Mans. Med. J. Vol. 51. Issue 4, 2022, Pages 187-194

Mansoura Medical Journal

(Official Journal of Mansoura Faculty of Medicine)

pISSN: 1110-211X; **eISSN:** 2735-3990

Assessment of Serum Levels of Interleukin-27 and Interleukin-37 in Patients with Non-Segmental Vitiligo

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DOI: 10.21608/mjmu.2022.157523.1136

Submit Date: 21 Aug. 2022
Accept Date: 15 Sept. 2022
Available online: 01 Dec. 2022

Keywords

- Interleukin-27
- Interleukin-37
- Non-Segmental Vitiligo

Abstract

Background: Vitiligo is a multifactorial disorder characterized by a deficient or absent skin pigmentation because of melanocyte loss or inactivity in the basal layer of epidermis or mucosa. Numerous studies revealed that the complex interaction between genetic, environmental, biochemical and immunologic factors favours a loss of melanocytes in vitiligo. Cytokines found to have a significant role in pathogenetic process of the disease. Methods: A case-control study enrolled 40 vitiligo patients and 40 healthy age- and sex-matched controls. VASI and VIDA were calculated. IL-27 and IL-37 were measured in venous blood samples using ELISA. Results: Higher IL-27 level was detected among controls, however, higher IL-37 level was detected among patients. A statistically significant difference existed between patient and control groups regarding IL-27 and IL-37 (P<0.001). A statistically significant positive association between IL-37 and VASI and VIDA were detected while a non statistically significant negative relationship was noticed between IL-27 and VASI. Median IL-27 (ng/ml) in controls was 0.327 versus 0.095 in patients. Median IL-37 (pg/ml) was 21.99 among controls versus 277.56 among patients. Conclusions: Cytokines have significant role in vitiligo onset and progression. They can act as biomarkers for identification of patients with progressive disease. IL-37 had important role in vitiligo pathogenesis as well an important predictor for vitiligo severity while IL-27 had immunomodulatory effects in vitiligo, no relationship was found between IL-27 values and patients' demographic data, disease severity and vitiligo type. IL-27 should be considered as a novel target for manipulation of the immune response in vitiligo.

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Introduction

Vitiligo is characterized by a defective or absent skin pigmentation because of melanocyte loss or inactivity in the basal layer of epidermis or mucosa [1]. It was recently described as basal melanocyte detachment [2].

Over the past years, numerous studies reported that the complex interaction among genetic, environmental, biochemical, and immunologic factors produces a micro-environment that favours loss of melanocytes in vitiligo [3, 4].

Interleukin-27 (IL-27), a heterodimeric immunologic factor of IL-12 family, is composed of p28 and Epstein-Barr virus–induced gene 3 (EBi3) subunits. It is predominantly released by antigen-presenting cells upon activation of innate immunity [5, 6].

IL-37, a newly discovered member of IL-1 family, has the capacity to reduce inflammatory and immune responses through decreasing the release of pro-inflammatory cytokines, and to improve the inflammation-related fatigue through the induction of metabolic reprogramming and the reduction of the metabolic effects of inflammatory response [7].

Human and experimental studies offered significant evidence about the autoimmune response pattern in the pathogenetic process of vitiligo. Humoral and cellular immune responses have been found to have a role in the aetiology of vitiligo [8]. Evidence also proposes a key role of cell-mediated pathways, such as Th1/Th17 and Tc1 cells in disease pathogenetic process [9].

IL-27 can be classified as a pro-inflammatory or anti-inflammatory cytokine. Various IL-27 concentrations have been reported in many immune-mediated and skin diseases. Although earlier research recognized the pro-inflammatory activity of IL-27, there is evidence suggesting that IL-27 inhibits the proliferation of a variety of immune cell as well as cytokine release [10].

It was found that IL-27 causes immunomodulation in vitiligo; it must be considered as a novel target for manipulating immune responses in different autoimmune diseases [11].

IL-37, whose anti-inflammatory properties were recognized to have a key role in the pathogenetic process of many disorders, has the ability to inhibit innate immunity by inhibiting the release of inflammatory cytokines, which maybe stimulated by Toll-like receptor agonists [12, 13].

