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GENETIC AND METABOLIC DISORDERS IN PATHOGENESIS OF VITILIGO, AND DEVELOPMENT OF METHODS FOR THEIR CORRECTION

14.00.11 – Dermatology and venereology (medical sciences)

ABSTRACT OF DISSERTATION OF DOCTOR (DSc) OF MEDICAL SCIENCES

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INTRODUCTION (annotation of doctoral dissertation)

Topicality and relevance of the theme of doctoral dissertation. The efforts of researchers and dermatologists worldwide are fostered to elucidate the mechanism of onset and progression of vitiligo and to develop efficient methods for its correction. Vitiligo is a disease associated with the skin pigmentation's abnormality characterized with the rapid reduction or absence of melanocytes synthesizing melanin, a pigment in the skin, and appearance of distinct white or milky white spots of various forms, size, quantity and localization. Vitiligo is a widely spread human skin disease with the prevalence in the general population of 1-2%. Recent epidemiological studies clearly indicate intensive growth of patients with vitiligo worldwide, and specifically, in Uzbekistan. Recently observed increase in prevalence of vitiligo among children, young men and persons of working age, as well as decline of the patients' quality of life add the dermatosis a social implication. Despite all above, pathogenesis of vitiligo remains unestablished; there are no efficient methods of treatment of the disease. Thus, it is urgent and challenging to study pathogenesis of vitiligo and to develop novel efficient medical approaches to its treatment.

In the days of independence in our country due to improvement in sci-tech provision and comfortable facilities for research considerable progress in solutions to technically scientific problems in dermatology, vitiligo inter alia, was achieved. Epidemiological studies estimated vitiligo prevalence among population of Uzbekistan to be 1.2%. Up-to-date methods of molecular-genetic analysis were applied to dermatology to establish association between polymorphism in various genes with skin tissue pathology; marker genes responsible for a person's susceptibility to vitiligo were determined, and a number of fundamental world-class findings pertaining to a mechanism of vitiligo onset and progression were obtained.

At the present time, study on mechanisms of the skin depigmentation and development of systems providing biosynthesis of functionally active melanocytes is an extremely urgent problem. According to some authors, the skin depigmentation in patients with vitiligo results from intensification of lipid peroxidation processes and suppression of an organism's antioxidant protection, which includes the focus of damage, to cause accumulation of toxic products destructing melanocytes (so called theory of self-destruction). The association between lipid peroxidation processes and concentrations of phospholipids, essential substrates for lipid peroxidation in the patients' skin, remains an unexplored problem in pathogenetic mechanism of vitiligo onset. Disorders in microelement composition of skin have a special place in the pathogenesis in question. Copper is known to be indispensable for activation of tyrosinase, iron is mandatory for activation of catalase. Zinc and calcium are active in the synthesis of melanin. Of note, there are only few publications on the role of micro- and macro-elements in the onset and progression of vitiligo, so further study on composition and concentrations of chemical elements in the skin of patients with vitiligo holds both theoretical and practical significance. Despite multiple studies,

etiopathogenesis of vitiligo is thought to be explored incompletely; methods for its efficient therapy meeting requirement of practical medicine have not been developed. This makes complex clinical-biochemical study on mechanism of vitiligo onset and progression and development of novel pathogenetic approaches in up-to-date medical science based on the findings from the study above an urgent and challenging problem. It should be emphasized that no structural-metabolic studies on the skin tissue, in vitiligo inter alia, have been ever conducted. There are no data on studies of the skin phospholipids, structurally basic for membranes of pigment cells of skin. It is destruction of melanocyte membrane structure under various effects, such as oxidative stress, autoimmune processes, etc., that is thought to be the main factor of vitiligo pathogenetic mechanism. Thus, insufficient extent of prior investigation of vitiligo etiopathogenesis and absence of efficient methods for its therapy determine extreme urgency of all-sided study in the direction.

To a certain extent, the dissertation has been made in pursuance of tasks outlined in the Republic of Uzbekistan President's Resolution (No. 1652, dated November 28, 2011) "Concerning further intensification of public health system's reforming" to provide wide accessibility of population to high quality medical care according to up-to-date requirement and standards, and in other statutory and regulatory documents adopted in the sphere.

Relevance of the research to the priority areas of science and technology development of the Republic The dissertation meets the priority of research and development of science and technology in the Republic of Uzbekistan titled "Medicine and pharmacology"

Review of international researches on the topic of the dissertation The studies on role of genetic and metabolic disorders in mechanism of vitiligo onset and progression as well as on methods of its therapy are under way in the leading centers and higher educational institutions worldwide, to name University of Colorado Denver (USA), Instituto Dermatologico San Gallicano (Italy), Department of Dermatology at Fudan University (China), Anhui Medical University (China), University of New Mexico (USA), Erasmus MC University Medical Center, Rotterdam (the Netherlands), University of Zagreb (Croatia), Center for Theoretical Problems of Physicochemical Pharmacology, Russian Academy of Sciences (Moscow), Astana Medical University (Kazakhstan), the Johnson and Johnson Skin Research Center (USA).

Studies conducted in the sphere of epidemiology, etiopathogenesis and therapy of vitiligo yielded a number of novel and unique findings, which include the fact that vitiligo prevalence among population worldwide is 0.5-1.5% in the average, but in some ethnic groups it seems to be quite different. Vitiligo is triggered by a complex of ecological, genetic, immunological factors and oxidative stress to unambiguously contribute to the destruction of melanocytes resulting in appearance of typical depigmentated areas on the skin (University of Colorado Denver, USA; Instituto Dermatologico San Gallicano, Italy). Vitiligo was found to onset in susceptible people only upon combination of predisposing and promoting factors; "marker genes" responsible for susceptibility to vitiligo was identified.

Among multiple marker genes associated with autoimmune disorders TYR was found to be the most special for predisposition to vitiligo (Fudan University, China, University of New Mexico, USA and University of Colorado Denver, USA).

Wide-scale studies on molecular mechanisms of onset and progression of vitiligo conducted worldwide include elucidation of role of free radical processes in appearance of the depigmentated skin areas with the demonstration that it is imbalance between oxidative effects and antioxidant protection of the skin that results in oxidative stress; accumulation of high H_2O_2 concentrations takes place in the epidermis of patients with vitiligo along with significant reduction in the activity of catalase. In its turn, oxidative stress causes damage of melanocytes. The role of disorders in microelement composition of the skin as well as the one of phospholipids in mitochondria of melanocytes in the pathogenesis of vitiligo was confirmed. To efficiently treat vitiligo liposomal formulations were produced.

The degree of study of the problem Foreign researchers, such as Spritz S., Jin Y., Picardo M. and Taieb A., Shen Ch. and Ko R., Zhang Z and Xiang L.F., Salinas-Santander M., and Wu D., are among those who greatly contributed to genetic study of vitiligo. They managed to perform the mapping of chromosome loci to identify specific genes responsible for predisposition to vitiligo, tumor necrosis factor-α (TNF-α) gene is among those. The gene encoding TNF-α is of paramount importancein pathogenesis of autoimmune disorders, including vitiligo. Some authors demonstrated obvious association of TNF-α gene polymorphism with vitiligo (Namian A.M., Laddha N.C., Al-Harthi F.); the findings of others were quite opposite (Yazici A.C., Wu D.). Hence, further studies are needed to confirm the association between TNF-α gene polymorphism and predisposition to vitiligo.

Recently, scientists from Italy (Picardo M., Dell Anna M.L.), Great Britain (Schallreuter K.U., Rokos H. et al.), the Russian Federation (Kosrunskaya I.M., Zhavoronkova E.V., Lomonosov K.M.) and other countries have been intensively researching oxidant-antioxidant system in patients with vitiligo. Accumulation of free oxygen radicals and suppression of antioxidant enzymes' activity causing oxidative stress both in the skin and blood serum is thought to be a leading pathogenetic factor for onset of the dermatosis. Reactive oxygen intermediates, H_2O_2 inter alia, act as endogenous toxic agents, suppress functional activity of melanocytes and induce death of these cells. Efficiency of various therapies for vitiligo is widely discussed. Efficiency of topical therapy was conclusively proved (Sharafutdinova L.A., Lomonosov K.M.). Topical corticosteroids are essential for inflammatory manifestations of vitiligo to be arrested. Immunodepressive activity of glucocorticosteroids is known to be their typical peculiarity. New data on successful use of liposomes for topical therapy of dermatological disorders, vitiligo inter alia, is emerging (J. de Leeuw et al., Z. Vanic). High degree repigmentation of the affected skin areas was found in therapy of vitiligo with kellin-encapsulated lecithin liposomes.

Contribution of researchers in the Republic of Uzbekistan to study on vitiligo is worthy. Epidemiological studies conducted by Prof. Arifov S.S. with

colleagues demonstrated that vitiligo prevalence in Uzbekistan is 1.2%; it is 8.2% among skin diseases. Skin microelement composition of patients with vitiligo has been studied by Tadjibaev T.T., VaisovA.Sh. and Khasanov D.S., who demonstrated rapid decline in concentrations of copper in the skin depigmentated areas of the patients. By virtue of their findings, Professor VaisovA.Sh. concluded that copper deficiency is of paramount importance in pathogenesis of vitiligo. Some advantages in therapy of vitiligo were achieved (Khasanov D.S., Abdullaev M.I., Vaisov A.SH., Rakhmatov A.B.). Thus, complex PUVA-therapy (Psoralens + Ultra-Violet A) in combination with cupirum and tactivin was developed to significantly improve processes in the skin of patients with vitiligo. Professor Kapkaev R.A. et al. developed complex therapy for vitiligo with corticosteroids; results of the therapy allowed concluding that its efficiency is far superior compared with the one of conventional therapy.

This dissertation for the first time presents the findings from complex study on basic pathogenetic factors involved in mechanism of onset and progression of vitiligo, and novel multicomponent liposomal formulation, lipovitilin, intended for pathogenetic therapy of the dermatosis.

Connection of the theme of dissertation with the scientific-research works of the higher educational institution, where the dissertation is conducted The work was performed in the frames of the State Research and Development Program -9 (2006-2008), A-9-169 Project “Overall clinical-biochemical study on mechanism of vitiligo onset and progression and development of novel methods of its pathogenetic therapy”, K11-004 Project (2009-2011) “Study on biochemical processes in the skin in therapy of vitiligo and generation of liposomal formulation” and IK-2013-16 Project (2-13-2014) “Exploration of technology for generation of liposomal formulation for treatment of vitiligo”.

The aim of the research The work was initiated to study role of genetic and metabolic disorders in mechanism of vitiligo onset and progression, and to develop novel method for pathogenetic therapy of the disease on the basis of the findings from the study.

The main tasks of the study include

- study on molecular-genetic mechanisms of vitiligo pathogenesis, study on association between TNF-α gene 308 G/A polymorphism and risk of vitiligo onset;
- study on association between TYR gene polymorphism and predisposition to vitiligo;
- study on composition and concentrations of phosphoglycolipids in the skin and blood serum of healthy subjects and patients with vitiligo;
- study on role of oxidative stress in pathogenesis of vitiligo;
- measurement of lipid peroxidation intensity and condition of antioxidant system in the skin and blood serum in normal conditions and vitiligo;
- comparative analysis of micro- and macro-element composition of the skin and scalp hair in normal conditions and vitiligo;
- development of novel method for pathogenetic therapy of vitiligo by means of liposomal technology on the basis of the findings from the study above;

- histomorphology of the skin of patients with vitiligo and assessment of oxidative stress in patients with vitiligo under therapy with liposomal formulation.

