



Study Of Detection Of Serum CXCL10 In Vitiligo Patients

Abd-El Aziz El- Rifaie^a, Rehab Mohamed Naguib^a, Laila Ahmed Rashed^b and Marwa Fathelbab Ahmed^c

^a Dermatology department, Faculty of Medicine, Beni-Suef University, Egypt

^b Medical Biochemistry & Molecular Biology Department, Faculty of Medicine, Cairo University, Egypt

^c Dermatology department, Maghagha General Hospital, Ministry of Health, Egypt

Abstract:

The goal of this study was to detect the level of CXCL10 in the serum of the vitiligo patients as compared to the normal control persons to investigate the possible role of CXCL10 in the pathogenesis of this disease. The present study included 30 Egyptian vitiligo patients, their age ranged from (20 to 50 years). They were recruited from Dermatology outpatient clinic at Beni-Suef University hospital. Thirty unrelated apparent healthy controls with similar demographic (matched age and sex) were taken during the period from (1st May 2018 to 1st May 2019). 5ml blood sample was taken from patients and controls and was left to clot for 30 minutes then serum was separated by centrifugation and kept frozen at -80c till analysis of CXCL10 by ELISA. The patients and controls were subjected to estimation of the serum level of CXCL10. The result of the study showed that increased levels of CXCL10 in the vitiligo patients when compared to controls and the patients in the progressive stage showed higher expression of CXCL10 than those in the stable stage, suggesting a mechanistic role of CXCL10 in vitiligo.

Keywords: Vitiligo; T cell immune response; IFN- γ ; CXCL-10.

1. Introduction:

Vitiligo is an acquired, progressive, multifactorial, depigmenting disorder characterized by the appearance of circumscribed milky white macules in the skin because of chronic, progressive loss of the functional melanocytes in the epidermis [1]. It may have devastating psychological and social consequences. Vitiligo is a common depigmenting disorder with profound psychosocial impacts [2].

The etiology of vitiligo is unknown yet, and several hypotheses (including autoimmune, neural, radical, self-destruction and inherent defect theories) have been proposed to explain its pathogenesis [3].

Chemokines(greek-kinos,movement) are a family of small cytokines, or signaling proteins secreted by cells. their name is derived from their ability to induce directed chemotaxis in nearby responsive cells; they are chemotactic cytokines [4,5].

Chemokines were divided into four families. The 2 largest families are the CXC family, in which these two cysteines are separated by any single amino acid, and the CC family, in which the first two cysteines are adjacent [6,7].

CXCL10 is a small molecular weight protein (10 kDa) which was functionally described as an inflammatory chemokine. Moreover , the lack of the ELR tripeptide (Arg-Leu-Glu) motif in the vicinity of (CXC) residues characterizes CXCL10 as an inhibitor of the neovascularization; hence, it acts as an 'angiostatic'(antiangiogenic) chemokine [8].

T-cell-dependent immune response contributes to the onset and evolution of the vitiligo. Direct damage to melanocytes is considered to be primarily caused by T cells especially CD8+T cell [9].

Thus, the migration of T lymphocytes to areas of melanocyte damage is a critical component in the activation of the immune system and inflammatory processes [10].

Depigmentation is accompanied by expression of type 1 cytokines such as interferon (IFN γ) and tumour necrosis factor (TNF α). IFN γ and TNF- α a genetic variants were associated with vitiligo [11,12]. IFN- γ is the most important cytokine, that is associated with the Th1 immune response [13]. CXCL10 is a chemokine produced under the influence of IFN- γ and binds to

its receptor, CXCR-3, present on lymphocytes inducing T cells homing [14], leading to their attraction to the sites of the vitiligo lesions and perpetuation of IFN- γ production in a continuous positive loop [15].

CXCL10 plays a role in directed migration of T cells within the skin and it was required for T cell function beyond simple recruitment [16].

