SKIN

IN-DEPTH REVIEW

Serum and Tissue CXCL9 Levels in Patients with Vitiligo Before and After Phototherapy: A Case-Control Study

Marwa Abdallah, MD[1], Ebtihal Hassan, MBBCh[1], Ahmed Elhawatky, MD[2], Samah Ibrahim, MD[1]

[1] Department of Dermatology, Venereology and Andrology, Ain Shams University, Cairo, Egypt
[2] Department of Dermatology, Venereology and Andrology, National Research Centre, Cairo, Egypt

ABSTRACT

Background: Vitiligo is a common pigmentation disease that affects 1–2% of the global population. It is a genetic disease that is triggered by an environmental factor resulting in an autoimmune disease. The predominance of the T-helper 1 (Th1) pattern favors the development of vitiligo. Interferon-gamma (IFN-γ) is the most important cytokine that is associated with the Th1 immune response. IFN-γ induces the release of chemokine which is called CXCL9.
Objectives: Evaluation of the effects of narrow band ultraviolet B (nbUVB) phototherapy on the serum and tissue levels of CXCL9 among vitiligo patients.
Patients and methods: We recruited in our study two groups; one group of twenty patients complaining of nonsegmental vitiligo and the other is a control group composed of another age and sex-matched twenty healthy controls, we assessed the serum level of CXCL9 in all subjects before the study and the patient group was reassessed after treatment with nbUVB for 12 weeks. We also determined tissue level of CXCL9 in patients before and after the phototherapy for 12 weeks from suction blister fluid.
Results: We detected that serum levels of CXCL9 were higher in patients with vitiligo compared to healthy matched controls. nbUVB sessions for twelve weeks were done, and we found that serum and tissue levels of CXCL9 after treatment were decreased.
Conclusion: Serum and tissue CXCL9 can be used as a marker for disease activity and potentiality for the response to treatment. Therefore, targeting the inhibition of the Interferon-γ-chemokine axis may help in treating the disease activity.

INTRODUCTION

Vitiligo is a chronic, acquired, multi-factorial depigmenting disease with serious psychological and emotional disturbances.[1,2] Hair in the affected skin also becomes white.[3] Nose and mouth cavities may also be involved.[4,5] The melanocytes of vitiliginous patients have morphologic abnormalities including enlargement, fragmentation, extracellular granular material, and dilated rough endoplasmic reticulum.[6]

The autoimmune theory has been the leading hypothesis for vitiligo. This theory has been supported by the clinical association of vitiligo with several other autoimmune disorders such as autoimmune thyroid diseases,[7] alopecia areata,[8] Addison's disease,[9] psoriasis,[10] and systemic lupus erythematosus.[11] Likewise, the association of

vitiligo with halo naevus can occur because of an autoimmune process against the melanocytes.[12]

The positive response to topical immunosuppressive therapy (topical steroid and tacrolimus) supports the autoimmune hypothesis.[13] Other types of cells also play a role in active disease, but less importantly than T helper (Th) cells, natural killer (NK) cells and macrophages.[13]

In vitiligo lesions, there is a reduction in the expression of Granulocyte-Macrophage Colony-Stimulating Factor (GM-CSF), Stem Cell Factor (SCF), and Basic Fibroblast Growth Factor (bFGF) which have a stimulatory effect on melanocytes. In contrast, Interleukin-6 and TNF-α are inhibitory cytokines for melanogenesis. These inhibitory cytokines were found to be significantly highly expressed in lesional skin.[14] IFN-γ is a pro-inflammatory cytokine produced by CD8+ T cells.[15] The expression of IFN-γ is associated with melanocyte destruction in the active phase of vitiligo lesions.[16]

UVB exerts a direct phototoxic effect on T-lymphocytes in the dermo-epidermal junction. In addition, nbUVB decreases the expression of ICAM-1, which is important for normal antigen presentation, activation of helper T lymphocytes, and inflammation. UVB radiation induces the release of a variety of immunosuppressive cytokines from keratinocytes, e.g., interleukins (6, 8, 10, 12, and 15), GM-CSF, and prostaglandins.[17] Phototherapy also stimulates hair follicular-stem cells to differentiate into melanocyte lineage and increase melanin formation and tyrosinase expression.[18]

Chemokines are a group of small cytokines. Cytokines are a big family of small proteins that are important in cell signaling and communication between cells to accomplish their basic activities. The name of chemokines is derived from their ability to induce chemotaxis in nearby responsive cells. Chemokines have been classified into four main subfamilies according to the number and spacing of two conserved N-terminal cysteine residues: CXC, CC, CX3C, and XC.[19]

