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CLINICAL AND IMMUNOGYCAL ASPECTS OF PATHOGENESIS AND COMPLEX THERAPY OF VITILIGO

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Abstract
Vitiligo is the most common depigmentation disorder where the selective destruction of functioning melanocytes causes depigmentation of the skin, hair and mucosal surfaces. It affects approximately 0.5% to 1% of the population, with an average age of onset at about 24 years, its prevalence appears to be equal between men and women and there is no difference in the rate of occurrence according to skin type or race. Several etiological factors have been suggested for which the most compelling evidence involves a combination of environmental, genetic and immunological factors interacting to contribute to autoimmune melanocyte destruction. Vitiligo is a common skin disorder characterized by depigmented white patches in the skin due to loss of melanocytes. It remains unclear what causes damage or death to the melanocytes, there are many potential pathophysiological theories involving autoimmune, neural, autocytotoxic, biochemical, oxidative stress, melanocytorrhagy, and decreased melanocyte survival hypotheses. Autoimmune theory is more prominent in generalized vitiligo, which is considered a complex disorder involving combined pathogenic effects of multiple susceptibility genes and unknown environmental factors that lead to autoimmune destruction of melanocytes.

Keywords: Vitiligo, depigmentation, repigmentation, cytostatic, cytokine, UVB-therapy 311 nm.

Introduction

Vitiligo is defined as a specific, common, often heritable, and acquired dermatological disorder characterized by well-circumscribed, milky-white cutaneous macules and patches devoid of identifiable melanocytes (10). Vitiligo affects 0.1–2% of the world's population. It usually begins in childhood or adolescence, with peak onset at 10 to 30 years, but it may occur at any age. Both sexes are equally affected (11). It is believed that vitiligo is a multifactorial polygenic disorder with a complex pathogenesis. Although several theories (that include autoimmune, auto cytotoxic, biochemical, neural, and genetic mechanisms) have been proposed to explain the loss of epidermal melanocytes in vitiligo, the precise cause remains unknown (11). At present, the autoimmune theory is most plausible (12). Over the years there have been various reports. Mainstay of treatment in unstable vitiligo are systemic steroids and phototherapy.Some studies provided evidence that in vitiligo skin a significantly higher

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expression of TNF-α was detected, compared with perilesional, non-lesion and healthy skin (13). Thus, it seems that TNF-α is a key step in the development of vitiligo. As steroids have many complications, phototherapy being cost prohibitive and loss of patient compliance due to regular visit to hospital twice or thrice weekly. Methotrexate is tried as steroid sparing therapy in present study. Methotrexate is an antimetabolite and anti-folate used to treat certain cancers, Rheumatoid arthritis, Pemphigus and other autoimmune diseases (14). Methotrexate results in decreased number of T cells capable of producing TNF alfa (15). Therefore, it may help in stopping progress and bringing down the diseaseprocess under control. Hence, we undertook this study to look the efficacy of methotrexate in unstable vitiligo who are resistant and failureto other modalities of treatment.

In this context, IFN-γ, TNF-α and several members of interleukin-10 family cytokines (IL-10, IL-22, IL-24) and their receptors (IL10RA, IL10RB) have previously been demonstrated to be associated with vitiligo pathogenesis (16,17). As mentioned earlier in the etiology section, patients with vitiligo have increased levels of IL-10, TNFα, and INFγ. (18) Topical calcineurin inhibitors (tacrolimus and pimecrolimus) are topical immunomodulators; calcineurin is an intracellular protein in lymphocytes and dendritic cells and it acts as transcription factor for cytokines, such as IL-2, TNFα. (19)IgG anti-melanocyte antibodies may play a role in in stimulation and inappropriate expression of HLA-DR and induction of ICAM-1 on melanocytes, and also increase of IL-8 production, MHC II complex molecules expressed in melanocytes can present antigens to $CD4^+$ cells and initiate an immune response and ICAM-1 with its role in the adhesion of immune cells can also play a role in immunological reactions and inflammation resulting in melanocytotoxicity(20).