CXCL9 and CXCL10 have been suggested to promote melanocyte-specific CD8+T cells to infiltrate into the basal layer of epidermis to attack melanocytes, resulting in the deficiency of melanin [16]. [16] reported that CXCL10 is elevated in both serum and lesional skin from patients with vitiligo, and it is critical for the progression and maintenance of depigmentation in a mouse model of vitiligo. [16] reported that serum CXCL9 and CXCL10 levels were significantly elevated in vitiligo, especially in active vitiligo. [17]

2. Patients and Methods:

The present study included 30 Egyptian vitiligo patients, their age ranged from (20 to 50 years). They were recruited from Dermatology outpatient clinic at Beni-Suef University hospital. Thirty unrelated

apparent healthy controls with similar demographic (matched age and sex) were taken during the period from (1st May 2018 to 1st May 2019). The studied subjects were divided into two groups as follows: Group I: (n = 30) vitiligo patients and Group II: (n = 30) unrelated apparent healthy controls.

2.1 Inclusion criteria:

- Age between 20 to 50.
- Age and sex matched apparently healthy controls.
- Patients with nonsegmental vitiligo in regard to surface area.
- Both males and females will be included.

2.2 Exclusion criteria:

- Age below 20 and above 50.
- Patients with other types of vitiligo.
- Patients receiving systemic vitiligo treatment in the last three months.
- Patients with other autoimmune diseases.
- Patients with associated systemic or dermatological diseases.

2.3 Controls:

Controls will be chosen randomly from any other outpatient clinic.

2.4 Data collection methods and tools: All patients were subjected to:

- Medical history was taken from each patient.
- ❖ Course of illness.
- ❖ Age of onset of the disease.
- ❖ Mode of presentation (Initial symptoms).
- ❖ Positive family history.
- ❖ Positive psychological stress.
- ❖ Duration of last lesion.
- Full clinical examination was done for each patient to determine the site, extent, and type of vitiligo.
- The study protocol was explained to the patients and controls and an informed consent was obtained from them.
- One blood sample, 5ml was taken from each patient. One blood sample, 5ml was taken from each control. All the blood samples were left to clot for 30 minutes then the serum will be separated by centrifugation and kept frozen at -80c till analysis of CXCL10 by ELISA.
- A thorough dermatological examination was carried out to determine the approximate percentage of body surface area involved using the "rule of nine".

Vitiligo Disease Activity Score (VIDA):

The Vitiligo Disease Activity (VIDA) Score is a 6-point scale for assessing vitiligo activity. It helps to assess effectiveness of interventions to halt and reverse the extension of depigmentation. Lower VIDA scores indicate less activity (Table 1) [18].

Scoring:

Based on the individual's own opinion of the present disease activity overtime. Active vitiligo involves either one of the following aspects: Expansion of existing lesions or Appearance of new lesions.

Table (1): The VIDA scoring system [18].

Vitiligo Activity	Time Period	VIDA Score
Active	6 weeks or less	+4
Active	6 weeks to 3 months	+3
Active	3 - 6 months	+2
Active	6 - 12 months	+1
Stable	1 year or more	0
Stable with spontaneous repigmentation	1 year or more	-1

Vitiligo Area Severity Index (VASI):

Its name is an adoption from PASI score in psoriasis. The percentage of vitiligo involvement is calculated in terms of hand units. One hand unit is approximately equivalent to 1% of the total body surface area. The degree of pigmentation is estimated to the nearest of one of the following percentages: 100% - complete depigmentation, no pigment is present; 90% - specks of pigment present; 75% - depigmented area exceeds the pigmented area; 50% - pigmented and depigmented areas are equal; 25% - pigmented area exceeds depigmented area; and 10% - only specks of depigmentation present [19,20].

Statistical analysis:

The collected data were coded then entered and analyzed using the SPSS version 22 (Statistical package for social science).

The descriptive statistics were done for categorical variables by frequency and percentage, and for numerical variables in the form of mean and standard deviation (mean ± SD).

- Suitable statistical tests of significance were used:
- Independent Sample t-test for two unrelated samples.
- Chi-Square (χ^2) test for categorical data.
- Mann Whitney U Test for two independent non-parametric samples.
- Spearman Correlation.

