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Research Article

Influence of Oral Supplementation of Vitamins A and E on the Effectiveness of Vitiligo Treatment

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Oxidative stress plays an important role in the development of vitiligo. The aim of our study was to assess the effect of oral vitamin supplementation with antioxidant activity on the effectiveness of the vitiligo treatment. The study group consisted of 46 patients suffering from nonsegmental vitiligo for over a year. Before and after the therapy, the clinical advancement of the disease (VASI) and impairment of quality of life (DLQI) were assessed in all patients, and the activities of selected antioxidant enzymes (SOD, CAT, and GPx), lipid peroxidation products (MDA), and vitamins A and E were determined. Each patient was randomly assigned to one of three therapeutic groups: FOTO (UVB therapy at 311 nm, 3x a week for 4 months), WIT (oral antioxidant supplementation-vitamin A + E at a dose of 5000 IU of retinol and 400 mg of tocopherol), and FOTO + WIT (combination therapy). After the treatment, an increase in all antioxidant enzymes and a decrease in the concentration of malondialdehyde in patients were noted, regardless of the therapeutic method used. The greatest improvement in the repigmentation of skin lesions was achieved in patients treated with combined therapy (VASI -6.95 ± 4.69, $p < 0.01$, DLQI -1.90 ± 2.77, $p = 0.011$). The burden of patients with risk factors for oxidative stress turned out to be associated with a greater severity of the disease process, and a longer period of disease was positively correlated with a reduction in the quality of life in patients.

1. Introduction

Vitiligo is the most common depigmentation skin disease, affecting 0.5–2% of the general population [1]. Clinical symptoms result from the dysfunction and destruction of pigment cells. These processes lead to development of depigmented patches of skin of various shapes. They are most often located on the face, the backs of the hands, and in the mammary, axillary, sacral, inguinal, navel, and anus areas [2].

The diagnosis of vitiligo is based mainly on the clinical course. Laboratory determinations and specialist consultations are necessary only to exclude the accompanying disorders [3]. Wood's lamp turned out to be helpful in diagnostics, especially with people with light complexion in order to assess the extent of skin lesions [4]. Histopathological preparations taken from lesions show a lack of melanocytes and melanin in the region of the basement membrane [3]. Moreover, a superficial infiltration of mononuclear lymphocytes with CD 4+ and CD 8+ is present in the marginal zone of vitiligo [5].

Vitiligo is a multifactorial disease. There are various theories explaining the causes of skin lesions, including genetic, oxidative stress, autoimmune, autoinflammatory, neurogenic, melanocytorrhagy, apoptotic, and multifactorial [6].

Currently, the most widely accepted theory is the multifactorial theory. Individuals with a genetic predisposition develop skin lesions following exposure to particular environmental factors [7]. Scientific reports indicate that in approximately 20% of patients, at least one first-degree relative is also affected [8]. The risk of developing vitiligo for these people is increased by about 7–10 times. Thanks to many years of observation of patients and genomic association studies (GWAS), susceptibility genes were discovered that are related to melanin biosynthesis, the antioxidant system, and the regulation of the immune system [9, 10].

In genetically predisposed people, in the case of additional environmental conditions, the disease may develop. Puberty, pregnancy, severe infections, mental stress, and skin injuries are factors that trigger symptoms of the disease in patients [7]. Exogenous stimuli are also of great importance in the synthesis of excess oxidative by-products. Among them, exposure to environmental factors such as ultraviolet radiation, cytotoxic chemicals, certain drugs, and diseases (cancer, severe infections, and nervous disorders) predominates.

The process of destroying melanocytes is probably initiated by oxidative stress, which is considered to be one of the first elements in the development of vitiligo. It is responsible for the induction of the inflammatory process and the recruitment of immune cells that lead to the progressive process of melanocyte apoptosis [11, 12].

Under the influence of cellular stress, the so-called damage-associated molecular patterns (DAMPs) are formed. They are endogenous molecules released following cell damage that activates nonspecific immunity mechanisms by interacting with pattern recognition receptors (PRPs). These include the NLRP1 receptor, overexpression of which leads to the activation of the inflammasome and the induction of the transition from pro-IL-1β to active IL-1β [13]. Overproduction of reactive oxygen species causes the secretion of exosomes by melanocytes, which, in addition to heat shock proteins, contain antigens specific for melanocytes. Exosomes, containing mesosomal proteins, are released from the pigment cells and presented by Langerhans cells to T lymphocytes. They form neoantigens that stimulate the process of the authoritative induction of melanocyte apoptosis by recruiting cells of the immune system.

