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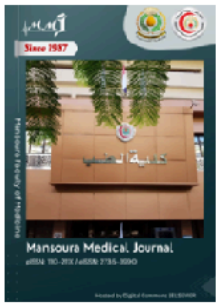

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Assessment of Serum Levels of CXCL10 in Patients with Non-Segmental Vitiligo

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ORIGINAL STUDY

Assessment of Serum Levels of C-X-C Motif Chemokine Ligand 10 in Patients With Nonsegmental Vitiligo

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Abstract

Background: **Vitiligo is the most common skin depigmentation disorder. Destruction of melanocytes results in depigmented skin lesions. Pathogenesis is not fully understood and mostly is multifactorial. However, the autoimmune theory is the most widely accepted one. Interaction between genetic, environmental, biochemical, and immunologic factors contribute to the disease. Cytokines have an important role in the development of the disease and its progression.**

Aim: **The aim of the study is to measure the blood levels of C-X-C motif chemokine ligand 10 (CXCL10) in vitiligo and to correlate it with disease activity and severity.**

Patients and methods: **This is a case−control study that enrolled 60 vitiligo patients (30 had active vitiligo and 30 had stable vitiligo) and 30 healthy age-matched and sex-matched controls. Vitiligo extent score (VES) was calculated. CXCL10 was measured in venous blood samples using enzyme-linked immunosorbent assay.**

Results: **CXCL10 levels were significantly higher among vitiligo patients compared with controls ($P < 0.001$). Also, CXCL10 levels in vitiligo patients were significantly higher in the active group compared with the stable group ($P < 0.001$). There was statistically significant positive correlation between CXCL10 levels and VES score ($P = 0.003$). Median CXCL10 (pg/ml) in controls was 2.38 versus 8.13 in patients. Furthermore, in vitiligo patients, the median CXCL10 (pg/ml) was 9.5 in the active group versus 7.6 in the stable group.**

Conclusions: **Serum levels of CXCL10 were significantly higher in vitiligo patients compared with healthy controls. Furthermore, patients with active vitiligo had higher levels of CXCL10 compared with the stable group. Moreover, patients with higher VES had higher CXCL10 serum levels.**

Keywords: CXCL10, Nonsegmental vitiligo, Vitiligo Extent Score (VES)

1. Introduction

Vitiligo is an acquired immune-mediated disorder of pigmentation clinically characterized by well-defined depigmented or chalk-white macules and patches on the skin. The prevalence of vitiligo varies by geographical area, affecting 0.5−2% of the population. The disease imposes a significant psychological burden due to its major impact on patients' social and emotional aspects of life (Hlača et al., 2022).

Vitiligo is classified as nonsegmental vitiligo (NSV), segmental vitiligo, and unclassified/undetermined vitiligo. NSV is characterized by depigmented macules that vary in size from a few to several centimeters in diameter. Macules may be present on both sides of the body and tend to have symmetrical distribution. Segmental vitiligo is characterized by

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depigmented macules strictly involving one or more segments of the body. These lesions never cross the midline (Ezzedine et al., 2012).

Although theories on the pathogenesis of vitiligo are diverse, including stress, infection, psychological damage, and hereditary susceptibility, the definitive pathogenesis remains unknown (Lambe et al., 2006). At present, many studies have shown that vitiligo is an autoimmune response targeting melanocytes (Ongenae et al., 2003; Richmond et al., 2013).

Many kinds of immune cells as well as cytokines participate in this response, the most important of which are melanocyte-specific CD8+ T cells (Van Den Boorn et al., 2009).

Immunohistochemistry showed a significant T-cell response, with pronounced dermal infiltrates of CD8+ T cells in vitiligo; and chemokines play an important role in regulating the homing of immune cells (Vazirinejad et al., 2014). A significantly increased expression of the chemokine receptor CXCR3 and its ligands C-X-C motif chemokine ligand 10 (CXCL10) were found in skin lesions of progressive vitiligo demonstrating a major role of the CXCL10/CXCR3 axis in T-cell recruitment in vitiligo (Yang et al., 2018).