The objects of the research 395 patients with various forms of vitiligo referred to the Republican Specialized Scientific-Practical Medical Center of Dermatology and Venereology, 135 healthy subjects as the controls as well as blood and biopsy materials from the skin of the patients and healthy subjects. Biopsy samples of the unaffected and depigmentated skin areas from the patients with vitiligo were analyzed separately.

The subject of the research spans association between TNF-α gene 308 G/A polymorphism and vitiligo onset risk, association between TYR gene polymorphism and predisposition to vitiligo, composition and concentrations of phospholipids in the skin and blood serum of patients with vitiligo and healthy subjects, intensity of lipid peroxidation and condition of antioxidative system in the skin and blood serum in normal conditions and vitiligo, comparative analysis of micro- and macro-element composition in the skin and scalp hair in normal conditions and vitiligo as well as development of novel methods for pathogenetic therapy of vitiligo with liposomal formulation, lipovitilin.

The methods of the research work to conduct the study clinical-anamnestic, biochemical, molecular-genetic, morphological and statistical methods of study were used.

Scientific novelty of the research for the first time

- presence of association between TNF-α gene 308 G/A polymorphism with vitiligo onset risk was proved; contribution of changes in distribution of some alleles and genetic variants of the polymorphism above to pathogenesis of the disease was established;
- frequency distribution of alleles and G/A polymorphism genotypes (rs 1393350) of TYR gene were identified in patients with vitiligo and healthy subjects; association of the polymorphism with a person's predisposition to vitiligo was established;
- qualitative and quantitative compositions of phospholipids and cerebrosides in the comparative aspect were identified; significant shifts in concentrations of some phospholipid fractions were found in the skin of patients with vitiligo as compared with the parameters in healthy subjects;
- high level of oxidative stress in the skin and blood serum of patients with vitiligo playing a special role in pathogenesis of onset and progression of the dermatosis was established; imbalance in microelement composition of the skin and scalp hair is typical of vitiligo;
- an unparalleled novel multicomponent liposomal formulation, lipovitilin, was developed for therapy of vitiligo.

Practical results of the research

- disorders in frequency of alleles and genotypes in polymorphism of TNF-α and TYR genes established in the study and conclusiveness of associations between polymorphisms of the genes and vitiligo onset risk are significant contributions to pathogenesis of the dermatosis;

- changes in fraction composition of phosphoglycolipids and aggravation of oxidative stress in the skin of patients with vitiligo seen as novel fundamental finding unambiguously enriching the understanding of vitiligo pathogenesis;

- marked imbalance in concentrations of essential micro- and macro-elements in the skin and scalp hair of patients with vitiligo should be considered as a special factor in vitiligo onset;

- simultaneous intensification of lipid peroxidation and antioxidant system in the skin and blood serum of patients with vitiligo confirms customary suggestion that vitiligo is a systemic disease of an organism with topical manifestation of the pathological process;

- practical value of the study is governed by a multicomponent formulation for topical application in pathogenetic therapy of vitiligo developed on the basis of liposomal technology. Ingredients of liposomes have membrane- reparating, antioxidant, membrane-modifying and melanogenesis- stimulating properties, and are capable of eliminating factors hampering melanogenesis. Altogether, it contributes to improvement of repigmentation in the affected skin areas and clinical cure of patients with vitiligo.

The reliability of the obtained resultsis confirmed by the fact that they were obtained by means of up-to-date molecular-genetic, biochemical, clinical-anamnestic, morphological and statistical methods. Statistic processing of the results was performed by means of the Statistics 6.0 software package. Validity of the findings is confirmed by expert evaluations of specialists, by practical realization of the findings, discussions at the republican and international conferences and symposia as well as by the peer-reviewed scientific publications and acquisition of a patent.

Theoretical and practical significance of the research results lies in the fact that the disorders of phospholipid composition, intensity of lipid peroxidation and antioxidant system activity as well as changes in chemical elements in the skin of patients with vitiligo established in the course of the study form the basis for development of novel efficient liposomal formulation intended for pathogenetic therapy of the dermatosis. Practical value is confirmed by the fact that the liposomal formulation, lipovitilin, was used for treatment of the limited contingent of patients with vitiligo in combination with conventional therapy of vitiligo.

Implementation of the research results.

The findings from the study were formalized as an information letter “Method for therapy of vitiligo” and methodic recommendations “Use of liposomal form of medication for therapy of vitiligo” “Up-to-date aspects of etiology and pathogenesis of vitiligo” affirmed for Uzbekistan Public Health Ministry (No.8, dated 02.09.2008) and reduced to medical practice of the Republican Specialized Scientific-Practical Medical Center of Dermatology and Venereology, in particular, as well as of the Tashkent Regional Dermatovenereological dispensary. The liposomal formulation is covered by Republic of Uzbekistan Patent No. IAP 04292 dated 24.01.2011. Production and release of lipovitilin in the spray form was performed in OOO TOP FARM SERVICE. The liposomal formulation was registered in the National Register under No. 1629741. Lipovitilin was recognized

as an up-to-date, multicomponent liposomal formulation with reconstructing, antioxidant, transporting, membrane modifying and melanogenesis stimulating properties to increase clinical efficacy of vitiligo therapy, normalize biochemical parameters in the skin melanocytes, help preventing recurrences of the disease and have less side effects than conventional methods of therapy.

Testing of the research results Key aspects set forth in the dissertation were presented and discussed at the scientific-practical conferences, to name "Condition and problems of dermato-venereological and cosmetological service in the Republic of Uzbekistan" in Tashkent, Uzbekistan (2011), XII Congress of dermatovenereologists and cosmetologists in Moscow, the Russian Federation (2012), Republican Conference of young scientists "Upon the path of scientific discoveries" in Tashkent, Uzbekistan (2013), III Euroasian Congress of Dermatologists and Venereologists in Astana, Kazakhstan (2013), "Condition and problems of dermatovenereological and cosmetological service in the Republic of Uzbekistan" in Bukhara, Uzbekistan (2014), VIII Republican fair of groundbreaking ideas, technology and projects in Tashkent, Uzbekistan (2015), International scientific-practical conference "Urgent problems in dermatology and medical esthetics" in Tashkent, Uzbekistan (2015), IX Republican fair of groundbreaking ideas, technology and projects in Tashkent, Uzbekistan (2016), 1stInternational Congress "Problems of medical esthetics and dermatology" in Tashkent, Uzbekistan (2016), 13th Spring EADV Symposium in Valencia, Spain (2015), V Congress of physiologists and biochemists from CIS countries in Sochi, the Russian Federation (2016), XXV EADV Congress in Vienna, Austria (2016) and Vitiligo International Symposium in Rome, Italy (2016).

Publications of the research results On the subject of the dissertation one patent has been granted; there were 33 relevant publications, including 15 journal articles published abroad in journals recommended by the Higher Attestation Commission, Republic of Uzbekistan, as appropriate for publication, 18 abstracts, 1 methodic recommendations and 1 information letter.

The structure and volume of the dissertation Containing 200 pages of computer-aided typesetting text, the dissertation has introduction, five chapters, review of literature, material and methods, results, conclusions, practical recommendations and a list of references including 341 titles, 126 and 215 published in Russian and English, respectively, among them. There are 1 figures, 21 tables and 4 pictures.

THE MAIN CONTENT OF THE DISSERTATION

Introduction establishes topicality and relevance of the doctoral dissertation subject; sets forth objective, tasks of study and main principles presented for defense; presents conformity of the doctoral dissertation to priorities in development of science and technology in the Republic of Uzbekistan; characterizes its novelty and practical value of the findings; validates the findings from the research; discloses theoretical and practical value of the findings from the research; provides information on reduction to practice, publications and

presentations of the findings from the research, extent and structure of the dissertation.

The first chapter titled "**Time-sensitive understanding of etiopathogenesis and therapy of vitiligo**" describes results from foreign and native studies on spread, mechanisms of onset and progression of vitiligo in detail and without bias. Pathogenetic links of vitiligo onset, including genetic aspects, oxidative stress, microelement composition and role of phosphoglycolipids in mechanism of vitiligo onset are described in a step-wise manner. Liposomes obtained by means of nanotechnological methods to treat dermatoses, vitiligo inter alia, are given pride of place to.

The second chapter titled "**Time-sensitive approaches to exploration of biological objects**" describes clinical observations on patients with vitiligo, biochemical study on their skin and blood serum, molecular-genetic and morphological studies, methods to obtain lipids from biological objects and to manufacture liposomal formulations.

There were 395 patients with vitiligo aged from 6 to 70 years, 221 (56%) men and 174 (44%) women among them examined in all. A vulgar form was found among patients with the generalized vitiligo more frequently (43%), while a focal form was more frequent in patients with the localized vitiligo (40.7%). The disease duration was from 1month to 35 years; the one of up to 5 years was found in 260 (65.8%) patients, from 6 to 10 years was observed in 87 (22,0%) patients, from 11 to 30 years was found in 41 (10.5%) and the disease duration more than 30 years was observed in 7 (1.7%) patients.

Most patients (n=205, 52.5%) noticed first signs of vitiligo or its progression during spring-summer period, while in 128 (32.5%) patient's white spots on the skin appeared during autumn-winter period; 62 (15%) patients could not associate vitiligo onset and progression with any season. 135 apparently healthy subjects were included into the control group.

Clinical and laboratory examination and monitoring of patients was performed by means of special medical record containing personal details, history, results of physical examination of the skin and inner organs, laboratory parameters including total blood and urine count, stool ova and parasites test, arterial pressure and therapeutic measures.

The third chapter titled "**Molecular-genetic aspects of vitiligo pathogenesis**" describes study on association between TNF-α gene 308 G/A polymorphism and vitiligo risk.

Comparative analysis of allele and genotype frequencies for TNF-α gene 308 G/A rs1800629 polymorphism in patients with vitiligo and the controls demonstrated significant differences. Unfavorable A allele of the polymorphism was found more frequently in patients with vitiligo than in the controls (23.7% versus 10.8%; X^2 =9.23; P=0.002; OR=2.56; 95% CI 1.38-4.75) (Table 1). Frequency of A allele in patients with the localized form of vitiligo was 19.8% versus 10.8% in the controls (χ^2 =3.60; P=0.06; OR=2.03; 95% CI 0.97-4.27), its frequency in those with the generalized form was 27.0% versus 10.8% in the controls (χ^2=10.91; P=0.001; OR=3.05; 95% CI 1.54-6.03). Frequency of G allele

in patients with vitiligo was 76.3% versus 89.2% in the controls, that is, 15% less. Frequency of G allele in patients with the localized and generalized forms and in the controls was 80.2%, 73% and 89.2%, respectively. Frequency of TNF-α gene 308 G/A polymorphism G allele can be seen significantly lower in both patients with vitiligo in all and patients with the localized and generalized forms than the one in the controls.

