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Treatment of Non-Segmental Vitiligo with Narrowband UVB Phototherapy (311 Nm): Clinical Efficacy and Mechanisms of Action

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Abstract

Objective: To estimate efficacy of narrowband UVB phototherapy (311 nm) in non-segmental vitiligo and its effect on subpopulations of T-cells and dendritic cells in vitiligo lesions.

Methods: 56 patients with progressive non-segmental vitiligo underwent narrowband UVB phototherapy (311 nm). 11 patients had biopsies before and after therapy from lesional (depigmentation area), marginal, and perilesional skin for immunohistochemical studies of CD4+, CD8+, CD1a+ and CD83+ cells in epidermis and derma.

Results: Median number of UVB procedures per course was 71, median total radiation dose-91.8 J/cm^2, median percent of repigmentation-49%. Repigmentation of >25% of the affected area was reached in 67.9% of patients and repigmentation of 76-100% in 19.6% . VIDA index decreased from 2 to 1 post-treatment (P<0.035).

In a subgroup who underwent immunohistochemical studies median number of UVB procedures per course was 88, median total dose of radiation-140.2 J/cm^2, and median percent of repigmentation-50%. Significant activation of T-cellular immune reactions was found in lesions before treatment. There was a statistically significant increase in CD8+ lymphocytes and CD1a+ dendritic cells in marginal and perilesional normally pigmented skin in epidermis. There was an increase in CD4+ and CD8+ lymphocytes and CD83+ dendritic cells in all three zones in derma. Normalization of CD8+ and CD1+ cells in epidermis of the affected skin was observed post-treatment. There was only partial reduction of CD4+, CD8+ and CD1+ cells in dermis.

Conclusions: Narrowband UVB phototherapy (311 nm) is an effective method of treatment of nonsegmental vitiligo. T-lymphocytes and Langerhans cells play a critical immunoregulatory role in narrowband UVB phototherapy (311 nm)-induced immune suppression. Lack of full normalization of immunological parameters in the skin after treatment indicates a need for optimization of narrowband UVB phototherapy (311 nm): conduction of longer courses including 150-200 procedures, or combinations of this treatment with immunosuppressive drugs.

Introduction

Vitiligo is an acquired disease characterized by the formation of areas of depigmentation of the skin due to reduction in the number of melanocytes. The prevalence of vitiligo in the general population is ranging from 0.5 to 2% [1]. The etiology of the disease is unknown. There are several hypotheses regarding the mechanis of the development of the disease-genetic, autoimmune, neuro-humoral, oxidative stress, melanocytorrhagia, auto-cytotoxic, and convergent. According to many experts the leading role in the damage of melanocytes and the disturbance of melanogenesis in vitiligo skin is played by autoimmune mechanisms, including the disturbance of T-cell immunity [2-7]. Immune responses in areas of vitiligo involve a complex interaction of melanocytes, keratinocytes, and lymphocytes. Skin dendritic cells which are responsible for recognizing and binding a foreign antigen and initiation of immune responses have significant importance in vitiligo pathogenesis as well [8,9].

Treatment of vitiligo is a complex problem because existing therapies are not very effective. One of the most common treatments of vitiligo around the world is a narrowband UVB phototherapy with a wavelength of 311 nm. It is mostly used for patients with non-segmental vitiligo with affected area more than 10-20% of the body surface. Repigmentation after narrowband UVB phototherapy (311 nm) may vary from 41.6% to 100% [10]. Double-blind randomized study showed a higher efficiency of narrowband phototherapy in vitiligo in comparison with PUVA therapy: repigmentation of more than 50% of the lesions was observed in 64% and 36% of patients, respectively [11]. According to different authors, the number of narrowband UVB phototherapy (311 nm) procedures for one course can vary from 19 to 100 or more. At the same time, the optimal duration of treatment and the number of phototherapy treatments required to achieve maximal clinical effect has not been established.

Narrowband UVB phototherapy (311 nm) offers one of the most effective treatment modalities for vitiligo but its mechanisms of action are not well understood. The aim of our study was to evaluate clinical efficacy of UVB phototherapy (311 nm) in non-segmental vitiligo patients and to investigate its effect on the contents of main subpopulations of T-cells (CD4+ and CD8+ lymphocytes) and dendritic cells (CD1a+ and CD83+ cells) in vitiligo lesions.