- P-values equal to or less than 0.05 were considered statistically significant.
- Simple graphs were used to illustrate some information

3. Results:

Table (2): Age Distribution Of Studied Population; (N=60):

	Mean ±SD	Minimum	Maximum	Range	<i>p-value</i> ^a
Cases	34.0 ±9.3	22	48	26	0.903
Controls	33.77 ±9.5	20	49	29	

^a *p-value >0.05 is considered non-significant by independent sample t-test.*

As illustrated in table (2); the average patient age was **34.0 ±9.3** (SD) years. There was no statistically significant difference between cases and control groups regarding age (see figure-1)

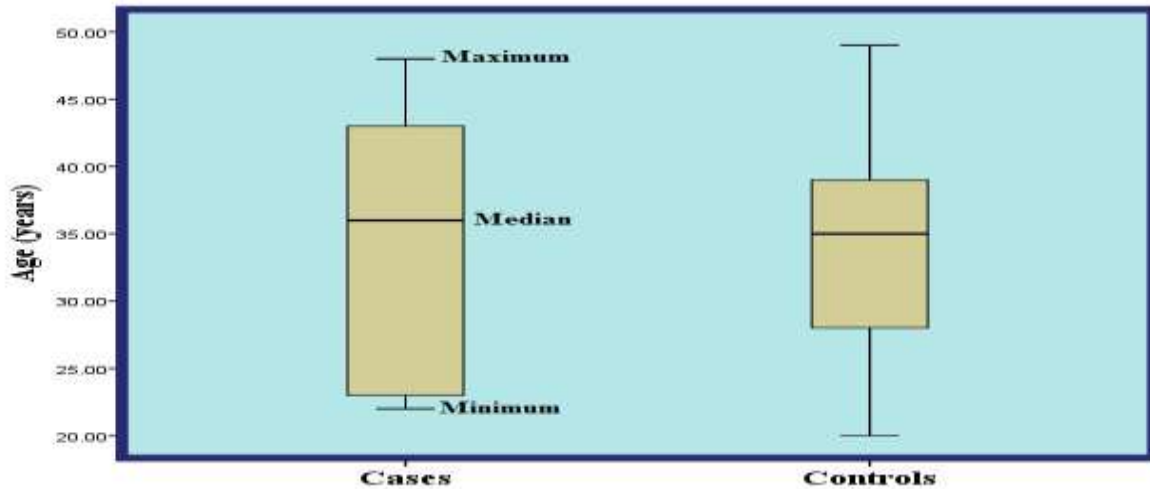


Figure (1): Age distribution between vitiligo cases and controls.

Table (3): Sex Distribution Of Studied Population; (N= 60):

		N (%)			<i>p-value</i> ^a
		Cases N= 30	Controls N= 30	TOTAL	
Sex	Female	19 (63.3)	16 (53.3)	35 (58.3)	0.601
	Male	11 (36.7)	14 (46.7)	25 (41.7)	

p-value >0.05 is considered non-significant by Chi-Square test.

Table (3) demonstrates sex distribution of studied patients; **63.3%** of them were females and **36.7%** were males (*see figure-2*). There was no statistically significant difference between cases and control groups regarding sex.

Table (4): Disease Characters Of Studied Vitiligo Cases; (N= 30):

	N	%
Vitiligo Type		
Segmental	0	0%
Non-Segmental	30	100%
Vitiligo Activity		
Active	20	66.7%
Stable	10	33.3%
Vitiligo disease activity (VIDA) score		
Mean ±SD	1.83 ±1.6	
Range (Minimum-Maximum)	5 (4 – (-1))	
(VASI) Score		
Mean ±SD	94.00 ±57.8	
Range (Minimum-Maximum)	190 (200 – 10)	
Duration of Vitiligo Disease (years)		
Mean ±SD	4.23 ±2.3	
Range (Minimum-Maximum)	8 (9 – 1)	

Duration of Last Lesion (months)	
Mean ±SD	12.02 ±14.3
Range (Minimum-Maximum)	47.30 (48 – 0.20)

Table (4) illustrates that; all the studied vitiligo cases were with non-segmental type (100%). Of them 20 cases (66.7%) were with active disease while 10 cases (33.3%) were with stable activity of the disease (see figure-3). Vitiligo disease activity (VIDA) score ranged from (-1) to (+4) with a mean of 1.83 ± 1.6 (SD), while (VASI) Score ranged from (10) to (200) with a mean of 94.00 ± 57.8 (SD).

