Role of chemokine ligand 22 in narrow-band ultraviolet B-induced pigmentation in vitiligo: an immunohistochemical study

Azza G. Antar Farag[a], Mostafa A. Hammam[a], Dalia R. Al-Sharaky[b], Reem A. Hassan[a], Eman N. ElShafey[a], Nehal A. Ali[c]

Departments of [a]Dermatology, Andrology and STDs, [b]Pathology, Faculty of Medicine, Menoufia University, Shebeen El-Kom, [c]Department of Dermatology at Ministry of Health, Ashmounn, Menoufia, Egypt

Correspondence to Azza Gaber Antar Farag, MD, Department of Dermatology, Andrology and STDs, Faculty of Medicine, Menoufia University, Shebeen El-Kom 32511, Egypt. Tel: +20 109 778 7204; fax: +20 248 222 6454; e-mail: azzagaber92@yahoo.com

Received 10 June 2018
Accepted 3 November 2018

Journal of the Egyptian Women's Dermatologic Society 2019, 16:97–104

Background
Chemokine ligand 22 (CCL22) is a heparin-binding immunomodulator protein that plays an important role in a variety of autoimmune diseases. Narrow-band ultraviolet B (NB-UVB) therapy was considered a cornerstone in the treatment of vitiligo. However, the mechanism of action of NB-UVB in vitiligo has not been completely elucidated.
Objective
To study the hypothesized role of CCL22 in vitiligo etiopathogenesis and to detect its possible role in NB-UVB-induced pigmentation in vitiligo through its lesional immunohistochemical evaluation in patients with vitiligo before and after ultraviolet B phototherapy.
Patients and methods
A total of 33 patients with vitiligo versus 20 patients of age-matched, sex-matched, and skin phototype-matched healthy controls were enrolled in this case–control study. Patients were treated with NB-UVB three sessions weekly for 12 weeks. Vitiligo Area Scoring Index score was evaluated before and after NB-UVB sessions. For patients with vitiligo, baseline CCL22 immunohistochemical staining was estimated, and compared with that of controls and with its posttreatment data in those patients.
Results
Baseline CCL22 immunohistochemical studied parameters were insignificantly lower in patients with vitiligo than controls except its cellular localization ($P<0.001$). After 12 weeks of NB-UVB, these CCL22 immunohistochemical parameters were significantly up-regulated ($P<0.001$). Although there was a negative correlation between the improvement in Vitiligo Area Scoring Index score and CCL22 *H* score, this correlation could not reach level of significance ($r=0.086$, $P=0.653$).
Conclusion
Although we could not confirm that CCL22 protein has an active role in the pathogenesis and development of vitiligo, we concluded that CCL22 chemokine may take part in photo-induced melanogenesis. Yet, the mechanism of NB-UVB-induced pigmentation is still far from being clarified, and further studies are needed.

Keywords:
chemokine ligand 22, narrow band ultraviolet B, vitiligo

J Egypt Women's Dermatol Soc 16:97–104

1687-1537

Introduction

Vitiligo is a common noncontagious disorder that is characterized by progressive patchy loss of skin pigmentation. It affects nearly 0.5–2% of the population worldwide, irrespective of ethnic origin or race, with significant decrease in the quality of life of affected cases [1].

Owing to its multifactorial nature, the pathogenesis of vitiligo is complex. Several pathogenic theories have been proposed including autoimmune mechanisms [2]. However, the precise cause behind melanocytes destruction remains unknown [3]. Chemokine ligand 22 (CCL22) is a secreted protein that induces migration and extravasation of chronically activated Th2 cells into the skin through binding to G protein-coupled receptor (CCR4). In turn, this binding increases intracellular Ca^{2+} mobilization that affects cytoskeleton-induced movement and increases the affinity of targeted cells to adhesion molecules [4].

CCL22 has a preferential effect on CD4+ regulatory T lymphocytes (Tregs), which have a role in immune homeostasis [5]. Autoimmune diseases like vitiligo have poor lesional-specific expression of CCL22 and

so lower regulatory T cells infiltrating the affected area than normal healthy skin [6].

Narrow band ultraviolet B (NB-UVB) therapy has been considered as a cornerstone in the treatment of vitiligo because of its safety and efficacy. However, the mechanism of action of NB-UVB in vitiligo has not been completely understood [7].

Therefore, we aimed in this study to investigate the hypothesized role of CCL22 in vitiligo etiopathogenesis and to detect if CCL22 has a role in NB-UVB-induced pigmentation in vitiligo through its lesional immunohistochemical evaluation in patients with vitiligo before and after ultraviolet B (UVB) phototherapy, and controls.