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Materials and Methods

Patients age seven and above with a typical clinical picture of non-segmental vitiligo that had no contraindications to ultraviolet therapy were included in the study. All patients had disseminated lesions including face, neck, trunk, axillae, and extremities including feet and palms. Patients who had lesions only on their hands and feet were excluded from the study as well as patients who had showed no signs of improvement after 20 procedures. Narrowband UVB phototherapy (311 nm) was peformed in the ultraviolet cabin Waldmann UV 7001K "Herbert Waldmann GmbH and Co. KG" (Germany) using the TL-01 lamps emitting in the range 310-315 nm with a maximum emission at a wavelength of 311 nm. Before determining the initial dose the skin type was determined. Irradiation was carried out 2-3 times a week. This treatment was prescribed as monotherapy. Only moisturizers were used as additional preparations. Treatment efficacy was evaluated after treatment by the changes in the affected areas with the calculation of the percentage of repigmentation. In addition, activity of the disease in all patients before and after treatment was evaluated by VIDA index [12].

Immunohistochemical studies of the patients' skin were carried out on paraffin sections using an indirect immunoperoxidase method with pretreatment in unmasking solution (citrate buffer pH-6,0) in pressure cooker and using detection system Novostain super ABC Kit (universal) and DAB visualized system. Mouse monoclonal antibodies (Novocastra Laboratories Ltd, UK) were used for immunophenotyping. These antibodies were specific to antigen CD4-subpopulation T-helpers/inductors marker, CD8-marker of suppressor-cytotoxic T cells subpopulation, CD1a-Langerhans cells marker, CD83-marker of mature and activated dendritic cells. Ready-to-use monoclonal antibodies were used for determination of CD4, CD8 and CD1a markers. Monoclonal antibodies in typical working dilution of 1:30 were used for determination of CD83 marker. Prepared specimens were studied by means of light microscopy with Nikon Eclipse E 600. Images were recorded with a digital camera Nikon D 100.

The number of cells in the epidermis was determined in a field of view based on 100 basal keratinocytes. The study of dermis cells was conducted with selection of 20 cells from 5 perivascular infiltrates. After that the number of labeled cells was counted, and then mean percentage of cells in each preparation was calculated.

Statistical analysis was performed using the software package Statistica 6.1 (StatSoft, Inc., USA). Descriptive statistics of quantitative traits was presented as medians and quartiles (Me [Q1; Q3]). Mann-Whitney U-test was used for comparison of unrelated groups by both quantitative and ordinal traits. Wilcoxon test was used to compare related groups (analysis of the dynamics of signs). In testing of hypotheses differences were considered statistically significant at $P<0.05$.

Results

Narrowband UVB therapy (311 nm) was used in 56 non-segmental vitiligo patients (40 women and 16 men) aged 8 to 60 years (median age-22 years) with II and III skin phototypes. The duration of the disease ranged from three months to 42 years (median-6 years). All patients had progressive disease stage (median VIDA index was 2 points).

The initial dose ranged from 0.05 to 0.36 J/cm², the maximum dose-from 0.56 to 3.66 J/cm². The number of UVB therapy procedures per course ranged from 20 to 126 (median-71 procedures) with a total radiation dose from 9.0 to 244.2 J/cm² (median-91.8 J/cm²).

Treatment resulted in a positive effect in the form of repigmentation of more than 25% of the lesions in 38 patients (67.9%): 26-50% of repigmentation lesion was observed in 14 patients (25%), 51-75%-13 patients (23, 3%), 76-100%-11 patients (19.6%). The median repigmentation percentage was 49%. After a course of phototherapy the VIDA index decreased from 2 to 1 point ($P<0.035$).

Phototherapy was well tolerated by most patients; it did not cause a sharp contrast between skin areas, and looked cosmetically acceptable (Figure 1). Slight swelling of the eyelids during the treatment was observed in 5 patients, 6 patients had light skin peeling, 8 patients had itching, 4 patients complained of dry skin. In 6 patients with vitiligo hyperpigmentation formed around the lesions which disappeared in a few months after the end of phototherapy.