Increased pro-inflammatory and anti-inflammatory cytokines was demonstrated in vitiligo. Cytokines can initiate in the onset and mediate the progression of vitiligo. They might thus serve as biomarkers of progressive disease that requires aggressive therapy [14].

This study enrolled 40 patients with non-segmental vitiligo and 40 healthy controls to assess relation between IL-27 and IL-37 in patients as well as their role as biomarkers of disease activity and severity.

At the same time to compare level of IL-27 and IL-37 between patients and control subjects.

Vitiligo Area Severity Index (VASI) score and Vitiligo Disease Activity (VIDA) score were calculated to evaluate disease severity and activity, respectively [15].

Subjects and Methods:

This case-control study enrolled 40 patients suffering from non-segmental vitiligo. In addition, 40 apparently healthy individuals of matched age and sex with no skin disease were chosen as a control group. Patients who received systemic treatment as steroid compounds, non-steroidal

anti-inflammatory drugs (NSAIDs) and immunosuppressants within one month and those with systemic diseases, especially autoimmune ones were excluded.

Methods:

Each patient was subjected to the followings:

1. Detailed history taking and complete general and skin examination.
2. Description of vitiligo including; site, symmetry, clinical type, activity and stability.
3. VASI score and VIDA score calculation.
4. Venous blood samples were obtained from all participants to estimate the IL-27 and IL-37 level using Enzyme-Linked Immunosorbent Assay (ELISA).

Statistical analysis: Data were analysed by IBM SPSS Corp. Released 2013. IBM SPSS Statistics for Windows, V22.0. Armonk, NY: IBM Corp. Qualitative data were represented as numbers and percents. Quantitative data were represented as medians (min. and max., interquartile ranges) for non-normally distributed data and means, standard deviations for normally distributed data after testing normality by Kolmogrov-Smirnov test. Significance of a result was judged at the (0.05) level.

Results:

Table (1): VASI score distribution among patients.

	N=40	**%**
VASI **Median (range)**	1.25(0.25-98)	

Table (1) illustrates that median VASI score is 1.25 ranging from 0.25 to 98 in patients.

Table (2): VIDA score distribution among patients.

	N=40	**%**
VIDA		
+1	2	5.0
+2	9	22.5
+3	8	20
+4	21	52.5

Table (2) shows that 52.5% of patients had +4 VIDA score , 22.5% had +2 VIDA score, 20% had +3 VIDA score and 5% had +1 VIDA score.

Table (3): comparison of IL-27 & IL-37 between patients & controls.

	Patients N=40	**Controls N=40**	**Test of significance**	
IL-27 (ng/ml)	0.095(0.02-0.56)	0.327(0.06-1.54)	Z=4.05 P<0.001*	Mann Whitney U test
IL-37 (pg/ml)	277.56(29.61-1987.8)	21.99(1.5-94.61)	Z=7.24 P<0.001*	Mann Whitney U test

Parameters represented as medians (min-max)(Interquartile range)

*statistically significant , Z: Mann Whitney U test p: probability , p≤0.05 is statistically significant

The obtained results as shown in table (3) revealed that median IL-27 (ng/ml) in controls was 0.327 versus 0.095 in patients. Median IL-37 (pg/ml) was 21.99 among controls versus 277.56 among patients. A statistically significant difference existed between patients and controls as regards IL-27 &IL- 37 (P<0.001). IL-27 was greater among controls while IL-37 was greater in patient group.

Table (4) validity of IL-27 , IL-37 in differentaing patients from controls.

	AUC (95%CI)	P-Value	Cut-off point	Sensitivity %	Specificity %	PPV%	NPV%	Accuracy %
IL-27 (ng/ml)	0.786 (0.688-0.883)	<0.001*	≤0.2875	82.5	54.1	66.0	74.1	68.8
IL-37 (pg/ml)	0.987 (0.965-1.0)	<0.001*	≥76.86	97.1	92.5	97.4	91.9	94.7

AUC: Area under curve , PPV: Positive predictive value NPV: Native predictive value

The obtained results in table (4) clarifies that area under curve for IL-27 was fair in differentiating patients from controls (AUC=0.786) with 82.5% sensitivity , 54.1% specificity and 68.8% accuracy , while area under curve for IL-37 was excellent in differentiating patients from controls (AUC=0.987) with 97.1% sensitivity , 92.5% specificity and 94.7% accuracy.