Patients and methods

A total of 33 patients with different degrees of vitiligo severity were included in this case–control study. They were recruited from the Dermatology Outpatient Clinic, Faculty of Medicine, Menoufia University Hospital, Shebin El-Kom, Egypt. This case–control study was recruited during spring from March 2016 to June 2016.

Additionally, 20 apparently healthy, vitiligo free, and age-matched, sex-matched, occupation-matched and Fitzpatrick skin phototype-matched volunteers were included as a control group. The enrolled patients were instructed to stop treatment of their vitiligo 1 month before joining the study. Patients having any other autoimmmune/inflammatory diseases or those having history of photosensitivity to ultraviolet rays were excluded from the study. The study protocol was approved by the Research Ethics Committee of Faculty of Medicine, Menoufia University, which was in accordance with Helsinki Declaration in 1975 (revised in 2000). A desired proposal sheet and general examination were performed to detect any of excluding factor. Dermatologically, the studied patients were evaluated to assess their skin phototype [8], and to classify patients with vitiligo into segmental and nonsegmental types [9]. Vitiligo Area Severity Index (VASI) was calculated in the first visit and at week 12 (end of therapy) [10]. By Waldmann UV 1000 L (TL 01) (Family-run company, Villingen-Schwenningen, Baden-Württemberg, Germany), NB-UVB phototherapy for the studied patients with vitiligo was given three times/week on nonconsecutive days for 12 weeks. We started the irradiation by 300 mJ/cm^2 (the minimum erythema dose of Egyptian skin) [11]. Then the dose was increased by 20% on each subsequent session till just faint erythema appeared. If symptomatic erythema (burning or blisters) developed, phototherapy was pending till the lesions healed. At that time, irradiation was taking place again at a dose 20% lesser. Thereafter, the dose was increased by 10% on subsequent sessions [12].

During treatment, genitals were shielded and eyes were protected with UV safety glasses. From each patient, two punch skin biopsies 4 mm in size from the involved skin were taken under local anesthesia one before and one after NB-UVB phototherapy. In addition, one site-matched skin biopsy from every control patient was obtained. All biopsies were submitted to Pathology Department, Faculty of Medicine, Menoufia University. Sections were cut from the paraffin-embedded blocks and were stained with purified rabbit polyclonal antibody (cat. #YPA1195; SNF Medical Company, Chongqing, China; *http://www.biospes.comhttp://www.biospes.com*) raised against CCL22 that was received as concentrated 0.1 ml. The optimal dilution was 1 : 200, by using PBS. Negative control slides were prepared, by omitting the primary antibody from the staining procedure, whereas tissue sections prepared from lymph node tissue were used as a positive control for CCL22.

Procedure of IHC staining was done according to received datasheet of the used antibodies. Immunohistochemically, CCL22 expression is confirmed by cytoplasmic, nuclear, and/or membranous staining and was evaluated in both epidermis and dermis. In case of positively expressed cells, the percentage of the positive cell was assessed at ×200 magnification field [13]. Intensity of the stain was graded subjectively as mild, moderate, or strong. Histo-score (*H* score) was calculated (*H* score=1×% of mildly stained cells+2×%moderately stained cells +3×%of strongly stained cells) [14].

CCL22 stain distribution pattern was categorized as either patchy or diffuse, and its cellular localization was assigned as either cytoplasmic, nuclear, and/or membranous.

Statistical analysis

The results were collected, entered, and processed on IBM-PC compatible computer using SPSS software (version 20.0; SPSS Inc., Chicago, Illinois, USA). Descriptive statistics were expressed as percentage, mean, and SD. Analytic statistics included Student's *t*-test and Mann–Whitney *U*-test to compare quantitative data according to its distribution.

Fisher's exact test was used in the analysis of 2×2 contingency tables when at least 25% of cells has expected number less than 5. McNemar's test of significance was used on paired (e.g. pre and post) qualitative data. Marginal homogeneity test was used on paired (e.g. pre and post) qualitative data when a category of the sample is more than two. χ^2-Test was used for qualitative data and Spearman's correlation to assess correlation. *P* value up to 0.05 was considered the cutoff value for significance.

Results

This case–controls study included 33 patients with vitiligo, with 15 (45.5%) males and 18 (54.5%) females, having male : female ratio of 1 : 1.2. Their age ranged from 15 to 50 years, with a mean value of 34.9±11.39 years and median of 34 years. Regarding Fitzpatrick skin phototype, nine (27.3%) cases were type II, 14 (42.4%) cases were type III, and 10 (30.3%) cases were type IV. Additionally, 14 (42.4%) cases had outdoor occupations and 19 (57.6%) cases had indoor ones. The control group included 20 healthy volunteers, with 11 (55%) males and nine (45%) females, having male : female ratio of 1.2 : 1. Their age ranged from 15 to 50 years, with a mean value of 37.35±10.82 years and median of 35 years. Regarding Fitzpatrick skin phototypes, four (20%) patients were type II, eight (40%) patients were type III, and eight (40%) patients were type IV. Additionally, nine (45%) patients had outdoor occupation and 11 (55%) patients had indoor occupation. There were insignificant differences regarding age, sex, skin phototypes, and occupation between patients with vitiligo and their controls ($P>0.05$ for all) (Table 1).