Vitiligo disease duration was ranged from 1 to 9 years with a mean of 4.23 ± 2.3 , while the duration of last lesion was ranged from 48 to 0.2 months with a mean of 12.02 ± 14.3 .

Table (5): Association With Psychological Stress In The Studied Cases; (N=30):

	N	%
Psychological Stress		
No	13	43.3%
Yes	17	56.7%

Of the studied cases; 17 cases (56.7%) reported associated psychological stress while 13 cases (43.3%) hadn't associated psychological stress.

Table (6): Association With Family History In The Studied Cases; (N=30):

	N	%
Family History		
Negative	21	70%
Positive	9	30%

Of the studied cases; 21 cases (70%) reported negative family history of vitiligo while 9 cases (30%) had positive family history.

Table (7): The Serum Level Of CXCL10 In Vitiligo Patients As Compared To Normal Control Persons; (N= 60):

	Cases N =30	Controls N= 30	<i>p-value</i>
Mean ±SD	157.11 ±37.7	50.16 ±20.6	< 0.001*
Range	95.10	31.40	
Maximum	217.20	116.30	
Minimum	77.68	84.90	

** P-value ≤ 0.05 is considered significant by (independent sample t-test).*

Table (7) demonstrates that the serum level of CXCL10 in vitiligo patients was significantly higher as compared to normal control persons (p-value < 0.001); where the mean scores were (157.11 vs. 50.16) in cases and controls respectively (see figure -2).

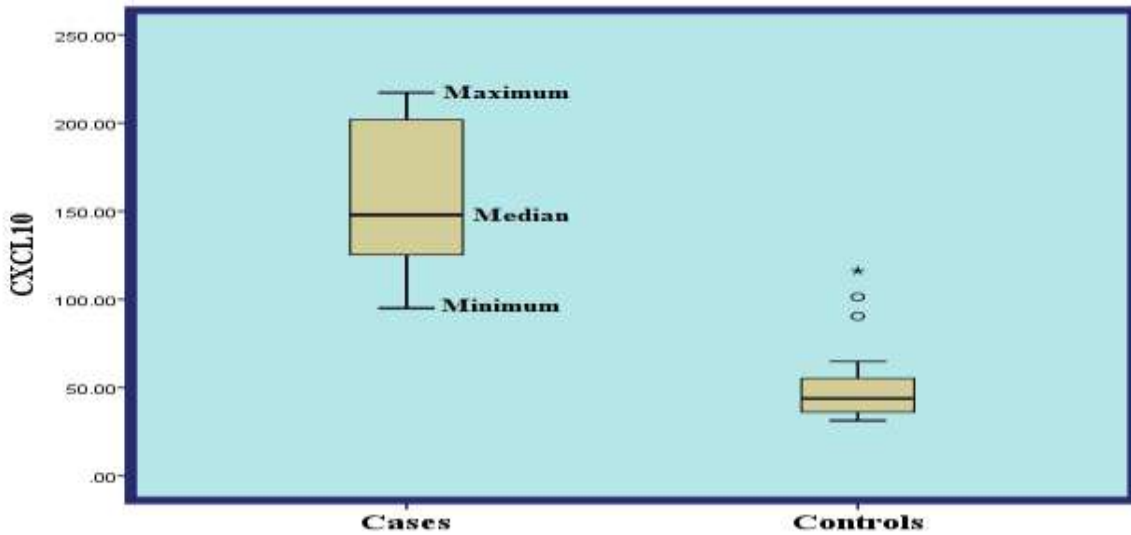


Figure (2): The serum level of CXCL10 in vitiligo patients as compared to normal control persons.