Table (5): Association between IL-27 , IL-37 and severity and activity index among patients.

	IL-27 (ng/ml)		IL-37 (pg/ml)	
	R	p	R	P
VASI(%)	**-0.803**	**<0.001***	**0.344**	**0.03***
VIDA	-0.233	0.148	**0.317**	**0.046***

r: Spearman correlation coefficient , p: probability , p ≤ 0.05 is statistically significant

Table (5) shows statistically significant positive correlation between IL-37 and both VASI ((r=0.344) (p=0.03)) and VIDA ((r=0.317) (p=0.046)), these findings indicated that IL-37 can be a valuable marker for activity and severity of vitiligo. A non statistically significant negative relationship existed between IL-27 and VASI((r=-0.803) (p<0.001)).

Table (6): Association between IL-27 and IL-37 in patients.

	IL-37 (pg/ml)	
	R	P
IL-27 (ng/ml)	**-0.435**	**0.005***

r:Spearman correlation coefficient , p:probability , p≤0.05 is statistically significant

Table (6) illustrates statistically significant negative association between IL-37 and IL-27 among patients (r=-0.435) (p=0.005).

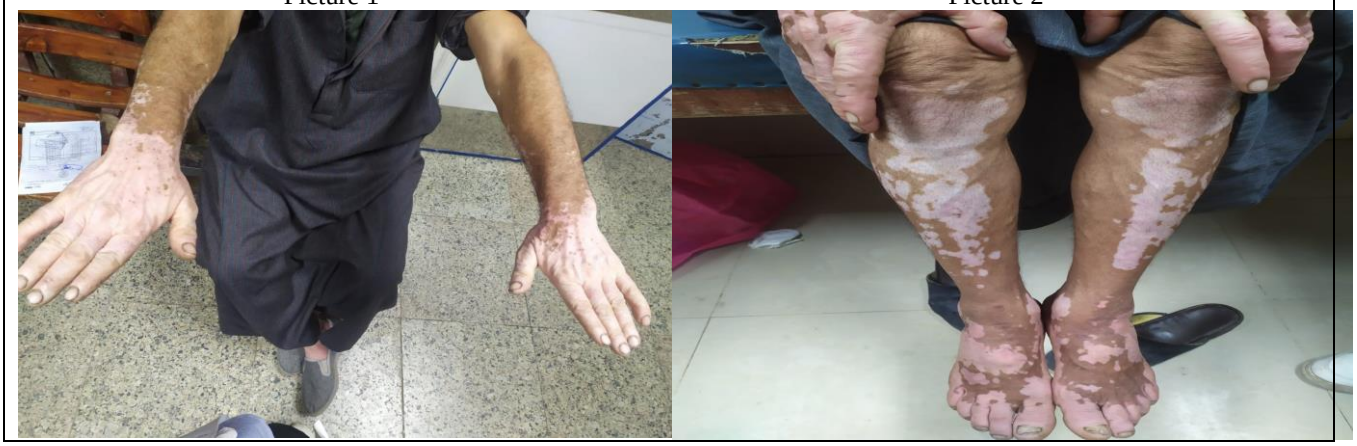
Table (7) Association between IL-27 and IL-37 in controls.

	IL-37 (pg/ml)	
	R	P
IL-27 (ng/ml)	**0.125**	**0.474**

r:Spearman correlation coefficient , p:probability , p≤0.05 is statistically significant

Table (7) illustrates a non-statistically significant relationship between IL-37 and IL-27 among controls (r=0.125) (p>0.05).

Picture 1 Picture 2



Pictures 1 and 2 shows examples of patients with non-segmental vitiligo included in the study.