The cytotoxic CD8+ T lymphocytes, which express the CXCR3 receptor on their surface, through which they migrate to the epidermis, are mainly involved in the process of melanocyte apoptosis [14]. It is a receptor shared by the C-X-C motif chemokine ligands such as CXCL9 and CXCL10. It was found that TCD8 + IFN-γ produced by TCD8 + lymphocytes recruited JAK1 and JAK2 kinases upon binding to the receptor [3]. Activated JAK kinases cause STAT phosphorylation and nuclear translocation. The JAK-STAT signaling pathway stimulates keratinocytes to produce CXCL9 and CXCL10 [15]. Then, a positive feedback loop is created, the effect of which is the recruitment of cytotoxic lymphocytes and the induction of melanocyte apoptosis [16].

2. Materials and Methods

The study was approved by the Medical Bioethics Committee No. 640/2015 on September 25, 2015. The study group consisted of 46 patients suffering from vitiligo under the care of the Clinic of Dermatology, Sexually Transmitted Diseases, and Immunodermatology in Bydgoszcz. The control group consisted of 20 healthy volunteers, matched appropriately for age and sex.

Adult patients suffering from nonsegmental vitiligo for over a year were qualified for the study. Criteria for excluding patients from the study were as follows: vitamin K deficiency, fat malabsorption syndrome, severe liver failure, obstruction of the bile ducts, the period of breastfeeding, pregnancy, condition after anastomosis of the jejunum and the ileum, photodermatosis, use of photosensitizing drugs, a set of atypical birthmarks, past malignant skin cancers, claustrophobia, epilepsy, circulatory failure III-IV NYHA, reaching the cumulative dose of UVB 311, eye diseases (cataracts, keratitis, macular degeneration).

After signing the informed consent for voluntary participation in the study, an interview was conducted with each patient, taking into account accompanying diseases (ischemic heart disease, hypercholesterolaemia, hypertension, neurodegenerative disease, hyperthyroidism/hypothyroidism, diabetes, obesity, neoplastic diseases, generalized atherosclerosis, bronchial asthma), medications (doxorubicin, cisplatin, paracetamol, diclofenac, rifampicin, chloramphenicol, gentamicin, penicillins, amphotericin, nystatin, natamycin, chlorpromazine, iproniazid, hydralazine, gossypol), nicotynism/alcoholism, exposure to stress, UVR, and heavy metals. Based on the interview, the patient's exposure to oxidative stress factors was assessed (each factor = 1 point). Then, photographic documentation of skin lesions was made and the clinical stage of the disease was determined (VASI scale). Patients were also asked to complete the DLQI scale to assess the impact of the disease on their quality of life.

Blood samples were taken twice from vitiligo patients (before and after NB-UVB therapy) and once from the control group. In the laboratory of the Department of Biology and Medical Biochemistry, the activity of selected antioxidant enzymes, lipid peroxidation products and vitamin A and E concentrations were assessed on the collection day. The activity of superoxide dismutase (SOD), catalase (CAT), glutathione peroxidase (GPx), and the concentration of substances reacting with thiobarbituric acid (TBARS) were determined by spectrophotometry (Table). The concentration of malondialdehyde (MDA) and vitamins A and E in the blood plasma was measured using high-performance liquid chromatography.

Patients were randomly assigned to three therapeutic groups: the FOTO group treated with UVB 311 nm phototherapy (3 times a week for 4 months), the WIT group treated with oral antioxidant supplementation over-the-

counter vitamin A + E preparation at a dose of 5000 IU of retinol and 400 mg of tocopherol, and the FOTO + WIT group, who received combined therapy (UVB 311 nm phototherapy 3 times a week for 4 months with simultaneous oral supplementation of vitamin A + E at a dose of 5000 IU of retinol and 400 mg of tocopherol).

A 311 nm UVB device (Waldmann UV7001K, Germany) was used for phototherapy. As recommended, the initial dose was 200 mJ/cm^2, regardless of the skin phototype. It was increased by 10–20% during the therapy, which was carried out 3 times a week. The duration of treatment was set at 48 irradiations.

