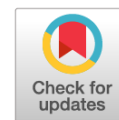


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Original study article



Determination of melanin levels in patients with vitiligo

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ABSTRACT

BACKGROUND: Vitiligo is an urgent problem for both patients and the scientific community of dermatologists. In this regard, there are many studies aimed at finding new methods of therapy for this disease, providing repigmentation of foci and stabilization of the process, as well as available methods of treatment control to objectify the results in dynamics.

AIM: To assess the melanin level in hypopigmentation foci in vitiligo patients before and during treatment using mexametry, as well as on the surrounding healthy skin, to study the possibility of using this method as a control of response to therapy.

MATERIALS AND METHODS: 17 vitiligo patients (10 female, 7 males, mean age 37.9 ± 2.9 , disease duration 16.5 ± 2.3 years) participated in the dynamic follow-up. Patients were measured melanin level in depigmentation foci and on the surrounding healthy skin in 19 localizations by mexametry before treatment and after 3 months of therapy. The study was carried out on the Soft plus device. The degree of repigmentation was also assessed clinically using the visual analog scale of repigmentation (G0–G4).

RESULTS: According to mexametry data, the most significant differences between the melanin level in vitiligo foci and on the surrounding healthy skin of the upper and lower extremities, on the skin of the trunk ($p < 0.001$). In 3 months after therapy a significant increase of melanin in vitiligo foci according to mexametry data was observed in the elbows — from 2 u to 13 u, shoulders — from 2 u to 17 u and knees — from 5 u to 18 u. Increased melanin levels according to mexametry correlated with the clinical picture. Thus, these areas showed a G4 excellent (repigmentation over 75%) and G3 very good response (repigmentation 50–75%) on the visual analog scale of repigmentation. There were no correlations between skin melanin levels and age, sex.

CONCLUSION: In the course of treatment there was an increase in melanin level in vitiligo foci according to mexametry, which correlated with the clinical picture, and testified to the effectiveness of therapy. On the basis of the obtained results we can conclude that mexametry is a good objective method of monitoring the effectiveness of therapy in patients with vitiligo in dynamics.

Keywords: vitiligo; treatment; mexametry; melanin.

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Оригинальное исследование

Определение уровня меланина у пациентов с витилиго

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АННОТАЦИЯ

Обоснование. Витилиго — актуальная проблема как для пациентов, так и научного сообщества дерматологов, в связи с чем проводится множество исследований, направленных на поиск новых методов терапии заболевания, обеспечивающих репигментацию очагов и стабилизацию процесса, а также доступных способов контроля лечения для объективизации результатов в динамике.

Цель исследования — оценка уровня меланина в очагах гипопигментации у пациентов с витилиго до и в процессе лечения с помощью мексаметрии, а также на окружающей здоровой коже; изучение возможности применения данного метода в качестве контроля ответа на терапию.

Материалы и методы. В динамическом наблюдении участвовало 17 пациентов с витилиго (10 лиц женского пола, 7 лиц мужского пола; средний возраст $37,9 \pm 2,9$ года; длительность заболевания $16,5 \pm 2,3$ года). Содержание меланина в очагах депигментации и на окружающей здоровой коже в 19 локализациях измеряли методом мексаметрии до начала лечения и спустя 3 месяца терапии с помощью прибора Soft Plus. Оценка степени репигментации проводилась клинически с использованием визуальной аналоговой шкалы репигментации (G0–G4).

Результаты. По данным мексаметрии, наиболее значительными были отличия концентрации меланина в очагах витилиго и на окружающей здоровой коже верхних/нижних конечностей и коже туловища ($p < 0,001$). Через 3 месяца после терапии значимое увеличение меланина в очагах витилиго, по данным мексаметрии, наблюдалось в области локтей (с 2 до 13 у.е.), плеч (с 2 до 17 у.е.) и коленей (с 5 до 18 у.е.). Повышение концентрации меланина, по данным мексаметрии, коррелировало с клинической картиной. Так, в данных областях, согласно визуально-аналоговой шкале, степень репигментации соответствовала уровням G4 (отличный ответ; репигментация более 75%) и G3 (очень хороший ответ; репигментация 50–75%). Корреляционные связи концентрации меланина в коже с возрастом и полом отсутствовали.

Заключение. В процессе лечения наблюдалось повышение содержания меланина в очагах витилиго по данным мексаметрии, что коррелировало с клинической картиной и свидетельствовало об эффективности проводимой терапии. На основании полученных результатов можно сделать вывод, что мексаметрия является хорошим объективным методом контроля эффективности терапии у пациентов с витилиго в динамике.

Ключевые слова: витилиго; лечение; мексаметрия; меланин.