One member of the CXC chemokine family is CXCL9, also known as monokine induced by interferon-gamma (MIG).[20] It is secreted by different types of cells including immune cells such as T lymphocytes, natural killer cells,[21] dendritic cells,[22] macrophages,[23] eosinophils,[24] endothelial cells, tumor cells, and fibroblasts.[25,26]

CXCL9 stimulates the T cells to multiply, differentiate, and migrate to tissues.[20] CXCL9 attracts the Th1 at the site of inflammation through CXCR3 and activates it.[27] The upregulation of CXCR3 ligands and the increased number of CXCR3+ lymphocytes was also observed in chronic inflammatory diseases such as rheumatoid arthritis, multiple sclerosis, autoimmune thyroiditis, Graves' disease, other HCV immune-mediated disorders, type 1 diabetes, systemic lupus erythematosus, systemic sclerosis, psoriasis or psoriatic arthritis and in sarcoidosis.[28-31]

CXCL9 plays two opposite roles in malignant tumors. It can enhance the migration of tumor cells and promote cancer metastasis.[32] On the other hand, CXCL9 also suppresses tumor cell spread by recruiting CD8+ T cells and NK cells to attack and inhibit tumor growth.[33]

Melanocyte-specific, cytotoxic CD8+ T cells are included in the destruction of melanocytes in vitiligo. An increase in serum and tissue levels of cytotoxic CD8+ T cells

was detected in vitiligo patients compared to healthy controls.[34] The intensity of CD8+ T cell reaction has been found to correlate with disease severity. T cells in the lesional skin have a recognizing specific melanocytes antigen and when migrated to melanocytes of normal skin they lead to induction of apoptosis in melanocytes.[35]

CD8+ T cells produce several cytokines such as tumor necrosis factor-alpha (TNF-α) and interferon-gamma (IFN-γ) that are primarily included in melanocyte destruction.[15] IFN-γ has an important role in adaptive immunity in vitiligo as IFN-γ inhibits melanogenesis and directly induces melanocyte apoptosis.[36] CXCL9 is reported to be increased in the serum of vitiliginous patients.[37] It is mainly produced by keratinocytes in vitiligo.[38] CXCL9 is one of the chemokines that attract cytotoxic T cells to attack the melanocytes in the epidermis.[37] The level of CXCL9 in progressive stages is higher than that in stable stages.[39] Some clinical trials measure CXCL9 directly in the skin as an early marker of treatment response.[40]

METHODS

Our study included two groups: a patient group recruited from the dermatology outpatient clinics including twenty patients complaining of nonsegmental vitiligo and a control group composed of twenty healthy controls with similar ages and sex. Age ranged from 18 to 65 years old.

The study protocol was approved by the Research Ethical Committee (MS 468/2019). All the patients were informed about the procedure including the blood sampling, suction blister technique, possible adverse effects, and photo documentation. The all signed an informed consent.

Inclusion Criteria

- Patients having active NSV (VIDA 3 & 4) with moderate disease progression
- Patients who did not receive any treatments for vitiligo in the last 3 months
- Both sexes

Exclusion Criteria

- Segmental vitiligo
- Stable vitiligo
- Pregnant and lactating females.
- Patients with other systemic diseases (e.g., SLE, ischemic heart disease, lung disease, and anemia)
- Patients with a history of solid or hematological malignancies (e.g., breast cancer, leukemia, etc.)

Methods

A full history of the disease (onset of disease, course of disease, duration, symptoms) and complete general examination to exclude other systemic diseases were performed. Each patient underwent the following:

VIDA Score: Evaluation of the disease activity by using VIDA score as it is a six-point scale for evaluating vitiligo activity. VIDA score is based on the patient's opinion. VIDA score included 6 stages (+4 = activity of 6 weeks or less--- to -1= stable at least for 1 year).[41]

Assessment for the activity markers per Benzekri et al. 2017 included: confetti-like lesions, hazy/blurred/trichrome margin, and Koebner phenomenon (type 2B).

VES score: Evaluation for the vitiligo body surface area (BAS) involvement by using Vitiligo Extent Score (VES) online calculator. VES was done before and after the phototherapy for 12 weeks.