In 11 of 56 patients aged 24 to 47 years skin biopsies taken

A)

B)

Figure 1: Patient B, 13 years old. A) Vitiligo lesions on the left lateral surface of the trunk before treatment. B) The same vitiligo lesions after 49 procedures of narrowband UVB phototherapy (311 nm).

from 3 zones of the lesions: lesional skin (area of depigmentation), the marginal zone (bordering the area), and area of perilesional normally pigmented skin before and after the course of phototherapy. Immunohistochemical studies were performed for determination of content and distribution of CD4+ and CD8+ lymphocytes as well as CD1a+ and CD83+ dendritic cells in the epidermis and dermis. In this subgroup the median duration of the disease was 12 years, the median number of phototherapy procedures-88 (range-38 to 104), the median total dose-140.2 J/cm², and the median percentage of repigmentation-50%. The control group consisted of 10 healthy people. Groups of vitiligo patients and healthy subjects did not differ statistically by age and sex (P>0.05).

Figure 2: Results of immunohistochemical studies of skin of a healthy volunteer and a patient with vitiligo before treatment

(**a**) CD1a+ cells in skin of a healthy person ×400, (**b**) CD1a+ cells in lesional skin of a patient with vitiligo×200, (**c**) CD1a+ cells in marginal skin of a patient with vitiligo×200, (**d**) CD1a+ cells in perilesional normally pigmented skin of a patient with vitiligo×200.

(**e**) CD83+ dendritic cells in skin of a healthy person x100, (**f**) CD83+ dendritic cells in lesional skin of a patient with vitiligo×400, (**g**) CD83+ dendritic cells in marginal skin of a patient with vitiligo×400, (**h**) CD83+ dendritic cells in perilesional normally pigmented skin of a patient with vitiligo×400.

(**i**) CD4+ lymphocytes in skin of a healthy person ×100, (**j**) CD4+ lymphocytes in lesional skin of a patient with vitiligo×400, (**k**) CD4+ lymphocytes of marginal skin of a patient with vitiligo×400, (**l**) CD4+ lymphocytes in perilesional normally pigmented skin of a patient with vitiligo×400.

(**m**) CD8+ lymphocytes in skin of a healthy person ×400, (**n**) CD8+ lymphocytes in lesional skin of a patient with vitiligo×200, (**o**) CD8+ lymphocytes in marginal skin of a patient with vitiligo×400, (**p**) CD8+ lymphocytes in perilesional normally pigmented skin of a patient with vitiligo×400.

Immunohistochemical studies showed that in the epidermis of healthy individuals there were only CD1a+ cells, whereas the CD4+, CD8+ and CD83+ cells were absent (Figure 2). Study of patients' skin prior to treatment showed that there were single CD8+ lymphocytes in the basal layer of epidermis in all three zones of the lesions (mainly in the area of depigmentation). CD1a+ cells were also detected. The largest accumulation of these cells was observed in the basal layer of the epidermis and spinosum layer of epidermis. CD4+ cells and CD83+ cells were absent in the epidermis. Populations of all study cells were detected in the dermis in both healthy and patients.

Statistical analysis showed significant increase in CD8+ lymphocytes in all zones of epidermis of vitiligo patients in comparison with those in healthy people (Table 1). Amount of CD1a+ cells was increased in the marginal and perilesional normally pigmented zones (P<0.001). At the same time the number of these cells was lower in depigmentation zone (lesional skin) compared to the number in healthy people (which may be due to their migration from the skin to the draining lymph nodes).

