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ORIGINAL ARTICLE

Serum Level of Interleukin -33 in Vitiligo.

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ABSTRACT

Background: Vitiligo is an acquired skin depigmentation condition due to the loss of active melanocytes or melanin in the epidermis, mucosa or hair follicles. Interleukin-33 (IL-33), a member of the IL-1 family, has been reported to function as an alarm due to its release following necrosis or damage of tissues.

Objectives: to evaluate whether serum levels of IL-33 is increased in vitiligo patients, and evaluate its relation with disease severity.

Methods: A case-control study included 44 subjects who were divided into two groups; Group A: included 22 vitiligo patients [15 males, 7 females] with age ranging from 7-62 years old. Severity of vitiligo was assesed by VASI score. Group B: included 22 apparently healthy persons [17 males, 5 females] with age ranging from 15-60 years old. Serum IL- 33 level was measured by ELIZA in both vitiligo and control group.

Results: Serum IL-33 levels were significantly higher in vitiligo patients than control group and its level was significantly related to disease severity measured by VASI score. There was no significant correlation between gender, age, duration of the disease or family history and serum IL-33 levels.

Conclusion: IL-33 may have an important role in the pathogenesis of vitiligo.

Keywords: Interleukin-33,Vitiligo, Pathophysiology.

INTRODUCTION

Vitiligo is a common skin depigmentation disease, characterised by presence of areas of milky white macules or patches, associated with loss of active melanocytes or melanin in the epidermis , mucosa or hair follicles. The disease has a slowly progressive course [1].

Vitiligo affects nearly 0.5-1% of people worldwide. It may occur at any age, but most patients have vitiligo before the age of 20. Its occurance is not affected by sex, type of skin or race. However, some studies suggest that vitiligo develops more in younger women and that there is marked geographical differences. [1].

The pathophysiology of vitiligo is becoming increasingly clarified. There are several pathophysiologic theories; including genetic, autoimmune, neurohumoral, autocytotoxic and oxidative stress hypothesis[2].

Cilinical and laporatory studies show that the autoimmune hypothesis is the most supported, especially for non-segmental vitiligo pathogenesis. This is evidenced by the high correlation between vitiligo and other autoimmune disorders like: autoimmune thyroid disorder, psoriasis, type I diabetes mellitus, alopecia areata, pernicious anaemia, and rheumatoid arthritis. Vitiligo can occur as part of type 1(APS1) and 2(APS2) autoimmune syndromes, or as part of other autoimmune syndromes [1].

Interleukin- 33 belongs to the IL-1 family. It has a receptor known as interleukin 1 receptor-like 1 protein (IL-1RL1) (also referred to as ST2 suppression of tumorgenesis)[3].

IL-33 is expressed mainly in cells that have barrier function, like epithelial and endothelial cells. It has been reported that IL-33 has an alarm function and released after cell death [4].

It has been observed that keratinocytes secrete IL-33 and that it is transferred from the nucleus to the keratinocyte cytoplasm in patients with vitiligo. In addition, keratinocyte-derived IL-33 can modulate the function of neighbouring keratinocytes by inhibiting stem cell factor (SCF) expression and basic fibroblast growth factor (bFGF) expression, both of which are vital for melanocyte growth, while increasing the expression of tumour necrosis factor α (TNF-α) and IL-6. Suggesting that IL-33

can cause melanocyte death by controlling cytokines in the microenvironment of the cells[5]. Vitiligo patients show high expression of IL-33 and its receptor ST2 in lesional skin and also high serum levels of IL-33. Furthermore, there was high expression of IL-33 gene in lesional and perilesional skin of vitiligo patients[5]**.**

This study was conducted to evaluate whether serum level of IL-33 is increased in vitiligo patients, and show if it has relation with disease severity, that may suggest future therapeutic modalities for controlling vitiligo**.**

Methods

This study was conducted in the Dermatology, Venerology and Andrology department and Clinical Pathology department, Faculty of Medicine, Zagazig University Hospitals. This study included 44 subjects who were divided into two groups; Group A: included 22 vitiligo patients, [15 males, 7 females] with age ranging from 7-62 years old, with active vitiligo (appearance of new depigmented lesions within the past 3 months) who did not use both topical or systemic treatment, at least 4 weeks before collection of blood samples. Group B: included 22 apparently healthy persons, [17 males, 5 females] with age ranging from 15-60 years old, with no previous or family history of vitiligo and no history of other autoimmune diseases. Approval for performing the study was obtained from Zagazig University Institutional Review Board (IRB). The study was performed in compliance with the World Medical Association Code of Ethics (Decleration of Helsinki) for research involving humans.