CXCL10, an interferon-gamma (IFN-γ) induced chemokine (Groom and Luster, 2011), was found to be elevated in the serum of patients with vitiligo, and CXCR3, its cognate receptor, was upregulated on autoreactive T cells in the blood and skin of a vitiligo mouse model and in patients with vitiligo (Yang et al., 2018; Rashighi et al., 2014; Wang et al., 2016). The IFN-γ/CXCL10 axis was demonstrated to be functionally required for both progression and maintenance of the disease in a mouse model, and therefore can be therapeutically targeted to reverse depigmentation. Harris abd colleagues, Rashighi and colleagues, and Maouia and colleagues found that CXCL10 in the serum of patients with vitiligo was significantly elevated compared with healthy controls. They further found that CXCL10 serum levels were higher in patients in the progressive stage compared with those in the stable stage of vitiligo, but they did not find any correlation between the serum level of CXCL10 and the Vitiligo Area Scoring Index score (Rashighi et al., 2014; Harris et al., 2012; Maouia et al., 2017).

Hypothesis. the levels of CXCL10 in the circulating blood are increased in vitiligo (subsequently, naive CD8+ T cells are attracted to the lesion and activated into effector T cells to attack autologous melanocytes).

In this study, we measure the blood levels of CXCL10 in 60 patients with NSV comparing it with the levels in 30 healthy controls. Also, we correlate CXCL10 with disease activity and severity.

2. Patients and methods

This case–control study enrolled 60 patients suffering from NSV (30 had active vitiligo and 30 had stable vitiligo). In addition, 30 apparently healthy individuals of matched age and sex with no skin disease were chosen as a control group. Patients who received systemic treatment as steroid compounds, NSAIDs, and immunosuppressants within 1 month and those with systemic diseases, especially autoimmune ones were excluded. Pregnant and lactating females were excluded. The patients were recruited from the Dermatology Outpatient Clinic of Mansoura University Hospital during the period from August 2020 to October 2021. The study protocol was approved by the Institutional Research Board (MS.20.06.1157).

2.1. Methods

Each patient was subjected to the following:

(1) Detailed history taking and complete general and skin examination.
(2) Skin examination to assess site, symmetry, clinical type, activity, and stability of vitiligo. Active vitiligo is defined as patients with an appearance of new lesions or a progression of old lesions within the last 12 months. Lesions that did not progress within 12 months were considered stable vitiligo (Ezzedine et al., 2012).
(3) Vitiligo extent score (VES) calculation using website https://www.vitiligo-calculator.com.
(4) Venous blood samples were obtained from all participants to estimate the CXCL10 level using enzyme-linked immunosorbent assay according to manufacturer recommendations (www.bt-laboratory.com; Bioassay Technology Laboratory, Shanghai, China).

2.2. Statistical analysis

Data were analyzed by IBM Corp. Released (2017) (IBM SPSS Statistics for Windows, Version 22.0.; IBM Corp., Armonk, New York, USA) Qualitative data were represented as numbers and percentage. Quantitative data were represented as medians (minimum and maximum, interquartile ranges) for nonnormally distributed data and means, SDs for normally distributed data after testing for normality using the Kolmogorov–Smirnov test. Significance of a result was judged at the 0.05 level.

3. Results

The mean age of the vitiligo group was 39.4 years; there were 21 (35%) males and 39 (65%) females in addition to 30 healthy control group of matched age and sex ($P > 0.05$ for each; Table 1). The vitiligo group was further divided into stable and active subgroups, with no significant differences between them regarding age and sex ($P > 0.05$ for each). Furthermore, comparing control to stable or active subgroups revealed no significant differences between the control and each subgroup regarding age and sex ($P > 0.05$ for each) (Table 2, Fig. 1):