Statistica 13 software and MS Excel from the Office 360 suite were used for the analysis of the results. The quantitative results were presented as mean values with standard deviation ($\overline{x} \pm \mathrm{SD}$). The compliance of the distribution of results with the normal distribution was checked using the Shapiro–Wilk test, separately for each group. The comparison of the results with a normal distribution between the groups was performed using the one-way ANOVA test, and the analysis of two continuous variables was performed using the Pearson correlation test. The analysis for dependent variables (before and after therapy) was performed with Student's t-test for dependent variables. Data not normally distributed were compared using the Kruskal–Wallis test for the three groups. The correlations of two variables where there is no normal distribution were performed using the Spearman rank correlation test. In the case of nonparametric decomposition data compared before and after treatment, the Wilcoxon test was used. Statistically significant differences between the groups were considered when p value <0.05. Correlated variables were considered when $R > 0.3$ while $p < 0.05$ for the statistical test.

3. Results

Comparative analysis in terms of age, gender distribution in the study and control groups, and the initial concentration of biochemical parameters did not indicate any statistically significant differences. The share of women and men in the control group (55% and 45%) was similar to that in the study group (60.9% and 39.1%) (Table 1).

Age distribution and disease duration in different therapeutic groups in patients with vitiligo did not differ statistically significantly (Table 2).

The results between the study groups do not differ statistically significantly in terms of biochemical parameters, except for vitamin E concentration, which is the highest in the FOTO group (8.8 ± 8.12) and the lowest in the combined group (2.83 ± 2.75) $p = 0.043$ (Table 3).

The results of biochemical analyses showed an increase in the concentrations of all assessed antioxidant enzymes after the applied therapy. The largest difference in catalase activity was observed in patients treated with the combined method despite no significant differences ($p = 0.287$) (Table 4). Measurement of glutathione peroxidase concentration in patients in this treatment group also showed the highest increase, which shows a significant difference ($p = 0.023$) (Table 4). The concentration of superoxide dismutase increased proportionally to its baseline values in patients, regardless of the treatment method used. However, no statistically significant differences between the analyzed groups of patients were observed ($p = 0.987$).

Table 1: Analysis of the distribution of sex, age, and biochemical parameters between the study group and the control group.

Variables	Control group	Study group	p
Sex, N (%)			
Female	11 (55%)	28 (60.9%)	0.656^{X}
Male	9 (45%)	18 (39.1%)	
Age (lata)	40.2 (7.5)	41.5 (10.3)	0.605^{T}
CAT ($10e^4 \times$ IU/g Hb)	71.3 (10.2)	68 (11.9)	0.283^{T}
SOD (U/g Hb)	770.9 (107.8)	759.4 (116)	0.630^{U}
GPX (U/g Hb)	8.05 (2.64)	8.2 (4.55)	0.691^{U}
Plasma MDA (nmol/ml)	0.45 (0.09)	0.44 (0.08)	0.815^{T}
MDA of erythrocytes (nmol/g Hb)	22.91 (5.18)	26.81 (11.04)	0.202^{U}

N, number; mean (SD), standard deviation. ^{T}Analyzed by the independent t-test; ^{U}analyzed by the Mann–Whitney U test; ^{X}analyzed by the chi-squared test.

In all patients, regardless of the therapeutic method used, a decrease in the concentration of malondialdehyde was observed, which reflects the degree of severity of oxidative stress in them. The highest decrease in the final value of MDA in plasma was found in patients after the use of isolated phototherapy (-0.05 ± 0.10 nmol/ml) and its combination with oral antioxidant supplementation (-0.04 ± 0.14 nmol/ml) (Table 5). However, no statistically significant differences were found between the studied groups ($p = 0.748$). In terms of malondialdehyde concentration in erythrocytes, the highest difference between the initial and final concentration was found in the group of patients who received combined therapy (-6.65 ± 15.41 nmol/ml). The results of these studies also showed no statistical significance in comparison between the groups ($p = 0.968$) (Table 5).

After the therapy, an increase in the concentration of vitamin E in the blood plasma of patients in all treatment groups was observed after treatment. The highest difference in values was observed in the case of patients undergoing combination therapy. In this group, the observed changes in vitamin E concentration were statistically significant ($p = 0.001$), similar to the patients treated with the oral supplement ($p = 0.004$) (Table 4).

After treatment, there was also a statistically significant increase in vitamin A concentration in the blood plasma in patients undergoing combination therapy and its decrease as a result of the applied phototherapy (Table 4). The differences between the groups after treatment were statistically significant ($p = 0.007$) (Table 5).

Regarding the studied clinical parameters, the greatest improvement in the repigmentation of skin lesions was observed in patients treated in a combined manner, with a decrease in VASI of 6.95 ± 4.69 ($p < 0.001$) (Table 4). Scoring on the scale showed statistically significant differences in this respect between individual groups of patients ($p < 0.001$) (Table 5). No adverse effects of the applied therapies were observed.