VES plus score: This score is the same as VES score but with an added perifollicular repigmentation scale (from 5% to 90%). VES plus score was done after treatment with nbUVB for 12 weeks to evaluate treatment efficacy.[42]

Laboratory Investigations

- Assessment of serum level of CXCL9 in all subjects by ELISA
- Reassessment of serum level of CXCL9 in patients after treatment with nbUVB for 12 weeks by ELISA
- Determination of tissue level of CXCL9 in patients before and after phototherapy sessions for 12 weeks by ELISA from suction blister fluid

Fluid Sampling: Venous blood: 3 ml venous blood was collected from each subject and then centrifuged. For measuring serum chemokine we used Luminex 200 (Millipore, Cat.No:E0049Hu, China, Shanghai). The minimum detection limit was 31.2 pg/mL for CXCL9.

Blister fluid: A blister was induced at the margin of the active vitiligo lesion using a 10 ml syringe by negative suction pressure. The blister usually took 1.5 hours to develop. The blister fluid was obtained by a 1-mL insulin syringe (**Figure 1**). This was done with extreme caution not to induce bleeding inside the blister. Finally, the blister fluid was stored in a tube and was measured as the serum. Only clear blister fluid was taken, blood-tinged fluid was not used.

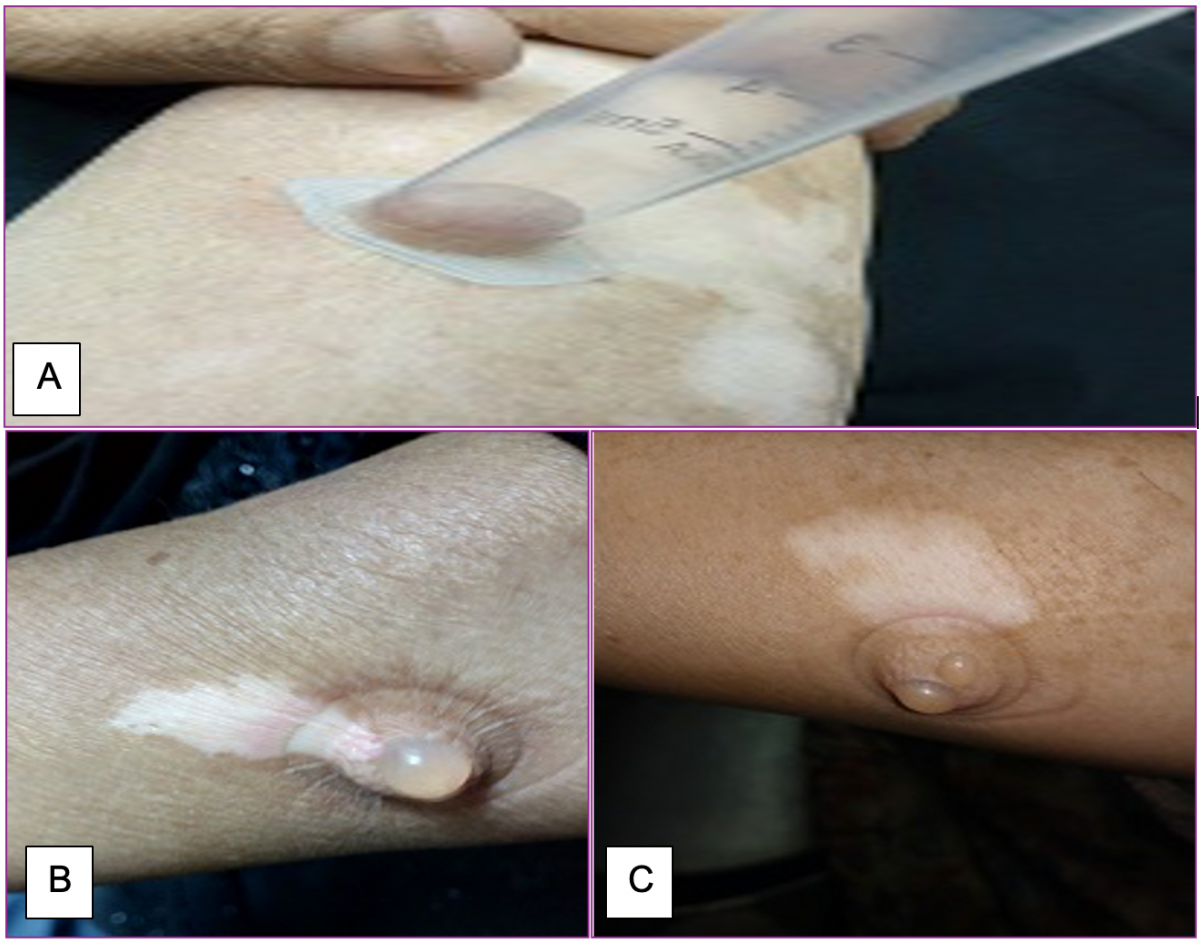

Figure 1. (A) (B) and (C) Blister formation

All mentioned steps were repeated with cases after treatment with nbUVB at week 12 to evaluate the changes in CXCL9 levels.