In the patients' dermis there was statistically significant increase in CD8+ and CD4+ lymphocytes and CD83+ cells (Table 1). The greatest accumulation of these cells was noted in the marginal and perilesional normally pigmented zones. CD4+ lymphocytes were detected only in the perivascular infiltrates of the skin, whereas CD8+ cells were found in the perivascular infiltrates and in the papillary dermis. They were often located near the basal layer of the epidermis in close contact with the epithelial cells and in the epithelial lining. We have not found any difference in the dermis in the content of CD1a+ cells between patients and healthy people.
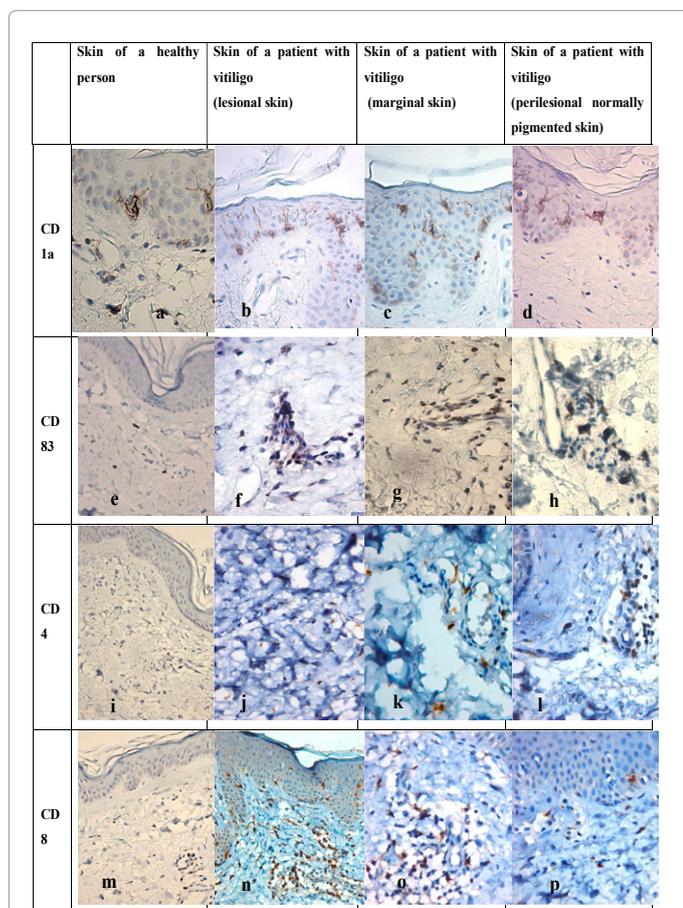
The data analysis after treatment showed that the improvement of the clinical picture of the disease after narrowband phototherapy (311 nm) was accompanied by positive changes in immunological indicators in the patients' skin. After a course of phototherapy in the epidermis a statistically significant reduction of CD8+ lymphocytes was observed in the lesional and perilesional normally pigmented skin, as well as the level of CD1a+ cells in all three zones of the lesions (Table 2). In perivascular infiltrates the number of CD4+ lymphocytes significantly decreased in perilesional normally pigmented skin, the number of CD8+ lymphocytes-in the marginal and perilesional normally pigmented zones and the number of CD1+ cells-in depigmentation zone (lesional skin) and in the marginal zone.

Comparison of cell populations in healthy individuals and patients after treatment did not reveal statistical differences in the number of CD8+ lymphocytes and CD1a+ cells in the marginal and perilesional normally pigmented skin of the epidermis, but normalization of these indicators in the area of depigmentation (lesional skin) was not observed (Table 3). Furthermore, phototherapy did not lead to the normalization in the subpopulations of lymphocytes and CD83+ cells in the perivascular infiltrates of the skin.

Discussion

Our data show efficacy of narrowband UVB phototherapy (311 nm) in patients with non-segmental vitiligo. There was a positive effect in the form of lesions repigmentation of more than 25% in 38 patients (67.9%). However, the good effect (repigmentation of 76-100% of the lesions) was observed only in 11 patients (19.6%). After a course of phototherapy there was a significant decrease in the VIDA index (double as compared with the index value before treatment) that indicates a decrease in the disease activity due to treatment.

In this work we evaluated the influence of narrowband UVB

Populations of cells	Patients with vitiligo (n=11)						
	Healthy volunteers (n=10)	Lesional skin	Marginal skin	Normally pigmented skin	P	P_1	P_2
Epidermis (cells)							
CD4+	0.0 (0.0; 0.0)	0.0 (0.0; 0.0)	0.0 (0.0; 0.0)	0.0 (0.0; 0.0)	-	-	-
CD8+	0.0 (0.0; 0.0)	0.4 (0.0; 0.4)	0.2 (0.0; 1.0)	0.2 (0.0; 0.4)	0.001	0.003	0.001
CD4+/CD8+	0.0 (0.0; 0.0)	0.0 (0.0; 0.0)	0.0 (0.0; 0.0)	0.0 (0.0; 0.0)	-	-	-
CD83+	0.0 (0.0; 0.0)	0.0 (0.0; 0.0)	0.0 (0.0; 0.0)	0.0 (0.0; 0.0)	-	-	-
CD1a	9.3 (8.8; 9.8)	6.0 (4.0; 9.0)	13.6 (12.4; 14.4)	13.2 (12.8; 14.8)	0.032	0.0003	0.0001
Derma (percent)							
CD4+	6.0 (5.0; 8.0)	10.0 (9.0; 12.0)	13.0 (9.0; 15.0)	14.0 (11.0; 18.0)	0.011	0.001	0.0002
CD8+	9.0 (7.0; 13.0)	22.0 (15.0; 28.0)	25.0 (20.0; 39.0)	26.0 (23.0; 29.0)	0.003	0.0001	0.0001
CD4+/CD8+	0.65 (0.60; 0.71)	0.48 (0.38; 0.72)	0.45 (0.38; 0.56)	0.55 (0.42; 0.58)	0.090	0.004	0.016
CD83+	6.5 (6.0; 10.0)	11.0 (9.0; 18.0)	21.0 (12.0; 26.0)	17.0 (12.0; 22.0)	0.016	0.001	0.007
CD1a+	6.5 (5.0; 8.0)	6.0 (4.0; 9.0)	5.0 (3.0; 6.0)	7.0 (5.0; 13.0)	1.0	0.523	0.943

