Research Article

Estimation of the Level of Interlukien-33 and Tumour Necrosis Factor-α in Lesional Tissues of Patients with Vitiligo

Abd E. El-Rifaie*, Laila A. Rashed, Ahlam H. Abd Allah* and El-Shaimaa S. A. El-Ashry***

* Department of Dermatology, Faculty of Medicine, Beni-Suef University, Egypt
**Department of Biochemistry, Faculty of Medicine, Cairo University, Egypt

Abstract

Background: Vitiligo is a common acquired immune disorder of the skin characterized by the presence of milky white depigmented macules in various distributions. It is an autoimmune disorder caused by the destruction of melanocytes in the skin. Its immunopathogenesis is not fully understood, but there are inflammatory alterations in the skin microenvironment**. Aim of the work:** This study aims to estimate the level of IL-33 and TNF–alpha in lesional tissues of patients with vitiligo in comparison with healthy controls**. Subjects and methods:** this is a case control study that was conducted on out-patients attending Beni-Suef University Hospital, 30 patients with vitiligo and 30 healthy controls, Tissue samples was be collected by punch biopsy (4mm) in diameter from Lesion (vitiliginous areas) of the patients and normal skin from the healthy controls. **Results:** TNF-α and IL-33 tissue levels were significantly increaesd in patients with vitiligo compared with healthy controls. No correlation between Level of Interlukien-33 and level of Tumor Necrosis Factor-α in Vitiligo Lesions among Studied Cases; (p-values> 0.05). **Conclusion:** Tissue IL-33 levels in patients with vitiligo were significantly increased compared with healthy controls. IL-33 could be considered a reliable marker for the disease. These results also might partly explain the mechanism for TNF-α inhibitor efficacy in patients with vitiligo. Tumour necrosis factor (TNF)-α, a proinflammatory cytokine central to many autoimmune diseases, has been implicated in the depigmentation process in vitiligo, as it is also increased in patients with vitiligo compared with healthy controls.

Keywords: Vitiligo,IL-33, TNF–alpha, case control

Introduction

Vitiligo is a common acquired immune disorder of the skin characterized by the presence of milky white depigmented macules in various distributions. It is an autoimmune disorder caused by the destruction of melanocytes in the skin.

Its immunopathogenesis is not fully under-stood, but there are inflammatory alterations in the skin microenvironment [(1).]

There are two main types: generalized non-segmental vitiligo, the common symmetrical form, and segmental, affecting only one side of the body **[(2)].**
Worldwide, the prevalence of vitiligo ranges from 0.4 to 2.0%, with regions of greater or lesser prevalence.

Most studies demonstrate slightly greater prevalence in females and 50 % onset in childhood, but exceptions to these rules exist. Childhood vitiligo has been associated with halo nevi, and family history of vitiligo and autoimmunity. Post-pubescent vitiligo has been associated with greater acrofacial disease and thyroid disease, and early diagnosis supports reduced non-melanoma and melanoma skin cancer risk **[(3)].**

A healthy skin is required for a person's physical as well as mental health. Skin disor-ders are thus a major cause of depression amongst sufferers and that too Vitiligo which compels the person to feel embarrassed. Stephen Rothman said that "Some diseases do not take life, but they just **ruin** it." Almost all patients having vitiligo suffer from depression**[(4)]**

There is considerable evidence that depigmentation may be an important cutaneous marker for immunological based diseases. The depigmentation of vitiligo is seen in halo naevus and Vogt-Koyanagi-Harada syndrome. In patients with halo naevus, vitiligo surrounds a melanocytic naevus, which is a localized developmental abnormality of the skin with increased number of melanocytes. Halo naevus is considered as an immune process and vitiligo can occur at remote sites.

Various hypotheses have been proposed which include genetic predisposition, neural theory, autocytotoxic mechanism and autoimmune hypothesis, followed by reactive oxygen species model, cellular and biochemical alterations accounting for loss of functioning melanocytes in vitiligo[5].