Clinical data of the studied vitiligo cases

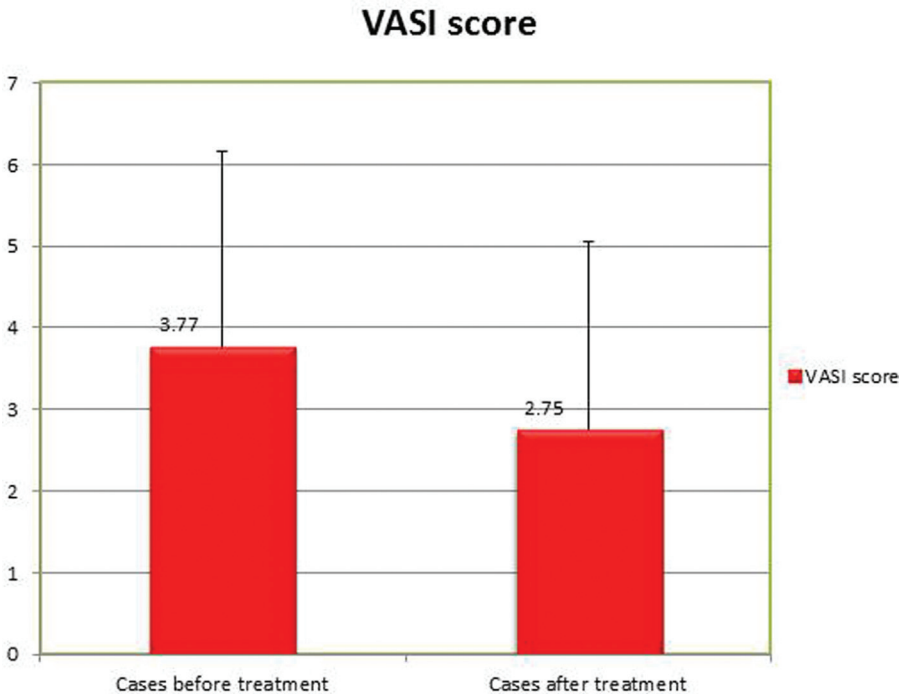
The result of clinical data in this study showed that most of our patients had negative family history (93.3%) and only two patients with vitiligo had

Table 1 Personal and clinical data of the studied participants

Evaluated data	Studied group (*N*=53)		Test of significance	*P* value
	Patients (*n*=33)	Control (*n*=20)		
Age (years)				
Mean±SD	34.9±11.39	37.35±10.82	*U*=0.751	0.456
Range	15.00–50.00	15.00–5.00		
Median	34.0	35.0		
Sex [*n* (%)]				
Male	15 (45.5)	11 (55.0)	χ^2=0.454	0.500
Female	18 (54.5)	9 (45.0)		
Occupation				
Outdoor	14 (42.4)	9 (45.0)	0.034	0.854
Indoor	19 (57.6)	11 (55.0)		
Skin type				
II	9 (27.3)	4 (20.0)	0.631	0.729
III	14 (42.4)	8 (40.0)		
IV	10 (30.3)	8 (40.0)		
Family history of vitiligo				
Positive	2 (6.1)	–	–	
Negative	31 (93.9)			
Type of vitiligo				
Segmental	13 (39.4)	–	–	
Nonsegmental	20 (60.6)			
Disease duration (years)				
Mean±SD	3.58±5.82	–	–	–
Range	0.25–24.0			
Median	1.0			
Age of onset (years)				
Mean±SD	31.61±0.8	–	–	–
Range	14.0–50.0			
Median	31.0			
VASI score before phototherapy				
Mean±SD	3.77±2.38	–	–	–
Range	0.9–10.8			
Median	3.6			

U, Mann–Whitney test; VASI, Vitiligo Area Severity Index.

