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Serum Interleukin-15 Evaluation in Vitiligo Patients

D.K.Ahmed[1], K.M.Monib [1], M.S.Hussein [1] and E.S.Ahmad [2]
[1]Dermatology, Venereology and Andrology Dept., Faculty of Medicine, Benha University, Benha, Egypt.
[2] Clinical and Chemical Pathology Dept., Faculty of Medicine, Benha University, Benha, Egypt
E-Mail: daliakaram8916@gmail.com

Abstract

Background: Immune dysregulation has been linked to vitiligo, a skin condition marked by areas of uneven pigmentation. The role of serum interleukin-15 (IL-15) levels in the development of vitiligo is not yet understood. This research set out to compare the blood IL-15 levels of healthy controls to those of vitiligo sufferers. Methods: Sixty people with vitiligo and twenty healthy people served as controls in this case-control study. The ELISA kits were used to test the levels of serum IL-15. A thorough evaluation was carried out, which included the Vitiligo Extent Tensity Index (VETI). The outcomes are: The median blood IL-15 levels in the patients were substantially greater than those in the controls (94 pg/ml compared to 25.4 pg/ml; P<0.001). The length of the illness had a positive correlation with VETI scores (r = 0.772, P< 0.001), whereas the age of vitiligo beginning had a negative correlation (r = -0.358, P = 0.005). A favorable correlation was seen between IL-15 and the VETI score (r = 0.757, P< 0.001), the duration of the condition (r = 0.713, P< 0.001), and the age at which vitiligo first appeared (r = 0.324, P = 0.011). Vitiligo was successfully diagnosed by serum IL-15 (AUC = 0.917, 95% CI: 0.852-0.981). The independent predictor for vitiligo was shown to be IL-15 by multivariate logistic regression (OR = 1.06, 95% CI: 1.035-1.086, P< 0.001). Final thoughts: One possible diagnostic indicator for vitiligo is an increase in serum IL-15 levels. The fact that IL-15 levels are correlated with disease severity measures implies that it plays a role in the course of vitiligo. IL-15 has great promise as a diagnostic tool and a means of delving more into the pathophysiology of vitiligo.

Keywords: Disease Severity, Vitiligo, Interleukin-15, Sera Levels, Diagnostic Marker, Immune Dysregulation.

1. Introduction

Vitiligo causes a gradual loss of melanocytes at the cutaneous level, leading to the gradual emergence of depigmented skin lesions; it is an inflammatory autoimmune skin condition. Before a new patch appears, some individuals feel itching. More than half of all cases of vitiligo occur in persons less than 20 years old, and it may afflict people of any age or ethnic background [1].

Genetic susceptibility, environmental factors (such as friction or chemicals), intrinsic abnormalities of melanocytes, changes in metabolism, and dysregulation of inflammatory and immunological responses all play a role in the development of vitiligo, a complicated illness [2].

Innate immunological stimuli and type 1 interferons trigger the production of interleukin (IL)-15 in a variety of cell types. Natural killer cells and certain subsets of T lymphocytes linked to mucosal lymphoid organs rely on it for their growth, differentiation, and survival. IL-15 promotes a strong immunological response by activating macrophages and increasing dendritic cell survival [3].

An immuno-regulatory cytokine, interleukin-15 acts on many types of immune cells to accomplish a wide range of immunological tasks. They are produced, function, and survive because of IL-15. It is required for T helper (Th)-1 and -17 immune responses and also works on cells of innate immunity, such as mast cells, neutrophils, natural killer cells, and macrophages [4].

The purpose of this research was to compare the serum IL-15 levels of people with vitiligo to those of healthy controls.

2. Methods

Patients:

This In a case-control research conducted from May 2020 to April 2021 at Benha University Hospital's Outpatient Clinic, sixty patients presenting with vitiligo symptoms were compared to twenty healthy volunteers who were chosen based on age and sex.

The research followed all of the rules laid forth in the Declaration of Helsinki and was approved by the Research Ethical Committee of the Benha Faculty of Medicine. Each participant gave their informed permission before blood samples were taken. Patients were able to discontinue participation in the trial at any moment without penalty, and the confidentiality of their information was preserved throughout the whole process. All information gathered was used just for the purpose of this research.

Patients with vitiligo and healthy individuals who were at least 18 years old and willing to take part in the research were included.