Lerner proposed the self-destruction theory of vitiligo. He said that melanocytes are destroyed by the same factors that are required for melanogenesis. He proposed that a melanin precursor might have a toxic lethal effect when the naturally occurring protective mechanisms are lost. There is usually a selective destruction of melano-cytes in vitiligo.

The two known mechanisms for the destruction of cells are necrosis and apoptosis. Some workers reviewed available data to find out which of the two mechanisms was operative in vitiligo. The histological data and some laboratory data suggested apoptosis rather than necrosis as the mechanism for the removal of melano-cytes. Apoptosis can be induced by a variety of factors including immune cytokines [6].

Autoimmune theory is supported by the frequent association of vitiligo with disorders that have an autoimmune origin, including Hashimoto's thyroiditis, Graves disease, type 1 insulin-dependent diabetes mellitus and Addison's disease. As cytokines are important mediators of immunity, there is evidence to suggest that they play a major role in the pathogenesis of autoimmune diseases [7].

Cytokines regulate immune response and inflammation and play an important role in depigmentation process of vitiligo. As it is an inflammatory disorder associated with increased expression of inflammatory cytokines in the skin and blood. A cytokine imbalance in the skin of vitiligo patients suggesting their prominent role in autoimmune pathogenesis. The melanocytes are targeted by multiple aggressions leading to marked reduction and destruction of pigment cells in vitiligo patients[8].

Cytokines are protein molecules that include Interferons (IFNs), Interleukins (ILs), various Colony Stimulating Factors (CSFs) and Tumor Necrosis Factors (TNFs) which are key molecules in mediating inflammatory and cytokine reactions. Their response due to imbalance or deficiency in the cytokine network may largely determine autoimmune disease susceptibility and severity. Alteration in the concentration of various proinflammatory and anti-inflammatory cytokines such as IL-6, IL-8, IL-10, IL-2, IL33, TNF-α, and IFN-γ has been associated with various autoimmune disorders[9]

IL-33 is a cytokine, encoded by the IL-33 gene, which is a member of the IL-1 family that drives the production of T-helper-2 (Th-2)-associated cytokines[10].
There is a positive correlation between serum IL-33 levels and disease activity, but there is no correlation with the clinical type of vitiligo. This explains a possible syste-mic role of IL-33 in the pathogenesis of vitiligo[11].

Tumor necrosis factor-alpha (TNF-alpha) is a proinflammatory cytokine central to many autoimmune diseases. Inhibition of TNF-alpha has proven to be an effective therapy for patients with inflammatory disorders including psoriasis, generalized progressive vitiligo, psoriatic arthritis, rheumatoid arthritis and ankylosing spondylitis [12].

Several findings reported that pro-inflammatory stimuli, such as IFN-γ and TNF-α, as well as IL-17, can induce IL-33 expression in normal human epidermal keratinocytes [13].

Patients and Methods

Type of the study: case-control study.

Site of the study: Dermatology out-patient clinic at Beni-Suef university hospital.

Study population: out-patients attending Beni-Suef University Hospital, 30 patients with vitiligo and 30 healthy controls.

Sample number: 60; 30 lesional tissue samples from patients with vitiligo, 30 tissue samples from relatively healthy individuals as control.

Type of sampling technique: patients will be chosen randomly according to inclusion and exclusion criteria.

Inclusion criteria: Age between 20 to 50 years old. Patients with vitiligo. Both males and females will be included.

Exclusion criteria: Age below 20 and above 50 years old. Patients who are suffering from any infection. Patients with skin malignancies or any other tumors. Patients who received any systemic, local treatment or photo-therapy for vitiligo within the last 4 weeks prior to taking the biopsies.

❖ Controls will be chosen randomly from any other out-patient clinics.

Data collection methods and tools :

All the patients and healthy controls will be suspected to the following:

- Informed consent for participation in the study.
- History taking: name, age, sex, number, extent, site of lesions of vitiligo, duration, previous treatments, recurrence, other medical conditions and history of drug intake.
- Tissue samples will be collected by punch biopsy (4mm) in diameter from: Lesion (vitiliginous areas) of the patients. Normal skin from the healthy controls.
- These samples will preserved in sterilized tubes in frozen state at -20°C until assayed using ELISA technique.
- Disease Severity was evaluated by VASI score.

