CXCL10 Serum Level in Relation to Clinical Scoring in Vitiligo Patients

F. K. Badran[1], K. F. El Mulla[1], N. F. Agamia[1], R. A. Ghazala[2], A. H. Ibrahim[3*]

Abstract

Background: Vitiligo stability is a vital prerequisite in the management of vitiligo. Vitiligo status based on patient history is an inaccurate tool to evaluate disease stability. Vitiligo disease activity score (VIDA) is considered as an indicator for disease activity. Recently, serum C-X-C motif chemokine ligand 10 (CXCL10) serve as a crucial indicator used for evaluating disease-related activity/stability. **Objective:** This study aimed to evaluate serum CXCL10 in relation to clinical assessment and VIDA score, to ascertain whether serum CXCL10 could be potential dependable serum marker for vitiligo activity and to define a serum CXCL10 level for activity. **Methods:** Clinical assessment and VIDA score were performed on one hundred patients diagnosed with the disease of vitiligo included in this cross-sectional research study. We measured the serum level of CXCL10 in vitiligo patients using the enzyme-linked immunosorbent assay (ELISA) method and a serum CXCL10 level for activity was defined. **Results:** The current study revealed that CXCL10 was significant in relation to clinical assessment and VIDA score. The sensitivity and specificity of measured biomarker was evaluated using the ROC curve. Serum level of CXCL10 more than 102 ng/L, the vitiligo lesion was considered active. **Conclusion:** Serum CXCL10 could be candidate as a biomarker to evaluate vitiligo activity. There is a positive relationship to disease activity, indicating that CXCL10 may play a significant role in the pathogenesis of vitiligo.

Key Words: CXCL10; VIDA; activity; vitiligo.

DOI Number: 10.14704/nq.2022.20.9.NQ44758 **Neuro Quantology 2022; 20(9):6471-6476**

Corresponding author: Ahmed Hassan Ibrahim

Affiliations:

1. Department of Dermatology, Andrology and Venereology, Faculty of Medicine, University of Alexandria, Alexandria, Egypt
2. Department of Medical Biochemistry, Faculty of Medicine, University of Alexandria, Alexandria, Egypt
3. Department of Dermatology, Andrology and Venereology, Alexandria Military Hospital, Alexandria, Egypt.

Relevant conflicts of interest/financial disclosures:
The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Introduction

Vitiligo is a depigmentation disorder that develops as a result of multiple etiologies, causing severe emotional and psychological distress (1). Numerous pathogenetic connected elements, including neuro associated theory, amount of hereditary impact, and inadequate antioxidative defense, have been proposed for future inquiry in order to evaluate the vitiligo illness linked genesis. Multiple events, which may be genetically, environmentally, biochemically, or immunologically connected, combine to create a permissive complex milieu. As a result, the melanocyte loss seen in vitiligo is the product of numerous processes working in concert (2).

The incidence of vitiligo ranged from 1 to 2% of the overall population. It might impact people of various colors, ages, and socioeconomic backgrounds. Almost half of persons suffering with vitiligo are younger than 20 years old (3). Vitiligo is clearly visible as macules or patches of depigmentation with no symptoms of irritation in the surrounding normal skin regions. The visible macules appear as a well-defined milky-white, circular, oval, or linear shaped area. The patch size varies between a few millimeters and a few centimeters. The face, neck, hands dorsum, scalp, or trunk are the most common areas where vitiligo is seen. Lesions on the face were discovered to be perioral and periocular. Vitiligo lesions can also appear in areas that are often exposed to trauma, such as the knees and elbows. Koebnerization occurs in around 20% to 60% of vitiligo sufferers (4).

Vitiligo stability is seen as an essential need before having a vitiligo surgical intervention and is a crucial care guideline for diagnosed individuals. The medical history of a patient cannot be used to identify the severity of the vitiligo condition (5). Many studies have examined the clinical characteristics that assist determine the stability of vitiligo as well as the ultrastructural, serological, and biochemical evaluating criteria to distinguish between stable and active vitiligo. Understanding the illness pathophysiology and its widely linked theories has led to the development of the majority of these parameters (6). The distinction between the illness's stability and its activity in vitiligo patients is first determined to be necessary in order to make an effective treatment choice, particularly when discussing surgical therapy and for early management of disease development. Additionally, one of the disease-related prognostic factors that may be considered is the stability or activity of the vitiligo condition (7).