Table (8): Correlation Between The Serum Level Of CXCL10 In Vitiligo Patients And Age Of The Studied Cases; (N=30):

	Age of the studied cases	
Serum Level of CXCL-10	$r = 0.027$	$p\text{-value} = 0.840$

r Spearman correlation coefficient

Table (8) demonstrates no detected correlation between the serum level of CXCL10 in vitiligo patients and age of the studied cases; where (p-value > 0.050).

Table (9): Relation Between The Serum Level of CXCL10 In Vitiligo Patients And Sex Distribution Of The Studied Cases; (N=30):

	Females N= 19	Males N= 11	P-value
Mean ±SD	155.70 ±38.3	195.35 ±38.7	0.794
Minimum	95.10	103.50	
Maximum	217.20	215.30	
Range	122.10	111.80	

* $P\text{-value} \leq 0.05$ is considered significant by (Mann–Whitney U test).

There were no detected relation between sex and the serum level of CXCL10 in vitiligo patients; p-value >0.05.

Table (10): Relation Between The Serum Level Of CXCL10 In Vitiligo Patients And Disease Activity Of The Studied Cases; (N=30):

	Active N= 20	Stable N= 10	P-value
Mean ±SD	171.44 ±35.6	128.45 ±23.7	0.002*

Minimum	121.50	95.10
Maximum	217.20	163.70
Range	85.70	68.60

* $P\text{-value} \leq 0.05$ is considered significant by (Mann–Whitney U test).

Mean serum level of CXCL10 in vitiligo patients with active disease was significantly higher as compared with mean serum level in stable vitiligo cases (171.44 vs. 128.45) in active and stable disease activity respectively; $p\text{-value} = 0.002$.

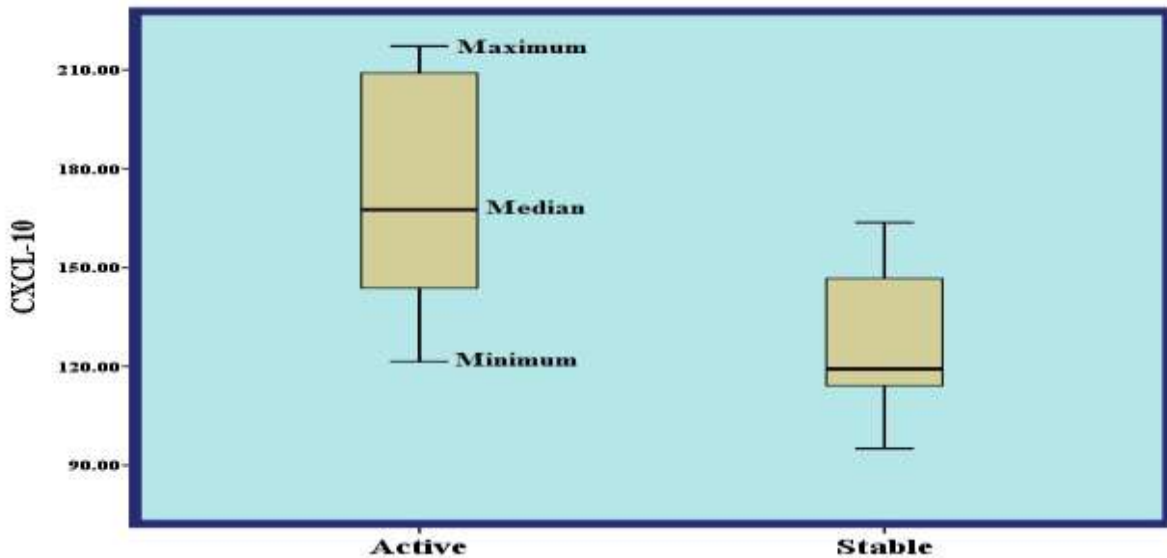


Figure (3): Relation between the serum level of CXCL10 in vitiligo patients and disease activity of the cases.