Ethical considerations:

Explain the study to the participant. Informed consent from the participant. Approval of the ethical committee.

A. Human IL-33 ELISA Procedure:

Sample Preparation:

Sample Handling:

- Culture supernatants tested in this ELISA.
- 50µl per well of culture supernatant required.
- Stored samples to be assayed within 24 hours at 2-8°C. For long- term storage, aliquot and freezed samples at -70°C.
- Avoided repeat freeze-thaw cycles when storing samples.
- Test samples and standards assayed in Duplicate each time the ELISA is performed.
- Gradually equilibrated samples to room temperature before beginning assay. Did not use heated water baths to thaw or warm samples.
- Mixed samples by gently inverting tubes.
- If samples were clotted, grossly hemolyzed, lipemic or microbially contaminated, or if there is any question about the integrity of a sample, we made a note on the template and interpret results with caution.

B. Human TNF-α ELISA Procedure:

Sample Preparation

- Culture supernatant tested in this assay; 50µL per well of culture supernatant is required.
- Stored samples to be assayed within 24 hours at 2-8°C. For long-term storage, aliquot and freezed samples at -70°C. Avoided repeated freeze-thaw cycles when storing samples.
- Samples and standards must be assayed in duplicated each time the assay is performed.
- Equilibrated samples gradually to room temperature before beginning the assay. Did not use a heated water bath to thaw or warm samples.
- Mixed samples by gently inverting the tubes.
- If samples were clotted, grossly hemolyzed, lipemic or contaminated, made a note on the template and interpreted results with caution.
- If the TNFα concentration of a sample exceeded the highest point of the standard curve (i.e., 1000 pg/mL), prepared one or more 5-fold dilutions of the sample. Prepared a 5-fold dilution by adding 50µL of sample to 200µL of Sample Diluent and mix thoroughly. Prepared all sample dilutions using the Sample Diluent provided.

F. Calculation of Results:

- The standard curve is used to determine human IL-33 amount in an unknown sample. We generated the standard curve by plotting the average absorbance obtained for each Standard concentration on the vertical (Y) axis vs. the corresponding human IL-33 concentration (pg/ml) on the horizontal (X) axis.

- Calculated results using graph paper or curve-fitting statistical software. The human IL-33 amount in each sample was determined by interpolating from the absorbance value (Y axis) to human IL-33 concentration (X axis) using the standard curve.
- If the test sample was diluted, we multi-plied the interpolated value obtained from the standard curve by the dilution factor to calculate pg/ml of human IL-33 in the sample.

G. Calculation of Results:

- Generated the standard curve by plotting the average absorbance (450nm minus 550nm) obtained for each Standard concentration on the vertical (Y) axis vs. the corresponding TNFα concentration on the horizontal (X) axis.
- Calculated results manually using graph paper or with a curve-fitting statistical software package. If using curve-fitting software, plotted a four-parameter logistic curve fit. Alternatively, a point-to-point curve fit may be used. Determined the amount of TNFα in each sample by interpolating from the TNFα concentration (X axis) to the absorbance value (Y axis).
- If the sample was diluted, we multi-plied the interpolated value obtained by the dilution factor to determine amount of TNFα in the sample.
- Absorbance values obtained for Duplicates should be within 10% of the mean value. Carefully considered duplicate values that differ from the mean by greater than 10%.