Table 2: Analysis of the age, duration of the disease, and the degree of exposure to oxidative stress in the studied groups of people.

Variables	FOTO	FOTO + WIT	WIT	p
Patient's age	2.7 (1.1)	2.4 (0.6)	2.1 [1]	0.321^{H}
Duration of illness (years)	10.7 (5.5)	6.7 (4.2)	8.5 (8.5)	0.071^{H}
Degree of exposure to oxidative stress (points)	43.9 [13]	38.3 (6.1)	42.1 (10.1)	0.266^{H}

Median (p25–p75). ^{H}Analyzed by the Kruskal–Wallis H test.

Table 3: Analysis of biochemical parameters and VASI and DLQI scales before therapy.

Variables	FOTO	FOTO + WIT	WIT	p
CAT (U/g Hb $\times 10^4$)	69.83 (12.45)	64.79 (12.7)	69.19 (10.65)	0.457^{A}
SOD (U/g Hb)	771 (122.1)	740.4 (96.9)	766.2 (131.7)	0.911^{H}
GPX (U/g Hb)	7.35 (4.23)	7.22 (3.82)	10.09 (5.2)	0.172^{H}
wit. A (μg/l)	0.57 (0.17)	0.66 (0.43)	0.57 (0.15)	0.992^{H}
wit. E (μg/l)	8.8 (8.12)	2.83 (2.75)	4.48 (3.39)	0.041^{H}
Plasma MDA (nmol/ml)	0.53 (0.33)	0.58 (0.31)	0.49 (0.29)	0.494^{H}
MDA of erythrocytes (nmol/g)	0.58 (0.3)	0.49 (0.31)	0.53 (0.27)	0.719^{H}
VASI (points)	19.13 (10.53)	18.08 (5.46)	16.1 (14.37)	0.299^{H}
DLQI (points)	8.13 (3.9)	10.4 (4.63)	8.13 (5.48)	0.210^{H}

Mean (SD), standard deviation. ^{H}Analyzed by the Kruskal–Wallis H test; ^{A}analyzed by the ANOVA test.

Table 4: Comparison of biochemical parameters and VASI and DLQI scales before and after treatment.

Variables	Group	Before treatment	After treatment	p
CAT (U/g Hb $\times 10^4$)	FOTO	69.83 (12.45)	71.28 (12.51)	0.597^{T}
	FOTO + WIT	64.79 (12.7)	68.73 (11.39)	0.287^{T}
	WIT	69.19 (10.65)	69.35 (9.29)	0.963^{T}
SOD (U/g Hb)	FOTO	771 (122.1)	787.5 (155.5)	0.721^{T}
	FOTO + WIT	740.4 (96.9)	749.6 (72.4)	0.658^{T}
	WIT	766.2 (131.7)	781.8 (108.6)	0.609^{W}
GPX (U/g Hb)	FOTO	7.35 (4.23)	28.7 (61.63)	0.233^{W}
	FOTO + WIT	7.22 (3.82)	11.37 (5.47)	0.023^{W}
	WIT	10.09 (5.2)	9.78 (4.25)	0.836^{T}
wit. A (μg/l)	FOTO	0.57 (0.17)	0.53 (0.28)	$<0.001^{W}$
	FOTO + WIT	0.66 (0.43)	0.85 (0.18)	$<0.001^{W}$
	WIT	0.57 (0.15)	0.68 (0.18)	0.059^{T}
wit. E (μg/l)	FOTO	8.8 (8.12)	9.05 (7.93)	0.605^{W}
	FOTO + WIT	2.83 (2.75)	16.65 (15.62)	0.001^{W}
	WIT	4.48 (3.39)	11.02 (7.8)	0.004^{W}
Plasma MDA (nmol/ml)	FOTO	0.53 (0.33)	0.55 (0.29)	0.730^{W}
	FOTO + WIT	0.58 (0.31)	0.69 (0.6)	0.691^{W}
	WIT	0.49 (0.29)	0.47 (0.27)	0.733^{W}
MDA of erythrocytes (nmol/g)	FOTO	0.58 (0.3)	0.8 (0.35)	0.048^{T}
	FOTO + WIT	0.49 (0.31)	0.67 (0.35)	0.256^{W}
	WIT	0.53 (0.27)	0.75 (0.35)	0.065^{T}
VASI (points)	FOTO	19.13 (10.53)	11.97 (7.17)	0.001^{W}
	FOTO + WIT	18.08 (5.46)	11.13 (4.12)	$<0.001^{T}$
	WIT	16.1 (14.37)	15.86 (14.1)	0.018^{W}
DLQI (points)	FOTO	8.13 (3.9)	6.5 (3.25)	0.002^{W}
	FOTO + WIT	10.4 (4.63)	8.5 (4.22)	0.013^{W}
	WIT	8.13 (5.48)	7.8 (5.05)	0.138^{W}

Mean (SD), standard deviation. ^{W}Analyzed by the Wilcoxon test; ^{T}analyzed by the dependent t-test.