Table (11): Correlation Between The Serum Level Of CXCL10 In Vitiligo Patients And Vitiligo Disease Activity (VIDA) Score Of The Studied Cases; (N=30):

	Vitiligo disease activity (VIDA) score	
Serum Level of CXCL-10	$r = 0.575$	$p\text{-value} = 0.001^*$

r Spearman correlation coefficient

Table (11) demonstrates the serum level of CXCL10 in vitiligo patients was moderately positive correlated with vitiligo disease activity (VIDA) score of the studied cases; where ($r=0.575$, $p\text{-value}=0.001$).

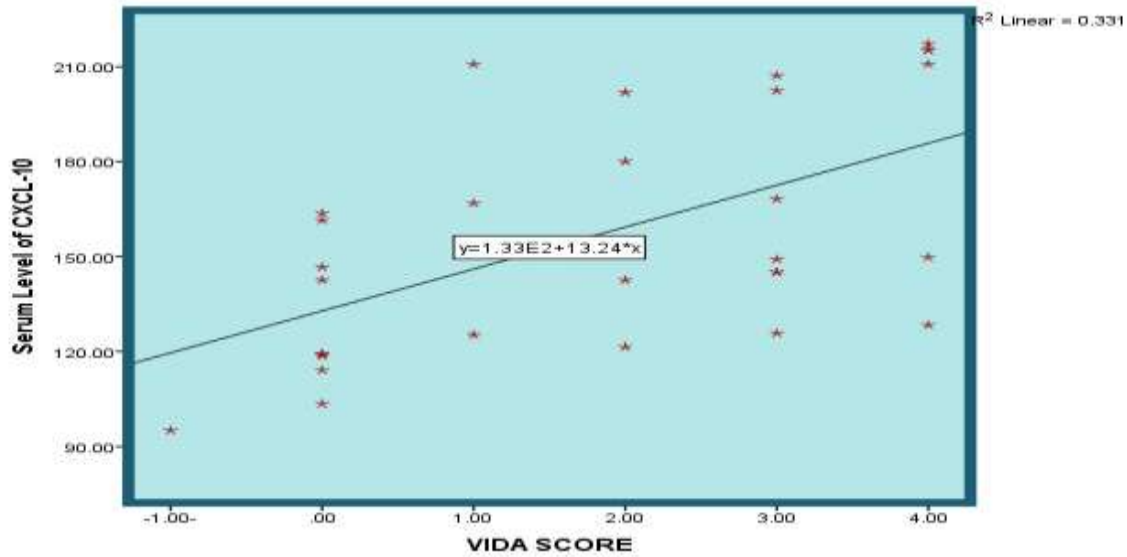


Figure (4): Correlation between the serum level of CXCL10 in vitiligo patients and vitiligo disease activity (VIDA) score of the studied cases.

Table (12): Correlation Between The Serum Level Of CXCL10 In Vitiligo Patients And (VASI) Score Of The Studied Cases; (N=30):

	(VASI) Score	
Serum Level of CXCL-10	$r = 0.655$	$p\text{-value} = 0.001^*$

r Spearman correlation coefficient

Table (12) demonstrates the serum level of CXCL-10 in vitiligo patients was moderately positive correlated with (VASI) score of the studied cases; where ($r=0.655$, $p\text{-value}=0.001$).