Note: P, P_1 and P_2–levels of statistical significance when comparing indicators in healthy volunteers and patients, respectively, regarding lesional skin, marginal skin, and perilesional normally pigmented skin (Mann-Whitney U-test).

Table 1: Presence of subpopulations of lymphocytes and dendritic cells in the skin of healthy volunteers and patients with vitiligo before treatment (medians and quartiles).

Populations of cells	Lesional skin		Marginal skin		Perilesional normally pigmented skin		P	P_1	P_2
	Before	After	Before	After	Before	After			
Epidermis (cells)									
CD4+	0.0 (0.0; 0.0)	0.0 (0.0; 0.0)	0.0 (0.0; 0.0)	0.0 (0.0; 0.0)	0.0 (0.0; 0.0)	0.0 (0.0; 0.0)	-	-	-
CD8+	0.4 (0.0; 0.4)	0.0 (0.0; 0.2)	0.2 (0.0; 1.0)	0.0 (0.0; 0.2)	0.2 (0.0; 0.4)	0.0 (0.0; 0.2)	0.03	0.141	0.028
CD4+/CD8+	0.0 (0.0; 0.0)	0.0 (0.0; 0.0)	0.0 (0.0; 0.0)	0.0 (0.0; 0.0)	0.0 (0.0; 0.0)	0.0 (0.0; 0.0)	-	-	-
CD83+	0.0 (0.0; 0.0)	0.0 (0.0; 0.0)	0.0 (0.0; 0.0)	0.0 (0.0; 0.0)	0.0 (0.0; 0.0)	0.0 (0.0; 0.0)	-	-	-
CD1a	6.0 (4.0; 9.0)	5.0 (3.0; 7.0)	13.6 (12.4; 14.4)	10.0 (9.2; 11.0)	13.2 (12.8; 14.8)	10.0 (9.0; 10.8)	0.03	0.003	0.003
Derma (percent)									
CD4+	10.0 (9.0; 12.0)	10.0 (8.0; 14.0)	13.0 (9.0; 15.0)	11.0 (8.0; 13.0)	14.0 (11.0; 18.0)	10.0 (7.0; 12.0)	1	0.051	0.017
CD8+	22.0 (15.0; 28.0)	21.0 (17.0; 26.0)	25.0 (20.0; 39.0)	22.0 (21.0; 28.0)	26.0 (23.0; 29.0)	23.0 (20.0; 24.0)	0.894	0.037	0.025
CD4+/CD8+	0.48 (0.38; 0.72)	0.47 (0.33; 0.84)	0.45 (0.38; 0.56)	0.50 (0.33; 0.59)	0.55 (0.42; 0.58)	0.42 (0.40; 0.55)	1	0.878	0.155
CD83+	11.0 (9.0; 18.0)	22.0 (9.0; 26.0)	21.0 (12.0; 26.0)	21.0 (8.0; 22.0)	17.0 (12.0; 22.0)	21.0 (9.0; 26.0)	0.076	0.126	0.351
CD1a	6.0 (4.0; 9.0)	5.0 (3.0; 7.0)	7.0 (5.0; 13.0)	5.0 (3.0; 6.0)	6.0 (5.0; 7.0)	5.0 (3.0; 6.0)	0.042	0.013	0.185

Table 2: Presence of subpopulations of lymphocytes and dendritic cells in the skin of patients with vitiligo before and after narrowband UVB phototherapy (311 nm) (n=11, medians and quartiles).