Even though there are several ways for determining the presence of vitiligo, it is still difficult to confirm, and the procedures that are now in use are either not accurate or even impractical for use in routine clinical practice (8). The lack of new apparent lesions over the previous two years, the absence of old lesions progressing, the absence of the Koebner phenomenon, and the repigmentation of lesions as a result of medically administered treatments are among the clinically significant criteria used to assess the stability extent of incident vitiligo (7).

Vitiligo activity and stability over time have been evaluated using a six-point scoring system called VIDA. Based on the patient's diagnosis, the VIDA score is calculated (7). Lower VIDA scores determine less vitiligo activity (9). However, because it is based on the patient's own words, judgment, and memories, the findings of this subjective scoring are seen as being erroneous. Despite various clinical signs being highly predictive of vitiligo activity/stability in connection to clinical assessment, there is no reliable scoring system to confirm the condition's clinical activity or stability. However, relying exclusively on the patient's recollection or perception is problematic since there are generally few objective signs that evaluate the activity or stability of the condition. The presence of CD8+ cytotoxic cells in active perilesional skin has been shown in several studies to be a sign for activity and a bad prognosis (10).

The requirement for skin biopsies, however, is a real barrier to precise disease activity detection. Therefore, it seems like a more workable option to look for activity indicators in the serum (11). There is evidence that suggests cytokines have a significant role in the pathophysiology of autoimmune illness and the enhancement of disease activity. There is no agreement on the relationship between the number of cytokines studied and the severity of the condition, such as interleukin-2 (IL-2), IL-4, IL-6, IL-17, tumor necrosis factor (TNF), and interferon (IFN) (12).

CXCL-10 has been more prominent in recent years as a potential vitiligo activity marker. IFN-induced chemokine CXCL-10 attracts lymphocytes to the areas of vitiligo lesions and binds to its receptor, CXCR-3, which is present on lymphocytes, causing

IFN-production to continue in a continual positive loop (13).

Methods:

Study involved design and ethics:

This observational cross-sectional research study was including about one hundred patients clinically diagnosed with vitiligo at the dermatology department of Alexandria University, Egypt. The local ethics committees gave their approval for the study [*IRB NO: 00007555; FWA NO: 00015712*] and performed in accordance with the Helsinki Declaration. All the participants were asked for their written informed permission prior to their actual involvement in the study. Patients with vitiligo of both genders, found to be 18 to 60 years old with VASI score ranged from 10% to 15% and patients with active lesions only or stable lesions were only included while patients with active and stable lesions at same time were excluded and also patients diagnosed with other detected hypopigmentation or depigmentation related disorders as well. All the participating patients were subjected to a) Full documentation of their history involving their personal history (age, sex); present vitiligo related history (onset age, course, and disease duration); history of prior illnesses, prior therapies, history of vitiligo in the family, or history of any autoimmune diseases. b) A general clinical examination to rule out conditions such diabetes mellitus, hyperthyroidism, and autoimmune disorders as well as conditions that can affect CXCL10 levels. Clinical full documentation to the patient's history was done, with clinical assessment for vitiligo stability as lack of detecting new lesions within the previous 2 years, no of pre-existing lesion extensions meanwhile and lack of the phenomenon of Koebner.

VIDA score was recorded as follows:

VIDA score	Disease activity
+4	Active in the past 6 weeks.
+3	Active in the past 3-6 months.
+2	Active in the past 6-12 months.
+1	Active in the past year.
0	Stable for at least one year.
-1	Stable for at least one year with spontaneous repigmentation.

Each patient who was participating in the study had a blood sample taken in order to evaluate the serum CXCL10 level using an ELISA kit (*MyBioSource, San Diego, CA, USA. Catalog # MBS824608*). An ELISA kit was used to measure the concentration of human CXCL10 in serum using the traditional sandwich ELISA method. The human CXCL10 sample serum level is evaluated using ELISA kits that use a double-antibody sandwich ELISA technique. A human CXCL10-specific antibody is used in this test.