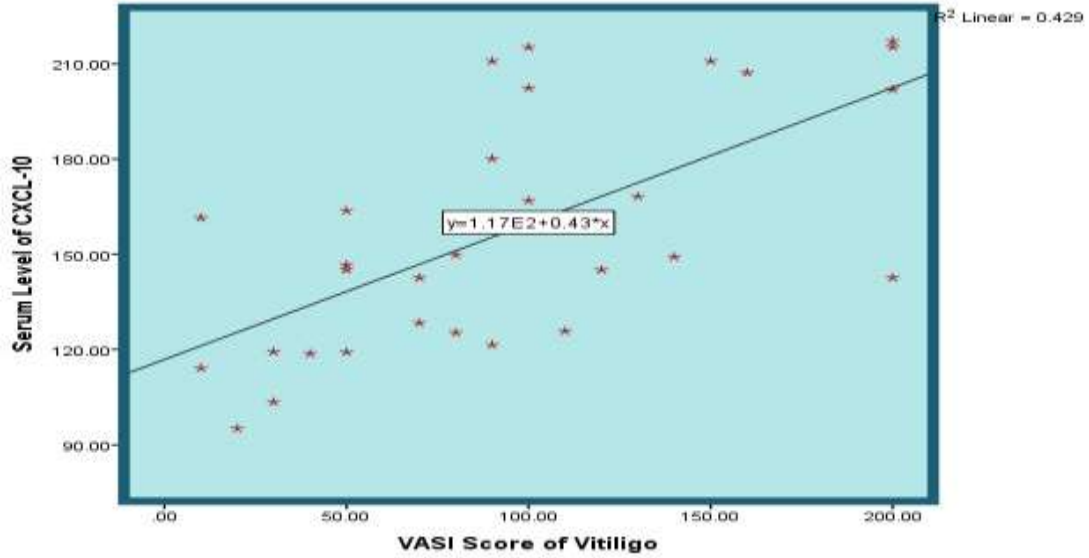


Figure (5): Correlation between the serum level of CXCL10 in vitiligo patients and (VASI) score of the studied cases.

Table (13): Correlation Between The Serum Level Of CXCL10 In Vitiligo Patients and Disease Duration Of The Studied Cases; (N=30):

	Disease Duration	
Serum Level of CXCL10	$r = 0.063$	$p\text{-value} = 0.704$

r Spearman correlation coefficient

Table (13) demonstrates no detected correlation between the serum level of CXCL10 in vitiligo patients and duration of disease of the studied cases; where (p-value > 0.050).

Table (14): Relation Between The Serum Level Of CXCL10 in Vitiligo Patients And Association With Psychological Stress Of The Studied Cases; (N=30):

	No N= 13	Yes N= 17	P-value
Mean ±SD	152.86 ±35.2	160.35 ±40.4	0.600
Minimum	114.20	95.10	
Maximum	215.30	217.20	
Range	101.10	122.10	

* *P-value ≤ 0.05 is considered significant by (Mann–Whitney U test).*

There were no detected relation between association with psychological stress and serum level of CXCL10 in vitiligo patients; p-value > 0.05.

Table (15): Relation Between The Serum Level Of CXCL10 In Vitiligo Patients And Family History Of The Studied Cases; (N=30):

	Negative N= 21	Positive N= 9	P-value
Mean ±SD	155.86 ±35.7	160.01 ±44.4	0.789
Minimum	95.10	103.50	
Maximum	215.20	217.20	
Range	120.10	113.70	

* *P-value ≤ 0.05 is considered significant by (Mann–Whitney U test).*

There were no detected relation between association with family history and serum Level of CXCL10 in vitiligo patients; p-value >0.05.

4. Discussion:

Vitiligo is a disorder of pigmentation characterized by the presence of depigmented skin macules due to chronic and progressive loss of the melanocytes from the cutaneous epidermis. Large population surveys have shown a worldwide incidence of 0.5-2%, with the disease beginning before the age of 20 years in 50% of cases. Susceptibility to vitiligo is not thought to be linked to gender, but 6-38% of the patients have family members with the disease, indicating a hereditary factor [21].

The discovery of a T-cell infiltrate in the lesion margins in inflammatory vitiligo was the first clue indicating participation of cellular immunity in the pathogenesis of vitiligo. Both helper and cytotoxic T cells promote Th1 response with the production of TNF- α and IFN- γ [22].

Melanocyte-specific CD8⁺ T cells were demonstrated at a higher frequency in both the blood and skin of patients with vitiligo and were capable of killing melanocytes in vitro [23].

Chemokines play an important role in regulating the homing of immune cells [24].

CXCL10 binds to its specific receptor, chemokine (C-X-C motif) receptor (CXCR)3, and regulates immune responses by the recruitment and activation of T cells, monocytes and natural killer cells. CXCR3 is expressed not only by the immune cells but also by endothelial cells, mesangial cells, thyrocytes and other epithelial cells. Recently, it has been shown that the tissue expressions of CXCR3 and CXCL10 are increased in various autoimmune diseases, and that they play fundamental parts in leukocyte homing into the inflamed tissues and contribute to the process of tissue damage [25].