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BACKGROUND

Vitiligo is a relatively common acquired pigmentation disorder, affecting 0.004%–9.98% of the global population [1]. The condition is characterized by the development of well-demarcated depigmented macules on the skin, resulting from the loss of melanocytes [2]. The main histopathological feature of vitiligo is the complete absence of functional melanocytes, accompanied by total loss of epidermal pigmentation [3]. However, Elsherif et al. [4], using Masson–Fontana staining, found that melanin is present in both affected and unaffected skin of patients with vitiligo. Furthermore, the presence of melanocytes in affected skin was confirmed by immunohistochemical staining (pigmented cells contain the Melan-A protein, which, upon interaction with specific antibodies, produces a color change visible under a microscope). The detected melanocytes were degenerated and contained a certain quantity of melanosomes, melanin granules, and autophagosomes [4].

Achieving repigmentation of vitiligo lesions remains a relevant clinical challenge. According to Russian and European clinical guidelines, ultraviolet B (UVB) phototherapy at 311 nm is considered the gold standard treatment for vitiligo. Current therapeutic regimens also include topical glucocorticoids and calcineurin inhibitors; however, available repigmentation methods remain suboptimal [5, 6].

A relatively novel approach for vitiligo treatment is microneedling. Various therapeutic strategies have been reported, both as monotherapy and in combination with pharmacological agents [7]. Microneedling combined with pharmaceutical agents represents a transdermal drug delivery method: locally controlled skin injury enhances the penetration of active ingredients into the deeper dermal layers at higher concentrations, bypassing the upper epidermal barrier [8, 9], thereby increasing treatment efficacy. Several investigations have confirmed the effectiveness of microneedling combined with topical tacrolimus ointment [10–12]. For example, Neinaa et al. [13] reported significant clinical improvement of vitiligo lesions following microneedling with subsequent application of 0.005% latanoprost solution in combination with UVB 311 nm phototherapy. There are also reports on the efficacy of combined microneedling with topical betamethasone and calcipotriol [14]. Single reports describe the combination of microneedling with 5-fluorouracil solution [15, 16], a method of interest for further investigation. According to published reports, the repigmentation mechanism of 5-fluorouracil is associated with its pro-pigmentary effect (direct stimulation of follicular melanocytes migrating during epithelialization to induce pigmentation), selective cytotoxicity toward keratinocytes, and activation of the CXCL12/CXCR4 axis, resulting in chemotactic melanocyte migration [17]. The microneedles induce a strong inflammatory response and local edema, increasing intercellular spaces in the basal layer. Active melanocytes migrate from pigmented epidermis through these spaces. Inflammatory mediators, such as

leukotrienes C4 and D4 and matrix metalloproteinases from keratinocytes, facilitate melanocyte migration and proliferation.

In our study, vitiligo treatment was performed using a combined method: microneedling with 5-fluorouracil solution and UVB 311 nm phototherapy.

During treatment, islands of repigmentation visible to the naked eye were observed. Several methods exist to assess repigmentation of vitiligo lesions before and during therapy, including international scores (VASI, VETF), repigmentation index (G0–G4), and dermatoscopy. However, only mexametry provides a quantitative assessment based on absorption and reflection of specific wavelengths by melanin and hemoglobin.

The study aimed to assess melanin levels in hypopigmented lesions and surrounding healthy skin in patients with vitiligo before and during treatment using mexametry, and to evaluate the method as an objective tool for monitoring response to therapy.

MATERIALS AND METHODS

Study Design

An open-label, randomized, prospective, single-center, controlled study.

Eligibility Criteria

Inclusion criteria: men and women aged 18–70 years with stable vitiligo, not receiving systemic or topical treatment within one month prior to study initiation, and with signed informed consent.

Non-inclusion criteria: age under 18 or over 70 years, presence of comorbidities (other autoimmune diseases, liver or kidney dysfunction, circulatory disorders), acute inflammatory conditions or exacerbation of chronic diseases at study entry, new or progressive lesions within the last 6 weeks (unstable vitiligo), presence of Koebner phenomenon, burns, or keloids in vitiligo lesions, tendency to form keloid scars, pregnancy or lactation, absence of signed informed consent.

Exclusion criteria: patient decision to withdraw from the study, pregnancy, noncompliance with the study protocol or prescribed examinations and treatment, or investigator judgment that further participation would be harmful to the patient's health.

Study Setting

The study was conducted at the Department of Skin and Venereal Diseases named after Rakhmanov, Sechenov First Moscow State Medical University, Ministry of Health of Russia (Sechenov University).

Study Duration

The study was conducted from 2023 to 2024.