Specific differences have been established in comparative analysis of various G308-A genotypes of TNF-α gene in the groups of patients with vitiligo and the controls. Frequency of wild rs 1800629* G/G genotype in patients with vitiligo was significantly lower than the one in the controls, indicating that it can protect its carriers from vitiligo risk. Of note, frequency of G/G genotype of TNF-α gene 308 G/A polymorphism in patients with the localized and generalized forms turned out significantly reduced as compared with the one in the controls (Table 1).

Table 1. Frequency of alleles and genotypes of TNF-α gene G-308A polymorphism in groups of patients

Group	N	Frequency of alleles				Frequency of TNF-α genotypes					
		G		A		G/G		A/G		A/A	
		n	%	n	%	n	%	n	%	n	%
Patients with vitiligo	93	142	76.3	44	23.7	53	57.0	36	38.7	4	4.3
Patients with the generalized form	50	73	73.0	27	27.0	26	52.0	21	42.0	3	6.0
Patients with the localized form	43	69	80.2	17	19.8	27	62.8	15	34.9	1	2.3
Control	74	132	89.2	16	10.8	58	78.4	16	21.6	-	0

In the group of patients with vitiligo frequency of unfavorable heterozygote rs1800629* genotype of TNF-α gene was twice higher than the one in the controls. G/A genotype could be seen in 36 of 93 patients (38.7%), it was observed in 21.6% of the controls (OR=2.29; p=0.007; χ^2 =9.88; 95% CI 1.14-4.58). Similarly, to the above, heterozygote G/A genotype was more frequently found in patients with the generalized (42%) and localized (34.9%) forms of vitiligo than in the controls (21.6%) (χ^2 =11.66; p=0.003; OR=2.63; 95% CI 1.19-5.78; χ^2 =4.44; p=0.11; OR=1.94; 95% CI 0.84-4.48, respectively).

Analysis of the findings unambiguously confirms presence of unfavorable A/A genotype of TNF-α gene G 308 A indicating possible overproduction of TNF-α cytokine in patients with vitiligo. Frequency of A/A genotype in patients with vitiligo was 4.3%, the genotype was found only in 4 of 93 patients. Frequency of rs1800628* A/A genotype of TNF-α gene in patients with the generalized form was 6% (3 of 50 patients), the genotype was found in only 1 of 43 patients with the

localized form (2.3%). Of note, mutant rs 1800629* A/A genotype of TNF-α gene was not found in the controls.

Thus, findings from the study on association between TNF-α gene 308 G/A polymorphism with vTNF-α gene 308 G/A polymorphism with various forms of vitiligo is a clear evidence for presence of association between carrying rs 1800629* A allele and rs1800629*G/A and rs1800629A/A genotypes with vitiligo risk.

Study on TYR gene rs 1393350 polymorphism and its association with vitiligo risk

The findings from the study on frequency of rs 1393350 polymorphism alleles and genotypes of TYR gene in patients with vitiligo and the controls can be seen in Table 2.

Table 2. Frequency of alleles and genotypes of TYR gene G/A polymorphism (rs 1393350) in groups of patients

Group	n	Frequency of alleles				Frequency of TNF-α genotypes					
		G		A		G/G		A/G		A/A	
		n	%	n	%	n	%	n	%	n	%
Patients with vitiligo	58	85	73.3	31	26.7	31	53.4	23	39.7	4	6.9
Control	40	64	80.0	16	20.0	26	65.0	12	30.0	2	5.0

Analysis of allele and genotype frequencies of TYR gene polymorphism in patients with vitiligo and the controls demonstrated significant differences. Lower frequency of wild G allele (by 10%) was found in patients with vitiligo as compared with the controls in the study of TYR gene rs 1393350 polymorphism alleles (73.3% versus 80%). Frequency of mutant unfavorable A allele of TYR gene polymorphism was significantly higher in the patients with vitiligo than the one in the controls, to be 26.7% and 20.0%, respectively. High level of mutant A allele TYR gene rs 1393350 polymorphism increases vitiligo risk by 1.5 times (OR=1.46; 96%CI 0.74-2.89).

Analysis of frequency for various TYR gene rs 1393350 polymorphism genotypes in patients with vitiligo and the controls demonstrated statistically significant differences. Frequency of wild homozygote G/G/ genotype of TYR gene polymorphism in patients with vitiligo was found significantly lower than the one in the controls to be 53.4% and 65%, respectively (OR=0.62; 95%CI -0.27-1.42). In patients with vitiligo frequency of unfavorable heterozygote G/A genotype in TYR gene rs 1393350 polymorphism was by more than 1.35 times higher than the one in the controls. G/A genotypes were found in 23 of 58 patients, to be 39.7% versus 30% in the controls (OR=1.53; 95%CI -0.65-3.61). The study on frequency of TYR gene rs 1393350 polymorphism genotypes helped establish high levels of mutant homozygote A/A genotype in patients with vitiligo (6.9% versus 5.0% in the controls) (OR=1.41; 95%CI -0.25-8.08). The data is the evidence for involvement of A/A genotype in predisposition to vitiligo; carriage of this genotype increases vitiligo risk almost by 1.5 times (OR=1.41).

Thus, our genetic study unambiguously confirmed presence of unfavorable G/A and A/A genotypesas well as A risk allele in TYR gene rs 1393350 polymorphism in patients with vitiligo. There are significant differences in frequencies of the allele and TYR gene polymorphism genotypes in patients with vitiligo and the controls.

The data allow concluding that A/G, A/A genotypes and A allele could serve as genetic markers for vitiligo risk in the Uzbek sample. This pathology risk in carries of A/A genotype is almost 1.4 times higher than in persons not carrying the genotype. In addition, in the Uzbek population presence of A-allele in TYR gene polymorphism increases the pathology risk by 1.46 times.

To sum up, study on TYR gene rs1393350 polymorphism in Uzbek patients with vitiligo is the authentic evidence for presence of association between carrier status of G/A and A/A as well as of A allele and risk of vitiligo onset and progression.

The fourth chapter titled "**Study on metabolic processes in vitiligo**" describes composition and concentrations of lipids in the skin of patients with vitiligo and healthy subjects as well as phospholipid composition of the skin and blood serum of patients with vitiligo and healthy subjects.

Composition and concentrations of lipids in the skin of patients with vitiligo and healthy subjects

Eight fractions of phospholipids were obtained by means of thin-layer chromatography on silica gel both in normal skin and in vitiligo (Table 3).

Table 3. Fractions of phospholipids in the normal skin and in vitiligo (%)

Phospholipidfractions	**Healthy skin (n=24)**	**Vitiliginous skin (n=28)**		**Statistical significance**
		Unaffected area	**Affected area**	
Lysophosphatidylcholine	3.4 ± 0.14	5.3 ± 0.26	5.26±0.24	P1-P2<0.001 P1-P3<0.001 P2-P3<0.1
Sphingomyelin	20.6 ± 0.90	18.2 ± 0.8	18.23±0.84	P1-P2<0.05 P1-P3<0.05 P2-P3<0.5
Phosphatidylcholine	37.6 ± 1.45	34.2 ± 1.8	34.25±1.83	P1-P2<0.05 P1-P3<0.25 P2-P3<0.5
Phosphatidylserine	3.34 ± 0.14	4.5 ± 0.2	4.48±0.21	P1-P2<0.001 P1-P3<0.001 P2-P3<0.001
Phosphatidylinositol	6.15 ± 0.27	7.9 ± 0.4	7.94±0.36	P1-P2<0.01 P1-P3<0.001 P2-P3<0.5

Phosphatidylethanolamine	22.99 ± 1.28	20.1 ± 1.2	20.08±1.18	P1-P2<0.02 P1-P3<0.1 P2-P3<0.25
Cardiolipin	2.21 ± 0.09	4.6 ± 0.2	4.56±0.21	P1-P2<0.001 P1-P3<0.001 P2-P3<0.25
Phosphatidic acid	3.71 ± 0.16	5.2 ± 02	5.20±0.24	P1-P2<0.001 P1-P3<0.001 P2-P3<0.001

Composition and concentrations of some phospholipid fractions in the skin of patients with vitiligo and healthy subjects can be seen in Table 3 and 4.

Table 4. Phospholipids in the skin of healthy subjects and patients with vitiligo

Phospholipid fractions	**Healthy skin**		**Vitiliginous skin**			
			Unaffected area		**Affected area**	
	µg P/g of dry tissue	**mg of PL per 1 g of dry tissue**	**µg P/g of dry tissue**	**mg of PL per 1 g of dry tissue**	**µg P/g of dry tissue**	**mmol of PL per 1 g of dry tissue**
Lysophosphatidylcholine	44.63± 1.96	1.11± 0.05	60.49± 2.68**	1.51± 0.06**	60.88± 2.73**	1.52± 0.07**
Sphingomyelin	273.09± 11.95	6.82± 0.29	185.96 ± 8.25*	4.65± 0.20*	211.0± 9.67*	5.27±0.24*
Phosphatidylcholine	498.1± 21.18	12.45± 0.52	341.55 ± 15.17*	8.53± 0.38	396.44± 18.15*	9.91± 0.45*
Phosphatidylserine	44.22± 1.93	1.10± 0.04	59.98± 2.64**	1.50± 0.06	51.88±2.38**	1.30±0.06**
Phosphatidylinositol	81.43± 3.56	2.03± 0.09	79.42± 3.51**	1.98± 0.08	91.9±4.21**	2.30±0.10**
Phosphatidylethanolamine	304.48± 13.32	7.61± 0.33	192.08 ± 8.51*	4.80± 0.21	232.41± 10.64*	5.81±0.26*
Cardiolipin	29.27± 1.26	0.73± 0.03	44.71± 1.99**	1.12± 0.04	52.08±2.41**	1.30±0.06**
Phosphatidic acid	1324.4± 57.9	1.22± 0.05	75.26± 1.96	1.88± 0.08	60.18±2.75	1.51±0.07**
Total sum of phospholipids	44.63± 1.96	33.11± 11.44	1039.5 ± 46.1*	25.99 ± 1.15	1157.0± 52.9	28.92±1.32*

Note: differences are significant in relation to normal *p<0.05; **p<0.001

Comparative study on concentrations of different phospholipid fractions in the bioptates of the skin of patients with vitiligo and healthy subjects demonstrated significant difference in the findings (Table 3 and 4). It is of interest that deviation from normal concentrations of phospholipids in the unaffected and depigmentated areas of patients with vitiligo is of unidirectional character.

Of note, as compared with those in the vitiliginous areas shaper increase in PA concentrations in the unaffected skin areas of patients can be seen ($p<0.001$).

The findings from our study demonstrate significant increase in the concentrations of cardiolipin in the skin of patients with vitiligo as well. Pathology increased concentrations of cardiolipin in the unaffected skin area by 54% and by more than 1.7 times in the vitiliginous one. Concentrations of other acidic phospholipid fractions, such as phosphatidylserine (PS) and phosphatidylinositol (PI), tended to increase both in the unaffected and depigmentated skin areas in patients with vitiligo. As compared with the control, PS concentrations in the unaffected and affected skin areas of patients with vitiligo increased by 1.73 and 1.34 times, respectively; both are statistically significant.

