(^{(19)}\). The function of IL-36 in the pathophysiology of the autoimmune disease primary Sjögren’s syndrome was reported by Ciccia et al. in 2015. He discovered that IL-36 was overexpressed in the blood and tissues\(^{(20)}\). The function of IL-36 in the aetiology of allergic contact dermatitis was reported by Matti et al. in 2013. Immunohistochemistry revealed that IL-36 agonists were expressed in epidermal layers, and that levels of all three IL-36 agonists were elevated in ACD-affected skin\(^{(8)}\). Derer et al., 2014, also corroborated a previous discovery that in arthritic joints, IL36 and IL36R mRNA levels are raised. IL36 and IL36R were previously discovered in the synovial tissues of patients with rheumatoid arthritis, psoriatic arthritis, and osteoarthritis; IL36 expression in the synovial tissue was higher in RA and psoriatic arthritis than in osteoarthritis, and CD138+ plasma cells were the primary source of IL36. IL36 also enhanced the secretion of IL6 and IL8 by activating nuclear factor B (NFB) and p38 mitogen-activated protein kinases. Derer et al., on the other hand, show that blocking IL36R signaling using an anti-IL36R antibody has no effect on arthritis progression\(^{(23)}\). More research on a wider scale with larger sample size is needed, and interleukin 36 levels in the skin of healthy people, as well as serum levels of interleukin 36 in patients with vitiligo.
kin 36 in vitiligo patients, are needed to better understand the function of interleukin 36 in the etiology of vitiligo.

**Conclusion**

The study concludes that higher expression of IL-36 in vitiligo patients was found in both lesional and nonlesional skin, suggesting a role of IL-36 in the pathogenesis of vitiligo. However, more research is needed to determine its specific involvement and the prospect of developing novel targeted medicines for the treatment of vitiligo in the future.

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**Conflicts of interest:** None

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**Figure 4: Moderate expression of IL36 in patients with vitiligo**  
**A:** H&E x200; section showed absent melanin in epidermis. Moderate inflammatory cells infiltrate (red arrow) around blood vessels (yellow arrow) in dermis. **B:** IL36 IHC x 400, showed moderate positive brownish stain (red arrow) in inflammatory cells infiltrate around blood vessels in dermis

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**References**


23. Derer, Anja; Groetsch, Bettina; Harre, Ulrike; et al. Blockade of IL-36 receptor signaling does not prevent from TNF-induced arthritis 2014; PLoS ONE 9, e101954.