Intervention

Melanin levels in depigmented lesions and surrounding healthy skin were measured using mexametry before treatment and after 3 months of therapy. Measurements were performed using the Soft Plus device (Callegari S.p.A., Italy). Because melanin absorbs light across a wide range of wavelengths—from ultraviolet to infrared—a specialized handheld sensor measured light at green ($\lambda = 568 \pm 3$ nm), red ($\lambda = 660$ nm ± 3 nm), and infrared ($\lambda = 870$ nm ± 10 nm) wavelengths. The wavelength was selected to correspond to the spectral absorption peak of melanin.

Measurements were performed at 19 anatomical locations: face and neck (forehead, eyelids, lips, chin, cheeks, neck); trunk (chest, axilla, back, buttocks, inguinal folds); upper extremities (shoulders, elbows, forearms, dorsum of the hands); and lower extremities (thighs, knees, shins, dorsum of the feet).

Measurement values ranged from 0 to 100 arbitrary units (AU). Before treatment, at each site, the sensor was placed three times in the depigmented lesion and on visibly healthy skin 3–5 cm away from the vitiligo lesion, and the mean melanin levels were calculated. The same procedure was repeated after 3 months of combined therapy with microneedling and 5-fluorouracil solution plus UVB 311 nm phototherapy.

Treatment included microneedling using a Dermapen device with 5-fluorouracil solution and UVB 311 nm phototherapy. Microneedling was performed at 7-day intervals, during which repigmentation was assessed. UVB 311 nm phototherapy was administered three times per week (20 procedures per course, two courses in total with a 30-day interval). Treatment started at a dose of 0.1 J/cm², with incremental increases of 0.1 J/cm² per session. Microneedling was performed perpendicularly to the skin using an electronic Dermapen (Dermapen P17 Mesoderm), moving horizontally, vertically, and diagonally until uniform pinpoint bleeding appeared. Needle depth was adjusted according to lesion location, ranging from 1 mm on the face and acral areas to 2.5 mm on the trunk. Following microneedling, 5-fluorouracil solution (50 mg/mL in ampules) was applied, and the microneedling procedure was repeated. On the same day, UVB 311 nm phototherapy was also performed.

Outcomes Registration

Repigmentation was evaluated clinically using a visual analog scale (G0–G4): G4, excellent response (repigmentation >75%); G3, very good response (repigmentation 50–75%); G2, good response (repigmentation 25–50%); G1, satisfactory response (repigmentation <25%); G0, poor response (no repigmentation).

Statistical Analysis

The study sample size was based on previous investigations using similar vitiligo therapies. The number

of included patients was sufficient for a study of moderate precision.

Data were analyzed using grouping, tabulation, correlation analysis, and sampling with Microsoft Excel software.

RESULTS

Participants

Seventeen patients with vitiligo were included in the case follow-up, including 10 women and 7 men. The mean age was 37.9 ± 2.9 years, and the mean disease duration was 16.5 ± 2.3 years.

Primary Results

Table 1 shows the distribution of melanin levels across all 19 locations in patients with vitiligo before and during treatment, as measured by mexametry. Melanin levels above 1 AU were observed in nearly all vitiligo lesions. The highest melanin levels in vitiligo lesions were detected on the chin (32 AU), forearms (24 AU), and knees (21 AU). Melanin levels in surrounding healthy skin varied by location: the highest levels were found on the dorsum of the feet (22.9 ± 9.7 AU), and the lowest on the chest (6.7 ± 8.2 AU). No significant differences in melanin levels between vitiligo lesions and surrounding healthy skin were observed on the buttocks (7.3 ± 7.4 AU vs 2.5 ± 2.6 AU, respectively). The most pronounced differences were observed in lesions and surrounding healthy skin on the upper and lower extremities and on the trunk ($p < 0.001$).

A significant increase in melanin levels in vitiligo lesions after 3 months of therapy, as measured by mexametry, was observed on the elbows (from 2 to 13 AU), shoulders (from 2.2 ± 1.5 to 17 AU), and knees (from 11.8 ± 10.5 to 18.0 ± 17.2 AU). The increase in melanin levels correlated with the clinical outcome. In these areas, excellent (G4, repigmentation >75%) and very good (G3, repigmentation 50%–75%) responses were observed according to the visual analog repigmentation scale.

No correlations were found between melanin levels in the skin and patient age or sex.

Adverse Events

Mild local reactions, including transient erythema, dryness, and pruritus, resolved quickly and did not require discontinuation of therapy.