Statistics/data analysis:

The outcome counts and percentages were used to summarize and display categorical data and means and standard deviations were calculated for numerical variables based on their summaries and assessments. The median with range or interquartile range was calculated for data that were normally distributed, but the Kolmogorov-Smirnov was used to confirm the normality of the distribution for data that were not regularly distributed. Additionally, sensitivity and specificity were both determined using a 95% confidence interval (CI). Spearman coefficient was used to correlate between CXCL10 with clinical assessment and VIDA score. Significance of the obtained results was judged at the 5% level. The area under the receiver operating characteristic curve (AUROC) and its 95% confidence interval (CI) were calculated after a receiver operating characteristic (ROC) curve was plotted to predict the quantitative activity level of serum CXCL10 against the clinical assessment and VIDA score. IBM SPSS software package version 20.0 was used to conduct the statistical analysis. (*Armonk, NY: IBM Corp*).

Results:

- **Patient demographics:**

The study involved 100 patients with vitiligo was compromising about 58 female and also 37 men having age ranges from 18 to 58 years old with a calculated mean of 31.0 ± 10.54 SD. 36 patients were found having skin type III while 64 patients were investigated having a skin type IV whereas 16 vitiligo diagnosed patients (16%) confirmed a positive family history.

- **Assessment of vitiligo status:**

The stability/activity of vitiligo was evaluated by applying two approaches: clinical assessment and VIDA score

1. Clinical assessment: according to clinical assessment, 60% of cases were clinically active while 40% of cases had stable lesions.

2. VIDA score: according to VIDA score, the disease was found to be stationary (stable) in course among 36 (36%) patients. Meanwhile, 64 patients (64%) showed a progressive (active) course.

- **Assessment of Serum CXCL10 level:**

Regarding CXCL10 serum level in selected patients, the range was from 49 to 471 ng/L with median 114.8 ng/L with mean and standard deviation 132.6 ± 74.42.

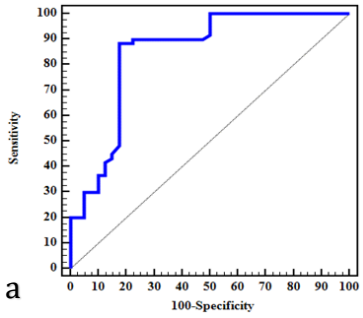
Evaluation of Serum CXCL10 level in relation to vitiligo activity parameters and cutoff activity level determination:

1- The ROC curve analysis for CXCL10 serum level to assess activity of vitiligo patients in relation to clinical assessment and its validity and a cutoff point determination for stability reveals sensitivity 88.33%, specificity 82.50%, 95% C.I = 0.763 – 0.933 and the cutoff CXCL10 activity level was >102 ng/L. **(Figure 1a).**

2- The ROC curve analysis for CXCL10 serum level to assess activity of vitiligo patients in relation to VIDA score and its validity and a cutoff point determination for stability reveals sensitivity 92.19%, specificity 97.22%, 95% C.I = 0.929 – 1.000 and the cutoff CXCL10 activity level was >102 ng/L. **(Figure 1b).**

From previous data, we denote that VIDA score had the higher specificity and sensitivity in relation to serum CXCL10 level and the cutoff activity level was >102 ng/L in relation to the two activity predictors where 58% of cases were active and 42% of cases were stable.

AUC	p	95% C.I	Cut off	Sensitivity	Specificity	PPV	NPV
0.987	<0.001*	0.763 – 0.933	>102#	88.33	82.50	88.3	100.0



AUC	p	95% C.I	Cut off	Sensitivity	Specificity	PPV	NPV
0.9 6	<0.001*	0.929–1.000	>102#	92.19	97.22	98.3	93.1

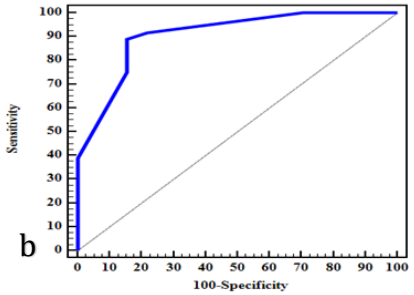


Figure (1): ROC curve for CXCL10 level for discriminating the activity of vitiligo among the patients according to clinical assessment **(a)** and VIDA score **(b).**