Discussion:

The present study found that median IL-37 (pg/ml) was 21.99 ranging from 1.5 to 94.61 among controls versus 277.56 ranging from 29.61 to 1987.8 pg/ml. Higher median IL-37 is detected among patients than controls. A statistically significant difference existed between patients and normal subjects as regard IL-37. Reciever Operating chracteristics curve was used to detect best cut-off point of the IL-37 to differentiate patients from controls and illustrates excellent AUC (0.987 , 95% CI ;0.965-1.0). yielding sensitivity of 97.1% , specificity 92.5% and total accuracy 94.7%. These current results are in agreement with the data of Karagün and Baysak, in 2020 who announced that the Mean ± SD of IL-37 (ng/ml) was 17.09 ± 17.17 in patient group and 6.25 ± 5.30 in control group. Vitiligo cases had significantly greater concentrations of IL-37 than in the control group which draw attention to the significance of this cytokine in vitiligo pathogenesis. Moreover, in Karagün and Baysak study no association was revealed between IL-37 values and disease parameters like vitiligo severity and activity while in our study we found a significant relationship with VASI and VIDA [14].

Our study demonstrated that, median IL-27 (ng/ml) was 0.327 ranging from 0.06 to 1.54 among controls versus 0.095 ranging from 0.02 to 0.56 ng/ml. Higher median IL-27 is detected among controls than patients. A statistically significant difference existed between patients and controls as regard IL-27. Reciever Operating chracteristics curve was utilized to detect best cut-off point of the IL-27 to differentiate patients from controls and illustrates fair AUC Roc (0. 786 , 95% CI ;0. 688-0.883). yielding sensitivity of 82.5% , specificity 54.1% and total accuracy 68.8%. These results are in agreement with the data obtained by Hosseini et al., in 2020 who found decreased serum IL-27 values in 79 cases with generalized and localized vitiligo as compared to 45 age and sex-matched controls. A significant difference in serum IL-27 values was found between patients and control individuals [11].

These findings indicated possible relation between IL-27 and IL-37 in the pathogenetic process of non-segmental vitiligo.

Concerning the VIDA score between our cases; 52.5% had (+4 VIDA score), 22.5% (+2 VIDA score), 20% (+3 VIDA score) and 5% (+1 VIDA score). While Mou et al., in 2016 reported a non-statistically significant difference between the 3 groups in their study regarding vitiligo activity prior to treatment [16].

Our study showed revealed a statistically significant positive association between IL-37 and VASI and VIDA indicating that IL-37 might be a useful marker for activity and severity of vitiligo. A non-statistically significant negative relationship between IL-27 and VASI was found. Earlier studies reported that serum IL-27 values were high in pemphigus and psoriatic subjects. They demonstrated that IL-27 concentrations significantly associated with the IgG auto-antibody titers in pemphigus, as well as the onset and severity of psoriasis (Ps). Also, there was increased IL-27 expression in lesional eczematous skin [17, 18]. This supports that IL-27 has a pro-inflammatory activity. In spite of skin diseases in which serum IL-27 levels are high, its concentration is decreased in many auto-immune disorders including Vogt-Koyanagi-Harada

syndrome, Behçet's disease, as well as SLE [19, 20].

Yuan and co-workers, in 2019 revealed that IL-37 concentrations increased among RA patients and were linked to disease activity [21]. IL-37 concentrations were found to be significantly greater in the serum of SLE cases when compared with control subjects [22, 23]. Teng et al., in 2014 reported that the enhanced IL-37 expression among psoriatic plaques significantly decreased the inflammation in mice and proposed that IL-37 inhibits the expression of pro-inflammatory cytokines in Ps [24].

In Conclusion, increased pro-inflammatory and anti-inflammatory cytokines have been reported in vitiligo pathogenesis. Cytokines are significant in the development and progression of the disease. They might thus act as biomarkers for identification of patients with progressive disease who require aggressive therapy. IL-37 had important role in vitiligo pathogenesis as well an important predictor for vitiligo severity while IL-27 has immunomodulatory effects in vitiligo, there was no relationship between serum IL-27 value and demographic data, disease severity and the type of vitiligo. IL-27 has 2 roles and must be considered as a novel target for manipulation of the immune response in different auto-immune diseases.

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