Participants were not allowed to participate if they had the following

conditions: a history of active cancer, a current immunosuppressive medication, segmental vitiligo, another chronic dermatological disease, a known septic focus, diabetes mellitus, cardiovascular disease, vitiligo, or were receiving systemic treatment for vitiligo within the month before the study.

Two groups were formed from all of the participants: Sixty vitiligo sufferers made up Group A, whereas twenty healthy volunteers made up Group B.

Research Approach:

All subjects were evaluated using the following tools:

I. Thoroughly documenting the past: Information about the individual, including their name, age, marital status, and any serious health conditions they may have had (such as smoking, for example). Current circumstances, including the beginning, progression, and length of vitiligo. Extensive medical history, including autoimmune disorders in particular. A history of vitiligo per family. Drug history, including past treatments for vitiligo.

Section II: External and Internal Evaluations:

Final test: In order to rule out any systemic disorders, individuals underwent clinical examinations. In order to determine the body mass index (BMI), the participants' height and weight were measured. The body mass index (BMI) was determined in the following ways: underweight (<18), normal weight (185-24.9), overweight (25-29.9), and obese (BMI 30 and above).

Local examination: A skin test was used to check for patches of discoloration, and the Vitiligo Extent Tensity Index (VETI) score was used to measure the severity of the condition [5].

To measure the severity of vitiligo, the VETI score uses a numerical scale derived from the rule of nines used in burn evaluation. The five phases of disease severity are used to provide ratings based on evaluations conducted at five main sites: the head, upper limbs, trunk, lower limbs, and genitalia. From normal skin (Stage 0) to hypopigmentation (Stage 1), full depigmentation with various hair pigmentation patterns (phases 3, 4), and total depigmentation with considerable hair whitening (Stage 5) are the disorders that are delineated by these phases [6].

After taking into consideration the contributions from every part of the body, the following formula was used to determine total body VETI: The VETI score is calculated by adding the percentages of head involvement and trunk involvement, then dividing the result by the intensity grade. 4+ (Divide the percentage of the upper limbs that are involved by the intensity grade). The results are 2+ for the lower limbs and 4+ for the genitalia, calculated by dividing the percentage of participation by the intensity grade. 0.1.

The rule of nines was used to derive the coefficients in this formula, which are based on the proportion of skin surface. The given coefficients were as follows: genitalia = around 0.1 (1:9 = 0.1), lower limb and trunk = 4 (36:9 = 4), upper limb = 2 (18:9 = 2), and head = 1 (9:9 = 1). A VETI score of 55.5 is considered maximum [5].

The vitiligo disease activity score (VIDA) was used to evaluate the stability of vitiligo lesions. If no new lesions nor enlargements of existing ones occurred within a year, the lesions were deemed stable [7].

Section III: Laboratory Investigations: Five milliliters of venous blood per subject was collected for serum samples. To prepare the serum for the experiment, the samples were centrifuged at 2000-3000 r.p.m. for 15 minutes after clotting for 30 minutes. The serum was then kept at -20 °C.

Assessing the Concentration of IL-15: Biokit Co., Ltd.'s ELISA kits were used to assess IL-15 levels in the serum. A double-antibody sandwich ELISA method was used in the experiment. At first, a monoclonal antibody enzyme that had been previously coated with a human IL-15 monoclonal antibody was given IL-15. After that, an immunological complex was created by adding IL-15 antibodies that had been tagged with biotin and streptavidin-horseradish peroxidase. Chromatin solutions A and B were added after incubation and washing to remove unbound enzyme. The sample's color changed from blue to yellow, which corresponded with the quantity of IL-15.

The assay procedure included diluting the standard reagent according to the manufacturer's instructions in order to create different standard concentrations. Sample wells included sample, IL-15 antibody, and streptavidin-horseradish peroxidase; standard wells contained a combination of standard and streptavidin-horseradish peroxidase; and blank wells had no substance. The optical density was measured at a wavelength of 450 nm after incubation and the following processes, which included washing, adding chromogen solutions, and a stop solution. We calculated the IL-15 concentration in the sample using the standard curve regression equation, which is based on standard concentrations and their associated optical densities.