Concentrations of sphingomyelin (SPH) and other neutral phospholipids, such as phosphatidylcholine (PC) and phosphatidylethanolamine (PEA), in the unaffected and affected skin areas of patients with vitiligo were significantly lower than the parameters in the healthy skin. SPH concentrations in the unaffected and the affected areas of patients with vitiligo were found to reduce by 1.3 and 1.2 times, respectively. As compared with normal values, average reduction of SPH concentrations in the unaffected and affected skin areas of patients with vitiligo was 31.6% and 20.5%, respectively. In both cases differences between the parameters in vitiligo and normal values was statistically significant ($p<0.05$). As to phosphatidylethanolamine, its concentrations in the unaffected and affected skin areas of patients with vitiligo, as compared with the controls, was found to reduce by 37% and 23.7%, respectively, the data were statistically significant.

Analysis of concentrations of phospholipid fractions in the unaffected and depigmentated skin areas of patients with vitiligo demonstrated some decline in concentrations of neutral fractions along with the significant increase in the acidic ones. Thus, if the average proportion of neutral phospholipids in the skin of healthy subjects is 81.22 ± 3.63% of total sum of phospholipids, the values in the unaffected and affected skin areas of patients with vitiligo were 69.23 ± 3.27% and 72.56 ± 3.85%, respectively. Mean total sums of acidic phospholipids in the healthy skin, in the unaffected and depigmentated skin areas of patients were 18.78 ± 0.8%, 30.77 ± 1.56% and 27.44 ± 1.26%, respectively. Acidic-neutral phospholipids ratios in the healthy skin, unaffected and vitiliginous skin areas were 0.23, 044 and 0.38, respectively. The increase in the parameter in vitiligo takes place mainly due to increase in concentrations of acidic phospholipid fractions.

Thus, the findings allow concluding that in vitiligo content of phospholipids in the skin undergoes considerable changes.

It should be noted that the findings from our study are unique, because there are no data on the human skin phospholipid content and composition in the normal and pathological conditions ever published.

Phospholipid composition of blood serum in patients with vitiligo

We have measured concentrations of total phospholipids, quantitative and qualitative content in blood serum of 12 healthy subjects and 18 patients with vitiligo. The results can be seen in Tables 5 and 6.

Table 5. Phospholipids in blood serum of healthy subjects and patients with vitiligo

Phospholipid fractions	Normal (n=12)		Vitiligo (n=12)		
	%	µg of lipid P/ml of serum	%	µg of lipid P/ml of serum	P
Lysophosphatidylcholine	6.62	4.94 ± 0.32	8.61	5.94 ± 0.35	<0.05
Sphingomyelin	12.64	9.44 ± 0.72	14.41	9.94 ± 0.75	>0.5
Phosphatidylcholine	65.95	49.25 ± 3.45	62.62	43.22 ± 3.28	>0.1
Phosphatidylserine	3.62	2.7 ± 0.2	3.19	2.2 ± 0.17	>0.05
Phosphatidylinositol	4.97	3.71 ± 0.28	4.11	2.84 ± 0.21	>0.02
Phosphatidylethanolamine	2.01	1.5 ± 0.11	2.23	1.54 ± 0.12	>0.5
Cardiolipin	1.67	1.25 ± 0.09	0.70	0.48 ± 0.04	<0.001
Phosphatidic acid	2.52	1.88 ± 0.14	4.13	2.85 ± 0.21	<0.001
Total sum	100	74.67 ± 5.36	100	69.01 ± 5.16	>0.5

As it can be seen, total phospholipids in blood serum of patients with vitiligo tended to decline as compared with the normal values, but the results turned out statistically insignificant.

Study of blood serum by means of thin-layer chromatography on silica gel demonstrated presence of 8 phospholipid fractions both in patients with vitiligo and healthy subjects. Comparative analysis of the findings from the study demonstrated various concentrations of phospholipid fractions in blood serum of patients with vitiligo and the controls.

Table 6. Phospholipid composition of blood serum in the normal condition and vitiligo (mmol/l)

Phospholipid fractions	Normal	Vitiligo	P
Lysophosphatidylcholine	0.188 ± 0.009	0.226 ± 0.01	<0.05
Sphingomyelin	0.305 ± 0.023	0.321 ± 0.024	>0.5
Phosphatidylcholine	1.567 ± 0.099	1.375 ± 0.094	>0.1
Phosphatidylserine	0.087 ± 0.006	0.071 ± 0.005	>0.05
Phosphatidylinositol	0.119 ± 0.009	0.091 ± 0.006	>0.02
Phosphatidylethanolamine	0.48 ± 0.003	0.049 ± 0.003	>0.5
Cardiolipin	0.019 ± 0.001	0.007 ± 0.0005	<0.001
Phosphatidic acid	0.061 ± 0.004	0.092 ± 0.006	<0.001
Total sum	2.402 ± 0.173	2.219 ± 0.166	>0.5

In human blood serum concentrations of phosphatidylcholine fraction are the highest ones. Our findings demonstrate that in blood serum of healthy subject's PC proportion is 69.95 ± 4.61% of the total phospholipids to be 49.25 ± 3.45 μg of lipid phosphorus per ml of serum or 1.567 ± 0.09 mmol/l, on the average.

In patients with vitiligo insignificant decline in serum PC levels could be seen to be 62.6 ± 4.75% of the total phospholipids. The findings from our study demonstrated that PC mean concentration in blood serum of the controls was 12.64% of the total phospholipids. In blood serum of patients with vitiligo the proportion of this phospholipid fraction is higher than in the serum of healthy subjects. In contrast to normal, there was significant increase in concentrations of lysophosphatidylcholine and phosphatidic acid in blood serum of patients with vitiligo. Thus, if LPC concentrations in blood serum of healthy subjects were 6.62 ± 0.42%, 4.94 ± 0.32μg of lipid phosphorus per ml of serum or 0.188 ± 0.009 mmol/l, in serum of patients with vitiligo the parameters were 8.61 ± 0.5%, 5.94 ± 0.35 μg of lipid phosphorus per ml of serum or 0.226 ± 0.01 mmol/l. In blood serum of patients with vitiligo, LPC concentrations increase by 1.3 times, as compared with the normal values. In patients with vitiligo fraction of phosphatidic acid (PA),the most active minor one, changes most sharply. As compared with normal values, its concentrations in blood serum of patients with vitiligo were found to increase by 1.64 times. In vitiligo statistically significant decline in phosphatidylinositol (PI) and cardiolipin could be seen. Of note, concentrations of other phospholipid fractions, such as phosphatidylcholine and phosphatidylethanolamine were found not to change in blood serum of patients with vitiligo.

Our findings demonstrate that total sums of acidic and neutral phospholipid fractions in blood serum of healthy subjects were 19.4 ± 1.03% and 80.6 ± 6.1% of total phospholipids, respectively; acidic to neutral phospholipids ratio was 0.24. In

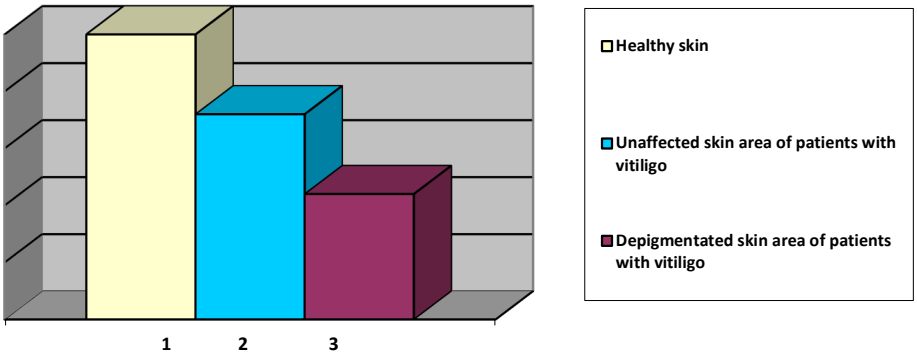
patients with vitiligo insignificant increase in total sum of acidic phospholipid fractions could be seen. Total sums of acidic and neutral phospholipid fractions were 20.74 ± 1.11% and 79.26 ± 5.46%, respectively; acidic to neutral phospholipids ratio was 0.26. In vitiligo, acidic to neutral phospholipids ratio can be seen to increase by 8.3%, as compared with the normal conditions.

Thus, our findings demonstrate that despite absence of significant differences in concentrations of total phospholipids in blood serum of healthy subjects, unambiguous proof for changes in fraction composition of phospholipids in vitiligo is demonstrated.

Study on cerebrosides in the skin of healthy subjects and patients with vitiligo

The findings from study on concentrations of cerebrosides in skin bioptates of healthy subjects and patients with vitiligo can be seen in Fig.1.

Figure 1. Proportions of cerebrosides in the normal and vitiliginous skin and (%)



As it can be seen in Figure 1, proportions of cerebrosides in the skin of patients with vitiligo declined, as compared with normal; the decline can be seen both in the depigmentated and unaffected skin areas to indicate that the changes in cerebrosides are of unidirectional character. In the skin of health subjects average concentrations of cerebrosides were 283.5 ± 12.8 μg per 1 g of tissue. In patients with vitiligo the parameter was 245.2 ± 11.4 μg per 1 g of tissue and 265.5 ± 11.9 μg per 1 g of tissue in the affected and unaffected skin areas. Thus, the findings from our study demonstrate that the decline in concentrations of cerebrosides in patients with vitiligo was more pronounced in the areas adjacent to a white spot. It should be noted that the findings from our study are unique, because there are no data on the human skin cerebrosides in the normal and pathological conditions ever published.

The data on changes in concentrations of cerebrosides in vitiligo suggest definite role of these lipids in pathogenesis of the dermatosis. Role of cerebrosides as a barrier to skin permeability taken into account, it should be noted that they are necessary for intracellular membrane transport, proliferation and survivability of cells as well as for various functions of immune system. Decline in cerebrosides in the vitiliginous skin can be a cause for suppression of the cell processes, eventually resulting in the death of skin cells, melanocytes, in particular. According to

literature, damage in metabolism of galactocerebrosides cause changes in the rate of breathing and ATP synthesis in the mitochondria of the liver and brain. Similar damages in functioning of mitochondria take place in the skin cells upon changes in proportions of cerebrosides. In its turn, this confirm hypothesis about damage of cerebrosides metabolism in the skin in vitiligo.

Lipid peroxidation and organism's antioxidant system in vitiligo

Intensity of lipid peroxidation and antioxidant system's condition in the skin of patients with vitiligo

Intensity of lipid peroxidation (LPO) was studied by accumulation of malondialdehyde (MDA) and condition of antioxidant system by catalase activity in the skin of healthy subjects and patients with vitiligo. Data on MDA concentrations in the skin of healthy subjects and patients with vitiligo can be seen in Table 7 and As it can be seen from Table 7, average MDA concentration was 3.78 ± 0.22 nmol/mg of protein/min.

Table 7. Concentrations of MDA in the skin of healthy subjects and patients with vitiligo (nmol/mg of protein/min)

Object of study	n	Patients	MDA	P
Healthy skin	10	1	3.78 ± 0.22	
Vitiligo: unaffected area	14	2	14.52 ± 0.97	P1:P2<0.001
Depigmentated skin area	14	3	12.51 ± 0.88	P1:P3<0.001 P2:P3<0.25

MDA concentrations were found increased both in the unaffected and depigmentated skin areas of patients with vitiligo. As compared with normal, MDA level in the unaffected and affected skin areas of patients with vitiligo increased by 3.84 and 3.31 times, respectively.

The findings from our study are the evidence for the intensification of reactive oxygen intermediates' generation and significant stimulation of lipid peroxidation.