Exclusion Criteria: Other autoimmune disease such as systemic lupus erythematosus, rheumatoid artheritis, autoimmune diabetes mellites andgravesdisease.Dermatological diseases other than vitiligo such as atopic dermatitis and psoriasis.

Method:

All patients were subjected to full history taking, complete clinical examination and disease severity assessment by the VASI score. Serum IL- 33 level was measured in both vitiligo patients and control group by sandwich Enzyme- Linked Immunosorbent Assay (ELISA) (Sunredcompany). Blood sample was collected from the studied groups; serum was separated and stored at -20° C until performing the test.

Principle of the assay:

The serum is incubated in a well, and each well contains a different serum. An anti-human Antibody IL-33 coating is absorbed into wells. Human IL-33 that is present in the sample or normal binds to the well-absorbed antibodies.

Unbound biological components are separated during a wash phase following incubation. A biotin-conjugated anti-human IL-33 anti-body is added and binds to the first antibody that catches human IL-33.

The unbound biotin-conjugated anti-human IL-33 anti-body is removed during a wash phase after incubation. Streptavidin-HRP is added and binds to the anti-human IL-33 antibody that is conjugated with biotin.

Streptavidin-HRP is extracted during a wash stage following incubation unbound, and substrate solution reactive with HRP is applied to the wells. In proportion to the amount of human IL-33 present in the sample or norm, a coloured product is produced. The reaction is terminated by adding acid, and the measurement of absorbance is 450 nm. A standard curve is prepared from the 7 standard human IL-33 dilutions and the determined human IL-33 sample concentration[6]**.**

Statistical Analysis:

The data collected were analysed by computer using the social services version 24 (SPSS) statistical kit, data were represented in tables and graphs, continuous quantitative variables were expressed as mean ± SD & median (range) e.g. age, and categorical qualitative variables were expressed as absolute frequencies (number) & relative frequencies (percentage). After being tested for normality, acceptable statistical measures of significance were used. When the relevant likelihood was less than 0.05 ($P < 0.05$), the findings were deemed statistically significant. P-value < 0.001 was considered to be highly statistically significant (HS) and P-value≥0.05 was deemed to be statistically insignificant (NS).

RESULTS

The duration of vitiligo among the studied group ranged from 1 month to 10 years with mean 1.87±2.29 years, most of the studied group having negative Family history of vitiligo (81.8%), while all of them (100%) suffering from appearance of new lesions during the past 3 months.Regarding history of other autoimmune disease, other dermatological diseases or receiving treatment during past 3 months, all the studied group are free **(Table1).**

Vitiligo Area Scoring Index (VASI) among the studied vitiligo group ranged from 2- 14.25 with mean 4.86±3.22 **(Table 2).**

The mean of VASI among the studied male vitiligo patient is 4.83±3.48, while in vitiligo female patients it is 4.93 ± 2.85 with no statistical significance **(Table 3).**

The mean serum level of IL-33 among the studied vitiligo group is statistically higher than the mean serum level of IL-33 in thecontrol group (31.08 ± 12.08) vs. (14.18 ± 4.85) respectively**(Table4).**

There was no statistical significant relationship between IL-33 serum level and age, sex, family history or duration of the disease**(Table 5, 6)**.