Figure 1



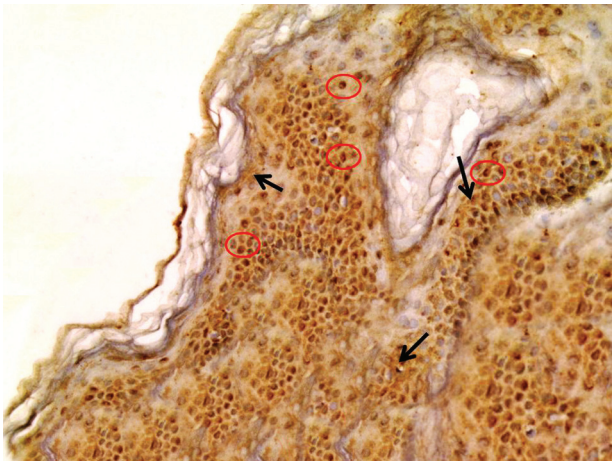
CCL22 immunohistochemical expression in normal skin biopsy showing moderate nuclear immunoreactivity (red circles) and cytoplasmic reactivity (black arrow) in the epidermis (immunoperoxidase ×400 HPF). CCL22, chemokine ligand 22.

positive family history. Regarding the type of vitiligo, 20 (60.6%) patients had nonsegmental vitiligo. Disease duration ranged from 0.25 to 24 years, with a mean value of 3.58±5.82 years and median of 1 year. Age of onset of vitiligo ranged from 14 to 50 years with a mean value of 31.61±10.8 years and median of 31 years. Concerning severity of vitiligo in studied patients, calculated VASI score ranged from 0.9 to 10.8 with a mean value of 3.77±2.38 and median of 3.6 (Table 1).

Baseline chemokine ligand 22 immunohistochemical staining in the studied groups

In controls (Fig. 1), CCL22 expression was positive in all sections (100%), which was mainly of nuclear localization (17.85%), and showed mild intensity in half of evaluated tissues (50%), with patchy distribution in 16 (80%) sections. Percent of CCL22 immunoreactivity ranged from 10 to 95, and its *H* score ranged from 10 to 285. However, in patients with vitiligo, CCL22 immunohistochemistry before phototherapy (Figs 2a and 3a) showed that CCL22 expression was positive in 30 (90.9%) patients, which was of patchy distribution in most of these sections (24, 80%) and showed mild intensity in nearly half of them (16, 53.3%). Moreover, its localization was mainly nucleocytoplasmic and cytoplasmic (11 and 36.7%, respectively). Percent of CCLL22 immunoreactivity ranged from 5 to 85, and its *H* score ranged from 5 to 255. There were no significant differences between patients with vitiligo and controls regarding all studied CCL22 immunohistochemical staining parameters except its cellular localization, which was mainly nuclear in controls (85 vs. 20%) ($P<0.001$) (Table 2).

Figure 2

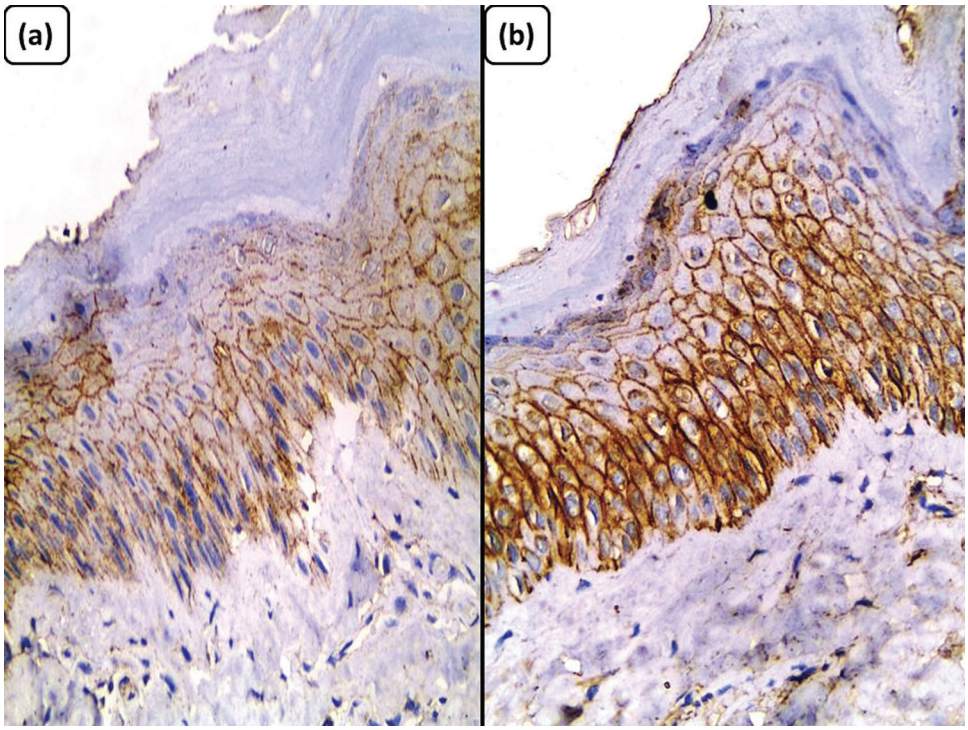


CCL22 immunohistochemical expression in a patients with vitiligo (a) before NB-UVB phototherapy showing mild CCL22 membranous expression and (b) after NB-UVB phototherapy demonstrating intense membranous CCL22 expression in the epidermis (immunoperoxidase ×400 HPF). CCL22, chemokine ligand 22; NB-UVB, narrow band ultraviolet B.