Catalase activity was measured in skin bioptates of healthy subjects and patients with vitiligo. The data in Table 8 demonstrate significant decline in activity of catalase, an enzyme of antioxidant protection, in the skin tissue. The decline could be seen both in the unaffected and depigmentated skin area of patients with vitiligo.

Table 8. Catalase activity in the skin of healthy subjects and patients with vitiligo (μmol H_2O_2/mg of protein/min)

Object of study	N	Patients	Catalase activity	P
Healthy skin	10	1	8.2 ± 0.57	
Vitiligo: unaffected area	14	2	6.57 ± 0.44	P1:P2<0.002
Depigmentated skin area	14	3	7.03 ± 0.36	P1:P3<0.05 P2:P3>0.5

As it can be seen, the deviation from normal values of catalase in the unaffected and affected skin areas in patients with vitiligo is of unidirectional character. As compared with the control values, catalase activity in the unaffected and vitiliginous skin areas declined by 19.9% and 14.3%, respectively.

No significant difference was found in parameters of catalase activity in the affected and unaffected skin areas in patients with vitiligo ($P<0.5$).

Thus, the findings from our study is unambiguous evidence for oxidative stress taking place upon onset of vitiligo and manifesting in sharp increase of MDA, and significant decline in catalase activity.

Lipid peroxidation and condition of antioxidant system in the skin of patients with vitiligo were comparatively analyzed by the disease duration. The data are presented in Tables 9 and 10. As it can be seen in Table 9 the highest MDA level both in the unaffected and depigmentated skin areas could be seen in patients with the disease duration less than 1 year. With increase in the disease duration gradual decline in lipid peroxidation could be seen in the skin of patients with vitiligo. In patients with the long disease duration (5 and more years) lipid peroxidation intensity was found to reduce by 22.5%, as compared with the parameter in patients with the disease duration less than 1 year. The results were statistically significant ($p<0.05$).

Table 9. MDA levels in the skin of patients with vitiligo by the disease duration

Groups of patients	**Number of patients**	**MDA level (nmol/mg of protein/min)**	
Control	10	3.78 ± 0.22	
Patients with vitiligo	28	Unaffected skin area	Depigmentated skin area
		14.52 ± 0.97 P1:P2<0.001	12.51 ± 0.88 P1:P2<0.001
With the disease duration less 1 year	10	15.95 ± 0.96 P1:P3<0.001	13.82 ± 0.71 P1:P3<0.001
With the disease duration from 1 to 5 years	9	14.44 ± 0.89 P1:P4<0.01 P3:P4>0.001	12.28 ± 0.64 P1:P4<0.001 P3:P4>0.25
With the disease duration 5 and more years	9	13.02 ± 0.87 P1:P5<0.001 P3:P5<0.05 P4:P5>0.25	11.27 ± 0.79 P1:P5<0.001 P3:P5<0.02 P4:P5>0.05

The similar findings were obtained in study on catalase activity in patients with vitiligo by the disease duration; activity of the enzyme tended to increase by the gradual increase of the disease duration. These changes were found to take place both in the affected and unaffected skin areas.

Mean catalase activity in the depigmentated skin areas in patients with the disease duration less than 1 year was 6.2 ± 0.07 μmol H_2O_2/mg of protein/min; the

values in patients with the disease duration from 1 to 5 years and those with the disease duration 5 and more years were 7.1 ± 0.12 and 7.8 ± 0.08 μmol H_2O_2/mg of protein/min, respectively. The shifts in catalase activity in the skin of patients of the groups above were found statistically significant. Parameters of catalase activity in patient with the prolonged disease were found to reach those in normal skin.

Decline in MDA in the skin of patients with vitiligo by the increase in the disease duration can be explained by the reduction in generation of reactive oxygen intermediated due to membrane destructive processes and rapid reduction in melanocytes and other cells in the skin, as well as by reduction in substrates of oxidation the focus of affection.

Table 10. Catalase activity in the skin of patients with vitiligo by the disease duration

Groups of patients	**Number of patients**	**Catalase activity (μmol H2O2/mg of protein/min)**	
Control	10	8.2 ± 0.57	
Patients with vitiligo	28	Unaffected skin area	Depigmentated skin area
		6.57 ± 0.44 P1:P2<0.001	7.03 ± 0.36 P1:P2<0.001
With the disease duration less 1 year	10	5.67 ± 0.13 P1:P3<0.001	6.22 ± 0.07 P1:P3<0.002
With the disease duration from 1 to 5 years	9	6.59 ± 0.09 P1:P4<0.01 P3:P4<0.001	7.11 ± 0.12 P1:P4>0.1 P3:P4>0.001
With the disease duration 5 and more years	9	5.55 ± 0.11 P1:P5>0.5 P3:P5<0.001 P4:P5<0.001	7.85 ± 0.08 P1:P5>0.5 P3:P5<0.001 P4:P5<0.001

Lipid peroxidation and antioxidant system in blood of patients with vitiligo

In blood of 10 healthy subjects and 30 patients with vitiligo levels of malondialdehyde (MDA), end product of lipid peroxidation, and activity of catalase were measured. The findings can be seen in Table 11 and 12.

Mean MDA concentration in blood serum of healthy subjects was 1.57 ± 0.15 nmol H_2O_2/mg of protein/min; MDA levels range from 0.93 to 2.48 nmol H_2O_2/mg of protein/min.

Mean MDA concentration in blood serum of patients with vitiligo was 2.60 ± 0.19 nmol H_2O_2/mg of protein/min; MDA levels range from 1.45 to 3.32 nmol H_2O_2/mg of protein/min. Significant increase in MDA in blood serum of patients with vitiligo as compared with the one in healthy subjects can be seen. The increase was 65%, significance of the results was $p<0.001$ (Table 11).

Table 11. MDA in blood serum of healthy subjects and patients with vitiligo

Objects of study	n	MDA (nmol H2O2/mg of protein/min)	P
Blood serum of healthy subjects	10	1.57 ± 0.15	
Blood serum of patients with vitiligo	30	2.60 ± 0.19	<0.001

Significant increase of MDA concentrations in blood of patients with vitiligo indicates overgeneration of reactive oxygen intermediates with subsequent accumulation of toxic radicals resulting causing changes of metabolic processes in an organism underlies the pathology.

Contrary to the data above, catalase activity in our study was significantly lower than the one in the controls (Table 12).

Table 12. Catalase activity in blood of healthy subjects and patients with vitiligo

Objects of study	n	MDA (nmol H2O2/mg of protein/min)	P
Blood serum of healthy subjects	10	60.15 ± 1.58	
Blood serum of patients with vitiligo	30	45.61 ± 2.49	<0.001

Activity of the enzyme in blood of healthy subjects in our study was 60.15 ± 4.74 mcat/l on the average; the parameter ranged from 53.47 to 66.34 mcat/l. In blood of patients with vitiligo average catalase activity was found to reduce by 1.32 times, as compared with the control ($p<0.001$). In blood of patients with vitiligo average catalase activity was 45.61 ± 2.49 mcat/; the parameter ranged from 29.17 to 57.79 mcat/l.

Next, MDA concentrations and catalase activity in blood of patients with vitiligo were comparatively analyzed by the disease duration. All patients (n=30) were divided into 3 groups. Patients with the disease duration less than 1 year were included into the 1st group, those with the disease duration from 1 to 5 years comprised the 2nd one and those with the disease duration 5 and more years were included into the 3rd group. The data are presented in Table 13. It can be seen that there are significant differences in MDA concentrations blood of patients from different groups.

The highest MDA concentrations were found in blood of patients with the disease duration less than 1 year (3.26 ± 0.21 nmol H_2O_2/mg of protein/min versus 1.57 ± 0.15 nmol H_2O_2/mg of protein/min which is normal). The disease duration increasing, MDA concentrations in blood of patients with vitiligo tended to decline.

In the second and third groups of patients average MDA concentrations in blood were 2.53 ± 0.09nmol H_2O_2/mg of protein/minand 1.85 ± 0.11, respectively.

MDA concentrations in blood of patients with the longest disease duration approach the parameters in the control group, but do not decline to normal ones.

Study on catalase activity in blood of patients with vitiligo with various duration demonstrated not uniform level of the enzymatic activity (Table 13). In patients of the 1st group (the disease duration less than 1 year) the lowest catalase activity was observed (40.04 ± 1.61mcat/l versus 60.15 ± 1.58mcat/l, which is normal). Duration of the pathology increasing, dynamic increase in catalase activity in blood of patients with vitiligo was observed. Differences in catalase activity between groups of patients with vitiligo turned out statistically significant.

The findings from our study are the evidence for the fact that by increase of the disease duration changes in parameters of lipid peroxidation and antioxidant system in blood of patients with vitiligo toward the normal ones. Thus, the data above demonstrate significant suppression of catalase activity and imbalance in lipid peroxidation – antioxidant system condition in vitiligo. Analysis of the findings allow concluding that the course of vitiligo is accompanied by oxidative stress onset in organism indicated by high MDA level and decline in catalase activity both in the skin and blood of the patients.

Table 13. Parameters of lipid peroxidation and antioxidant system condition in blood serum of patients with vitiligo by the disease duration

Groups of patients	Number of patients	MDA (nmol H2O2/mg of protein/min)	Catalase (mcat/l)
Control	10	1.57± 0.15	60.15 ± 1.58
Patients with vitiligo	30	2.60 ± 0.19 P1:P2<0.001	45.61 ± 2.49 P1:P2<0.001
With the disease duration < 1 year	10	3.26 ± 0.21 P1:P3<0.001	40.04 ± 1.61 P1:P3<0.001
With the disease duration from 1 to 5 years	12	2.539 ± 0.09 P1:P4<0.001 P3:P4<0.01	45.22 ± 0.93 P1:P4>0.001 P3:P4>0.01
With the disease duration 5 and more years	8	1.85 ± 0.11 P1:P5>0.1 P3:P5<0.001 P4:P5<0.001	53.16 ± 1.02 P1:P5<0.002 P3:P5<0.001 P4:P5<0.001

Oxidative stress manifesting in intensification of lipid peroxidation and decline in antioxidant system activity is a key pathogenetic factors affecting onset, course and outcome of many diseases.

Intensification of reactions of free radical oxidation of lipid substrates result in destruction and destabilization of cell membranes and subcellular organelles, damage of their membrane-receptor, transport and enzymatic functions further exacerbating metabolic abnormalities in organs and tissues. Based on our findings and the results of other authors, oxidative stress can be suggested as a pathogenetic mechanism of melanocyte degradation in vitiligo.

Intensification of reactive oxygen intermediates generation in patients with vitiligo was demonstrated in our study. High level of free radical formation (MDA) both in the unaffected and depigmentated skin areas of patients with vitiligo was established. In blood of patients with the dermatosis we found increase in MDA concentrations by 65% as compared with the normal. Our findings are consistent with other authors' data demonstrating increase in hydrogen peroxide (H_2O_2) formation along with low activity of catalase, an antioxidant enzyme, in the skin of patients with active form of vitiligo. Recognizing key role of oxygen free radicals in vitiligo onset, Korsunskaya et al. (2003) indicated H_2O_2 high levels in blood serum, epidermis and tissue fluid in the depigmentated skin areas of patients with vitiligo. Reactive oxygen intermediates and hydrogen peroxide act as endogenous toxic agents suppressing functional activity of melanocytes and inducing death of the cells. Significant inhibition of catalase activity in the skin and blood serum of patients with vitiligo was demonstrated in our study as well. Our findings demonstrated decline in catalase activity in the unaffected and vitiliginous skin areas by 20%and 14.3%, respectively, the decline in blood was 32% as compared with the values in the controls.