Statistical analysis:

Data IBM SPSS version 25 (Armonk, New York, United States) was used for management and statistical analysis. In order to determine whether the quantitative data was normally distributed, we used the Shapiro-Wilk test for controls, the Kolmogorov-Smirnov test for patients, and direct data visualization techniques for both sets of data. We used medians and ranges, or standard deviations and means, to summarize the numerical data. Numeric and percentage representations were used for the categorical data. For numerical variables that did not follow a normal distribution, we used the Mann-Whitney U test, and for data that did, we used the independent t-test. A Chi-square test was used for the purpose of comparing categorical data. To distinguish between the two groups, we used ROC analysis on serum IL-15 levels. Diagnostic indices, optimal cut-off point, and area under curve (AUC) with 95% confidence interval (95%CI) were used in the calculations. We used Spearman's correlation to find the correlations. The Mann-Whitney U test was used to compare IL-15 according to various criteria. For the purpose of vitiligo prediction, logistic regression analysis was used, and odds ratios with a 95% CI were computed. A two-sided test was used for all statistical analyses, and a P value of less than 0.05 was considered significant.

3. Results

In terms of age (P = 0.292), gender (P = 0.796), body mass index (BMI) (P = 0.574), obesity (P = 0.698), and smoking (P = 0.694), there were no statistically significant differences between the patient and control groups (Table 1).

Patient and control group demographics are summarized in Table 1.

		Patients (n = 60)	Controls (n = 20)	Test	*P*
Age (years)	Mean ±SD	36 ±13	33 ±10	***t*** = -1.062	0.292
Gender	Male n (%)	32 (53.3%)	10 (50%)	X^2= 0.067	0.796
	Female n (%)	28 (46.7%)	10 (50%)		
BMI	Mean ±SD	27 ±5	26 ±5	X^2= -0.564	0.574
Obesity	N (%)	30 (50%)	9 (45%)	X^2 = 0.150	0.698
Smoking	N (%)	24 (40%)	9 (45%)	X^2 = 0.155	0.694

We used a t-test for independent variables (age and body mass index) and a chi-square test (X2) for categorical variables.

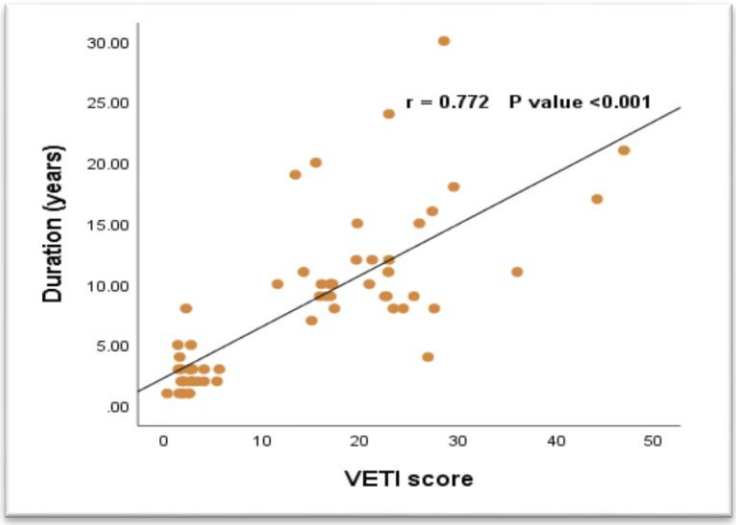
All patients and controls had their serum IL-15 levels tested. Table 2 shows that the median blood IL-15 level was considerably higher in the patients compared to the controls (Median= 94 pg/ml vs. 25.4 pg/ml; P <0.001).

Patients' and controls' serum IL-15 levels are shown in Table 2.

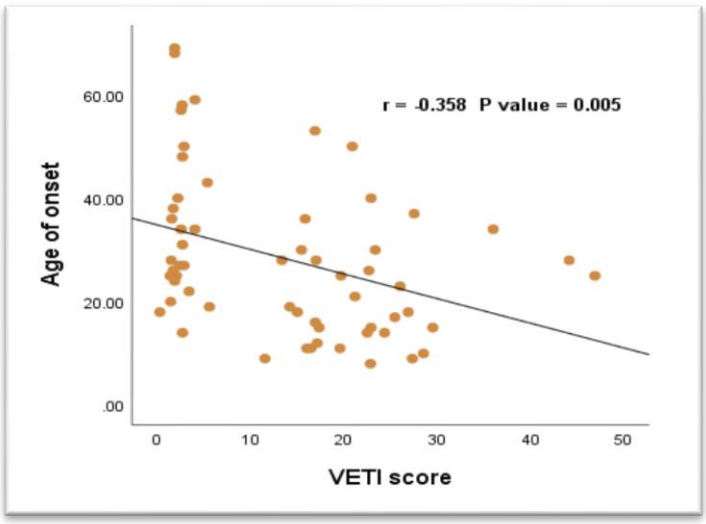
Serum IL-15 pg/ml	Patients (n = 60)	Controls (n = 20)	Z	*P*
Median (range)	94 (20.3 - 322.7)	25.4 (15.4 - 82.4)	-5.556	<0.001*

Z=Whitney, Mann *P<0.05 indicates statistical significance in the U test.