Results after phototherapy

CCL22 immunohistochemical expression in patients with vitiligo showed that after 12 weeks of NB-UVB

Figure 3



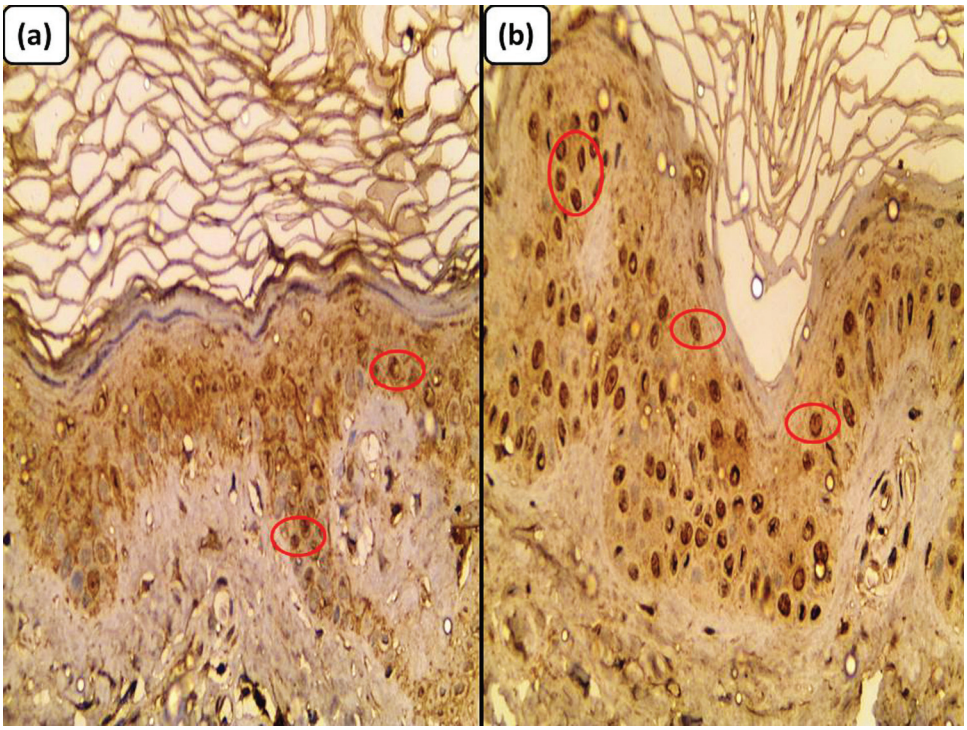
CCL22 immunohistochemical expression in a patients with vitiligo (a) before NB-UVB phototherapy showing moderate nuclear (red circles) and cytoplasmic CCL22 expression and (b) after NB-UVB phototherapy demonstrating intense nuclear (red circles) and moderate cytoplasmic CCL22 immunoreactivity (immunoperoxidase a, b ×400 HPF). CCL22, chemokine ligand 22; NB-UVB, narrow band ultraviolet B.

Table 2 Chemokine ligand 22 immunohistochemical staining of patients with vitiligo before and after phototherapy and controls

Immunohistochemical staining	Studied group (N=60) [n (%)]			Test of significance	P value
	Controls (n=20)	Patients before treatment (n=33)	Patients after treatment (n=33)		
Expression					
Positive	20 (100)	30 (90.9)	33 (100)	$t_1=\chi^2=1.93$	$P_1=0.282$
Negative	0	3 (9.1)	0	t_2=McNemar $\chi^2=3.14$	$P_2=0.250$
Localization					
Cytoplasmic	0	11 (36.7)	6 (18.2)	$t_1=\chi^2=21.70$	$P_1=0.001$*
Membranous	0	1 (3.3)	2 (6.1)	t_2=McNemar=1.24	$P_2=0.216$
Membrano-cytoplasmic	0	1 (3.3)	3 (9.1)		
Nucleocytoplasmic	3 (15.00)	11 (36.7)	19 (57.6)		
Nuclear	17 (85.00)	6 (20.0)	1 (3.0)		
Nucleomembranous	0	0	2 (6.1)		
Intensity					
Mild	10 (50.0)	16 (35.3)	5 (15.2)	$t_1=\chi^2=1.81$	$P_1=0.405$
Moderate	7 (35.0)	6 (20.0)	18 (45.5)	t_2=McNemar=1.98	$P_2=0.047$*
Strong	3 (15.0)	8 (26.7)	10 (30.0)		
Distribution					
Patchy	16 (80.0)	24 (80.0)	16 (48.5)	$t_1=\chi^2=0.00$	$P_1=1.00$
Diffuse	4 (20.0)	6 (20.0)	17 (51.5)	t_2=McNemar $\chi^2=1.44$	$P_2=0.115$
Percent					
Mean±SD	39.50±31.07	31.17±23.22	55.00±26.43	$t_1=U=0.836$	$P_1=0.403$
Range	10.0–95.0	5.0–85.0	10.0–90.0	t_2=Wilcoxon signed rank=4.71	$P_2=0.001$*
Median	30.0	22.5	60.0		
H score					
Mean±SD	86.00±96.53	51.83±64.80	137.58±87.63	$t_1=U=1.35$	$P_1=0.179$
Range	10.0–285.0	5.0–255.0	10.0–270.0	t_2=Wilcoxon signed rank =4.62	$P_2=0.001$*
Median	40.0	30.0	120		