Catalase is known as a key regulator of hydrogen peroxide metabolism. Imbalance of lipid peroxidation/antioxidant system activity we found in vitiligo suggested importance of oxidative stress in pathogenesis of the skin depigmentation in the dermatosis. The ratio of prooxidants and antioxidants probably changes leading in the onset of the disease to marked membrane destruction, active death of melanocytes and reduction of their quantity. In its turn that results in damage of adequate functioning of melanocytes, their responsiveness to melanocyte-stimulating hormone and in damages of melanogenesis.

Thus, the structural and functional changes in membrane structures caused by oxidative stress can be seen as primary biochemical abnormality arising in vitiligo responsible for reduction in survivability and death of melanocytes.

Comparative analysis of micro- and macro-element composition of human skin and scalp hair in normal condition and in vitiligo

Neutron activation analysis was used to determine element composition of the skin and scalp hair of healthy subjects and patients with vitiligo. Study on chemical elements in the skin bioptates of healthy subjects and patients with vitiligo allowed determining presence of 20 elements (Table 14).

As it can be seen in Table 14, in healthy subject's proportions of chlorine (Cl), sodium (Na), potassium (K) and calcium (Ca) turned out to be the highest. Thus, mean concentrations of Cl, Na, K, and Ca were respectively 7837.3, 6530, 3165 and 570 μg/g of dry skin tissue. Proportion of iodine was rather high too (137.7 μg/g of dry skin). Mean concentrations of iron (Fe), zinc (Zn) and copper (Cu) were respectively 109.3, 24.5 and 23.4 μg/g of dry skin tissue. Mean concentrations of bromine (Br), chromium (Cr), rubidium (Rb) and manganese (Mn) were respectively 2.73, 2.15, 2.83 and 0.5 μg/g of dry skin tissue. Concentrations of lanthanum (La), mercury (Hg) and silver (Ag) were the lowest;

amounts of these elements could be considered as trace. Concentrations of other chemical elements in the skin of healthy subjects ranged from 0.01 to 0.047 μg/g of dry skin tissue.

Table 14. Mean concentrations of chemical elements in the skin of healthy subjects and patients with vitiligo (μg/g of dry tissue)

Chemical elements	Skin of healthy subjects, M ± m n=33	Skin of patients with vitiligo	
		Apparently undamaged area, M± m n=34	Depigmentated area, M ± m n=34
I	137.7 ± 10.9	10.4 ± 0.8	10.9 ± 1.9
Cl	7837.3 ± 759.5	9140.0 ± 202.2	8453.3 ± 291.2
Mn	0.5 ± 0.01	0.87 ± 0.09	0.9 ± 0.03
Na	6530.0 ± 568.3	5250.0 ± 69.8	5106.0 ± 38.6
K	3165.0 ± 267.06	2556.6 ± 50.6	2903.3 ± 93.4
Ca	570.0 ± 45.1	876.6 ± 62.9	886.6 ± 76.03
Cu	23.4 ± 1.3	11.9 ± 0.8	9.5 ± 0.6
Au	0.016 ± 0.002	0.013 ± 0.0005	0.016 ± 0.001
Br	2.73 ± 0.2	2.56 ± 0.07	3.1 ± 0.067
La	0.01 ± 0.002	0.022 ± 0.006	0.02 ± 0.003
Se	0.23 ± 0.005	0.38 ± 0.01	0.35 ± 0.017
Hg	0.001 ± 0.0002	0.017 ± 0.002	0.006 ± 0.001
Cr	2.15 ± 0.48	3.53 ± 0.16	5.5 ± 0.09
Ag	0.015 ± 0.003	0.026 ± 0.007	0.014 ± 0.002
Sc	0.047 ± 0.0001	0.012 ± 0.001	0.0075 ± 0.00051
Rb	2.83 ± 0.3	2.84± 0.05	3.0 ± 0.07
Fe	109.3 ± 3.6	133.7 ± 7.3	154.3 ± 3.9
Zn	24.5 ± 1.3	41.2 ± 3.2	61.6 ± 10.9
Co	0.044 ± 0.003	0.062 ± 0.004	0.071 ± 0.002
Sb	0.004 ± 0.001	0.038 ± 0.006	0.023 ± 0.002

As compared with healthy controls, significant differences in macro- and microelement composition of the skin in patients with vitiligo could be clearly seen. Differences in concentrations of iodine were the most significant. Thus, in patients with vitiligo mean concentrations of iodine in the skin bioptats from the depigmentated and apparently undamaged areas were 10.8 and 10.4 μg/g of dry skin tissue, respectively, while in the skin of healthy subjects mean iodine concentration was 137.7 μg/g of dry skin tissue. In the skin of patients with vitiligo significant reduction in concentrations of sodium, potassium and copper could be observed. The levels of these elements declined by 27.8%, 9% and 246.3%, respectively, in the depigmentated skin areas and by 24.3%, 23.8% and 196.6%, respectively, in the apparently undamaged ones. Copper can be seen to decline in vitiliginous skin more sharply. In contrast to normal parameters, in both

depigmentated and apparently undamaged areas of the skin in patients with vitiligo concentrations of chlorine, manganese, calcium, chrome and zinc increased. Of special note, in vitiligo concentrations of heavy metals, such as mercury (Hg), zinc (Zn), cobalt (Co) and antimony (Sb) were found to increase significantly. Concentrations of other chemical elements in the skin of patients with vitiligo turned out to be in the normal limits. It should be emphasized that upon comparison of composition and concentrations of chemical elements in the depigmentated and apparently undamaged skin areas of patients with vitiligo the parameters were found to have changed as compared with those in healthy subjects more significantly in the depigmentated ones.

Neutron activation analysis allowed determining twenty-three chemical elements both in healthy subjects and patients with vitiligo (Table 15).

Table 15. Mean concentrations of chemical elements in the scalp hair of healthy subjects and patients with vitiligo (μg/g)

Chemical elements	**Scalp hair of healthy subjects, M ± m n=33**	**Scalp hair of patients with vitiligo, M ± m n=37**
Na	170.0 ± 11.40175	335.0 ± 27.24335
Cl	740.0 ± 32.24903	841.8 ± 17.87288
Ca	500.0 ± 25.0998	1070.0 ± 43.93177
Sc	0.0046 ± 0.000346	0.00588 ± 0.000508
Cr	0.26 ± 0.030332	3.68 ± 0.185472
Mn	0.6 ± 0.028284	4.7 ± 0.2
Fe	26.2 ± 2.764055	33.4 ± 1.32665
Co	0.04 ± 0.003033	0.0216 ± 0.001208
Ni	5.0 ± 0.250998	5.0 ± 0.250998
Cu	17.0 ± 0.707107	13.0 ± 1.140175
Zn	182.8 ± 7.317103	163.0 ± 3.962323
K	175.0 ± 24.18677	310.0 ± 9.082951
Se	0.5 ± 0.0251	0.3 ± 0.022804
Br	0.3 ± 0.022804	0.81 ± 0.034205
Rb	0.5 ± 0.032249	0.5 ± 0.042778
Ag	0.25 ± 0.026646	0.1 ± 0.006892
Cd	0.0294 ± 0.00275	0.084 ± 0.054019
Sb	0.02 ± 0.001517	0.04 ± 0.003033
I	3.04 ± 0.150333	1.6 ± 0.121326
La	0.0208 ± 0.002223	0.02 ± 0.001517
Au	0.0412 ± 0.004543	0.029 ± 0.00228
Hg	0.03 ± 0.003225	0.0698 ± 0.002223
U	0.274 ± 0.038678	0.1066 ± 0.02603

Next, we studied chemical composition in scalp hair of healthy subjects and patients with vitiligo to determine concentrations of chemical elements there.

Similarly, to concentrations of chemical elements in the skin, those of chlorine, sodium, potassium, calcium, iron, copper and zinc in the scalp hair of healthy subjects were found to be the highest, while concentrations of scandium (Sc), cobalt (Co), cadmium (Cd), antimony (Sb), lanthanum (La) and gold (Au) were the lowest. Thus, mean concentrations of chlorine were 740.0 ± 32.2 μg/g (range: 650-850 μg/g). Mean concentrations of zinc and iron were respectively 182.8 ± 7.3 μg/g (range: 160-260 μg/g) and 26.2 ± 2.7 μg/g (range 18-34 μg/g). Mean levels of cobalt and lanthanum in the scalp hair of healthy subjects were respectively 0.04 ± 0.003 μg/g (range: 0.003-0.048 μg/g) and 0.02 ± 0.00022 μg/g (range: 0.014-0.027 μg/g).

As compared with healthy controls, significant differences in macro- and microelement composition of scalp hair in patients with vitiligo could be clearly seen. First of all, in scalp hair of patients with vitiligo concentrations of chromium (3.6 μg/g *vs* 0.28 μg/g) and manganese (4.7 μg/g *vs* 0.6 μg/g) were found sharply increased. In addition, as compared with concentrations in the scalp hair of healthy subjects, concentrations of chlorine, calcium, potassium, cadmium and mercury were found higher in vitiligo, while copper, selenium, cobalt, iodine and silver were deficient. Copper is a vital chemical element and a constituent of some vitamins and hormones, participating in the metabolic processes and cell respiration. It is a constituent of essential enzymes, to name cytochrome oxidase, tyrosinase, ascorbic oxidase and others. A cofactor of superoxide dismutase, copper in human organism is present in the system of antioxidant defense participating in neutralization of oxygen free radicals. Participation in the synthesis of melanin by activation of tyrosinase, a copper-dependent enzyme, is essential function of copper. In its turn tyrosinase converts tyrosine into melanin. Iodine deficiency is known to underlie the thyroid function abnormality, as well as onset and progression of the goiter.

As our findings demonstrate, in vitiligo cobalt deficiency is the most pronounced one. Thus, concentrations of this microelement were found two times lower than in the healthy controls. Of note, concentrations of strontium, gold, lanthanum, cobalt and cadmium were the lowest in scalp hair of patients with vitiligo.

Our comparative study on micro- and macro-element composition of the skin and scalp hair of healthy subjects and patients with vitiligo helped determine marked imbalance in concentrations of key chemical elements in vitiligo, probably, to be a factor exacerbating the dermatosis. Our findings are quite intriguing from both the theoretical and practical point of view, and may contribute to development of novel efficient regimes for therapy of vitiligo.

The fifth chapter titled "**Development of pathogenetic methods for therapy of vitiligo by means of liposomal technology"** describes development of pathogenetic methods for therapy of vitiligo by means of liposomal technology.

The findings of the study demonstrate that a complex approach with key biochemical factors affecting melanogenesis taken into account is needed in development of novel efficient methods for therapy of vitiligo. It is expedient to

pay special attention to pathogenetic effect on the affected skin area aiming at correction of metabolic process links damaged.

Incorporation of large quantity of biologically active substances with pathogenetic effect into one medication by methods of nanotechnology (liposomal technology) is the perfect solution for the problem.