The association between VETI scores and other factors was examined using Spearman's correlation test. A strong positive connection (r = 0.772, P< 0.001) was seen between the VETI score and the duration of the illness (Figure 1 A). On the other hand, there was a statistically significant negative association between VETI score and age of vitiligo onset (r = -0.358 & P = 0.005) as shown in Figure 1 B. There were also non-significant correlations with patients' age (P = 0.726) and BMI (P = 0.996).



A)



B)

In Figure 1, we can see the correlations between the VETI score and the length of the illness (A) and the age of vitiligo onset (B) as shown in the figure.

Table 3 displays correlations between serum IL-15 and other variables. Clinical type of vitiligo showed a significant relation with serum IL-15, which was significantly higher in generalized vitiligo than acrofacial (P< 0.001). However, there were no significant relationships observed with gender (P = 0.354), obesity (P = 0.947), smoking (P = 0.582), family history (P = 0.929), history of other autoimmune diseases (P = 0.645), or course of vitiligo (P = 0.78).

Table 3: Relationships between Serum IL-15 and other factors

		IL-15 Median (range)	*P*
Gender	Males	95.6 (20.3 - 322.7)	0.354
	Females	87 (40.9 - 118.4)	
Obesity	Yes	95.1 (21.3 - 322.7)	0.947
	No	92.1 (20.3 - 120)	
Smoking	Yes	92.1 (20.3 - 260.9)	0.582
	No	97.2 (40.9 - 322.7)	
Family history of vitiligo	Yes	92.3 (21.3 - 322.7)	0.929
	No	94.3 (20.3 - 260.9)	
History of other autoimmune diseases	Yes	94.9 (20.3 - 322.7)	0.645
	No	93 (21.3 - 260.9)	
Clinical type	Acrofacial	74.3 (20.3 - 89.7)	<0.001
	Generalized	104.5 (88.6 - 322.7)	

The associations between IL-15 and other parameters were examined using Spearman's correlation test. There were positive and statistically significant relationships between serum IL-15 and the VETI score (r = 0.757, P <0.001), the duration of the illness (r = 0.713, P <0.001), and the age at which vitiligo first appeared (r =.324, P = 0.011) in Figure 2 A and 2 B, respectively. Contrarily, there was no statistically significant relationship between IL-15 and either patients' age (P = 0.589) or body mass index (P = 0.634).

