The Role of Innate Pro-Inflammatory Cytokines and Migration Inhibitory Factor (MIF) in Vitiligo Patients

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Abstract

Vitiligo is a common acquired autoimmune disease of the skin characterized by a prospective loss functional melanocytes. Genetic and non-genetic factors play a role in its pathogenesis. This study was conducted on 90 patient with vitiligo (40 males and 50 females). They were attended to the dermatology department in Al-Forat hospital during the period from 1st October 2020 to 1st March 2021. Cytokine profiles (IL-6, TNF-α, IL-17, and MIF) were done by using ELIZA technique.

The results showed that the Serum levels of cytokines IL-6 (169±48.1), TNF-α (59.8± ), IL-17 (84.2±48.1), and MIF (5.8±4.5) were increased significantly (P<0.05) in patients compared to control (95.6± , 52.1± , 57.4± , and 3.6± ). Also, serum levels of all these cytokines were significantly (p<0.05) raised in generalized vitiligo as compared to localized vitiligo. According to disease activity (active or stable), the level of all cytokines for both generalized and localized vitiligo was found to be significantly (p<0.05) elevated in active stage when compared with stabled stage.

Key words: vitiligo, cytokines, IL-6, TNF-α, IL-17, and MIF

Introduction

Vitiligo is a disorder of pigmentation characterized by the presence of depigmented skin macules caused by the chronic and progressive loss of melanocytes from the cutaneous epidermis. 1 It is characterized by the loss of melanocytes from the cutaneous epidermis and often associated with other autoimmune diseases, including autoimmune thyroid diseases, alopecia areata, and halo nevi. 2 Many factors have been implicated in the development of the disease, including infection, stress, neural abnormalities, melatonin receptor dysfunction, impaired melanocyte migration, genetic susceptibility and autoimmunity. 3

Cellular immunity is known to have a role in the pathogenesis of vitiligo. Both helper and cytotoxic T cells promote a T-helper (Th)1 response with secretion of tumour necrosis factor (TNF)-α and interferon (IFN). Immune responses to Th1 or Th17 are induced in autoimmune diseases, and these responses may be responsible for the development and progression of the disease.4

In vitiligo, TNF-α could contribute to keratinocyte apoptosis, which may result in autoimmune response and ultimately melanocyte disappearance.5 IL-6 causes polyclonal B-cell activation and subsequent increase in antibody production which ultimately results in immunological damage of

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In patients with vitiligo Th17 cells secrete IL-17, IL-6, IL-22, and TNF which in turn stimulate the release of IL-1α, IL-6, and TNF in keratinocytes. Macrophage migration inhibitory factor (MIF) was originally identified as a lymphokine that concentrates macrophages at inflammatory loci, it may also play a role in autoimmune skin diseases and is considered to play an important role in cell-mediated immunity.

As cellular immunity is implicated in the pathogenesis of the vitiligo, and autoimmunity has also a role in the etiology of vitiligo, we considered that pro-inflammatory cytokines and chemokines may have a role in the pathogenesis of vitiligo. Therefore, we aimed to determine the profile of cytokines in vitiligo patients. For this, we measured the serum level of IL-6, IL-17, TNF-α, and MIF in patients with vitiligo and compared with healthy controls.

Methods

Subjects:

This case-control study was carried out on 90 patients of vitiligo disease (69 localized vitiligo and 31 generalized vitiligo) with different degrees of disease severity. They were 40 males and 50 females. Their age ranged between 6 to 65 years with 23.5 as a mean value. This study was conducted in the dermatology unit / Al-Forat hospital in Al-Najaf city/ Iraq during the period from 1st October 2020 to 1st March 2021. The patients age group ranging between 10-60 years they were diagnosed clinically and were not on any topical or systemic treatment in the past 3 months. Patients with autoimmune disease, pregnant or lactating women, immunosuppression were excluded from study. All patients were subjected to complete general and clinical examination to detect any exploded factors and dermatological examination to evaluate the type and disease severity. Healthy control group included 50 individually with age and sex matching to patients were also collected.

Collection of samples:

Venous blood samples (5 ml) were collected from the patient and control groups under sterile conditions. After centrifugation at 3000 rpm for 10 min, the serum was separated and the samples were stored at 80°C until they were examined by ELISA.