Liposomes have high potential in terms of permeability into deep lying skin layers by substances incorporated into them.

In our work we used total phospholipids from the brain of cattle rich in phospholipids to form bilayer phospholipid membranes able to turn into continuous vesicular coats upon various physicochemical effects. These coats entrap a part of surrounding aqueous solution; phospholipid membranes forming them possesses properties of semi-permeable barrier giving way to water but obstructing diffusion of substances dissolved in the water. In addition, liposomes prepared from total sum of brain phospholipids were used to normalize phospholipid composition of the skin changed by vitiliginous damage. Interchange of phospholipids between cell membrane and liposome coat is known to take place upon interaction of liposomes with the cell. Modification of phospholipid composition in cell membranes with subsequent change in its functional properties takes place whereupon.

Phospholipid constituting liposomes facilitate reconstruction of lipid composition in membranes of melanocytes, influencing intracellular biochemical reactions and regulating biosynthesis of melanin. Cholesterol incorporated into liposomal formulation facilitates stability and steadiness of membranes.

Necessity to incorporate bioantioxidants into liposomes, to name α-tocopherol, was dictated by the fact that generation of reactive oxygen intermediates and oxidative stress is key in mechanism of inhibition of melanin synthesis and induction of apoptosis of the skin melanocytes, that is, in mechanism of onset and progression of vitiligo. Liposomes with bioantoxidants incorporated into them are capable of preventing excess formation of toxic products of superoxide radicals and death of melanocytes. A number of microelements, quantities of which significantly decline in the depigmentated skin in vitiligo, are necessary for the skin to function normally. All above taken into account, we included copper (a constituent of cupirum) into our liposomes. Ultrasound processing to disperse lipids allows obtaining small unilamellar liposomes with size of 25-100 nm.

These liposomes penetrate deep lying skin tissues easily. Efficient therapy of vitiligo requires delivery of medications in combination with ultraviolet to hair follicles in the skin where inactive, immature melanocytes unable to synthesize melanin are preserved. Repigmentation of the affected skin in therapy of vitiligo is performed by means of transformation of inactive melanocytes into active ones and their stimulation under effect of medications and ultraviolet processing. More deep penetration of medications into the skin necessary for high therapeutic effect can be facilitated by liposomes.

Therapy of vitiligo with liposomal formulation and dynamics of some biochemical parameters in the process of the therapy

We compared efficacy of therapeutic effect produced in a group of patients by phototherapy (PUVA and external photochemiotherapy) in combination with lipovitilin, our liposomal formulation, with the one produced in a group of patients receiving PUVA or external photochemiotherapy. For the purposes of the study all patients were divided into two groups. 153 patients were included into the 1st group to be divided into two subgroups. The 1st subgroup consisted of 70 patients (45.7%) patients with a restricted form of vitiligo who received external photochemiotherapy. The 2nd subgroup consisted of 82 (53.6%) patients with the expanded form of vitiligo who received PUVA-therapy only. The second group consisted of 42 patients – volunteers. To 19 (45.2%) of them with the restricted skin process photochemiotherapy was prescribed in combination with topical application of lipovitilin. The rest 23 patients (54.8%) with the expanded skin processes received lipovitilin along with PUVA-therapy. Patients in the first and second groupin addition to phototherapy and lipovitilin received vitamin therapy (A,E, B vitamins, C), microelements (cupirum, pyracinum) and medications to correct concurrent pathology. PUVA-therapy was performed thrice a week, 15-16 exposures per course of therapy with an interval between the courses of 21-30 days. Distribution of patients by the groups by the clinical forms of vitiligo can be seen in Table 16.

Table 16. Distribution of patients with vitiligo by the clinical forms of vitiligo

Clinical forms	Number of patients (n,%)		
	1st group	2nd group	Total
Localized	70 (45.7%)	19 (45.2%)	89 (45.6%)
Generalized	82 (53.6%)	23 (54.8%)	105 (53.8%)
Setton's nevus (halonevus)	1 (0.7)	-	1 (0.7)
Total	153 (100%)	42 (100%)	195 (100%)

Outcomes of the therapy were assessed by an area of repigmentation (in % of initial sizes of damage).

Repigmentation of 96-100% of foci was considered as clinical cure, the one of 60-95% was registered as significant improvement, repigmentation of 10-60% was considered as improvement, no effect was registered with the repigmentation of 0-10%, and appearance of new or enlargement of initial depigmentated foci was considered as the worsening (VaisovA.Sh., 1989; Arifov S.S., 1994).

Data on efficacy of conventional therapy for vitiligo by number of PUVA-therapy courses is presented in Table 17. As it can be seen, after first course of PUVA-therapy repigmentation could be observed in 33 patients (40.2%); significant improvement and improvement could be seen in 3 (3.6%) and 30 (36.6%) patients. No effect of the therapy could be seen in 47 (57.3%) patients. The skin process worsening was registered in 2 patients (2.4%).

After second course of therapy, proportion of patients with repigmentation increased to 61.5%. Of 70 patients clinical cure was observed in 3 (4.3%), significant improvement and improvement were registered in 14 (20%) and 26), respectively. No effect was found in 25 (35/7%) patients. In 2 patients the process worsening could be seen. Of 65 patients with vitiligo receiving the third course of PUVA-therapy repigmentation took place in 49 (75.4%); clinical cure was registered in 7 (10.7%), significant improvement and improvement in 23 (35.4%) and 19 (29.3%) patients, respectively. In 15 (23.1%) patients no effect of the therapy could be seen, in 1 (1.5%) the process worsening took place.

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Thus, of 82 patients with vitiligo receiving conventional therapy positive therapeutic effect was registered in 60 (73.2%). Among these patients clinical cure was registered in 9 (10.9%), significant improvement and improvement were found in 27 (32.9%) and 24 (29.4%) patients, respectively. No effect could be seen in 19 (23.1) patients, in 3 (3.7%) patients the pathological process worsening was found. In our study therapeutic effect of PUVA-therapy was found to depend to the disease duration.

Table 17. Outcomes of therapy for vitiligo by number of PUVA-therapy courses

Number of courses	Number of patients	Outcomes of therapy (n, %)				
		Clinical cure	Significant improvement	Improvement	No effect	Worsening
1	82	-	3 (3.6%)	30 (36.6%)	47 (57.3%)	2 (2.4%)
2	70	3 (4.3%)	14 (20%)	26 (37.2%	25 (35.7%)	2 (2.8%)
3	65	7 (10.7%)	23 (35.4%)	19 (29.3%)	15 (23.1%)	1 (1.5%)
Total	82	9 (10.9%)	27 (32.9%)	24 (29.4%)	19 (23.1%)	3 (3.7%)

Next, study on therapeutic efficacy of our method for therapy of vitiligo pertaining to PUVA-therapy in combination with topical application of lipovitilin was conducted. As it was indicated above, of 42 patients the expanded form of the disease was found in 23 (54.8%). The data on efficacy of lipovitilin in combination with PUVA-therapy can be seen in Table 18.

As it can be seen in Table 18, after first course clinical cure was registered in 2 of 23 patients (8.7%), significant improvement and improvement could be seen in 6 (26.1%) and 11 (47.8%) patients, respectively. No therapeutic effect could be seen in 4 (17.4%) patients. Thus, in 19 (82.6%) patients repigmentation of various degrees took place. After the second course, proportion of repigmentation significantly increased to reach 95%. Clinical cure was registered in 3 of 20 (15%) patients, significant improvement and improvement could be seen in 7 (35%) and 9 (45%) patients, respectively. No effect was found in 1 (5%) patient.

Repigmentation of various degrees was registered almost in all patients (n=16) receiving the third course of therapy. Thus, clinical cure was registered in 4 (25%) patients, significant improvement and improvement could be seen in 6

(37.5%) and 5 (31.4%) patients, respectively. No effect was found in 1 patient only.

As a whole, when lipovitilin was used in combination with PUVA-therapy clinical cure was registered in 34.8%, significant improvement and improvement could be seen in 39.1% and 21.8%, respectively. The proportion of repigmentation was 95.7%. No side effects were found.

Table 18. Outcomes of therapy for vitiligo by number of PUVA-therapy courses in combination with lipovitilin

Number of courses	Number of patients	Outcomes of therapy (n, %)				
		Clinical cure	Significant improvement	Improvement	No effect	Worsening
1	23	2 (8.7%)	6 (26.1%)	11 (47.8%)	4 (17.4%)	-
2	20	3 (15.0%)	7 (35.0%)	9 (45.0%)	1 (5.0%)	-
3	16	4 (25%)	6 (37.5%)	5 (31.4%)	1 (6.1%)	-
Total	23	8 (34.8%)	9 (39.1%)	5 (21.8%)	1 (4.3%)	-

External photochemiotherapy described above was performed in 70 patients of the 1st group with the restricted skin pathology. After three courses of the therapy of 70 patients clinical cure was registered in 9 (12.8%), significant improvement and improvement could be seen in 26 (37.1%) and 23 (32.9%), respectively. No effect could be seen in 10 (14.4%) patients, the pathological process worsening was registered in 2 (2.8%).

Table 19. Outcomes of therapy for vitiligo by number of external photochemiotherapy courses

Number of courses	Number of patients	Outcomes of therapy (n, %)				
		Clinical cure	Significant improvement	Improvement	No effect	Worsening
1	70	-	4 (5.8%)	31 (44.3%)	33 (47.1%)	2 (2.8%)
2	60	2 (3.3.0%)	5 (8.4%)	34 (56.7%)	17 (28.3%)	2 (3.3%)
3	50	4 (8%)	13 (26%)	29 (58%)	4 (8%)	-
Total	70	9 (12.8%)	26 (37.1%)	23 (32.9%)	10 (14.4%)	2 (2.8%)

In the second group of patients (n=19, 45.2%) with the restricted skin pathological process external photochemiotherapy was performed in combination with lipovitilin. After three courses of the therapy clinical cure was registered in 6 of 19 (31.6%) patients, significant improvement and improvement could be seen in 8 (42.1%) and 4 (21.1%) patients, respectively. No effect could be seen in 1 patient (5.2%).

Outcomes of therapy for vitiligo in the second group of patients by a number of courses of external photochemiotherapy in combination with lipovitilin can be seen in Table 20.

Table 20. Outcomes of therapy for vitiligo in the second group of patients by a number of external photochemiotherapy courses in combination with lipovitilin

Number of courses	Number of patients	Outcomes of therapy (n, %)				
		Clinical cure	Significant improvement	Improvement	No effect	Worsening
1	19	2 (10.5%)	5 (26.3%)	9 (47.4%)	3 (15.8%)	-
2	16	2 (12.5%)	6 (37.5%)	6 (37.5%)	2 (12.5%)	-
3	13	4 (30.6%)	5 (38.6%)	4 (30.7%)	-	-
Total	19	6 (12.8%)	8 (42.1%)	4 (21.1%)	1 (5.2%)	

As it can be seen, after therapy in combination with the liposomal formulation efficacy of both PUVA-therapy and external photochemiotherapy significantly increase, as compared with the outcomes in the controls who received PUVA-therapy or external photochemiotherapy without application of the formulation.

Thus, the findings from our clinical study on therapeutic efficacy of therapy for vitiligo with the liposomal formulation in combination with conventional methods demonstrate clear advantages of the former. This fact opens a prospect for extensive use of lipovitilin for therapy of vitiligo in the future.