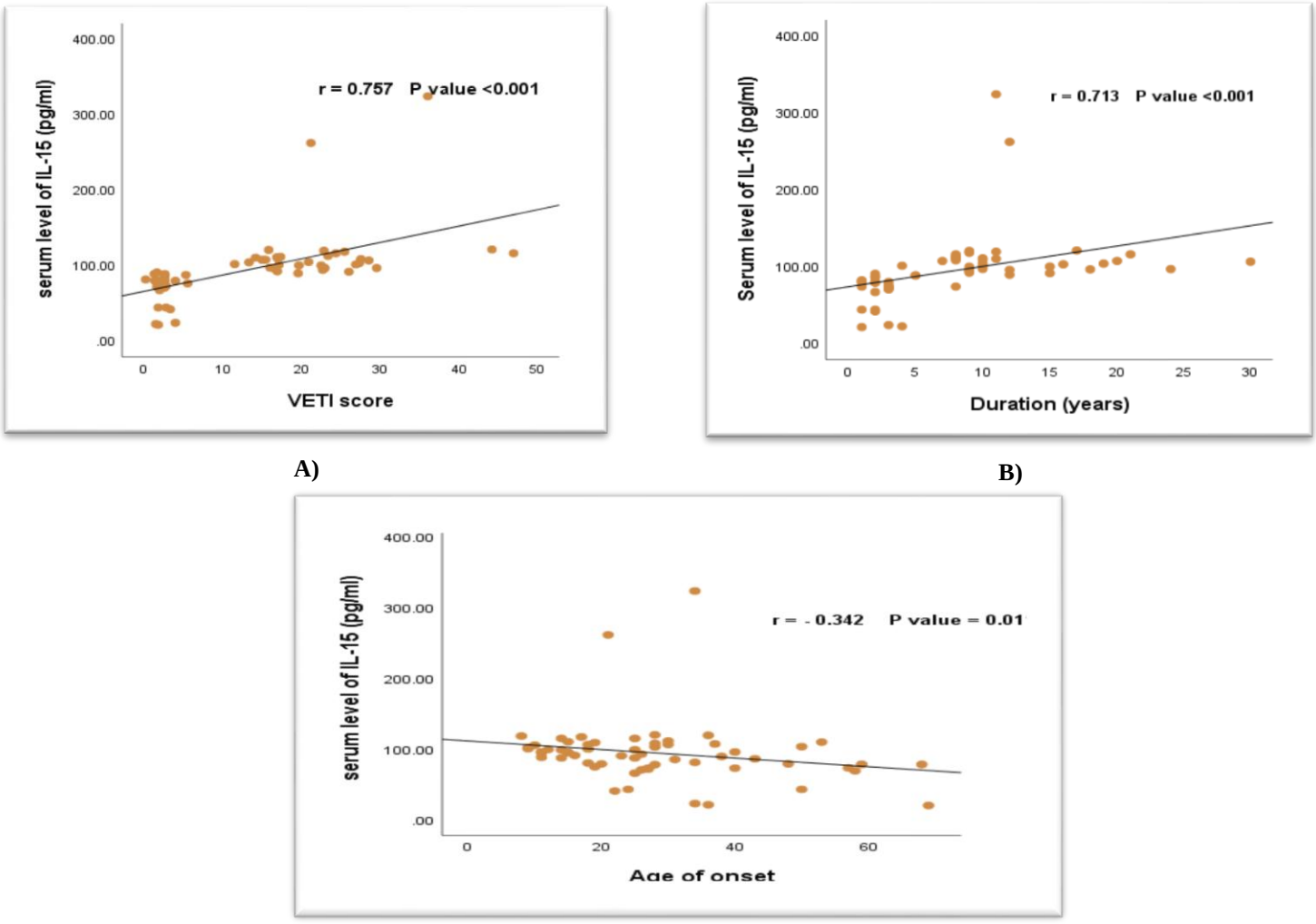


Fig. 2: A) IL-15 correlation with VETI score, B) IL-15 correlation with illness duration, and C) IL-15 correlation with age of vitiligo start.

4. Discussion

Vitiligo is a non-communicable pigmentation condition that develops over time; it is marked by the appearance of discrete white patches that may vary in size, shape, and quantity. In the histological image, white patches represent the loss of melanocytes and melanin, while the progressive boundaries of vitiligo are characterized by an irregular lympho-mononuclear infiltration. A depigmenting skin illness caused by the immune system's selective elimination of melanocytes, vitiligo may be emotionally and mentally debilitating. Vitiligo shows less inflammation than other autoimmune skin disorders including psoriasis and atopic dermatitis. Permanent depigmentation may result, however, from the failure to arrest melanocyte death in its early stages[8, 9].

Autoimmune illnesses such as inflammatory bowel disease, rheumatoid arthritis, and psoriasis rely heavily on IL-15, a cytokine belonging to the IL-2 family. One possible target for treating vitiligo is its effect on natural killer cells, dendritic cells, and tissue-resident T memory cells that express CD122 [10–12].

The purpose of this research was to compare the serum IL-15 levels of people with vitiligo to those of healthy controls.

The median blood IL-15 level was 94 pg/ml in patients and 25.4 pg/ml in controls, with a p-value of less than 0.001. Atwa et al. [13] found that IL-15 levels were significantly higher in vitiligo patients' sera compared to control subjects' (median = 39, 26 pg/ml respectively; P =.001), which is consistent with the current study and suggests that IL-15 plays a role in the pathogenesis of vitiligo by promoting the survival and maturation of NK cells, neutrophils, and DCs. It enhances the phagocytic activity of neutrophils and macrophages, NK cell cytotoxicity, and cytokine production such as IFN-γ and TNF-α. Through IL-15 trans-presentation, DCs also effectively control memory Tc cell growth and

survival. In addition, IL-15 promotes the expansion of Th17 cells that are reliant on T-cell receptors. The number ten.

In a study conducted by Gholijani et al. [14], it was discovered that innate immunity cytokines such as IL-8, IL-12, and IL-15 were elevated in vitiligo patients compared to controls (median =.39 - 3500 pg/ml; P <.04). Similarly, Hamza et al. [15] found that IL-15 serum levels were significantly higher in vitiligo patients compared to healthy controls (median = 154.88, 136.33 ng/L respectively; P =.003).