Material and Methods

The study included 60 patients with different forms of vitiligo (36 women, 24 men). The criteria for inclusion in this study were patients over 18 years of age, the presence of typical skin manifestations of disease, the absence of accompanying health and other ultraviolet diseases. In the course of the study, a standard complex of clinical examination of patients was carried out, including an in-depth analysis of complaints, anamnesis of life and illness, assessment of the dermatological status, examination of the foci with a Wood fluorescent lamp. The area of each loss rate was measured with the use of a line, summed up with other points and evaluated in terms of the relative amount of space. These anamnesis included information on the age and sex of the patients, the duration and nature of the course of the disease (stable or progressive), possibly the presence of a provoking factor, the rate of spread and and its localization (in accordance with the localization of vitiligo D. Mosher et al, 1979), the presence of an identical disease in other family members, an assessment of the effectiveness of the previously used methods of treatment. In order to exclude accompanying pathology and contraindications, examinations were carried out, including general clinical and biochemical blood analyzes, urine analysis, instrumental studies (Ultrasound of the thyroid gland, organs of the abdominal cavity, kidney, organs of the small pelvis, electrocardiography (ECG), if necessary, the patients were consulted by therapists, endocrinologist, gynecologist (urologist) and other specialists.

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To study the cytokine status of patients with vitiligo, we used an enzyme-linked immunosorbent assay with monoclonal antibodies, which were fixed in the wells of the plate from the sets of the IFA-BEST test systems. The blood turnover of patients with vitiligo was determined by the levelbasic provocative cytokines (IL-2, IL-6, IL-8, IL-10, FNOalfa). Determination of the cytokine status in blood serum was carried out in several stages. Before the start of the blood test, the wells of the plate were twice treated with a buffer solution, after which a solution of buffer C (10 μl each) was added, and additional buffer solution B (100 μl). The range of concentration of cytokines tended to change from 20 pkg to 0.3 IU / ml. During the analysis, the test serum was added (100 μl each). Then the incubation process was maintained under conditions of 60 minutes, at + 25C and constant continuous shaking. The next step was to remove the liquid from their wells and rinse with a buffer solution (washing frequency - 3 times). Then, 200 μl of a solution of two antibodies were added to the wells and the process of incubation was followed for one hour at a temperature of + 37C. This was followed by the process of washing with distilled water and aspiration of the wells, then a substrate with a dye (200 μl) was added to the dry wells and incubation was carried out for 10 - 15 minutes at a temperature of +22 C. The reaction was stopped with 50 μl of a solution of H2SO4 (sulfuric acid). After stopping the reaction, the results were introduced and analyzed using a spectrophotometer (wavelength 450 nm). In the studied cytokines, the concentration of receptor antagonists was determined, which was assessed using a calibration graph (unit of measurement - (pg / ml). The results of the immunological studies of the group were compared with the sample.

All patients were divided into two groups. In the 1st group (n = 30), the patients underwent combined therapy - UVB therapy 311 nm and tab methotrexate; in the 2nd group (comparison group) (n = 30), the patients received only photo therapy of 311 nm. The course of photo therapy in both groups lasted no more than 20 weeks. Photo therapy with UVB rays of 311 nm was carried out 4 times a week. The treatment was started without a minimum erythema dose. The initial dose was 0.05-0.1 J / cm2 with a subsequent increase, in the absence of erythema by 0.1 J / cm2, depending on the reaction of the skin to ultraviolet.

Methotrexate is tried as steroid sparing therapy in present study. Methotrexate is an antimetabolite and anti-folate used to treat certain cancers, Rheumatoid arthritis, Pemphigus and other autoimmune diseases (14). Methotrexate results in decreased number of T cells capable of producing TNF alfa (15). Therefore, it may help in stopping progress and bringing down the diseaseprocess under control. Hence, we undertook this study to look the efficacy of methotrexate in unstable vitiligo who are resistant and failureto other modalities of treatment. In study we present a case series of patients In the 1st group (n = 30) with vitiligo treated for 6 months with low-dose methotrexate (7.5-10 mg per week) with folic acid supplementation with clinically significant skin repigmentation, with response within 6 months in one case. There were no severe adverse effects reported.

Results

The effectiveness of the therapy was assessed after the end of the course of treatment according to the dynamics of the area of damage with the calculation of the percentage of repigmentation. For

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improvement, they took a repigmentation of the skin on an area that was not less than 25-50% of the initial area of use, a significant improvement in the remainder of 51-95% and a complete improvement of 96-95%. The lack of effect was determined when the pigmentation was restored on an area that occupied less than 15% of the initial area of damage. We also evaluated the distant results of the treatment, based on the persistence, formed in the process of photo therapy of pigmentation and the absence of progress. As a result of combination therapy, clinical cure (repigmentation over 96%) was observed in 21 patients (70%), significant improvement (repigmentation 51-95%) in 5 (16.7%), improvement - in 4 (13.3%).