Populations of cells	Patients with vitiligo (n=11)						
	Healthy Volunteers (n=10)	Lesional Skin	Marginal Skin	Normally Pigmented Cells	P	P1	P2
Edipdermis (cells)							
CD4+	0.0 (0.0; 0.0)	0.0 (0.0; 0.0)	0.0 (0.0; 0.0)	0.0 (0.0; 0.0)	-	-	-
CD8+	0.0 (0.0; 0.0)	0.0 (0.0; 0.2)	0.0 (0.0; 0.2)	0.0 (0.0; 0.2)	0.017	0.083	0.082
CD4+/CD8+	0.0 (0.0; 0.0)	0.0 (0.0; 0.0)	0.0 (0.0; 0.0)	0.0 (0.0; 0.0)	-	-	-
CD83+	0.0 (0.0; 0.0)	0.0 (0.0; 0.0)	0.0 (0.0; 0.0)	0.0 (0.0; 0.0)	-	-	-
CD1a	9.3 (8.8; 9.8)	5.0 (3.0; 7.0)	10.0 (9.2; 11.0)	10.0 (9.0; 10.8)	0.002	0.147	0.322
Derma (percent)							
CD4+	6.0 (5.0; 8.0)	10.0 (8.0; 14.0)	11.0 (8.0; 13.0)	10.0 (7.0; 12.0)	0.005	0.003	0.007
CD8+	9.0 (7.0; 13.0)	21.0 (17.0; 26.0)	22.0 (21.0; 28.0)	23.0 (20.0; 24.0)	0.0003	0.001	0.0001
CD4+/CD8+	0.65 (0.60; 0.71)	0.47 (0.33; 0.84)	0.50 (0.33; 0.59)	0.42 (0.40; 0.55)	0.105	0.006	0.001
CD83+	6.5 (6.0; 10.0)	22.0 (9.0; 26.0)	21.0 (8.0; 22.0)	21.0 (9.0; 26.0)	0.004	0.007	0.003
CD1a+	6.5 (5.0; 8.0)	5.0 (3.0; 7.0)	5.0 (3.0; 6.0)	5.0 (3.0; 6.0)	0.188	0.126	0.155

Note: P, P_1 and P_2–levels of statistical significance when comparing indicators of healthy volunteers and patients, respectively, regarding lesional skin, marginal skin and perilesional normally pigmented skin (Mann-Whitney U-test).

Table 3: Presence of subpopulations of lymphocytes and dendritic cells in the skin of healthy volunteers and patients with vitiligo after narrowband UVB phototherapy (311 nm) (medians and quartiles).

phototherapy (311 nm) on the presence of various types of immunocompetent cells in the skin of patients with vitiligo. Analysis of the presence of T-cells in skin of patients before treatment revealed the increased number of CD4+ and CD8+ lymphocytes in the lesions. Several other studies showed similar results [13,14]. Infiltration of skin by CD4+ and CD8+ lymphocytes is considered to be one of the indicators of the immune inflammation which is a pathogenetic basis of many chronic dermatoses, such as psoriasis, atopic dermatitis, lichen planus, etc., and it points to the involvement of T-cellular immunity in pathological process. CD8+ lymphocytes were found in the epidermis

while both CD4+ and CD8+ lymphocytes were present in derma; however, CD8+ lymphocytes dominated. Predominance of CD8+ lymphocytes in the lesions of vitiligo has also been found by others [14,15].

At this point it is not clear which of T-cells subpopulations of CD4+ or CD8+ play a leading role in the disease process. In a number of studies it has been established that destruction of melanocytes in the lesions of vitiligo was caused mainly by the impact of cytotoxic CD8+ T-lymphocytes [16-19].

We have found an increased number of CD1a+ dendritic cells in the epidermis in marginal and perilesional normally pigmented skin in patients with vitiligo. Similar data has been shown by others [8,20,21] and it points to the important role of this dendritic cell population in non-segmental vitiligo disease process. CD1a+ cells are antigen-presenting cells and represent one of the types of dendritic cells involved in the initiation of immune reaction in the skin. Moreover, we found a significant increase in the quantity of CD83+ dendritic cells in all three zones of vitiligo lesions. There are very few publications indicating involvement of CD83+ cells in vitiligo disease process [22]. CD83 molecule is a marker of the mature and activated dendritic cells [23]. Cells with antigen CD83 on their surface are capable of migrating with the lymph flow and to come into contact with naive T-cells (CD4+ and CD8+ lymphocytes), presenting an antigen. Subsequent cascade of signals between dendritic cells and T-cells initiates activation of naive T-cells and their subsequent differentiation [23,24]. N. Hirano *et al.* showed that active involvement of CD83+ cells in the presentation of an antigen can lead to proliferation of specific CD8+ lymphocytes [25]. The specified data indicates possible involvement of CD83+ cells in immunological mechanisms of melanocytes damage.