Data Management and Statistical Analysis

Data were collected, revised, coded, and entered into the Statistical Package for Social Science (IBM SPSS) version 23. The comparison between groups regarding qualitative data was done by using Chi-square test. Also, more than two groups were compared by using One Way ANOVA when parametric and Kruskall-Wallis test when nonparametric. The P value was considered significant as the following: P>0.05 = Non-significant, P<0.05= significant, and P<0.01= highly significant.

RESULTS

Our case-control study included two groups: the patient group comprised 20 patients (11 females and 9 males) suffering from non-segmental vitiligo (NSV), with ages ranging from 18 to 65 years (mean 40.75 ± 14.16 years), and the control group included 20 healthy controls with similar age and sex distribution (p=0.805, p=0.749), consisting of 8 males and 12 females, with ages ranging 16 to 65 years (mean 39.65 ± 13.83 years). Family history of vitiligo was positive in 5 patients (25.0%) and negative in 15 patients (75.0%).

The patient group included 12 individuals with Fitzpatrick's skin phototype III (60%), 5 with phototype IV (25%), and 3 with phototype V (15%). The control group included 10 individuals with skin phototype III (50%), 7 with skin phototype IV (35%), and 3 with skin phototype V (15%).

Regarding the involved areas of the disease, 3 patients (15%) had their lesions on the sun-exposed areas only, 1 patient (5%) had his lesions on the non-exposed areas only and 16 patients (80%) had their lesions on both sun-exposed and non-exposed areas.

The disease duration in vitiligo patients ranged from 6 months up to 35 years with a median duration of 7.5 years. Recent activity during the last 6 weeks (VIDA 4) was seen in 45% of the patients and from 6 weeks to 3 months (VIDA 3) in 55% of cases. Regarding the activity markers, confetti-like depigmentation was present in 1 patient (5%), Koebner phenomenon type 2B in 4 patients (20.0%), and hypochromic areas/borders in 3 patients (15.0%) (**Table 1**).

The serum level of CXCL9 was significantly higher in the patient group before nbUVB treatment compared to healthy controls (P<0.001) (**Table 2**).

When we compared the serum and tissue levels of CXCL9 in the patient group before nbUVB, we detected no statistically significant difference (P=0.296).

According to the sex, there was no statistically significant difference between female and male patients regarding the serum and tissue levels of CXCL9 before nbUVB (P=0.179 and P=0.446, respectively).

Serum levels of CXCL9 after the nbUVB sessions were statistically significantly decreased compared to before the sessions in the patient group (P<0.001).

Also, the tissue levels of CXCL9 after nbUVB sessions were statistically significantly decreased compared to before the sessions in the patients' group (P<0.001).

On comparing the serum and tissue levels of CXCL9 after nbUVB, a statistically significant

Table 1. Duration of vitiligo disease, family history, VIDA score, and the presence of activity markers among the patients group

		Patients group No. = 20
Disease Duration (years)	Median (IQR)	7.5 (4 – 15)
	Range	0.67 – 35
VIDA Score	+3	11 (55.0%)
	+4	9 (45.0%)
Confetti-Like Depigmentation	None	19 (95.0%)
	Present	1(5.0%)
Koebner Phenomenon Type 2B	None	16 (80.0%)
	Present	4 (20.0%)
Hypochromic Areas/Borders	No	17(85.0%)
	Present	3 (15.0%)

Table 2. Comparison between the serum levels of CXCL9 in control and patients group before NB-UVB

Serum CXCL9 before nbUVB	Control group No. = 20	Patients group No. = 20	Test value≠	P-value	Significance
Median (IQR)	150 (125 – 200)	1000 (800 – 1075)	-5.441	<0.001	Highly Significant
Range	100 – 250	400 – 3500			

Table 3. Comparison between serum and tissue levels of CXCL9 after NB-UVB in the patients group

	After NB-UVB		Test value≠	P-value	Significance
	Serum level of CXCL9	Tissue level of CXCL9			
Median (IQR)	250 (200 – 250)	150 (150 – 200)	-2.017	0.044	Significant
Range	150 – 300	50 – 350			

decrease in tissue levels of CXCL9 was detected compared to the serum levels (P=0.044) (**Table 3**).