P value: Probability value; **CI:** Confidence Intervals; **NPV:** Negative predictive value; **PPV:** Positive predictive value; ***:** Statistically significant at p ≤ 0.05; #Cut off was choose according to Youden index

As a result of previous results, regarding CXCL10 serum level, 60% of cases were more than 102 ng/L while 40% of cases were equal or less than 102 ng/L

Accuracy and comparison of serum CXCL10 level in relation to activity parameters:

The serum CXCL10 level accuracy in reference to the involved clinical assessment and VIDA score was 86% and 94% respectively with highest accuracy with clinical assessment. **(Table 1)**

Table (1): Accuracy of CXCL10 to discriminate activity status of vitiligo

	Total (n = 100)	CXCL10 ≤102 (Stable) (n = 40)		CXCL10 >102 (Active) (n = 60)		χ^2	p	Accuracy
	No.	No.	%	No.	%			
Clinical assessment								
Stable	40	33	82.5	7	17.5	50.174*	<0.001*	86%
Active	60	7	11.7	53	88.3			
VIDA								
Stable	36	35	97.2	1	2.8	76.743*	<0.001*	94%
Active	64	5	7.8	59	92.2			

X^2: Chi square test; P: P value for comparing between ≤102 (Stable) and >102 (Active); *: Statistically significant at p ≤ 0.05

Discussion

The course of the acquired illness vitiligo varies. Clinically, it is indicated by clearly defined, depigmented macules or patches that are assumed to result from melanocyte malfunction and loss. It is the most prevalent depigmentation condition, affecting 0.5% to 2.0% of people, and is neither gender- nor race-specific. Nonsegmental and segmental subtypes of vitiligo are distinguished,

with the latter affecting only a small proportion of people (5%–16%) (14).

Vitiligo's cause is not well known. Stress, trauma, sun exposure, infections, malignancies, neurological abnormalities, melatonin receptor failure, delayed melanocyte migration, certain medicines, endocrine illnesses, and cytotoxic substances have all been linked to the development of vitiligo. These causative variables may also work independently. The main elements targeting melanocytes in vitiligo are CTLs. The number of CTLs is noticeably higher in vitiligo patients, both in the peripheral blood and in skin lesions (15).

Increased interferon gamma and tumor necrosis factor alpha production in inflamed tissues is caused by recruited Th1 lymphocytes, which in turn trigger inflammatory cells to secrete CXCL10. By attracting and activating T cells and monocytes, CXCL10 modulates immunological responses by binding to its particular receptor, the chemokine CXCR3. It has been demonstrated that different autoimmune illnesses result in higher tissue expression levels of CXCR3 and CXCL10, which are crucial for leukocyte homing to inflamed tissues and for the development of tissue damage. It is uncertain, nevertheless, if CXCL plays a role in the etiology of vitiligo. In order to determine whether there is a potential connection between serum CXCL10 level and disease activity, we calculated the serum CXCL10 level in vitiligo patients (16).

In current study, we developed and validated a cutoff serum CXCL10 level denoting vitiligo status activity/stability. The reliability of this cutoff level was confirmed by its correlation and comparison with other vitiligo status parameters, which was tested in a large number of patients (n = 100) in which the patient age included was ranging from 18 -58-year-old with a mean 31.0 ± 10.54 SD. In agreement with many previous studies as **Paradisi et al.** (17) and Brazilian study (18), the female patients outnumbered the males, which may be explained by women are more likely to seek treatment, for the cosmetic disfigurement caused by vitiligo.

In the current study, the patient's skin phototypes were skin type III- IV. According to **Grimes et al.** (19), the disease displays no related racial, ethnic, or socioeconomic inclination. However, it is considered most abundant to occur among persons with highly pigmented skin. The family history was positive in range of 16% of our patients, a finding which is compatible with other previous studies as **Silverberg** (20) and **Vora** survey (21). Regarding clinical assessment in present performed study, about 60 patients (60%) were diagnosed active and other 40 patients (40%) were considered stable.