(1) Median baseline VES was 0.7, which ranged from 0.05 to 74.13 in all studied cases.
(2) Regarding stable subgroup, median baseline VES was 0.71, which ranged from 0.05 to 30.
(3) Regarding active subgroup, median baseline VES was 0.73, ranged from 0.07 to 74.13.
(4) No significant differences were found between active and stable cases regarding VES ($P > 0.05$; Table 3).
(5) The vitiligo group showed significantly higher levels of CXCL10 when compared with the control group (median = 8.13 vs. 2.38; $P1<0.001$).
(6) The stable subgroup showed significantly higher levels of CXCL10 when compared with the control group (median = 7.60 vs. 2.38; $P2<0.001$).
(7) The active subgroup showed significantly higher levels of CXCL10 when compared with the stable subgroup (median = 9.50 vs. 7.60; $P4<0.001$) (Fig. 2).
(8) CXCL10 levels showed significant positive correlation with VES ($P = 0.003$), while no significant correlation was found between CXCL10 levels with age of vitiligo patients ($P > 0.05$; Tables 4 and 5).
(9) CXCL10 levels showed significant positive correlation with VES in stable as well as in active vitiligo cases ($P = 0.039$, $P < 0.001$, respectively), but not with patients' age in both subgroups ($P > 0.05$ for each).

Table 2. Vitiligo extent score in all studied Vitiligo cases.

	Vitiligo			*P*
	Total ($N = 60$)	Stable ($N = 30$)	Active ($N = 30$)	
VES				
Median	0.71	0.71	0.73	
Minimum	0.05	0.05	0.07	0.684
Maximum	74.13	30	74.13	

VES, vitiligo extent score.
Mann–Whitney test was used for comparison.

4. Discussion

Many reports emphasize the critical role of immune cells and their mediators in the immunopathogenesis of vitiligo. Oxidative-stress-mediated activation of innate immunity cells such as dendritic cells, natural killer, and ILC-1 cells is thought to be a key event in the early onset of vitiligo. Innate immunity cells serve as a bridge to adaptive immunity cells including T helper 1 cells, cytotoxic T cells, and resident memory T cells. IFN-γ is the primary cytokine mediator that activates the JAK/STAT pathway, causing keratinocytes to produce the key chemokines CXCL9 and CXCL10. Complex interactions between immune and nonimmune cells finally result in apoptosis of melanocytes (Hlača et al., 2022).

Autoreactive cytotoxic $CD8^+$ T cells engage melanocytes and promote disease progression through the local production of IFN-γ, and IFN-γ-induced chemokines (C-X-C motif chemokine ligands 9, 10, and 11) are then secreted from the surrounding keratinocytes to further recruit T cells to the skin through a positive-feedback loop causing progressive destruction of more melanocytes as the disease spreads (Frisoli et al., 2020).

Vitiligo occurs equally in males and females; however, females tend to seek medical advice more than males (Bergqvist and Ezzedine, 2020). In this study, females represented 65% of the vitiligo group compared with 35% only of males.

Table 1. Comparison of demographic data between studied groups and subgroups.

	Control ($N = 30$)	Vitiligo			*P1*	*P2*	*P3*	*P4*
		Total ($N = 60$)	Stable ($N = 30$)	Active ($N = 30$)				
Age (years)								
Mean ± SD	40.6 ± 13.2	39.4 ± 12.1	40.4 ± 11.6	38.5 ± 11.3	0.700	0.947	0.550	0.596
Males								
n (%)	14 (46.7)	21 (35.0)	10 (33.3)	11 (36.7)	0.285	0.292	0.432	0.787
Females								
n (%)	16 (53.3)	39 (65.0)	20 (66.7)	19 (63.3)				

Student's *t* test was used for numerical parameters; χ^2 test was used for categorical parameters; *P*1, comparison between all studied cases and control groups; *P*2, comparison between stable vitiligo and control groups; *P*3, comparison between active vitiligo and control groups; *P*4, comparison between stable and active vitiligo cases.