The aim of the present study was to detect the level of CXCL10 in the serum of the vitiligo patients as compared to normal control persons to investigate the possible role of CXCL10 in the pathogenesis of this disease. The diagnosis was made on the existence of typical clinical picture of vitiligo. We performed this case-control study at dermatology outpatient clinic at Beni-Suef University hospital during the period from (1st May 2018 to 31 of May 2019) and the study included 60 participants; divided into 2 matched groups as 30 cases with vitiligo and 30 normal

controls. The average patient age was 34.0 \pm 9.3 (SD) years. In our study 63.3% of studied cases were females and 36.7% were males.

All the studied vitiligo cases were with non-segmental type (100%). Of them twenty cases (66.7%) were with active disease while ten cases (33.3%) were with stable activity of the disease. In our study 30% of the cases reported positive family history. And 56.7% of our studied cases reported associated psychological stress. The patients and controls were subjected to estimation of the serum level of CXCL10.

We evaluated the expression of CXCL10 in the serum and found that the serum level of CXCL10 in vitiligo patients was significantly higher as compared to normal control persons (p-value < 0.001), we also found that the patients in the progressive stage showed higher expression of CXCL10 than those in the stable stage (p-value = 0.002).

In consistent with our study [16] examined serum levels of CXCL10 and showed elevated CXCL10 levels in patients with vitiligo and also demonstrated that another treatment strategy for vitiligo would be to directly target CXCL10 or its receptor

CXCR3. This could possibly be a safer approach, as it interrupts the disease process further downstream without interfering with the other effectors of IFN- γ .

Our results were consistent with the study of [17] who reported that the serum CXCL10 levels were clearly elevated in both active and stable vitiligo patients compared with the healthy controls particularly in the progressive stages, introducing it as a possible activity marker and were positively correlated with Vitiligo Area Scoring Index (VASI) score. found that CXCL10, an IFN- γ induced chemokine, was elevated in the serum of the patients with vitiligo, and also found that CXCR3, its cognate receptor, was upregulated on autoreactive T cells in the blood and skin of patients with vitiligo and therefore can be therapeutically targeted to reverse depigmentation. [16,26]

It had been reported that the serum or tissue CXCL10 levels are increased in the organ-specific autoimmune diseases or systemic diseases [16,27].

In the present study we had no detected correlation between the serum level of CXCL10 in vitiligo patients and age of the studied cases, no correlation with the

duration of vitiligo disease, no detected relation with psychological stress and no relation with family history. Also no relation with gender of the studied cases.

However the serum level of CXCL10 in the Vitiligo patients was moderately positive correlated with the disease severity (VASI) score of the studied cases suggesting the potential utility of serum CXCL10 as a novel biomarker in monitoring the disease activity and useful indicator of therapeutic action in progressive vitiligo.

Also the serum level of CXCL10 in the vitiligo patients was moderately positive correlated with Vitiligo disease activity (VIDA) score of the studied cases suggesting an important role of CXCL10 in vitiligo.

The present study highlighted the potential role of CXCL10 as a reliable marker for the vitiligo activity: first by showing its significant elevation in the serum of patients compared to controls, next by detecting further significant rise in active versus stable cases.

5. Conclusion and Recommendations:

We concluded that the serum levels of CXCL10 might be a novel biomarker in monitoring disease activity and guiding treatment for progressive vitiligo and should be investigated further.

According to the present finding the following recommendation could be suggested.

- Using serum samples of CXCL10 as an accurate marker to monitor vitiligo disease activity and useful indicator of therapeutic action in progressive vitiligo.
- Additional studies focusing on new alternative therapeutic approach that target CXCL10 or its receptor and present a new and effective therapy for vitiligo.
- To date there are few studies concerning the role of CXCL10 in vitiligo. Further studies in a larger group of patients with longer follow up are required to address whether CXCL10 overexpression is a primary cause or a secondary event in vitiligo pathogenesis.

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