Results

Table (1): Age and Sex distribution of the studied cases; (N= 60):

	Patients with Vitiligo N= 30	**Healthy Control N= 30**	**p-value**
Age; (years)			
Mean ±SD	32.87 ±10.9	34.30 ±10.5	0.607[1]
Minimum	20.00	20.00	
Maximum	50.00	50.00	
Sex			
Males	10 (33.3)	14 (46.7)	0.430[2]
Females	20 (66.7)	16 (53.3)	

Table (2): Vitiligo disease activity score (VIDA) of the studied Vitiligo Cases; (N=30):

Mean ±SD	**2.27 ±1.5 0000**
(Minimum) – (Maximum)	(0) – (+4)
0	6 (20.0%)
+1	2 (6.7%)
+2	8 (26.7%)
+3	6 (20.0%)
+4	8 (26.7%)

Tissue samples had been collected by punch biopsy (4mm) in diameter from lesion (vitiliginous areas) of the patients. Fifty percent of biopsies were from back area, 16.7% from upper limb and 33.3% from lower limb.

Table (3): Comparison of Level of Interlukien-33 among Lesional Skin of studied Patients with Vitiligo and healthy control individuals; (N=60):

	Level of Interlukien-33		
	Patients with Vitiligo N= 30	Healthy Control N= 30	p-value
Mean ±SD	129.71 ±21.9	33.92 ±7.3	**<0.001***
Minimum	93.20	21.60	
Maximum	169.80	56.80	

Level of Tumor Necrosis Factor-α (TNF-α) in Patients with Vitiligo was significantly higher as compared with normal healthy controls where the mean values were (217.23 vs. 68.43) in Patients with Vitiligo and normal healthy controls respectively; (p-value <0.001).

Table (4): Comparison of Level of Tumor Necrosis Factor-α among Lesional Skin of studied Patients with Vitiligo and healthy control individuals; (N=50):

	Level of Tumor Necrosis Factor-α		
	Patients with Vitiligo N= 30	Healthy Control N= 30	p-value
Mean ±SD	217.23 ±28.7	68.43 ±18.7	**<0.001***
Minimum	142.60	42.70	
Maximum	290.50	110.20	

Level of Tumor Necrosis Factor-α was significantly lowest among studied lesional Vitiligo Skin Type (V) as compared with other skin types; (p-values≤ 0.05). However; no detected significant differences between other skin types.

Table (5): Correlation of Level of Interlukien-33 and Tumor Necrosis Factor-α in Lesional Vitiligo and Onset of Last New Lesion (months) of Studied Cases; (N=25):

	Onset of Last New Lesion (months)	
	r	p-value
Level of Interlukien-33	-0.097	0.610
Level of Tumor Necrosis Factor-α	0.208	0.269

No detected correlation between Level of Interlukien-33 and Tumor Necrosis Factor-α in Lesional Vitiligo and Onset of Last New Lesion (months); (p-values> 0.05).

Table (6): Correlation of Level of Interlukien-33 and Tumor Necrosis Factor-α in Lesional Vitiligo and Vitiligo disease activity score (VIDA) of Studied Cases; (N=25):

	Vitiligo disease activity score (VIDA)	
	r	p-value
Level of Interlukien-33	-0.241	0.200
Level of Tumor Necrosis Factor-α	0.166	0.382

No detected correlation between Level of Interlukien-33 and level of Tumor Necrosis Factor-α in Vitiligo Lesions among Studied.

Table (7): Correlation of Level of Interlukien-33 and Level of Tumor Necrosis Factor-α in Vitiligo Lesions of Studied Cases; (N=30):

	Level of Tumor Necrosis Factor-α	
	r	p-value
Level of Interlukien-33	-0.145	0.445

Discussion

Vitiligo is an acquired pigmentary disorder of the skin that is characterized by depigmentation caused by the loss of functioning melanocytes from epidermis and circumscribed, depigmented macules and patches **(14).**

There are two main types: generalized non-segmental vitiligo, the common symme-trical form, and segmental, affecting only one side of the body **(2).**

Tumour necrosis factor (TNF)-α, also known as cachectin, is a polypeptide hormone that plays a role in inflammatory, infectious and autoimmune processes in human disease. The TNF gene is located on chromosome 6, and its production is regul-ated at the transcriptional and post-transcriptional levels. TNF-α exists in two forms: a transmembrane protein and a soluble protein. Both are biologically active in vivo (15).