MH, marginal homogeneity test; t_2: McNemar χ; t_1 and P_1: comparison between patients before treatment and controls; t_2 and P_2: comparison between patients before and after treatment; U, Mann–Whitney test. *Significant.

Figure 4



VASI score mean values before and after NB-UVB phototherapy in patients with vitiligo. NB-UVB, narrow band ultraviolet B; VASI, Vitiligo Area Scoring Index.

phototherapy, CCL22 immunohistochemical staining (Figs 2b and 3b) showed increased positivity (30, 90.9 vs. 33, 100%) and significant elevation in CCL22 intensity (P=0.047). Furthermore, CCL22 expression percentage and *H* score revealed significant increase in post-treatment than pretreatment sections (55±26.43 vs. 31.17±23.22 and 137.58±87.63 vs. 51.83±64.80, respectively) (P<0.001 for both) (Table 2).

VASI score in patients with vitiligo showed that after phototherapy, there was observed clinical improvement in studied patients with vitiligo and a significant decrease in their VASI score mean values than before phototherapy (2.75±2.31 vs. 3.77±2.38) (P<0.001) (Fig. 4).

Correlations between the improvement in Vitiligo Area Scoring Index score and the improvement in chemokine ligand 22 *H* score among patients with vitiligo

There was a nonsignificant negative correlation between the improvement in VASI score and CCL22 *H* score (r=0.086, P=0.653).

Relation between the improvement of chemokine ligand 22 *H* score and studied personal and clinical data of patients with vitiligo

The relationship between the improvement in CCL22 *H* score and personal and clinical parameters of studied patients with vitiligo showed insignificant associations regarding all of these parameters (P>0.05 for all) (Tables 3 and 4).

Discussion

To best of our knowledge, this study is the first one that investigates the possible role of CCL22 in NB-UVB-induced pigmentation in vitiligo through its lesional immunohistochemical evaluation in patients with vitiligo before and after UVB phototherapy. From which, we suggested that CCL22 upregulation after NB-UVB phototherapy may partially participate in NB-UVB-induced pigmentation in patients with vitiligo. Yet, the mechanism of NB-UVB-induced pigmentation is still not well known, and further studies are needed

In normal situations, CCL22 was found in all sections from normal human tissues including skin [15], Confirming this finding, the present study demonstrated that CCL22 immunohistochemical staining of studied control patients had positive immunoreactivity in all examined sections (100%), in which CCL22 produced by monocyte-derived dendritic cell binds to CCR4 receptor inducing chemoattraction on Th2 lymphocytes with a preferential effect on CD4+regulatory T lymphocytes (Tregs) [16]. Thus, CCL22 has a role in controlling

Table 3 Relation between improvement in chemokine ligand 22 *H* score and personal and clinical parameters of patients with vitiligo

Personal and clinical data of vitiligo cases	*n* (%)	Improvement in chemokine ligand 22 *H* score (mean ±SD)	Test of significance	*P* value
Sex				
Male	15 (45.5)	52.38±31.42	1.73	0.084
Female	18 (54.5)	68.13±30.71		
Occupation				
Outdoor	14 (42.5)	64.60±30.37	0.440	0.660
Indoor	19 (57.5)	57.86±33		
Family history				
Positive	2 (6.06)	68.8±38.3	0.665	0.506
Negative	31 (93.9)	60.2±31.8		
Skin type				
II	9 (27.3)	55.4±30.1	1.03[a]	0.599
III	14 (42.4)	67.8±28.9		
VI	10 (30.3)	55.5±37.7		
Type of vitiligo				
Segmental	13 (39.4)	51.9±33.2	1.25[b]	0.212
Nonsegmental	20 (60.6)	66.7±29.8		

[a]*U*, Mann–Whitney. [b]*K*, Kruskal–Wallis test

Table 4 Correlation between improvement in chemokine ligand 22 *H* score and age of patients with vitiligo, age of onset of the disease, and disease duration among studied patients with vitiligo

	Improvement in chemokine ligand 22 *H* score	
	r	*P* value
Age of patients with vitiligo	0.138	0.343
Age of onset of vitiligo	0.281	0.133
Duration of the disease	0.031	0.871

the immune response to self- and foreign antigens, preventing autoimmune diseases [17].