There was positive correlation between serum level of IL-33, and VASI score among vitiligo patients which means that the higher disease severity the more elevated IL-33 serum level **Figure (1).**

Table (1): Present history and clinical picture among vitiligo patients

Item	Studied vitiligo cases (N=22)	
	No.	**%**
Duration of disease (years)		
Mean ± SD	1.87±2.29	
Median (Range)	1(0.08-10)	
Family history of vitiligo		
Positive	4	18.2
Negative	18	81.8
New lesions during past 3 months		
Positive	22	100.0
Negative	0	0.0
Clinical history		
Treatment during past 3months		
Positive	0	0.0
Negative	22	100.0
Other autoimmune disease		
Positive	0	0.0
Negative	22	100.0
Other dermatological diseases		
Positive	0	0.0
Negative	22	100.0

Table (2): The Vitiligo Area Scoring Index (VASI) among vitiligo patients.

Item	Studied vitiligo cases (N=22)
VASI score	
Mean ± SD	4.86±3.22
Median (Range)	4(2-14.25)

Table (3): The Vitiligo Area Scoring Index (VASI) among vitiligo patients according to sex.

VASI	Male (N=15)	Female (N=7)	Test	P-value
Mean ± SD	4.83± 3.48	4.93 ± 2.85	46.00	0.645 (NS)
Median (Range)	3.5(2-14.25)	4.5(2-11)		

Mann Whitney U test. NS: not significant.

Table (4): Serum level of IL-33 among the studied groups.

Serum level of IL-33(ng/l)	Vitiligo group (N=22)	Control group (N=22)	Test	P-value
Mean ± SD	31.08± 12.08	14.18 ± 4.85	19.500	0.000* (HS)
Median (Range)	27.5(16-55.6)	14.5(7-23.5)		

Mann Whitney Utest.
*HS: highly significant.

Table (5): Serum level of IL-33 among vitiligo patients according tosex and family history.

Item	Serum level of IL-33(ng/l)		Test	P-value
	Male (N=15)	**Female (N=7)**		
Sex				
Mean ± SD	32.05± 11.59	29.03 ± 13.76	41.000	0.417 (NS)
Median (Range)	28(21-55.6)	24(16-54.7)		
	Negative (N=18)	**Positive (N=4)**		

Item	Serum level of IL-33(ng/l)		Test	P-value
Family history				
Mean ± SD	32.24± 12.9	25.87 ± 5.72	32.000	0.733 (NS)
Median (Range)	27(16-55.6)	28(17.5-30)		

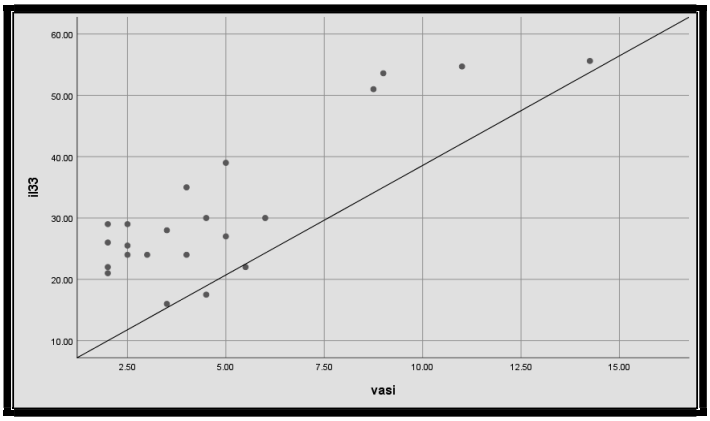
Mann Whitney U test. NS: not significant.

Table (6): Correlation between Serum level of IL-33, duration of disease and age of vitiligo patients.

Variable	Serum level of IL-33 (ng/l)	
	(r)	**p-value**
Duration of disease	0.150	0.506
Age (years)	0.115	0.610

*Correlation coefficient (r) is significant at the 0.05 level

Figure (1): Scatter diagram showing correlation between serum level ofIL-33, and VASI score among vitiligo patients* Statistically significant difference (P ≤ 0.05)



DISCUSSION

Vitiligo's pathophysiology is being increasingly explained. There are several pathophysiologic theories; including genetic, autoimmune, neurohumoral, autocytotoxic and oxidative stress hypothesis[2].

The autoimmune hypothesis is the most endorsed by reliable laboratory and clinical evidence, relevant for non-segmental vitiligo pathogenesis[1].

Interleukin-33 (IL-33) belongs to family IL-1. It is a legacy of the interleukin-1 receptor-like 1 protein (IL-1RL1) (also known as ST2 tumour suppression)[3].