Table-1

Treatment	Clinical cure	Significant improvement	Improvement
Combined therapy - UVB therapy 311 nm and tab methotrexate (n=30)	21 (70%)	5 (16,7%)	4 (13,3%)
UVB therapy 311 nm (n=30)	9 (30%)	10 (33,3%)	11 (36,7%)

We studied the level of cytokines in the blood serum of patients different forms of vitiligo in 2,5 months after photos and compared with the initial results. By defining the serum concentrations of cytokines, we have established. What is the content of pro-inflammatory cytokines in the blood serum of patientsvitiligo in both groups is significantly higher in comparison with the control group. We have also discovered and a significant drop anti-inflammatory cytokine IL-10 in patients with vitiligo of both groups. Results of conducted immunological studies, conducted in the background of a photo therapy, are presented in the table-2, indicate tendencies towards positive changes in the values of the studied indicators.

Dynamics of cytokine profile indicators before and after treatment

	Group I (n = 11)		GroupII (n=12)		Control group (n = 15)
	Before treatment	After treatment	Before treatment	After treatment	
IL 2	36,4±1,8	26,1+2,1	27,8±2,1*	15,6+1,7	15,4±2,43
IL 8	53,8±12,9	39,5+5,7	41,5±17,3	37,4+7,2	28,6±11,2
IL 10	3,4±1,5*	10,1+0,2*	3,9±1,1*	9,5+0,3*	10,82±3,1
FNOα	31,7±4,1*	12,8+2,1*	18,3±1,6*	10,7+0,6*	9,06±1,4

* - p <0.05 in relation to control

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Dynamics of cytokine profile indicators before and after treatment

	Group I (n = 11)		Group II (n=12)		Control group (n = 15)
	Before treatment	After treatment	Before treatment	After treatment	
IL - 8 / IL-10	15,8± 0,4	4,1±0,2	10,64±0,2	3,7±0,12	2,64±0,15
FNO-a/ IL-10	9,32±0,2	2,3 ±0,5	4,69±0,5	1,85±0,2	0,83±0,2

* - p <0.05 relative to control

Conducted comparative analysis of the results of the study of cytokinin status showed that on the background of different methods of photo therapy in patients with different vitamins
a decrease in comparison with the initial levels of the content in the blood serum of the blood FNO-alpha and positive, although statistically unreliable, there is a tendency towards a decrease in the content of IL-2 and IL-8 in the peripheral blood.

Thus, the level of pro-inflammatory cytokine - TNF-alpha in groups of patients suffering from vitiligo significantly exceeded the group's indicators healthy persons and compiled the combined method therapy 31.7 ± 4.1 pg / ml before and 18.3 ± 2.1 pg / ml (p <0.05) after treatment. In this way, in the result of the conducted treatment in each of the groups there was established statistically reliable decrease in the level of FNO-alpha to figures comparable with the indicators of healthy blood levels (p <0.05). Average the values of the results of measuring this cytokine in blood serum patients in the study groups who received a combined therapy 311 nm and tab methotrexate (12.8 ± 2.1 pg / ml; p> 0.05) and UVB-therapy 311 nm (10.7 ± 0.6 pg / ml; p> 0.05).

Discussion

The two most frequently used modalities in the treatment of vitiligo are PUVA and corticosteroids (21). In actively spreading vitiligo, PUVA therapy is not an ideal treatment. Corticosteroids and narrow-band UVB seems to be the most effective and safest therapies for localized and for generalized vitiligo respectively (22), but it may cause unwanted side-effects, such as skin atrophy, telangiectasia and striaedistensae, acne and local hypertrichosis. There have been a few reports on the use of methotrexate in vitiligotherapy.In a study conducted by Sandra et al a patient having both rheumatoid arthritis and unstable vitiligo responded well to methotrexate (23).Another study was conducted by Benerjee K with methotrexate showed no response in patients with unstable vitiligo.So,in our study we observed moderate re pigmentation in 70% of the patients and disease activity ceased in 90% of the cases.With regard to the site of the vitiligo sun exposed areas yielded better response(face and upperlimbs&upper trunk) .Patients with short duration of vitiligo got better results. Palms, soles and mucosa had poor outcome. Maximum response we observed is decrease in the body surface area by 30% and minimum was 0-1%.

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Conclusion

Methotrexate can be considered as a promising treatment inducing repigmentation and maintaining stability as an alternative to traditional therapy and phototherapy.

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