Changes in clinical picture of the disease in the process of therapy for vitiligo by means of two above methods correspond to results of biochemical investigations performed in the blood of the patients. The results can be seen in Table 21.

The data in Table 21 demonstrate tendency in oxidative stress to decline in patients treated with conventional methods and by method with use of lipovitilin. The effect of therapy was more pronounced when the liposomal formulation in question was used. As compared with the parameters prior to therapy, MDA concentrations in blood of patients with vitiligo treated conventionally and by means of liposome therapy were found to reduce by 5.7% ($p>0.5$) and 22.6% ($p<0.001$), respectively. As compared with the parameters prior to therapy, catalase activity in persons treated conventionally and those undergoing liposome therapy increased by 6.2 ($p>0.5$) and by 15.4% ($p<0.02$), respectively. We are inclined to think that the changes taking place in parameters of lipid peroxidation and antioxidant system activity in blood of patients with vitiligo towards normalization due to both conventional therapy and the liposome one are the result of antioxidant preparations' usage.

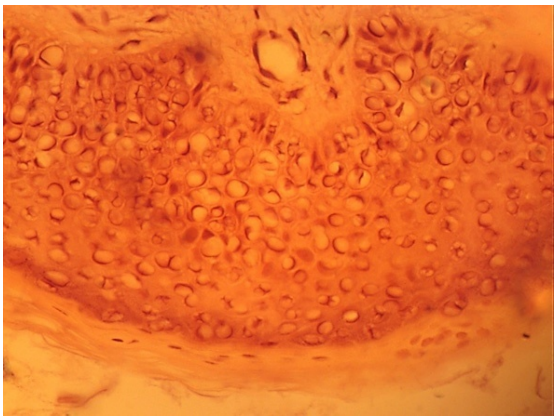
Table 21. Effects of conventional and liposome therapies on lipid peroxidation

Parameters under study	Normal values (n=10) (P)	Before therapy (n=30) (P1)	After therapy		Statistical significance
			Conventional method (n=18) (P2)	Liposome method (n=12) (P3)	
MDA (nmol/mg of protein/min)	1.57±0.15	2.60±0.19	2.46±0.13	2.12±0.11	P:P1<0.001 P:P2<0.001 P:P3<0.001 P1:P2>0.5 P1:P3<0.05 P2:P3<0.05
Catalase (mcat/l)	60.15±0.58	45.61±2.49	48.43±1.09	52.62±1.53	P:P1<0.001 P:P2<0.001 P:P3<0.002 P1:P2>0.5 P1:P3<0.02 P2:P3<0.05

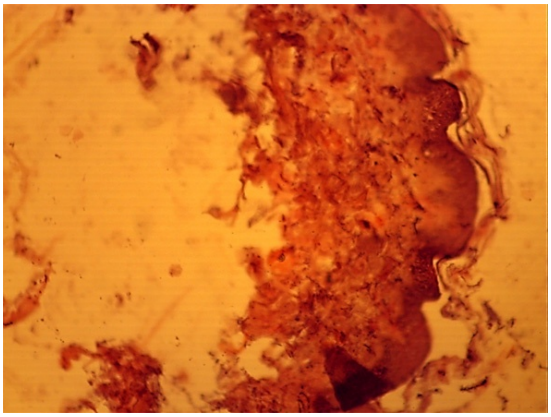
Findings from our study demonstrate that today the issue of penetration by liposomes through the skin is practically assured.

To sum up, the results from our study are the evidence for higher efficacy of lipovitilin, the liposomal formulation we developed, for therapy of vitiligo, as compared with the one of conventional therapy for the disease.

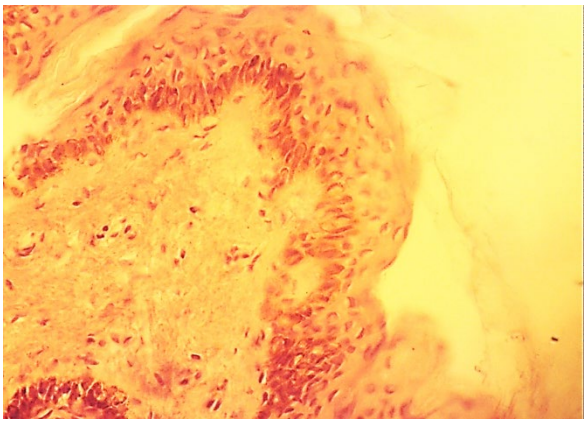
RESULTS OF HISTOMORPHOLOGICAL STUDY



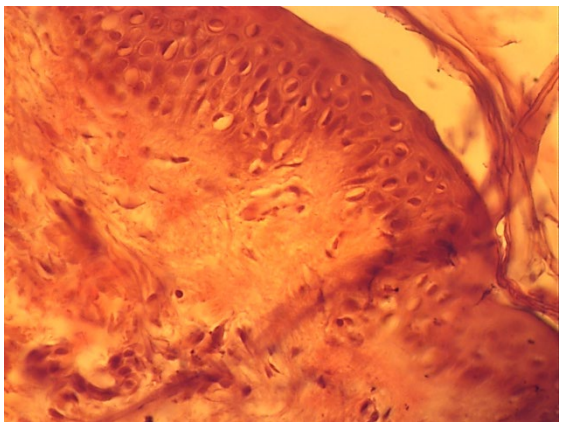
Picture 1. The plot of the skin tissue of the patient focus dipigmentizirovannogo vitiligo. Group 1. After the phototherapy. Otechny multilayered stratum corneum. Stained with hematoxylin eosin. Increased 400-x



Picture 2. The plot of the skin tissue of the patient focus dipigmentizirovannogo vitiligo. Group 1. After the phototherapy. Thick multilayered stratum corneum. Stained with hematoxylin eosin. Increased 400x



Picture 3. The plot of the skin tissue of the patient focus dipigmentizirovannogo vitiligo. Group 2. After conducting the combination therapy using the liposomal form "Lipovitilin" preparation. Melanin granules in the basal layer. Stained with hematoxylin eosin. Increased 400-x



Picture 4. The plot of the skin tissue of the patient focus dipigmentizirovannogo vitiligo. Group 2. After the combination therapy with ispolzovaniemliposomalnoy form of preparation "Lipovitilin". The mitotic activity of the cells. Stained with hematoxylin eosin. Increased 400x

CONCLUSIONS

1. The association of polymorphism (rs 1800629) of 308G/A gene TNF-α with the risk of vitiligo development was proved for the first time and there was established contribution of the changes in the frequencies of distribution of allele-A and genotypes G/A, A/A of this polymorphism into the pathogenesis of disease. In this case the frequency of the identification of unfavourable allele A of this polymorphism was significantly higher in the patients with vitiligo in comparison with control – 23,7% and 10,8%, respectively. Of 93 in 36 patients there was found unfavourable heterozygous genotype G/A, that accounted for 38,7%, and in control group – 21,6%. The incidence rate of mutant genotype A/A of the gene TNF-α polymorphism in the common group of patients with vitiligo accounted for 4,3%, that is, among 93 patients in 4 cases there was revealed this genotype, that was not found in control group.

2. The frequencies of incidence of alleles and genotypes of polymorphism G/A (rs 1293350) of gene TYR in healthy persons and patients with vitiligo were determined for the first time in Uzbekistan. There was revealed presence of allele A and genotype A/A in polymorphism (rs 1393350) of gene TYR. There was established association of this polymorphism with risk of vitiligo development. Of 58 in 23 patients with viitiligo there were unfavourable genotypes G/A that accounted for 39,7%< and in control group 30%, more than 1,35 times in comparison with control group. The frequency of occurrence of unfavourable mutant allele A of polymorphism of gene TYR was significantly higher in group of patients with vitiligo than among the persons of control group - 26,7% and 20,0% respectively. It was found presence at rather high level of mutant homozygous genotype A/A, the content of which made up 6,9%, and in control group – 50%

that indicated about predisposition to the development of vitiligo and its carrying increased risk of development of this disease almost 1,5 times.

3. The qualitative and quantitative contents of phospholipids as well as cerebrosides in the human skin in norm and in vitiligo were studied in he first time in comparative aspect. As distinct from norm in the skin of the patients with vitiligo there were revealed significant changes in the contents of some fractions of phosphoglycolipids. On the basis of reduction of neutral fractions of phospholipids of sphingomyelin, phosphatidylcholine and phosphatidyletanolamine there was noted reliable increase of acid fractions of phospholipids of lysophosphatidylcholine, phosphatidic acid and cardiolipin. It was established that in comparison with norm in the patients with vitiligo in the impaired skin site the concentration of cerebrosides reduced by 13,6%, and in the intact area – by 7%.

4. The oxidative stress, determined both in the skins and in the blood of the patients with vitiligo, is one of the leading pathogenic factors of occurrence and development of this dermatosis. Induced oxidative stress in the experimental animals sharply suppresses proliferative activity of the skin cells that expressed at lifting of mice to the height 4000 m, mitotic index (MI%) 0,36±0,12, apoptotic index (AI%) 0,3±0,03; at lifting to the height to 6000 m (MI%) 0,23±0,08, (AI%) 0,2±11 with increase of severity of oxidative stress the apoptotic and mitotic index reduced.

5. In the patients with vitiligo, in contrast to norm, there was observed marked imbalance in the contents of the most important macro- and microelements in the contents of skin and hair, that bring contribution into the pathogenesis of disease. It was established that in the skin of the patients with vitiligo there is noted reliable reduction in the contents of iodine (I) and cuprum (Cu), and in the hair reduction of iodine (I), cuprum (Cu), zinc (Zn), cobalt (Co) and Aurum (Au).

6. On the basis of results of the own investigations there has been developed and introduced into medical practice new without having analogues on a world scale multicomponent liposome drug designed to pathogenic treatment of vitiligo.

7. It is established that inclusion of liposome preparation “Lipovitilin” into the combined therapy of vitiligo provides increase in mitotic index and normalization of the processes of melanocytes proliferation in the depigmentated skin zone in patients with vitiligo.In this case there is observed suppression of intensity of oxidative stress in the body of patients that indicates about increase in effective therapy of vitiligo.

Practical recommendations:

1. The patients with limited forms of vitiligo and duration of disease not more than 5 years are recommended to use spray “Lipovitilin” as monotherapy or in association with UV radiation therapy with wave length 308-311 Nm. Lipovitilin is applied externally on the focuses of lesions during 2-5 minutes 2 times a day during 6-8 weeks.

2. The patients with the common form of vitiligo and duration of disease more than 5 years are recommended to use spray “Lipovitilin” externally on the focuses

of lsions 2-3 times a day during 8-12 weeks in association with traditional methods of therapy with external use of PUVA therapy with wave length 320-400 Nm for increase of efficacy of the therapy performed.

3. In the patients with vitiligo of Setton's disease are recommended externally the spray "Lipovitilin" as monotherapy without use of UV radiation on the focuses of lesions 2 times a day during 5-6 weeks.

4. "LIPOVITILIN" spray may be used both for the children from the first month of life and for the pregnant women and during lactation period, because "LIPOVITILIN" is spray of natural origin and designed only for external use which has recovering, antioxidant and melanogenesis stimulating effect and has no adverse effects.

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