This is why the study was selected to be conducted to estimate the level of IL-33 and TNF–alpha in lesional tissues of patients with vitiligo in comparison with healthy controls in Dermatology out-patient clinic at Beni-Suef university hospital. The study was case-control study included 60; 30 lesional tissue samples from patients with vitiligo, 30 tissue samples from relatively healthy individuals as control. The duration of the study had been from 6 to 12 months.

The study included sixty individuals divided as (30) patients with vitiligo and matched (30) normal individuals as healthy controls. Patient's ages ranged from 20 to 50 years old with an average of 32.87 ±10.9 with no statistically significant difference between cases and controls. Of the studied Patients with Vitiligo; 10 cases were males and 20 were females without a statistically significant difference between cases and controls.

It is characterized by progressive depigmentation and the appearance of white cutaneous macules, usually with no other clinical symptoms. Clinically, it can present as a non-segmental variant, with bilateral and generalized distribution or as a segmental variant, involving a single body region. Depigmentation is due to the loss of functioning melanocytes (16).

In the present study 20% of them had positive family history, 80% generalized type, 90% bilateral, 60% symmetrical, 50% of them had vitiligo vulgaris and 26.7% of them had mixed type and the Mean ±SD of the duration of last lesion was 5.41 ±5.3 months. Mean ±SD of VIDA was 2.27 ±1.5.

These results are in contrary of findings reported by Abdallah et al.,(17) as they reported that their disease duration was of an average of 8.03±7.18 years. Regarding Wang et al.,(18) the mean±SD of disease duration was 7.7± 5.1 in stable vitiligo and 4.9±3.1 in progressive vitiligo.

According to the Vitiligo Disease Activity score (sixpointscale), 22.5%patients had astable course, whereas 77.5% patients had a progr-essive course. The course of vitiligo is often unpredictable.The natural course of the disease involved slow progression,but it may stabilize or exacerbate rapidly. Vitiligo spreads either by appearance of new depigmented macules or by centrifugal enlargement of pre-existing lesions or both reported by (19).

Our results show that Tissue samples had been collected by punch biopsy (4mm) in diameter from lesion (vitiliginous areas) of the patients. Fifty percent of biopsies were from back area, 16.7% from upper limb and 33.3% from lower limb.

In the present study level of Interlukien-33 in Patients with Vitiligo was significantly higher

as compared with normal healthy controls where the mean values were (129.71 vs. 33.92) in Patients with Vitiligo and normal healthy controls respectively; (p-value <0.001).

Our results are in agreement with findings reported by Li et al.,[20] as they shown for the first time that IL-33 and ST2 expression is increased in the skin, and that serum IL-33 expression is raised in patients with vitiligo. They found that IL-33 is secreted by keratinocytes, and that it is transferred from the nucleus to the cytoplasm in keratinocytes from patients with vitiligo.

Furthermore, keratinocyte derived IL-33 may modulate the function of neighbouring keratinocytes by inhibiting expression of SCF and bFGF expression, both of which are vital for melanocyte growth, while increasing TNF-a and IL-6 expression. These results suggest that targeting IL-33 may represent a novel approach in vitiligo treatment. Regarding Vaccaro et al.,[11] IL-33 serum levels in patients with vitiligo were significantly increased than those in healthy controls.

Similarly Balato et al.,[13] found IL-33 serum levels in patients with vitiligo were significantly increased than those in healthy controls. Interleukin-33/ST2 signaling has been studied in a wide range of inflammatory skin conditions for its crucial role in immune responses and tissue homeostasis.
TNF-α, a paracrine inhibitor of melanocytes, is implicated in the pathogenesis of vitiligo because increased expression of TNF-α and its promoter polymorphisms correlate with disease progression, and result in greater susceptibility to the disease[5].

In the present study Level of Tumor Necrosis Factor-α (TNF-α) in Patients with vitiligo was significantly higher as com-pared with normal healthy controls where the mean values were (217.23 vs. 68.43) in Patients with vitiligo and normal healthy controls respectively; (p-value <0.001).