Detection of serum Cytokines:

The detection of IL-6, IL-17, TNF-α, and MIF levels in serum were performed by enzyme-linked immunosorbent assay (ELISA) strictly according to the manufacturer's instructions of the ELISA kit (Bioassay Technology Laboratory/china). The double-antibody sandwich ELISA was used to assay the target protein level in the samples. The target protein in the serum was placed in an enzyme well, which was precoated with target protein monoclonal antibody, for incubation. Washing was performed to remove the uncombined enzyme. Chromogen Solution A and B were then added; the color of the liquid changed to blue and finally became yellow due to the effect of acid. The chroma of the color and the concentration of the target protein in the samples were positively correlated.

Statistical analysis

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Data were analyzed statistically by SPSS for Windows (version 17.0). Measurement data are presented as mean ± standard deviation. t-test was used for the intergroup comparison.

Results & Discussion

In this study, the most important cytokines associated with vitiligo included IL-6, TNF-α, IL-17, and MIF were measured by using ELISA technique.

The levels of all measured cytokines in patients and healthy controls are summarized in Table 1. The serum levels of cytokines IL-6, TNF-α, IL-17, and MIF were increased significantly (P=0.05) in patients as compared to control (169±80 vs 95.6±52.5 (IL-6), 59.8±54.4 vs 52.1±26.1 (TNF-α), 84.2±48.1 vs 57.4±34.8 (IL-17), and 5.8±4.5 vs 3.6±1.5 (MIF)). Also, serum levels of all these cytokines were significantly (p<0.05) raised in generalized vitiligo as compared to localized vitiligo as follows: 223±36.3 vs 170±95.7 for IL-6, 61.6±60 vs 55.8±36.2 for TNF-α, 83.9±67 vs 79.4±32 for IL-17, and 5.96±3.3 vs 5.8±6.2 for MIF.

Table 1: Cytokine levels (pg/ml) in the sera of vitiligo patients in various groups compared to control group.

<table>
<thead>
<tr>
<th>Cytokine</th>
<th>Localized Mean ± SD</th>
<th>Generalized Mean ± SD</th>
<th>Control groups Mean ± SD</th>
<th>Comparison between groups (p value)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Localized vs. control</td>
</tr>
<tr>
<td>IL-6</td>
<td>172±79.6</td>
<td>227±36.3</td>
<td>95.6±52.5</td>
<td>172±79.6 vs 95.6±52.5</td>
</tr>
<tr>
<td>TNF-α</td>
<td>55.8±36.2</td>
<td>61.6±60</td>
<td>52.1±26.1</td>
<td>55.8±36.2 vs 52.1±26.1</td>
</tr>
<tr>
<td>IL-17</td>
<td>79.4±32</td>
<td>83.9±67</td>
<td>57.4±34.8</td>
<td>79.4±32 vs 57.4±34.8</td>
</tr>
<tr>
<td>MIF</td>
<td>5.8±6.2</td>
<td>5.96±3.3</td>
<td>3.6±1.5</td>
<td>5.8±6.2 vs 3.6±1.5</td>
</tr>
</tbody>
</table>

Assessment of serum levels of cytokines in relation to disease activity were appeared in figure (8). In both generalized and localized vitiligo the level of IL-6 was found to be significantly (p<0.05) elevated in active stage when compared with stabled one respectively (183.8±24 ng/ml vs 161.3±32 ng/ml) (235±32 ng/ml vs 219±25ng/ml) as in figure (8A). TNF-α were also found to be significantly elevated in active stage of generalized when compared with stabled form and non significant raised in active form of localized vitiligo (78.9±15 ng/ml vs 27.7±10 ng/ml) (56.2±15ng/ml vs 54.4±14ng/ml) as in figure (8B).
B). IL-17 show a significantly elevated in active stage when compared with stabled one respectively (85.9±7ng/ml vs 62.5±4 ng/ml) (83.±5 ng/ml vs 76.1±6ng/ml) figure (8 C). Finally, In both generalized and localized vitiligo the level of MIF were also found to be significantly elevated in active stage when compared with stabled one respectively (6.1±1.2 ng/ml vs 4.4±1 ng/ml) (6.1± 1.5ng/ml vs 5.7±1ng/ml) (8 D).