It is necessary to point out that abnormalites in the presence of T-cells and dendritic cells were found not only in pigmented and marginal skin, but also in normally pigmented skin. Our results confirm the observations of other authors that even normally pigmented skin (at sites distant from the afflicted area) is histologically abnormal [26-28]. Data analysis showed that improvement in the clinical picture of the disease under the influence of narrowband phototherapy (311 nm) was accompanied by positive dynamics of immunological parameters in patients' skin. After phototherapy in the epidermis normailzation in the number of CD8+ lymphocytes and CD1+ dendritic cells was observed in the depigmentation zone and perilesional normally pigmented skin. There was a partial reduction in the number of CD4+, CD8+ and CD1+ cells in the derma of patients. There are limited studies related to the effect of narrowband UVB phototherapy on T cells and dendritic cells in nonsegmental vitiligo and the results are contradictory.

Study conducted by B. Engin *et al.* where patients with vitiligo underwent narrowband UVB phototherapy (311 nm) did not reveal any significant changes in the amount of CD3, CD4 and CD8 cells in perilesional skin after treatment [29]. Probably, it can be explained by the fact that the dose of radiation received by patients in that study was twice lower than in our work. At the same time, there are publications about ability of UVB radiation to inhibit CD4 and CD8 cells and skin inflammation which are consistent with results of our studies regarding reduction of these cell populations in the skin of patients with vitiligo under the influence of phototherapy [30,31].

The reduction in the number of CD1+ cells found by us in vitiligo lesions observed after a course of phototherapy is consistent with data of F. Prignano et. al. [22] and also confirms data obtained by K. Taguchi et

al. [32] regarding an important role of these cells in immunosupressive effects of narrowband UVB phototherapy.

Thus, our studies showed that a course of narrowband UVB phototherapy (88 procedures) led to a partial reduction in CD4+, CD8+ and CD1+ cells in the skin of patients with vitiligo. However, full normalization of the studied indicators wasn't observed. At present the optimum narrowband UVB phototherapy parameters are not established. In particular, the optimum duration of treatment and also the maximum acceptable number of phototherapy procedures are not established. Considering theoretically possible risk of cancerogenic effect of high cumulative doses of the medium wave ultraviolet radiation, patients with I-III types skin should not undergo more than 200 procedures of narrow band phototherapy (311 nm) [33]. Lack of full normalization of immunological parameters in the skin of patients after treatment indicates the need in optimization of narrowband UVB phototherapy (311 nm): conduction of longer courses including 150-200 procedures, or combinations of this type of treatment with immunosuppressive drugs.

Conclusions

1. The use of narrowband UVB phototherapy (311 nm) is effective in the treatment of non-segmental vitiligo patients. Positive effect was observed in 67.9% of patients and good and excellent effect-in 19.6% of patients.

2. There was an increased number of CD4+ and CD8+ lymphocytes in areas of vitiligo compared to the skin of healthy individuals, indicating activation of T-cell immune responses. CD8+ lymphocytes were present in the epidermis and CD4+ and CD8+ lymphocytes, with predominance of the latter, were found in the dermis. Changes in the number of CD8+ lymphocytes were mostly seen in the marginal skin and perilesional normally pigmented skin.

3. In the epidermis of vitiligo patients there was a significant increase in the number of CD1+ cells (Langerhans cells) and in the dermis of the mature CD83+ dendritic cells, which shows the involvement of different populations of dendritic cells in the disease pathogenesis.

4. A course of narrowband UVB phototherapy (311 nm), which included 88 procedures, lead to normalization of CD8+ and CD1+ cells in the epidermis of the affected skin and partial reduction of the CD4+, CD8+ and CD1+ cells content in the patients' dermis. These findings indicate that T-lymphocytes and Langerhans cells play a critical immunoregulatory role in narrowband UVB phototherapy (311 nm)-induced immune suppression.

5. The lack of complete normalization of immunological parameters in the skin of patients after treatment indicates the need in optimization of UVB phototherapy (311 nm): conduction of longer courses including 150-200 procedures or combining this treatment with immunosuppressive drugs.

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