Our results are in agreement with findings reported by Wang et al.,[18] as they reported that Tumor Necrosis Factor-α (TNF-α) in Patients with vitiligo was significantly higher as compared with normal healthy controls. TNF-α could stimulate the melanoma mitogens IL-8 and CXCL1, inhibit pigmentation-related signaling and melanin production, and increase the production of b-defensin, an antagonist for MC1-R.

Regarding Camara-Lemarroy et al.,[21] TNF-α might play an important role in the development of vitiliginous lesions, although with some heterogeneity as to its precise relevance. Furthermore, molecular mechanisms of TNF-α mediated melanocyte dysfunction and death have been uncovered using in vitro methods. A dual role for TNF-α could explain the lack of clinical effectiveness of TNF-α inhibition therapy in vitiligo.

In the present study level of Interlukien-33 in vitiligo lesions had no significant differences in relation to different skin types; (p-values> 0.05). Level of Tumor Necrosis Factor-α was significantly lowest among studied lesional Vitiligo Skin Type (V) as compared with other skin types; (p-values≤ 0.05). However; no detected significant differences between other skin types.

Our results are in agreement with findings reported by Ebrahim et al.,[22] as they reported that regarding the clinical type of the disease, no significant difference was found in serum IL-33 levels in either types of vulgaris, acrofacial and focal types of vitiligo (P=0.340).

This suggests that the clinical type of the disease has no influence on serum IL-33 levels.
Our results are in contrary of findings reported by Vaccaro et al.,[11] as they reported that there was a positive corre-lation of IL-33 serum levels with extension of vitiligo and disease activity.

In the present study no detected association between Level of Interlukien-33 and Tumor Necrosis Factor-α in lesional vitiligo and family history; (p-values >0.05). No detected association between Level of Interlukien-33 and Tumor Necrosis Factor-α (TNF-α) in lesional vitiligo and sex of studied cases; (p-values> 0.05). No detected association between Level of Interlukien-33 and Tumor Necrosis Factor-α (TNF-α) in lesional vitiligo and laterality of lesion of studied cases; (p-values >0.05).

Our results are supported by findings reported by Ebrahim et al.,[22] as they reported that there

was no statistically significant correlation between seruml levels of IL-33 and extent of vitiligo (P=0.469). This finding is in contrast with the study by Vaccaro et al.,[11], who reported a significant positive correlation between serum IL-33 levels and extent of vitiligo (P=0.05). This can be explained by the fact that all patients in their study reported worsening of already existent as well as the development of new lesions during the previous 3 months.

In a study of eight cases, five had strong TNFα staining, whereas three did not, although TNFα staining intensity did seem to correlate to disease activity [23].

Another study showed that TNF-α serum levels were high in patients with generalized vitiligo as compared to localized vitiligo and suggested that TNF-α promoter polymorphisms may be genetic risk factors for susceptibility and progression of the disease [5].

Our results show that there was no detected association between level of Interlukien-33 and Tumor Necrosis Factor-α (TNF-α) in lesional vitiligo and symmetry of lesion of studied cases; (p-values> 0.05), age of studied cases and onset of last new lesion.

Our results are in line with findings reported by Ebrahim et al.,[22] as they reported that no significant correlations were found between sex, age, family history, smoking status, precipitated factors and serum IL-33 levels. This finding is in contrast with the study by El-Taweel et al.,[24] who suggested that there is no significant correlation between serum IL-33 levels in both moderate and severe cases of psoriasis and duration of disease. This can explain the fact that some of patients reported long durations of vitiligo with a stable course.

In the present study, no detected correlation between Level of Interlukien-33 and level of Tumor Necrosis Factor-α in vitiligo lesions among Studied Cases; (p-values> 0.05).

Our results are in contrary of findings reported by Ebrahim et al.,[22] as they reported that there was a positive correlation between IL-33 levels and TNF-α in vitiligo (P=0.001). This finding suggests that IL-33 is involved in the occurrence and development of vitiligo.