In cutaneous autoimmune disorders, it was suggested that loss of peripheral tolerance in the skin occurred in two steps, the induction and effector phases. In induction phase, the effector-autoreactive T cells were stimulated and expanded by CD4+T cells. These cells circulate in the blood but with no symptoms of autoimmunity, whereas in the effector phase, the skin microenvironment became favorable for escaping peripheral tolerance [18].

Herein, as previously reported [6], we observed that immunohistochemical evaluation of CCL22 showed downregulation of CCL22 protein in patients with vitiligo compared with their matched peers regarding all immunohistochemical staining parameters. However, this downregulation could not reach level of significance except for its cellular localization, which could be attributed to small sample size in our study.

In autoimmune microenvironment of vitiligo lesions, downregulation of CCL22 expression causes reduction in skin homing by functional Tregs and resulted in imbalance between effector and regulatory mechanisms, as an influx of effector T cells in vitiligo is not accompanied by an influx of Treg. In fact, resident Treg are reduced in nonlesional and lesional vitiligo skin. These data suggest that an ongoing immune response to self-antigens is not kept in check by the appropriate immune regulatory mechanisms within the skin [6].

After NB-UVB phototherapy sessions, in agreement with previous studies [7,11,19], we observed significant clinical improvement and decrease in VASI score mean values in our studied vitiligo cases.

Parallel to the clinical improvement, after NB-UVB phototherapy, CCL22 immunohistochemical staining showed increased positivity and significant elevation in CCL22 intensity, percentage, and H score mean values than pretreatment state.

Supporting our finding, Taguchi *et al.* [20] reported that UVB may cause CCL22 upregulation through Interleukin (IL)-4, prostaglandin E2, calcitonin gene-related peptide, a melanocyte-stimulating hormone, and platelet activating factor. Additionally, Langerhans dendritic cells upon exposure to UVB became unable to sensitize the body against selective antigen owing to DNA damage, which modifies Langerhans cells to have immunosuppressive properties. Furthermore, treatment with NB-UVB was capable of elevating transforming growth factor (TGF)-levels, which are suggested to be related to the stability of vitiligo [21].

In same context, we observed negative correlation between the improvement of VASI and the improvement of CCL22 *H* score mean values in our studied patients with vitiligo; however, this correlation could not reach level of significance, which could be a result of small sample size in our study. Therefore, we hypothesized that CCL22 may participate partially in vitiligo repigmentation after NB-UVB phototherapy. Induced overexpression of CCL22 in the skin will generate a chemokine gradient that can support Treg recruitment to the treatment site and provide a therapeutic platform to treat vitiligo [22]. Moreover, it has been recently reported that topical CCL22 may be used for the treatment of vitiligo in the animal models [23]. An advantage to treating vitiligo is that the target organ is external and can be directly accessed [22].

Based on the results of the current study and its discussion, we could not confirm that CCL22 protein has an active role in the pathogenesis and development of vitiligo. However, CCL22 chemokine may take part in photo-induced melanogenesis. Yet, the mechanism of NB-UVB-induced pigmentation is still far from being clarified, and further studies are needed.

Acknowledgements

The authors are grateful to administrative and technical staffs at Dermatology Clinic, and Pathology Department, Faculty of Medicine, Menoufia University, Egypt, who kindly helped throughout this study.

Financial support and sponsorship

Nil.

Conflicts of interest

There are no conflicts of interest.

References

1 Kumar CM, Sathisha UV, Dharmesh S, Rao AG, Singh SA. Interaction of sesamol (3,4- ethylenedioxyphenol) with tyrosinase and its effect on melanin synthesis. Biochimie 2011; 93: 562–569.

2 Farag AGA, Hammam MA, Habib Elnaidany NF, Kamh ME. Macrophage migration inhibitory factor as an incriminating agent in vitiligo. An Bras Dermatol 2018; 93:191–196.

3 Gauthier Y, Cario Andre M, Taïeb A. A critical appraisal of vitiligo etiologic theories. Is melanocyte loss a melanocytorrhagy? Pigment Cell Res 2003; 16:322–332.

4 Hyduk SJ, Chan JR, Duffy ST, Chen M, Peterson MD, Waddell TK, *et al.* Phospholipase C, calcium and calmodulin are critical for alpha4 beta1 integrin affinity up-regulation and monocyte arrest triggered by chemoattractants. Blood 2007; 109:176–184.