When we correlated the percentage change of serum and tissue levels of CXCL9 with the demographic data and calculated scores before and after nbUVB we detected that there was no statistically significant correlation between the percentage of the change in serum and tissue levels of CXCL9 with age (P=0.368), duration of the disease (P=0.457), VES before nbUVB (P=0.355), VES after nbUVB (P=0.209), VES plus (P=0.352), percentage change of VES after NB-UVB (P=0.360) and percentage change of VES plus (P=0.609).

There was no statistically significant relationship between the percentage change of tissue level of CXCL9 with gender (P=0.183), skin phototype (P=0.885), the involved areas of the disease (P=0.331), family history (P=0.290), the presence of activity markers (P=0.797) or VIDA score (P=0.159).

The suction blister technique is a useful method that is equivalent to or maybe better than skin biopsy. It is less invasive and unlikely to form scars. However, there were some drawbacks regarding this technique.

For example:

1. It was time-consuming and painful.
2. It was difficult to carry out. In 40 % of the patients, the technique was usually not successful from the first time.
3. It requires experience.
4. It was challenging to convince the patients of this new method of investigation.
5. 10% of the patients had blood inside their blisters which led to repeating the procedure.

DISCUSSION

Vitiligo significantly influences appearance and brings enormous psychological stress to patients.[43] CXCL9, by binding to CXCR3, has been suggested to promote infiltration of the epidermis by specific cytotoxic T cells attacking the melanocytes.[37]

We aimed in our study to find out the effect of Narrowband Ultraviolet B sessions on the serum and tissue levels of CXCL9 among vitiligo patients. Our study included 20 patients with active NSV with VIDA score +3 or +4 and another 20 healthy controls with similar age and sex distributions. We sampled the tissue level of CXCL9 in the blister fluid from the perilesional skin of patients by using the suction blister technique. The patient group received treatment with nbUVB for twelve weeks. We re-examined the serum and tissue levels of CXCL9 in the patient group after treatment with nbUVB.

Serum CXCL9 was high in the vitiligo group compared to the control one. This further supports the role of CXCL9 in the pathogenesis of vitiligo, since CXCL9 is one of the chemokines produced by IFN-gamma, that attract CD8+ T cells to attack the melanocytes in the epidermis. CXCL9 is also considered a marker of disease activity in vitiligo patients. In addition, CXCL9 may be expressed first in vitiligo lesions which fits with its role as a “recruit” signal.[38] We noticed a non-significant difference in serum and tissue CXCL9 between males and females in the patient group. Such a finding is consistent with the fact that vitiligo is equally affecting both sexes.[43]

Our results were comparable to those reported that serum CXCL9 was significantly higher in patients compared with healthy

controls. Wang et al. further found elevated CXCL 9 and CXCL 10 in active versus stable cases.[39,44]

On the other hand, Yang et al. showed that the serum level of CXCL9 did not significantly differ between stable and active vitiligo patients as well as vitiligo patients and the control group. They hypothesized that differences in results could be due to differences in detection methods. We suggest different degrees of activity, as our cases were active VIDA 3 and 4 (<3 months of activity), while Yang et al. included patients with up to 6 months of activity.[45]

The tissue levels of CXCL9 after nbUVB decreased more significantly than the serum level, which may give us a result of the importance and the more accurate findings of the results of treatments, like that of the biopsies compared to those of serum levels. Moreover, we detected high CXCL9 in blister fluid of active vitiligo, similar to Strassner et al. in 2017, who reported significantly higher CD8+ T cell number and CXCL9 protein concentration in active lesional compared to non-lesional skin. Those T-cells expressed high CXCR3, the receptor for CXCL9[46] previously detected strong expression of tissue CXCL9 in active versus non-progressive perilesional vitiligo skin using immunohistochemistry. These findings may highlight the usefulness of the suction blister technique using ELISA, which has similar results to tissue biopsy but is less invasive.

As we reassessed our patients after twelve nbUVB sessions, serum and tissue CXCL9 were decreased. nbUVB exerts a direct phototoxic effect on T-lymphocytes and decreases T-cell activation. In addition, nbUVB induces the release of immunosuppressive cytokines from keratinocytes and inhibits the T cells from production of the inflammatory cytokines.[17]

Our study on 20 patients supports the findings of Strassner et al., who showed a decrease in CXCL9 level in the blister fluid of one patient after nbUVB treatment.[40] Different modalities of treatment can affect the serum and tissue levels of CXCL9. This was detected by Liu et al., who noticed the decrease of CXCL9 in the lesional blister fluid after treatment with tofacitinib, further underscoring the importance of targeting CXCL9, among other chemokines, in future treatment of vitiligo.[47]