This finding is in agreement with the study of Li et al.,[20] who found that IL-33 may bind ST2 receptors on keratinocytes and stimulate TNF-α production. TNF-α is a paracrine inhibitor of melanocytes, resulting in the loss of pigmentation or loss of melanocytes, and its promoter polymerphisms correlate with disease progression; In the study by Xu et al., [25], which was carried out on patients with RA, serum IL-33 levels correlated with disease severity in RA. The study by Pastorelli et al.,[26] on patients with inflammatory bowel disease reported appositive correlation between activity of IBD and IL-33 levels.

Conclusion

Tissue IL-33 tissue levels in patients with vitiligo were significantly increased compared with healthy controls. IL-33 could be considered a reliable marker for the disease.

This explains a possible systemic role of IL-33 in the pathogenesis of vitiligo, and IL-33 serves as an alarmin in inducing melanocyte death in vitiligo skin. Inhibiting IL-33 activity might be a novel therapeutic strategy in the treatment of autoimmune inflammatory disease such as vitiligo. Also TNF-α tissue levels in patients with vitilgo were increased compared with healthy controls. These results might therefore partly explain the mechanism for TNF-α inhibitor efficacy in patients with vitiligo. Tumour necrosis factor (TNF)-α, a proinflammatory cytokine central to many autoimmune diseases, has been implicated in the depigmentation process in vitiligo, as it is also increased in tissues of patients with vitiligo compared with healthy controls.

References

1. Chang, A.Y., Wanat, K.A., and Seykora, J.T. (2014): Melanocytes and VitiLigo (and Hair Graying). In Pathobiology of Human Disease: A Dynamic Encyclopedia of Disease Mechanisms, (Elsevier Inc.), pp. 1148–1157.
2. Whitton, ME; Pinart, M; Batchelor, J; Leonardi-Bee, J; González, U; Jiyad, Z; Eleftheriadou, V; Ezzedine, K (2015): "Interventions for vitiligo". The Cochrane Database of Systematic Reviews (2): CD003263.doi:10.1002/14651858. CD003263. pub5. PMID 25710794.
3. Silverberg, N.B. (2015): The Epidemiology of Vitiligo. Current Dermatology Reports 4, 36–43.