as in figure

A

B
C

D
Cytokines are involved in auto immune diseases and thus may play important roles in the pathogenesis of vitiligo. A number of studies are ongoing to recognize the cytokines involved, and their role in the biology of vitiligo. Evidence suggests that stress in melanocytes may result in activation of innate immune mechanisms. The activation of innate immune system develops inflammation and finally leads to activation of the adaptive immunity. Adaptive immunity activation facilitates autoimmune destruction and disease progression.\(^4\sim9\) In a number of studies, abnormal immune responses and changes in the level of cytokines frequently observed in vitiligo patients suggesting that cytokine imbalance may be responsible for development of this autoimmune disease.\(^4\sim10\)

Many studies reported the significant elevation of IL-6, TNF-\(\alpha\), IL-17, and MIF in all patients with different types of vitiligo. these studies consist with the present results.\(^11\sim4\) studies of Farag et al.(2018) and Sushama et al.(2019) also shown that IL 6, IL17, and MIF level were significantly higher in patient with generalized vitiligo when compared to both patient with localized vitiligo and healthy controls.\(^11\sim12\). In the same studies the level of TNF A in localized patients were higher than generalized patient Yang et al.(2019) Acharya & Mathur (2020) shown that cytokines (IL6,IL17,TNF A ,MIF) level were significantly higher in patient with vitiligo at active stage when compared to both patient with vitiligo at stable stage and to healthy .\(^6\sim13\)

In vitiligo, TNF-\(\alpha\) could contribute to keratinocyte apoptosis, which may result in autoimmune response and ultimately melanocyte disappearance. IL-6 causes polyclonal Bcell activation and
sequent increase in antibody production which ultimately results in immunological damage of melanocytes.\textsuperscript{14}

IL-6, as a kind of stimulating factor, shows a variety of biological functions in vivo.\textsuperscript{15} It is speculated that the increase of IL-6 levels in vitiligo may be due to the destruction of cellular network in patients with vitiligo. When cells secrete IL-6 continuously, IL-6 can bind to the receptor and further stimulate the proliferation of cells, thereby leading to local skin damage. It was also found that the expression of serum IL-6 in patients with vitiligo is increased, the expression level of sICAM-1 is significantly related to IL-6, and the larger the skin lesion area of patients is, the higher the expression of IL-6 will be.

In this study TNF-\(\alpha\) was found to be significantly raised in all 90 patients of vitiligo (localized and generalized) which is consistent with earlier finding of Moretti et al.(2002) and Sushama et al.(2018).\textsuperscript{16,11} However, Yu et al(2013) reported decreased levels of TNF-\(\alpha\) in vitiligo.\textsuperscript{17} Sushama et al (2018) observed positive correlation of serum levels of TNF-\(\alpha\) with activity of vitiligo.\textsuperscript{11} Kai et al.( 1990) suggested that TNF-\(\alpha\) can cause melanocyte destruction as a result of autoimmune response, and this may explain increased activity in patients with raised serum TNF-\(\alpha\) levels.

IL-17 is mainly produced by T cells. It was found that the expression of serum IL-17 in patients with vitiligo is increased, and it is positively correlated with the skin lesion area. Some scholars speculate that IL-17 in the body may change the local microenvironment of patients, leading to melanocyte damage and vitiligo.\textsuperscript{18} In the current study, comparing serum levels of IL-17 in patients with generalized vitiligo and localized vitiligo, found that the levels were significantly raised in the generalized group as in Sushama et al.(2018) study.\textsuperscript{11}

MIF was originally characterized as a chemotactic lymphokine that attracts macrophages at inflammatory loci. MIF may also play a pivotal role in many autoimmune skin diseases, such as systemic lupus erythematosus, systemic sclerosis and atopic dermatitis.\textsuperscript{19} In addition, MIF is associated with the generation of cell-mediated immune responses. Accordingly, MIF has been shown to induce up-regulation of many cytokines, including IL-1\(\beta\), IL-8, IFN-\(\gamma\), TNF-\(\alpha\) and IL-6.\textsuperscript{20} TNF-\(\alpha\) and IL-6 are two inflammatory cytokines with an inhibitory effect on pigmentation.\textsuperscript{12}

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