On the other hand, in 2016, Wang et al. stated that the decrease seen in serum levels of CXCL9 before and after treatment with intramuscular injections of corticosteroids was not significant.[39] There was significant improvement in the BSA of the disease after treatment with phototherapy by calculating the VES and VES plus scores.[39]

The reduction in affected body surface area in patients after phototherapy sessions is due to the pigmentary effect of nbUVB in vitiligo through stabilization of the depigmentation process and stimulation of the hair follicle with the residual melanocytes to proliferate and produce melanin.[48]

There was no statistically significant correlation between the percentage change of serum and tissue levels of CXCL9 and the demographic data, the family history of vitiligo, VIDA score, Fitzpatrick's skin phototype, the involved areas of the disease, duration of vitiligo, the presence of activity markers and the calculated scores of BSA of the disease before and after nbUVB (VES score and VES plus score). Wang et al. similarly reported no correlation between VASI scores of the patients and the serum level of CXCL9.[39]

The modified suction blister technique adopted in the current study in collecting

tissue CXCL9 is a useful, minimally invasive technique, that measures cytokines in the interstitial skin fluid and is unlikely to form scars.[47] However, it was time-consuming to raise the blisters and patients felt pain. In 40% of the patients, the technique was not successful the first time and 10% of the patients got hemorrhagic blisters, which led to repetition of the procedure. During taking the blister fluid, one should be careful not to induce hemorrhage inside it. It needed experience and was also challenging to convince the patients of this new method of investigation.

CONCLUSION

CXCL9 is significantly elevated in patients with active NSV when compared to controls. It is also decreased after treatment with nbUVB. These findings mean that measuring CXCL9 may be beneficial in determining the disease activity and may be used as a marker for the therapy results. Drugs targeting the IFN-γ chemokine could be used in treating vitiligo. The suction blister technique is a useful, minimally invasive technique that may be equivalent to or better than skin biopsy. However, it needs experience in performing it.

Conflict of Interest Disclosures: None

Funding: None

Corresponding Author:
Ahmed Elhawatky
33 El Buhouth Street, Ad Doqi, Dokki
Cairo Governorate 12622 Egypt
Phone: +201065226628
Email: Dramfat@yahoo.com

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. *Dermatologic Therapy*. 2018; 31:e12620.
2. Taïeb A, Meurant JM. Should we prioritise psychological interventions in the management of vitiligo? *J Eur Acad Dermatol Venereol*. 2018; 32(12):2053–4.
3. Ezzedine K, Eleftheriadou V, Whitton M, van Geel N. Vitiligo. *Lancet*. 2015; 386(9988): 74–84.
4. Kanwar AJ, Parsad D, De D. Mucosal involvement in vitiligo: a comprehensive review of 241 cases. *J Eur Acad Dermatol Venereol*. 2011; 1361-3.
5. Nagarajan A, Masthan MK, Sankar LS, Narayanasamy AB, Elumalai R. Oral manifestations of vitiligo. *Indian J Dermatol*. 2015; 60(1):103.
6. Harris J. E. Chemical-Induced Vitiligo. Dermatologic clinics. 2017; 35(2): 151–161.
7. Sheth VM, Guo Y, Qureshi AA. Comorbidities associated with vitiligo: a ten-year retrospective study. *Dermatology*. 2013; 227:311–5.
8. Gan EY, Cario-Andre M, Pain C, et al. Follicular vitiligo: a report of 8 cases. *J Am Acad Dermatol*. 2016; 74:1178–84.
9. Laberge G, Mailloux CM, Gowan K et al. Early disease onset and increased risk of other autoimmune diseases in familial generalized vitiligo. *Pigment Cell Res*. 2005; 18:300–305.
10. Chen YT, Chen YJ, Hwang CY, et al. Comorbidity profiles in association with vitiligo: a nationwide population-based study in Taiwan. *J Eur Acad Dermatol Venereol*. 2015; 29:1362–9.
11. Gill L, Zarbo A, Isedeh P, Jacobsen G, Lim HW, Hamzavi I. Comorbid autoimmune diseases in patients with vitiligo: a cross-sectional study. *J Am Acad Dermatol*. 2016; 74:295–302.
12. Spritz RA. The genetics of generalized vitiligo: autoimmune pathways and an inverse relationship with malignant melanoma. *Genom Med*. 2010; 2(10):78.
13. Sandoval-Cruz M., Garcia-Carrasco M., Sanchez-Porras R. et al. Immunopathogenesis of vitiligo. *Autoimmune Rev*. 2011; 10(12): 762-765.
14. Laddha N.C., Dwivedi M., Begum R. Increased TNF-α and its Promoter Polymorphisms Correlate with Disease Progression and Higher Susceptibility towards Vitiligo. *PLoS ONE*. 2012; 7: e52298.