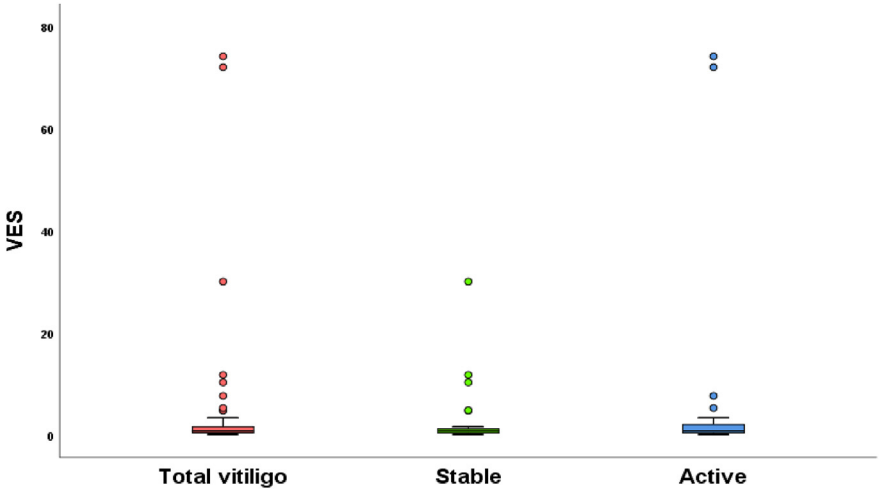

Fig. 1. VES score in vitiligo cases. VES, vitiligo extent score.

CXCL9 and CXCL10, in parallel with their receptor, CXCR3, are present in vitiligo and correlate with disease activity and severity. Infiltrating T cells in patients with progressive vitiligo are recruited at least in part due to overactivated CXCL10/CXCR3 signaling pathways (Putri et al., 2019).

This study revealed that the vitiligo group had significantly higher levels of CXCL10 when compared with the control group (median = 8.13 vs. 2.38; *P*1<0.001). Moreover, the active subgroup had significantly higher level of CXCL10 when compared with the stable subgroup (median = 9.50 vs. 7.60; *P*4<0.001). Our results agree with Maouia and colleagues who found that CXCL10 in the serum of patients with vitiligo was significantly elevated compared with healthy controls. They further found that CXCL10 serum levels were higher in patients in the progressive stage compared with those in the stable stage of vitiligo (Maouia et al., 2017).

Although this study did not reveal significant differences between active and stable cases regarding VES ($P > 0.05$), there was significant positive correlation between CXCL10 serum levels and VES ($P = 0.003$). Previous studies have shown the same correlation between CXCL10 levels and vitiligo indices expressing disease activity and severity, and they considered that serum CXCL10 can be a new biomarker in monitoring disease activity and severity (Wang et al., 2016; Putri et al., 2019). This needs to be proven through future studies on a larger number of patients.

Reliable phenotypic or clinical markers for vitiligo progression are scarce. The use of an objective marker for disease activity in vitiligo would help guide possible treatments (Speeckaert et al., 2017). CXCL10 is a potential biomarker that can demarcate between stable and active vitiligo. Trichrome sign, confetti-like depigmentation, and the Koebner phenomenon have been reported as clinical features of progressive vitiligo. Presence of these clinical markers was associated with significantly higher levels of CXCL10 (Zhang et al., 2020).

Targeting CXCL10 prevented disease development in mice and induced repigmentation in established lesions, implicating this chemokine as a critical player in the progression and maintenance of vitiligo. Results support that CXCL10 neutralization can be a base for targeted systemic therapeutic approach in the treatment of vitiligo (Rashighi et al., 2014).

Table 3. Comparison of C-X-C motif chemokine ligand 10 levels among cases and control groups and subgroups.