A non-significant difference in blood IL-15 levels between men and females was found in the present investigation. The findings were in line with those of Atwa et al. [13] and Hamza et al. [15], which also demonstrated that there was no statistically significant difference between the sexes in terms of serum IL-15 levels (P = 0.79 and P = 0.26, respectively).

According to sex, contrary to the current findings, Garsaud et al. [16] discovered that their patients were mostly female. Dogra et al. [17] also found that whereas men made up 47.8% of the patient population, females accounted for 52.2%. Nevertheless, a greater occurrence was noted in males by McBurney [18]. According to research conducted in the United States by Alkhateeb et al. [19], the occurrence of vitiligo seems to be about equal in boys and girls. The interest in cosmetic treatment and frequent visits with dermatologists by women may account for the observed prevalence [20].

The present study's findings demonstrate a strong correlation between serum IL-15 and clinical vitiligo types; specifically, the generalized type of vitiligo was shown to have a substantially higher serum IL-15 level than the acrofacial form. Clinical forms of vitiligo were not significantly correlated with serum IL-15 (P = 0.26), according to Atwa et al. [13].

The current investigation found a strong association between the length of time vitiligo has been present and blood levels of IL-15. The findings contradicted those of Atwa et al. [13], who discovered no statistically significant relationship between IL-15 blood levels and illness duration (P = 0.68). A link between serum IL-15 level and illness duration was observed by Hamza et al. [15], however it was judged to be non-significant (P = 0.058). Distinct sample sizes account for these variations.

The IL-15 level was much greater in the current study's unstable vitiligo patients compared to their stable vitiligo patients. Supporting these findings, Hamza et al. [15] found that patients with unstable vitiligo had a substantially higher mean serum IL-15 level compared to individuals with stable vitiligo (243.12±136.64 ng/L vs. 144.35±28.82 ng/L, respectively).

Eladl et al. [21] also looked at IL-15 blood levels in non-segmental vitiligo patients to see whether there was a correlation with the severity, length, and activity of the condition. The stable group had lower serum IL-15 levels (72.1 pg/ml) compared to the active vitiligo group (84.9 pg/ml), but, this difference was not statistically significant (p< 0.104).

The authors Atwa et al. [13] found no statistically significant relationship between IL-15 levels in the blood and the VIDA score. Both active and stable vitiligo patients contain resident memory T cells, which means that IL-15 will be produced in both types of vitiligo to help these cells survive. This is how they interpreted the data.

The present research demonstrated a statistically significant relationship between VETI score and serum IL-15 level. While Atwa et al. [13] utilized a different score (VASI) to evaluate vitiligo severity, they also discovered a substantial positive connection (P = 0.001) between IL-15 levels and vitiligo severity, which is in accordance with the current findings. There was a weak association (P = 0.214) between serum IL-15 and VASI score, according to Hamza et al. [15]. Researchers Elela et al. [22] discovered a favorable correlation between VASI and VIDA and IL-15, IL-17, and IL-22.

Results from a recent ROC analysis demonstrated the usefulness of serum IL-15 in the diagnosis of vitiligo. The results for serum IL-15 were very promising, with a sensitivity of 90% and specificity of 85%, and an area under the curve of 0.917 (P< 0.001). In line with the current findings, Eladl et al., [21] examined the specificity and sensitivity of serum IL-15 as a diagnostic indicator for vitiligo using a ROC curve. An area under the curve for IL-15 was 0.937, indicating good accuracy (P< 0.001), with a sensitivity of 98.3% and a specificity of 83.3%.

The results of this study raise the possibility that IL-15 is involved in the immunological etiology of vitiligo. Consequently, IL-15 might be a great target for vitiligo treatments. We did not investigate additional indicators of vitiligo activity, such as the ill-defined border or Koebner's phenomenon, since we depended on the VETI score for evaluation, which is one of the shortcomings of the research. The exact roles of IL-15 in vitiligo, its levels before and after

treatment, and whether it is expressed lesion- or non-lesionally need more research.

5. Conclusion

IL-15 The high amount of IL-15 in vitiligo patients' sera raises the possibility that it plays a key role in the development of the disease. A measure of the severity of vitiligo might be IL-15. Researchers hope that the results of this research may pave the way for the creation of novel treatments for vitiligo.

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