15. Boniface K, Jacquemin C, Darrigade AS, Dessarthe B et al. Vitiligo Skin Is Imprinted with Resident Memory CD8 T Cells Expressing CXCR3. *J Invest Dermatol*. 2018; 138(2):355-364.
16. Ezzedine S, El Rewiny EM, Abozied AA, Samna SM. Evaluation of Serum Interferon-gamma Level in Vitiligo Patients. *The Egyptian Journal of Hospital Medicine*. 2018; 73(10):7806-13.
17. Bulat V, Situm M, Dediol I, Ljubicic I, et al. The mechanisms of action of phototherapy in the treatment of the most common dermatoses. *Coll Antropol*. 2011; 35 (2): 147–151.
18. Dong D, Jiang M, Xu X, Guan M, et al. The effects of NB-UVB on the hair follicle-derived neural crest stem cells differentiating into melanocyte lineage in vitro. *J Dermatol Sci*. 2012; 66: 20–28.
19. Mélik-Parsadaniantz S, Rostène W. "Chemokines and neuromodulation". *Journal of Neuroimmunology*. 2008; 198 (1–2): 62–8.
20. Tokunaga R, Zhang W, Naseem M, Puccini A, Berger MD, Soni S, McSkane M, Baba H, Lenz HJ. "CXCL9, CXCL10, CXCL11/CXCR3 axis for immune activation - A target for novel cancer therapy". *Cancer Treatment Reviews*. 2018; 63: 40–47.
21. Smit MJ, Verdijk P, van der Raaij-Helmer EM, et al. CXCR3- mediated chemotaxis of human T cells is regulated by a Gi- and phospholipase C-dependent pathway and not via activation of MEK/p44/p42 MAPK nor Akt/PI-3 kinase. *Blood*. 2003; 102:1959-65.
22. Muthuswamy R., Urban J., Lee J. J., Reinhart T. A., Bartlett D., and Kalinski P. Ability of mature dendritic cells to interact with regulatory T cells is imprinted during maturation. *Cancer Res*. 2008; 68:5972–5978.
23. Ikeda A., Aoki N., Kido M., Iwamoto S., Nishiura H., Maruoka R., et al. Progression of autoimmune hepatitis is mediated by IL‐18‐producing dendritic cells and hepatic CXCL9 expression in mice. *Hepatology*. 2014; 60:224‐236.
24. Tworek D., Kuna P., Mlynarski W., Gorski P., Pietras T., and Antczak A. MIG (CXCL9), IP‐10 (CXCL10) and I‐TAC (CXCL11) concentrations after nasal allergen challenge in patients with allergic rhinitis. *Arc. Med. Sci*. 2013; 9:849‐853.
25. Antonelli A., Ferrari S. M., Fallahi P., Frascerra S., Santini E., Franceschini S. S., et al. Monokine induced by interferon gamma (IFNgamma) (CXCL9) and IFNgamma inducible T‐cell alpha‐chemoattractant (CXCL11) involvement in Graves' disease and ophthalmopathy: modulation by peroxisome proliferator‐activated receptor‐gamma agonists. *J. Clin. Endocrinol. Metabol*. 2009; 94:1803‐1809.
26. Vandercappellen J., Van Damme J., and Struyf S. The role of CXC chemokines and their receptors in cancer. *Cancer Lett*. 2008; 267:226–244.
27. Zohar Y, Wildbaum G, Novak R, Salzman AL, Thelen M, Alon R, Barsheshet Y, Karp CL, Karin N. "CXCL11-dependent induction of FOXP3-negative regulatory T cells suppresses autoimmune encephalomyelitis". *The Journal of Clinical Investigation*. 2014; 124 (5): 2009–22.
28. Lacotte S, Brun S, Muller S, et al. CXCR3, inflammation, and - autoimmune diseases. *Ann N Y Acad Sci*. 2009; 1173:310-7.
29. Chen, SC., de Groot, M., Kinsley, D. et al. Expression of chemokine receptor CXCR3 by lymphocytes and plasmacytoid dendritic cells in human psoriatic lesions. *Arch Dermatol Res*. 2010; 302: 113–123.
30. Su R, Nguyen ML, Agarwal MR, et al. Interferon-inducible chemokines reflect severity and progression in sarcoidosis. *Respir Res*. 2013; 14:121.
31. Antonelli A, Ferrari SM, Giuggioli D, et al. Chemokine (C-X-C motif) ligand (CXCL)10 in autoimmune diseases. *Autoimmun Rev*. 2014; 13:272-80.
32. Ding Q., Xia Y., Ding S., Lu P., Sun L., and Liu M. An alternatively spliced variant of CXCR3 mediates the metastasis of CD133+ liver cancer cells induced by CXCL9. *Oncotarget*. 2016; 7:14405–14414.
33. Clancy-Thompson E., Perekslis T. J., Croteau W., Alexander M. P., Chabanet T. B., Turk M. J., et al. Melanoma induces, and adenosine suppresses, CXCR3‐Cognate chemokine production and T‐cell infiltration of lungs bearing metastatic‐like disease. *Cancer Immunol. Res.* 2015; 3:956–967.
34. Lili Y, Yi W, Ji Y et al. Global activation of CD8+ cytotoxic T lymphocytes correlates with an impairment in regulatory T cells in patients with generalized vitiligo. *PLoS One*. 2012; 7:e37513.
35. Van den Boorn JG, Konijnenberg D, Dellemijn TA, et al. Autoimmune destruction of skin melanocytes by perilesional T cells