	Control ($N = 30$)	Vitiligo			*P*1	*P*2	*P*3	*P*4
		Total ($N = 60$)	Stable ($N = 30$)	Active ($N = 30$)				
CXCL10 (pg/ml)								
Median	2.38	8.13	7.60	9.50				
Minimum	1.27	2.97	2.97	8.10	<0.001	<0.001	<0.001	<0.001
Maximum	6.75	11.96	8.95	11.96				

CXCL10, C-X-C motif chemokine ligand 10.
Mann–Whitney test was used for comparison of numerical parameters; *P*1, comparison between all studied cases and control groups; *P*2, comparison between stable vitiligo and control groups; *P*3, comparison between active vitiligo and control groups; *P*4, comparison between stable and active vitiligo cases.

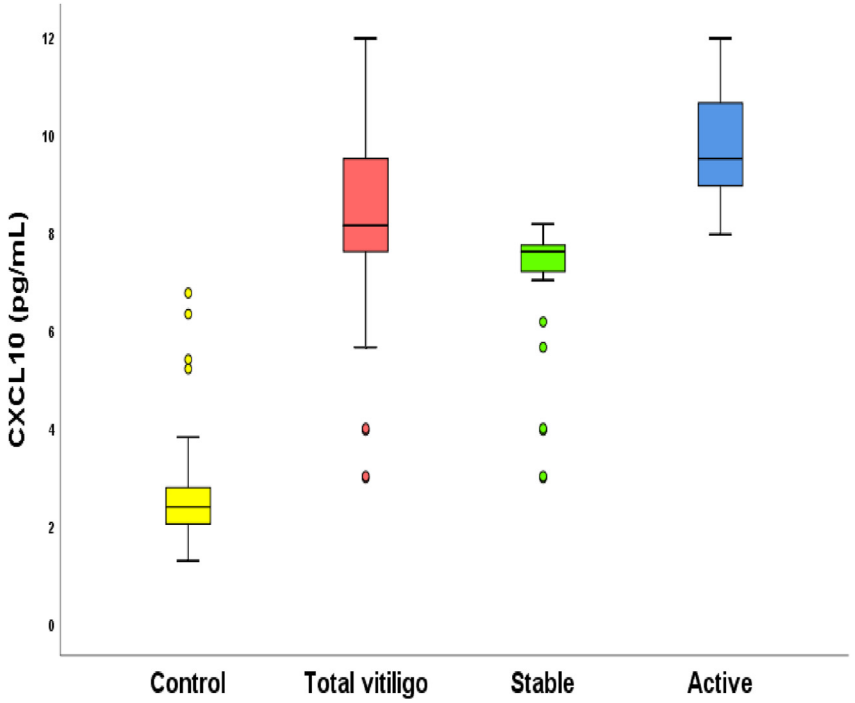

Fig. 2. Box plot for CXCL10 levels in control, vitiligo groups, and subgroups. CXCL10, C-X-C motif chemokine ligand 10.

Table 4. Correlations of C-X-C motif chemokine ligand 10 levels with age of vitiligo patients and vitiligo extent score.

	Vitiligo ($N = 60$)	
	CXCL10	
	r_s	P
Age	−0.192	0.142
VES	0.380	0.003

CXCL10, C-X-C motif chemokine ligand 10; r_s, correlation coefficient; VES, vitiligo extent score.

Table 5. Correlations of C-X-C motif chemokine ligand 10 levels with age and vitiligo extent score score in stable and active vitiligo cases.

	CXCL10			
	Stable vitiligo		Active vitiligo	
	r_s	P	r_s	P
Age	−0.099	0.602	−0.324	0.080
VES	0.299	0.039	0.599	<0.001

CXCL10, C-X-C motif chemokine ligand 10; r_s, correlation coefficient; VES, vitiligo extent score.

5. Conclusions

Serum levels of CXCL10 were significantly higher in vitiligo patients compared with healthy controls. Furthermore, patients with active vitiligo had higher levels of CXCL10 compared with the stable group. Moreover, patients with higher VES had higher CXCL10 serum levels.

CXCL10 might be an important predictor for vitiligo activity and severity. Targeting CXCL10 may limit vitiligo process.

Conflict of interest

None declared.

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