The analysis of the change in the quality of life index showed an improvement in the DLQI scale in the group of patients treated in a combined manner. As a result of the therapy, the DLQI index decreased by -1.90 ± 2.77 points. The differences between the individual treatment groups were statistically significant ($p = 0.011$) (Table 5).

With regard to the studied correlations of biochemical and clinical parameters, a relationship was found between the age of the patients and change in the concentration of malondialdehyde in their plasma after phototherapy ($r = -0.629$; $p = 0.009$) and isolated vitamin supplementation with antioxidant activity ($r = -0.551$; $p = 0.033$). A

Table 5: Analysis of differences obtained after therapy for biochemical parameters and VASI and DLQI scales.

Variables	FOTO	FOTO + WIT	WIT	p
CAT (U/g Hb × 10^4)	1.45 (10.74)	3.93 (13.77)	0.16 (13.45)	0.711^{A}
SOD (U/g Hb)	16.5 (181.7)	9.2 (79.2)	15.6 (125.5)	0.987^{A}
GPX (U/g Hb)	21.34 (63.63)	4.15 (6.35)	−0.31 (5.65)	0.154^{H}
wit. A (μg/l)	−0.04 (0.25)	0.19 (0.47)	0.11 (0.21)	0.007^{H}
wit. E (μg/l)	0.25 (4.93)	13.82 (14.16)	6.55 (8.46)	$<0.001^{H}$
Plasma MDA (nmol/ml)	0.02 (0.35)	0.11 (0.53)	−0.02 (0.35)	0.748^{H}
MDA of erythrocytes (nmol/g)	0.22 (0.4)	0.18 (0.52)	0.22 (0.42)	0.968^{A}
VASI (points)	−7.15 (5.7)	−6.95 (4.69)	−0.24 (0.35)	$<0.001^{H}$
DLQI (points)	−1.63 (1.41)	−1.9 (2.77)	−0.33 (0.82)	0.011^{H}

Mean (SD), standard deviation. ^{H}Analyzed by the Kruskal–Wallis H test; ^{A}analyzed by the ANOVA test.

similar relationship was found between the age of the patients and the change in plasma catalase concentration after exposure to UVB at 311 nm ($r = -0.582$; $p = 0.018$). In both cases, this relationship showed statistical significance and was inversely proportional (Table 6).

The statistical test showed a statistically significant correlation between the score of the scale of exposure to oxidative stress and the initial VASI ($r = -0.322$; $p = 0.029$) (Table 7).

4. Discussion

Vitiligo therapy remains one of the most difficult dermatological challenges. The aim of the treatment is to stop the disease's progression, stimulate the repigmentation of discolored macules and stabilize the disease process [17].

The current guidelines, developed by the Polish Dermatological Society, recognize the use of UVB 311 nm phototherapy in combination with topical steroids or calcineurin inhibitors as the most optimal treatment regimen. The therapy should be continued for up to 2 years if we observe stabilization of the disease and gradual repigmentation of skin lesions. In the case of rapid disease progression and no response to treatment after 3–6 months, the consensus is to use additional weekend mini-pulses from oral glucocorticosteroids. Stabilization of the disease and further lack of repigmentation suggests considering the use of surgical methods or depigmentation procedures.

Considering the important role of oxidative stress in the etiopathogenesis of vitiligo, its therapy should include the supplementation of compounds with antioxidant properties. However, there are no precise recommendations regarding the form, dose and duration of such therapy. Previous studies of their effectiveness in repigmentation of skin lesions in patients show contradictory results.

So far, preparations containing antioxidant enzymes such as superoxide dismutase, catalase, and pseudocatalase have been used in local therapy with varying success [18–20].

A greater achievement is the use of oral antioxidants. They include hydrophobic antioxidants that inhibit the process of lipid peroxidation, guarding the integrity of cell membranes. Numerous studies have confirmed the effectiveness of vitamins E and A, as well as plant extracts from *Polypodium leucotomos* [21] and *Ginkgo biloba* [22].