Interleukin-33, released after cell apoptosis or necrosis, has been documented to act as an alarm. It is expressed primarily in barrier working cells, such as endothelial and epithelial cells[4].

Interleukin-33 stimulates many forms of immune cells involved in type 2 immunity and allergic inflammation, including type 2 innate lymphoid cells (ILC2s), mast cells, Th2 cells, eosinophils, basophils, dendritic cells , and macrophages (AAM). In various tissues (lung, nasopharynx, skin) and diseases (asthma, atopic dermatitis, allergic rhinitis, chronic rhinosinusitis), the axis of IL-33 / ILC2 plays a crucial role. In addition, IL-33 plays a significant role in stimulating immune cells that are involved in type 1 immunity, infection, and chronic inflammation, such as Th1 cells, natural killer cells (NK), CD8 + T cells, neutrophils, macrophages, B cells, and natural T killer cells (NKT) [13].

IL33 has been reported to be essential for potent CD8 + T cell (CTL) responses, which can effectively destroy skin melanocytes.

There is compelling evidence that autoimmune attack of CD8 + T cells may cause damage and loss of melanocytes in vitiligo[7]. CD8 + T-cells unique to melanocytes can cause direct damage to melanocytes and apoptosis after they have reached the skin[8].

Specific CD4 + T cells can be stimulated alongside CD8 + T cells and can be differentiated into many subpopulations, amplifying immune reactions and potentiating the destruction of melanocytes. Th1 subpopulations and their cytokines IFN-γ and TNFα, and chemokines CCL5, CXCL9, 10 and 11 tend to have a dominant role in vitiligo as they recruit and activate CD8 + cells, macrophages, and NK cells, inducing a cellular immune response and contributing to melanocyte cytotoxicity[9].

The IL-33 serum level in this study was calculated by ELISA. IL-33 serum levels were found to be significantly higher in vitiligo patients than in the control group and were significantly linked to the severity of the disease as measured by the VASI score.

In line with this outcome, Vaccaro et al.[15] found that serum IL-33 levels were substantially increased relative to healthy people in patients with vitiligo. Also, they found that this increase is significantly correlated with extension of vitiligo.
Similar results were reported by **Li et al.**[5]who found that serum IL-33 levels in patients with vitiligo increased significantly compared to healthy controls. They also found that IL-33 and its receptor, suppression of tumorgenesity2 (ST2), expression was increased in lesional skin of vitiligo patients.
Also in our study there was no significant correlation between gender, age, duration of the disease or family history and serum IL-33 levels. This result was in agreement with **Vaccaro et al.**[15]**.**
Interleukin-33 is secreted by keratinocytes and, in response to combined stimulation of TNF-alpha and IFN-γ, induces the release of prionflammatory cytokines from activated keratinocytes. It was also found that IL-33 reduced both stem cell factor (SCF) and basic fibroblast growth factor (bFGF), but increased the expression of both IL-6 and TNF-α in primary keratinocytes; the latter two cytokines are responsible for melanocyte loss in vitiligo, indicating that IL-33 may induce melanocyte death by controlling cytokines in the cellular microenvironment[5].
Limitations of our study: This study can't explain the accurate causality between IL-33 and vitiligo growth. Thus, more longitudinal studies are needed to evaluate the developmental predictive power and course of vitiligo. Another drawback is that the findings were taken from the results of a small-size sample, which must be checked in a larger population.
Studying the level of serum and tissue IL-33 before and after treatment of vitiligo cases may clarifying more about the role of this interleukin and its precise involvement in the pathogenesis. Using biological therapy (anti IL-33 antibodies) and studying its effect in the treatment of cases may help to displace the use of systemic medications to avoid their side effects.

CONCLUSION

This study suggests that IL-33 plays a role in vitiligo pathogenesis. In the future, IL-33 could be a potential therapeutic target for vitiligo and other autoimmune diseases. Inhibiting IL-33 may improve the clinical course of vitiligo and the quality of life of patients.
Declaration of interest : The authors report no conflicts of interest. The authors along are responsible for the content and writing of the paper.
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