Regarding VIDA score in present study, about 64 patients (64%) were diagnosed active and other 36 patients (36%) were considered stable. In accordance to **Wang et al.** (22), **Gharib et al.** (23) and **Maouia et al.** (24) who considered the CXCL10 serum level as a potentially used vitiligo clinical marker that examined by flow cytometry, it was determining a positive correlation that is significant statistically among vitiligo activity and levels of CXCL10 where it was found higher in active versus stable vitiligo subgroup.

Regarding the relation between CXCL10 serum level and stability, **Abdallah et al.** (13) introduced a cutoff value for determining the stability/activity of vitiligo depending on ROC analysis, where CXCL10 level >100 ng/L was recognized to be active having a sensitivity of 83.3% and specificity of 96.0%. In present study, according to the CXCL10 serum level, 60 cases were active, while 40 cases were stable with a mean CXCL10 serum level was 132.6 ± 74.42 SD while in **Ibrahim et al.** (8) 57 patients (58.8%) diagnosed with active disease, and other 40 patients (41.2%) considered stable vitiligo with total mean CXCL10 serum level was 96.85 ± 27.65 SD, this difference may be explained by increased number of active cases in our study and patients selected in our study had active or stable lesion only (not both as in Ibrahim et al. study) to have more accurate results.

In **Gharib et al.** study (23), the serum level of CXCL10 ranged from 283 to 496 pg/mL in patients with active vitiligo and from 91 to 275 pg/mL in stable patients and a CXCL10 level of greater than 291 pg/mL had a sensitivity of 95% and specificity of 100%, with high statistical significance in predicting the activity state of vitiligo. **AL-Mousawi et al.** (25) define a CXCL10 serum level to differentiate between stable and active cases, at a cut-off value of 72.7, the sensitivity was 58.6% and specificity was 60.0%. The cutoff level of serum CXCL10 to discriminate activity of vitiligo patients was more than 102 ng/L according to the ROC curve done to assess the level of CXCL10 in serum regarding the performed clinical assessment and VIDA score.

In comparison to other studies, we added a new cutoff value to discriminate status of vitiligo patients. In current study, the cutoff level of serum CXCL10 more than 102 ng/L indicates activity of the lesion while in **Al-Mousawi et al.** (25), **Abdallah et al.** (13), **Gharib et al.** (23) studies, the cutoff level of serum CXCL10 more than 72.7 ng/L, 100 ng/L, 291 ng/L, respectively denotes activity of vitiligo lesion.

Conclusion

A potential possibility for a biomarker to assess the activity of vitiligo is serum CXCL10. CXCL10 may be important in the development of vitiligo as evidenced by the positive connection to disease activity. The authors express their gratitude to the patients who participated in this research investigation.