A 2007 study showed that an oral supplement containing alpha-lipoic acid, vitamins A and E, and polyunsaturated fatty acids increased the effectiveness of UVB 311 [23]. Patients in the study group received the preparation 2 months before and 6 months during phototherapy. Almost half of the patients treated in this way had over 75% repigmentation compared to 18% in the placebo group.

In a 2015 study, the authors gave patients an antioxidant containing 100 mg of *P. emblica*, 10 mg of vitamin E and 4.7 mg of carotenoids 3 times a day. [24]. Compared to the control group, treated topically and/or with UVB 311 nm without additional supplementation, after 6 months of treatment, they had a significantly higher percentage of whitewash repigmentation. Moreover, patients from the control group showed more severe symptoms of inflammation ($p = 0.002$), faster progression of lesions ($p = 0.039$), and a higher percentage of disease exacerbations ($p = 0.003$).

Similar results, indicating the advisability of combining phototherapy with oral vitamin E supplementation, were obtained by Elgoweini and Din [25]. As a result of the abovementioned treatment, the patients achieved a higher percentage of repigmentation in comparison to the patients using the isolated 311 nm UVB irradiation (72.7% vs. 55.6%). The therapy also resulted in a statistically significant reduction in the level of MDA in the plasma of patients from the study group.

There are several reports on the beneficial effect of oral supplementation with ginkgo biloba extract on the repigmentation of vitiligo lesions in the backs of the hands and face. In one study, patients from group A received gingko biloba extract in a dose of 40 mg three times a day, while patients from group B received a placebo [26]. In patients treated with ginkgo extract, statistically significant inhibition of disease progression was noted. Complete repigmentation was observed in 10 patients in group A; in only 2 patients in group B was a similar remission rate observed.

In a report from 2007, it was shown that the plant extract of *Polypodium leucotomos* has antioxidant and immunomodulatory effects, improves repigmentation in patients undergoing NB-UVB phototherapy [27]. 55 patients with vitiligo received orally 250 mg of the extract or placebo 3 times a day, in combination with NB-UVB twice a week for 25-26 weeks. As a result of the therapy, a higher percentage of facial and neck repigmentation was achieved in patients

Table 6: Statistical analysis of the correlation of age in relation to biochemical parameters with the division into the studied group.

Age of the patient	FOTO		FOTO + WIT		WIT	
	r	p	r	p	r	p
	The size of the obtained differences after the therapy					
CAT (U/g Hb × 10^4)	−0.582	0.018	0.263	0.344	−0.050	0.859
SOD (U/g Hb)	0.143	0.597	0.197	0.483	−0.456	0.088
GPX (U/g Hb)	0.133	0.624	−0.088	0.756	0.235	0.399
wit. A (μg/l)	−0.456	0.076	−0.170	0.545	−0.092	0.746
wit. E (μg/l)	0.382	0.144	−0.148	0.598	−0.214	0.445
Plasma MDA (nmol/ml)	−0.629	0.009	0.005	0.985	−0.551	0.033
MDA of erythrocytes (nmol/g)	−0.148	0.585	0.434	0.106	−0.133	0.637
VASI (points)	0.025	0.926	−0.123	0.664	−0.053	0.852
DLQI (points)	0.072	0.790	0.312	0.258	−0.234	0.402

r, analyzed by the Spearman's rank correlation.

Table 7: Statistical analysis of the correlation of the degree of exposure to stress in relation to the biochemical parameters obtained before the therapy for all participants of the study.

The degree of exposure to oxidative stress	All study participants	
	r	p
CAT (U/g Hb × 10^4)	−0.078	0.605
SOD (U/g Hb)	0.113	0.453
GPX (U/g Hb)	−0.223	0.136
wit. A (μg/l)	−0.018	0.906
wit. E (μg/l)	0.016	0.917
Plasma MDA (nmol/ml)	0.228	0.127
MDA of erythrocytes (nmol/g)	0.140	0.354
VASI (points)	0.322	0.029
DLQI (points)	0.026	0.866

receiving antioxidant supplementation, compared to the control group (44% vs. 27%, $p = 0.06$).

The results of the studies conducted so far indicate a beneficial effect of oral antioxidant supplementation on the effectiveness of the standardly recommended therapy. This is also confirmed by the results of our research. However, this issue requires further research in order to refine these recommendations.