DISCUSSION

Our results indicate that melanin levels exceeded 1 AU in all vitiligo lesions, except for lesions on the cheeks. This finding aligns with the study by Elsherif et al. [4], in which immunohistochemistry revealed a color change upon binding of specific antibodies to the Melan-A protein, indicating the presence of pigment (melanin) in

Table 1. Melanin concentration in the hypopigmentation foci and surrounding healthy skin of patients before and during treatment (standard units)

Location	Healthy skin	Vitiligo lesion	
		before treatment	after 3 months of therapy
<i>Face, neck</i>			
Cheeks	10.5±5.0	1.0±0.0	7.2±5.9
Eyelids	8.6±7.7	1.1±0.5	1.5±1.1
Forehead	15.1±7.6	2.3±2.2	7.3±7.1
Perioral area	11.9±3.5	8.4±4.9	10.6±8.2
Chin	13.7±4.9	12.1±6.6	13.4±7.6
Neck	12.4±6.5	1.3±1.4	4.2±3.1
<i>Trunk</i>			
Chest	6.7±8.2	1.5±2.2	3.1±3.9
Axilla	14.8±10.3	3.2±3.9	7.2±7.4
Back	13.2±10.4	2.1±2.5	5.4±5.1
Inguinal folds	14.3±7.5	4.1±4.3	9.7±8.6
Buttocks	7.3±7.4	2.5±2.6	5.6±5.0
<i>Upper extremities</i>			
Shoulders	16.2±11.4	2.2±1.5	17.8±15.4
Elbows	18.9±11.6	2.1±2.2	13.4±11.2
Forearms	20.0±10.5	3.1±5.2	6.3±5.1
Dorsum of hands	16.9±11.5	1.4±1.0	8.2±7.4
<i>Lower extremities</i>			
Thighs	16.9±11.8	4.7±3.2	16.3±15.8
Knees	21.5±10.6	5.5±5.3	18.0±17.2
Shins	17.5±10.3	3.2±3.0	12.5±11.2
Dorsum of feet	22.9±9.7	3.8±4.4	3.4±4.3

both affected and unaffected skin of patients with vitiligo. Comparisons were made between adjacent depigmented and pigmented areas due to the variable distribution of melanin across different lesions. Whiteman et al. [18] demonstrated that the number of melanocytes varies significantly depending on location. In adults, the highest melanocyte counts per 1 mm² were observed in the skin of the back and shoulders (17.1 ± 8.8 cells/mm²), upper extremities (12.6 ± 8.8 cells/mm²), and lower extremities (14.4 ± 5.9 cells/mm²), whereas the lowest counts were found in the anterior trunk (3.2 ± 2.4 cells/mm²).

It is also noteworthy that melanin levels in depigmented and pigmented skin within the same areas differ significantly. The most pronounced increases in melanin, as measured by mexametry, were observed at the elbows, knees, shoulders,

and trunk, which aligns with many publications describing patterns of vitiligo repigmentation. For example, Mina et al. [19], in a comparative investigation of microneedling combined with 5-fluorouracil or tacrolimus for vitiligo treatment, found that 5-fluorouracil induced a higher percentage of repigmentation than tacrolimus, particularly in acral areas. In another work evaluating microneedling with 5-fluorouracil, Zahra et al. [20] reported that repigmentation exceeding 75% was most frequently observed on the trunk, neck, and extremities.

The study by Hwang et al., in which vitiligo was treated with narrowband ultraviolet phototherapy, demonstrated that mexametry is sufficiently sensitive to detect subtle differences in pigmentation levels and provides satisfactory measurement reproducibility [21].

CONCLUSION

In this work, we evaluated the efficacy of a combined treatment method for vitiligo using the Soft Plus device—a narrowband reflectance spectrophotometer for measuring melanin levels. Previous investigations have demonstrated that mexametry is sufficiently sensitive to detect small differences in skin color and provides satisfactory reproducibility of measurements. Based on our results, mexametry can be considered a reliable, objective tool for monitoring treatment response in patients with vitiligo over time. This digitalized method allows for a more objective assessment of therapeutic outcomes.

ADDITIONAL INFORMATION

Authors' contributions. K.M. Lomonosov, L.N. Kayumova, K.V. Smirnov — scientific editing of the text, revision of the original text; E.A. Krotkova — collection and processing of literary sources, preparation and writing of the text, design of the reference list. Thereby, all authors provided approval of the version to be published and agree to be accountable for all aspects of the work in ensuring that questions related to the accuracy or integrity of any part of the work are appropriately investigated and resolved.

Ethics approval. The study protocol was approved by the local ethical committee of the Federal State Autonomous Educational Institution of Higher Education “I.M. Sechenov First Moscow State Medical University” of the Ministry of Health of Russia (protocol No. 23-23 of 01.12.2023). All study participants voluntarily signed an informed consent form before inclusion in the study.

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