5 Seneschal J, Clark RA, Gehad A, Baecher-Allan CM, Kupper TS. Human epidermal Langerhans cells maintain immune homeostasis in skin by activating skin resident regulatory T cells. Immunity 2012; 36:873–884.

6 Klarquist J, Denman CJ, Hernandez C, Wainwright DA, Strickland FM, Overbeck A, *et al.* Reduced skin homing by functional Treg in vitiligo. Pigment Cell Melanoma Res 2010; 23:276–286.

7 Farag AGA, Haggag M, Muharram NM, Mahfouz R, Elnaidany NF, Abd El Ghany HM. Is vitamin D a participant in narrow-band ultra violet induced pigmentation in patients with vitiligo? J Egypt Women Dermatol Soc 2018; 15:30–34.

8 Fitzpatrick TB. Mechanisms of phototherapy of vitiligo. Arch Dermatol 1997; 133:1591–1592.

9 Taïeb A, Picardo M. Vitiligo (clinical practice). N Engl J Med 2009; 360:160–169.

10 Hamzavi H, Jain D, McLean J, Shapiro H, Zeng, Lui H. Parametric modeling of narrowband UV-B phototherapy for vitiligo, using a novel quantitative tool: the vitiligo area scoring index. Arch Dermatol 2004; 140:677–683.

11 El-Mofty M, Mostafa WZ, Bosseila M, Youssef R, Esmat S, El Ramly A, *et al.* A large scale analytical study on efficacy of different photo(chemo) therapeutic modalities in the treatment of psoriasis, vitiligo and mycosis fungoides. Dermatol Ther 2010; 23:428–434.

12 Parsad D, Kanwar AJ, Kumar B. Psoralen-ultraviolet A vs. narrow-band ultraviolet B phototherapy for the treatment of vitiligo. J Eur Acad Dermatol Venereol 2006; 20:175–177.

13 Bahnassy AA, Zekri AR, El-Houssini S, El-Shehaby AM, Mahmoud MR, Abdallah S, *et al.* Cyclin A and cyclin D1 as significant prognostic markers in colorectal cancer patients. BMC Gastroenterol 2004; 23:4–22.

14 Smyth Jf, Gourley C, Walker G, Mackean MJ, Stevenson A, Williams AR, *et al.* Antiestrogen therapy is active in selected ovarian cancer: the use of letrozole in estrogen receptor-positive patients. Clin Cancer Res 2007; 13:3617–3622.

15 Chantry D, Romagnani P, Raport CJ, Wood CL, Epp A, Gray PW. Macrophage-derived chemokine is localized to thymic medullary epithelial cells and is a chemoattractant for CD3(+), CD4(+), CD8(low) thymocytes. Blood 1999; 94:1890–1898.

16 Sebastiani S, Allavena P, Albanesi C, Nasorri F, Bianchi G, Traidl C, *et al.* Chemokine receptor expression and function in CD4+T lymphocytes with regulatory activity. J Immunol 2001; 166:996–1002.

17 Bettelli E, Carrier Y, Gao W, Korn T, Strom TB, Oukka M, *et al.* Reciprocal developmental pathways for the generation of pathogenic effector TH17 and regulatory T cells. Nature 2006; 7090:235–238.

18 Steitz J, Wenzel J, Gaffal E, Tuting T. Initiation and regulation of CD8+T cells recognizing melanocytic antigens in the epidermis: implications for the pathophysiology of vitiligo. Eur J Cell Biol 2004; 83:797–803.

19 Yang YS, Cho HR, Ryou JH, Lee MH. Clinical study of repigmentation patterns with either narrow-band ultraviolet B (NBUVB) or 308 nm excimer laser treatment in Korean vitiligo patients. Int J Dermatol 2010 49:317–323.

20 Taguchi K, Fukunaga A, Ogura K, Nishigori C. The role of epidermal Langerhans cells in NB-UVB-induced immunosuppression. Kobe J Med Sci 2013; 59:E1–E9.

21 Tembhre MK, Sharma VK, Sharma A, Chattopadhyay P, Gupta S. T helper and regulatory T cell cytokine profie in active, stable and narrow band ultraviolet B treated generalized vitiligo. Clin Chim Acta 2013; 424:27–32.

22 Eby JM, Kang H, Tully ST, Bindeman WE, Peiffer DS, Chatterjee S, *et al.* CCL22 to activate treg migration and suppress depigmentation in vitiligo. J Invest Dermatol 2015; 135:1574–1580.

23 Le Poole C, Mehrotra S. Replenishing regulatory T cells to halt depigmentation in vitiligo. J Investig Dermatol Symp Proc 2017; 18:S38–S45.