4. Amer, A.A.A., Mc Hepange, U.O., Gao, X.H., Hong, Y., Qi, R., Wu, Y., Cai, Y., Zhai, J., and Chen, H.D. (2015): Hidden victims of childhood vitiligo: Impact on parents' mental health and quality of life. Acta Dermato-Venereologica 95, 322–325.
5. Laddha NC, Dwivedi M, Begum R. (2012): Increased tumor necrosis factor (TNF)-alpha and its promoter polymorphisms correlate with disease progression and susceptibility towards vitiligo. PLOS ONE; 7: e52298.
6. Huang, C.L., Nordlund, J.J., and Boissy, R. (2002): Vitiligo:
7. Xie, H., Zhou, F., Liu, L., Zhu, G., Li, Q., Li, C., and Gao, T. (2016): Vitiligo: How do oxidative stress-induced autoantigens trigger autoimmunity? Journal of Dermatological Science 81, 3–9.
8. Ala, Y., Pasha, M.K., Rao, R.N., Komaravalli, P.L., and Jahan, P. (2015): Association of IFN-γ: IL-10 cytokine ratio with nonsegmental vitiligo pathogenesis. Autoimmune Diseases.
9. Shimizu, J., Takai, K., Takada, E., Fujiwara, N., Arimitsu, N., Ueda, Y., Wakisaka, S., Suzuki, T., and Suzuki, N. (2016): Possible association of proinflammatory cytokines including IL1β and TNFα with enhanced Th17 cell differentiation in patients with Behcet's disease. Clinical Rheumatology 35, 1857–1863.
10. Miller, A.M. (2011): Role of IL-33 in inflammation and disease. Journal of Inflammation 8.
11. Vaccaro, M., Cicero, F., Mannucci, C., Calapai, G., Spatari, G., Barbuzza, O., ... & Gangemi, S. (2016): IL-33 circulating serum levels are increased in patients with non-segmental generalized vitiligo. Archives of dermatological research, 308 (7), 527-530.
12. Keystone, E.C., and Ware, C.F. (2010): Tumor necrosis factor and anti-tumor necrosis factor therapies. Journal of Rheumatology 37, 27–39.
13. Balato A, Lembo S, Mattii M et al., (2012): IL-33 is secreted by psoriatic keratinocytes and induces pro-inflammatory cytokines via keratinocyte and mast cell activation. Exp Dermatol; 21: 892–4.
14. Ezzedine K, Silverberg N (2016): A Practical Approach to the Diagnosis and Treatment of Vitiligo in Children. Pediatrics. 2016 Jul 138 (1):[Medline].
15. Webb, K. C., Tung, R., Winterfield, L. S., Gottlieb, A. B., Eby, J. M., Henning, S. W., & Le Poole, I. C. (2015): Tumour necrosis factor-α inhibition can stabilize disease in progressive vitiligo. British Journal of Dermatology, 173(3), 641-650.
16. Guerra L, Dellambra E, Brescia S, Raskovic D. (2010): Vitiligo: patho- 447 genetic hypotheses and targets for current therapies. Curr Drug 448 Metab.; 11: 451Ð6.
17. Abdallah, M., El-Mofty, M., Anbar, T., Rasheed, H., Esmat, S., Al-Tawdy, A., ... & Bassiouny, D. (2018): CXCL-10 and Interleukin-6 are reliable serum markers for vitiligo activity: A multi-center cross-sectional study. Pigment cell & melanoma research, 31(2), 330-336.
18. Wang CQ, Akalu YT, Suarez-Farinas M, Gonzalez J, Mitsui H, 604Lowes MA, Orlow SJ, Manga P, Krueger JG. (2013): IL-17 and TNF 605 synergistically modulate cytokine expression while suppressing 606melanogenesis: potential relevance to psoriasis. J Invest Derma 607tol.
19. Hann SK, Nordlund JJ. (2000): Clinical features of generalized vitiligo [Chapter 17]. In: Hann SK, Nordlund JJ, editors. Vitiligo. Oxford, UK: Blackwell Science; pp. 35–48.
20. Li, P., Ma, H., Han, D., & Mou, K. (2015). Interleukin-33 affects cytokine production by keratinocytes in vitiligo. Clinical and experimental dermatology 40(2), 163-170.
21. Camara-Lemarroy, C. R., & Salas-Alanis, J. C. (2013): The Role of Tumor Necrosis Factor-α in the Pathogenesis of Vitiligo. American Journal of Clinical Dermatology, 14(5), 343–350. doi:10.1007/s40257-013-0039-3.
22. Ebrahim AA, Hamed AM, Ahmed ES, Khudhair AS, (2018): Evaluation of serum interleukin-33 in nonsegmental vitiligo. Benha Med J; 35:54-9.
23. Kim NH, Torchia D, Rouhani P, Roberts B, Romanelli P, (2011): Tumor necrosis factor-alpha in vitiligo: direct correlation between tissue levels and clinical parameters. Cutan Ocul Toxicol; 30:225-7.
24. El-Taweel1 AI, El-Sawy1 FM, El Sayed OA, Amin NGZ. (2016): Assessment of serum interleukin-33 level in psoriatic patients. Int J Curr Microbiol App Sci; 5:11–17.

25. Xu D, Jiang HR, Kewin P, Li Y, et al., (2008): IL-33 exacerbates antigen-induced arthritis by activating mast cells. Proc Natl Acad Sci USA; 105: 10913–10918.
26. Pastorelli L, Garg RR, Hoang SB, Spina L, Mattioli B, Scarpa M, et al., (2010): Epithelial-derived IL-33 and its receptor ST2 are dysregulated in ulcerative colitis-sand in experimental Th1/Th2drive nenteritis.ProcNatlAcadSciUSA 107: 8017–8022.