REFERENCES

1. **Valle Y, Korobko I, Sigova J, et al.** Patient-reported outcomes: A 5-year long study reveals previously unreported therapeutic, demographic, socio-economic, and other correlations in vitiligo. Dermatol Ther. 2018;31(5):e12620.
2. **Vaccaro M, Cicero F, Mannucci C, et al.** IL-33 circulating serum levels are increased in patients with non-segmental generalized vitiligo. Arch Dermatol Res. 2016;308(7):527-30.
3. **Kim MS, Cho EB, Park EJ, Kim KH, Kim KJ.** Effect of excimer laser treatment on vitiliginous areas with leukotrichia after confirmation by dermoscopy. Int J Dermatol. 2016;55(8):886-92.
4. **Ahmed JN, Masood S. Vitiligo. Treasure Island, FL:** StatPearls Publishing, 2021.
5. **Nirmal B, Antonisamy B, Peter CVD, George L, George AA, Dinesh GM.** Cross-Sectional Study of Dermatoscopic Findings in Relation to Activity in Vitiligo: BPLeFoSK Criteria for Stability. J Cutan Aesthet Surg. 2019;12(1):36-41.
6. **Sahni K, Parsad D.** Stability in Vitiligo: Is there a Perfect Way to Predict it? J Cutan Aesthet Surg. 2013;6(2):75-82.
7. **Purnima NA, Gudivada T, Narasimharao TV. Dermoscopy -** A tool to Assess Stability in Vitiligo. Int J Contemp Med Res. 2017;4(10):2066-8.
8. **Ibrahim S, Hegazy RA, Gawdat HI, et al.** Differentiating active from stable vitiligo: The role of dermoscopic findings and their relation to CXCL10. J Cosmet Dermatol. 2022:doi.10.1111/jocd.14922.
9. **Feily A.** Vitiligo Extent Tensity Index (VETI) score: a new definition, assessment and treatment evaluation criteria in vitiligo. Dermatol Pract Concept. 2014;4(4):81-4.
10. **van Geel N, Grine L, De Wispelaere P, Mertens D, Prinsen CAC, Speeckaert R.** Clinical visible signs of disease activity in vitiligo: a systematic review and meta-analysis. J Eur Acad Dermatol Venereol. 2019;33(9):1667-75.
11. **Benzekri L, Gauthier Y, Hamada S, Hassam B.** Clinical features and histological findings are potential indicators of activity in lesions of common vitiligo. Br J Dermatol. 2013;168(2):265-71.
12. **Gupta LK, Singhi MK.** Wood's lamp. Indian J Dermatol Venereol Leprol. 2004;70(2):131-5.
13. **Abdallah M, El-Mofty M, Anbar T, et al.** CXCL-10 and Interleukin-6 are reliable serum markers for vitiligo activity: A multicenter cross-sectional study. Pigment Cell Melanoma Res. 2018;31(2):330-6.
14. **Kumar Jha A, Sonthalia S, Lallas A, Chaudhary RKP.** Dermoscopy in vitiligo: diagnosis and beyond. Int J Dermatol. 2018;57(1):50-4.
15. **Bambroo M, Pande S, Khopkar US.** Dermoscopy in the differentiation of Idiopathic Guttate Hypomelanosis (IGH). In: Khopkar U, (ed). Dermoscopy and Trichoscopy in Diseases of the Brown Skin: Atlas and Short Text. New Delhi: Jaypee Brothers Medical Publishers, 2012. 97-103.
16. **Lee DY, Kim CR, Park JH, Lee JH.** The incidence of leukotrichia in segmental vitiligo: implication of poor response to medical treatment. Int J Dermatol. 2011;50(8):925-7.
17. **Paradisi A, Tabolli S, Didona B, Sobrino L, Russo N, Abeni D.** Markedly reduced incidence of melanoma and nonmelanoma skin cancer in a nonconcurrent cohort of 10,040 patients with vitiligo. J Am Acad Dermatol. 2014;71(6):1110-6.
18. **Rao A, Gupta S, Dinda AK, et al.** Study of clinical, biochemical and immunological factors determining stability of disease in patients with generalized vitiligo undergoing melanocyte transplantation. Br J Dermatol. 2012;166(6):1230-6.
19. **Grimes PE.** New insights and new therapies in vitiligo. JAMA. 2005;293(6):730-5.
20. **Silverberg N.** The Epidemiology of Vitiligo. Curr Dermatol Rep. 2015;4(1):36-43.
21. **Vora RV, Patel BB, Chaudhary AH, Mehta MJ, Pilani AP.** A Clinical Study of Vitiligo in a Rural Set up of Gujarat. Indian J Community Med. 2014;39(3):143-6.
22. **Wang XX, Wang QQ, Wu JQ, et al.** Increased expression of CXCR3 and its ligands in patients with vitiligo and CXCL10 as a potential clinical marker for vitiligo. Br J Dermatol. 2016;174(6):1318-26.
23. **Gharib K, Gadallah H, Elsayed A.** Chemokines in Vitiligo Pathogenesis: CXCL10 and 12. J Clin Aesthet Dermatol. 2021;14(9):27-32.
24. **Maouia A, Sormani L, Youssef M, Helal AN, Kassab A, Passeron T.** Differential expression of CXCL9, CXCL10, and IFN-γ in vitiligo and alopecia areata patients. Pigment Cell Melanoma Res. 2017;30(2):259-61.
25. **AL-Mousawi ZR, Alattabi AS, Hamza DM, AL-Hussiny FA.** Serum Level of CCL20 and CXCL 10 in Patients with Vitiligo and their Association with Disease Activity. Indian Journal of Forensic Medicine & Toxicology. 2021 May 17;15(3):1147-56.