5. Conclusions

In conclusion, the overall results of our study show a clear involvement of oxidative-antioxidant homeostasis in the therapeutic process of vitiligo. The use of combination therapy, consisting of combining UVB 311 nm phototherapy with oral supplementation of vitamins A and E, was associated with the best results in terms of repigmentation of vitiligo spots. It turned out that the age of patients suffering from vitiligo who underwent NB-UVB 311 nm phototherapy negatively correlated with a decrease in plasma MDA concentration and an increase in CAT concentration in erythrocytes, which allowed the conclusion that the effectiveness of the therapy, which decreases with age, results from a lower antioxidant capacity of the organism. It was observed that the duration of the disease positively correlated with the concentration of CAT and SOD, which can be explained by an attempt to compensate for oxidoreductive disorders increasing over time. The longer the disease duration, the greater the reduction in patients' quality of life. The additional burden of the patients with oxidative stress factors turned out to be related to the greater intensity of the disease activity in the patients. The observations made confirm the system's attempts to maintain homeostasis in the field of oxidant-antioxidant balance, increasing with the duration of the disease, and the ability to repair membrane damage that deteriorates with age. It also significantly influences the higher degree of severity and extent of skin lesions in these patients (VASI scale) and the lower improvement in the quality of life obtained after treatment (DLQI scale). That is why, it is so important to implement the therapy at an early stage of the disease's development, which provides the best opportunities for repigmentation of skin lesions. Oral supplementation of vitamins A and E during phototherapy also seems justified, which seems to increase the effectiveness of the treatment.

Data Availability

The research results are available in the doctoral dissertation, "Evaluation of the oxidative-antioxidant balance and lipid peroxidation products in patients with vitiligo" in the collection of the Ludwik Rydygier Collegium Medicum Library in Bydgoszcz.

Conflicts of Interest

The authors declare that they have no conflicts of interest.

Authors' Contributions

Laura Nowowiejska, MD, PhD, was responsible for collecting material from patients, evaluating the results of the research and writing a dissertation based on them. Prof. dr hab. n. med. Rafał Czajkowski supervised the content of the entire study, giving invaluable help when editing the material for publication. Luiza Marek-Józefowicz, MD, PhD, was responsible for recruiting patients for the study. Prof. dr hab. n. med. Alina Woźniak and Prof. dr hab. Karolina Szewczyk-Golec assessed the oxidant-antioxidant balance in patients with vitiligo. Prof. Barbara Zegarska, MD, Marek Jankowski, MD, Anna Niezgoda, and MD Magdalena

Basałygo collaborated with the principal researcher in assessing the obtained biochemical parameters and their interpretation in the light of the latest research. All authors accessed the full text of the article, read, and approved its final form.

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References

[1] C. Kruger and K. U. Schallreuter, "A review of the worldwide prevalence of vitiligo in children/adolescents and adults," *International Journal of Dermatology*, vol. 51, no. 10, pp. 1206–1212, 2012.

[2] K. Ezzedine, V. Eleftheriadou, M. Whitton, and N. Van Geel, "Vitiligo," *The Lancet*, vol. 386, no. 9988, pp. 74–84, 2015.

[3] C. Bergqvist and K. Ezzedine, "Vitiligo: a review," *Dermatology*, vol. 236, no. 6, pp. 571–592, 2020.

[4] M. S.-G. Misterska and Ż. R. Aetiopathogenesis, "Clinical picture and treatment od vitiligo," *Post Dermatol Alergol*, vol. 4, pp. 212–223, 2009.

[5] A. Faria, R. Tarle, G. Dellatorre, M. Mira, and C. C. S. Castro, "Vitiligo--Part 2--Classification, histopathology and treatment," *Anais Brasileiros de Dermatologia*, vol. 89, no. 5, pp. 784–790, 2014.

[6] W. C. Placek and A. Chabior, "Bielactwo nabyte," *Dermatol Prakt*.vol. 3, pp. 9–18, 2009.

[7] R. P. Czajkowski, W. Placek, I. Flisiak et al., "Vitiligo. Diagnostic and therapeutic recommendations of the polish dermatological society," *Dermatology Review*, vol. 106, no. 1, pp. 1–15, 2019.

[8] S. Nath, P. Majumder, and J. Nordlund, "Genetic epidemiology of vitiligo: multilocus recessivity cross-validated," *The American Journal of Human Genetics*, vol. 55, no. 5, pp. 981–990, 1994.

[9] R. A. Spritz and G. Andersen, "Genetics of vitiligo," *Dermatologic Clinics*, vol. 35, no. 2, pp. 245–255, 2017.

[10] R. Czajkowski and K. Mecinska-Jundzill, "Current aspects of vitiligo genetics," *Advances in Dermatology and Allergology*, vol. 4, no. 4, pp. 247–255, 2014.