from vitiligo patients. *J Invest Dermatol*. 2009; 129(9):2220–32.
36. Yang L, Wei Y, Sun Y, Shi W, Yang J, Zhu L, Li M. Interferon-gamma Inhibits Melanogenesis and Induces Apoptosis in Melanocytes: A Pivotal Role of CD8+ Cytotoxic T Lymphocytes in Vitiligo. *Acta Derm Venereol*. 2015; 95(6):664-70.
37. Rashighi M., Agarwal P., Richmond J.M., Harris T.H. et al. CXCL10 is critical for the progression and maintenance of depigmentation in a mouse model of vitiligo. *Sci Transl Med*. 2014; 6: 223ra23.
38. Richmond JM, Bangari DS, Essien KI, Currimbhoy SD, Groom JR, Pandya AG, Youd ME, Luster AD, Harris JE. Keratinocyte-Derived Chemokines Orchestrate T-Cell Positioning in the Epidermis during Vitiligo and May Serve as Biomarkers of Disease. *J Invest Dermatol*. 2017; 137(2):350-358.
39. Wang X.X., Wang Q.Q., Wu J.Q., Jiang M., Chen L., Zhang C.F., and Xiang L.H. 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: 1318-1326.
40. Strassner JP, Rashighi M, Ahmed Refat M, et al. Suction blistering the lesional skin of vitiligo patients reveals useful biomarkers of disease activity. *J Am Acad Dermatol*. 2017; 76:847-55
41. Nagarajan A, Masthan MK, Sankar LS, Narayanasamy AB, Elumalai R. Oral manifestations of vitiligo. *Indian J Dermatol*. 2015; 60(1):103.
42. Van Geel N, Wolkerstorfer A, Lommerts JE, et al. Validation study of the Vitiligo Extent Score-plus. *J Am Acad Dermatol*. 2018; 78(5):1013-1015.
43. Grimes, P. E., & Miller, M. M. Vitiligo: Patient stories, self-esteem, and the psychological burden of disease. *International journal of women's dermatology*. 2018; 4(1): 32–37.
44. Maouia A, Sormani L, Youssef M, et al. Differential expres-sion of CXCL9, CXCL10, and IFN-γ in vitiligo and alopecia areata patients. *Pigment Cell Melanoma Res*. 2017; 30:259-61
45. Yang L, Yang S, Lei J, et al. Role of chemokines and the corresponding receptors in vitiligo: a pilot study. *J Dermatol*. 2018; 45: 31‐38.
46. Bhardwaj S, Rani S, Srivastava N, Kumar R, Parsad D. Increased systemic and epidermal levels of IL-17A and IL-1β promotes progression of non-segmental vitiligo. *Cytokine*. 2017; 91():153-161.
47. Liu, L. Y., Strassner, J. P., Refat, M. A., Harris, J. E., & King, B. A. Repigmentation in vitiligo using the Janus kinase inhibitor tofacitinib may require concomitant light exposure. *J Am Acad Dermatol*. 2017; 77(4): 675–682.e1.
48. Chahar, Y. S., Singh, P. K., Sonkar, V. K., Rajani, I., & Adil, M. Impact on Quality of Life in Vitiligo Patients Treated with Narrowband Ultraviolet B Phototherapy. *Indian Journal of Dermatology*. 2018; 63(5): 399–402.