[11] R. Speeckaert, J. Dugardin, J. Lambert et al., "Critical appraisal of the oxidative stress pathway in vitiligo: a systematic review and meta-analysis," *Journal of the European Academy of Dermatology and Venereology*, vol. 32, no. 7, pp. 1089–1098, 2018.

[12] Y. Wang, S. Li, and C. Li, "Perspectives of new advances in the pathogenesis of vitiligo: from oxidative stress to autoimmunity," *Medical Science Monitor*, vol. 25, pp. 1017–1023, 2019.

[13] C. Festa Neto, "Inflammasomes and Dermatology," *Anais Brasileiros de Dermatologia*, vol. 91, no. 5, pp. 566–578, 2016.

[14] J. Harris, "Cellular stress and innate inflammation in organ-specific autoimmunity: lessons learned from vitiligo," *Immunological Reviews*, vol. 269, no. 1, pp. 11–25, 2016.

[15] M. Rashighi, P. Agarwal, J. Richmond et al., "Cxcl10 is critical for the progression and maintenance of depigmentation in A mouse model of vitiligo," *Science Translational Medicine*, vol. 6, no. 223, Article ID 3007811, 2014.

[16] L. Yang, Y. Wei, Y. Sun et al., "Interferon-gamma inhibits melanogenesis and induces apoptosis in melanocytes: a pivotal role of Cd8+ cytotoxic T lymphocytes in vitiligo," *Acta Dermato-Venereologica*, vol. 95, no. 6, pp. 664–670, 2015.

[17] J. Richmond, J. Strassner, M. Rashighi et al., "Resident memory and recirculating memory T cells cooperate to maintain disease in A mouse model of vitiligo," *Journal of Investigative Dermatology*, vol. 139, no. 4, pp. 769–778, 2019.

[18] I. Kostovic and M. Judas, "Transient patterns of cortical lamination during prenatal life: do they have implications for treatment?" *Neuroscience & Biobehavioral Reviews*, vol. 31, no. 8, pp. 1157–1168, 2007.

[19] A. Hossani-Madani and R. Halder, "Topical treatment and combination approaches for vitiligo: new insights, new developments," *Giornale Italiano di Dermatologia e Venereologia*, vol. 145, no. 1, pp. 57–78, 2010.

[20] M. Soliman, N. Samy, M. Abo Eittah, and M. Hegazy, "Comparative study between excimer light and topical antioxidant versus excimer light alone for treatment of vitiligo," *Journal of Cosmetic and Laser Therapy*, vol. 18, no. 1, pp. 7–11, 2016.

[21] E. Reyes, P. Jaen, E. Heras et al., "Systemic immunomodulatory effects of Polypodium leucotomos as an adjuvant to puva therapy in generalized vitiligo: a pilot study," *Journal of Dermatological Science*, vol. 41, no. 3, pp. 213–216, 2006.

[22] O. Szczurko and H. Boon, "A systematic review of natural health product treatment for vitiligo," *BMC Dermatology*, vol. 8, no. 1, p. 2, 2008.

[23] M. Dell'anna, A. Mastrofrancesco, R. Sala et al., "Antioxidants and narrow band-uvb in the treatment of vitiligo: a double-blind placebo controlled trial," *Clinical and Experimental Dermatology*, vol. 32, no. 6, pp. 631–636, 2007.

[24] R. Colucci, F. Dragoni, R. Conti, L. Pisaneschi, L. Lazzeri, and S. Moretti, "Evaluation of an oral supplement containing Phyllanthus emblica fruit extracts, vitamin E, and carotenoids in vitiligo treatment," *Dermatologic Therapy*, vol. 28, no. 1, pp. 17–21, 2015.

[25] M. Elgoweini and N. N. E. Din, "Response of vitiligo to narrowband ultraviolet B and oral antioxidants," *The Journal of Clinical Pharmacology*, vol. 49, no. 7, pp. 852–855, 2009.

[26] D. Parsad, R. Pandhi, and A. Juneja, "Effectiveness of oral ginkgo biloba in treating limited, slowly spreading vitiligo," *Clinical and Experimental Dermatology*, vol. 28, no. 3, pp. 285–287, 2003.

[27] M. Middelkamp-Hup, J. D. Bos, F. Rius-Diaz, S. Gonzalez, and W. Westerhof, "Treatment of vitiligo vulgaris with narrow-band uvb and oral Polypodium leucotomos extract: a randomized double-blind placebo-controlled study," *Journal of the European Academy of Dermatology and Venereology*, vol. 21, no. 7, pp. 942–950, 2007.