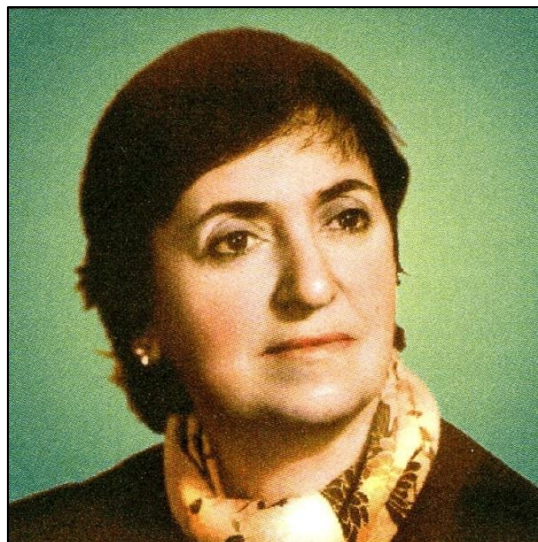


## **An Outstanding Ophthalmologist-Scientist and Organizer of Science: to the 100th Anniversary of Academician Zarifa Aliyeva**

### **FOREWORD**

An outstanding ophthalmologist-scientist, founder of the Azerbaijan Scientific School of Ophthalmology, academician Zarifa Aziz gizi Aliyeva (1923-1985), who made a huge contribution to the development of not only domestic, but also world science of ophthalmology. After graduating from the Azerbaijan Medical Institute with honors in 1947 she completed a specialty course in ophthalmology at the Central Institute of Advanced Training for Doctors in Moscow. In 1959, Zarifa Aliyeva defended her PhD dissertation on the topic "The treatment of trachoma with synthomycin in combination with other therapeutic methods" and in 1976, Zarifa Aliyeva defended her doctoral dissertation on "The conditions of the visual organs of employees of a number of chemical enterprises of Azerbaijan" at the Helmholtz Moscow Research Institute of Eye Diseases. In 1979, she was awarded the scientific title of professor by the Higher Attestation Commission under the Cabinet of Ministers of the USSR. In 1983, Professor Zarifa Aliyeva was elected a full member of the Academy of Sciences of the Azerbaijan SSR, taking into account her important achievements in scientific and scientific-organizational activities and personnel training, as well as their socio-economic importance.

The main directions of Zarifa Aliyeva's multifaceted scientific activity were trachoma clinic, physiology of the organ of vision, prevention, diagnosis, and treatment of eye pathologies caused by the effects of occupational diseases, condition of the organ of vision in employees of the chemical industry enterprises, herpetic eye diseases, viral damage to the visual organ, severe viral conjunctivitis, physiological-anatomical characteristics of the hydrodynamic system of the eye, treatment of watery eyes with modern surgical methods, eye diseases during diabetes, therapeutic ophthalmology, basics of iridodiagnostics, glaucoma problems, etc.



The eminent scientist left an indelible mark on the world medical science as the author of about 200 scientific articles, 14 monographs, and 12 effective recommendations related to various areas of ophthalmology.

Zarifa khanim Aliyeva was also an excellent teacher who inspired many generations of students with his enthusiasm and knowledge.

Zarifa Aliyeva's rich scientific heritage and scientific school, her research in various fields of ophthalmology, her important achievements, and the fundamental works she created on the basis of them are bright pages of Azerbaijani medical science. Her ideas increase their relevance over time and make real contributions to people's health by developing relevant fields of science and spreading light to the eyes.

The current issue of the Journal "Life Sciences and Biomedicine" is dedicated to the 100<sup>th</sup> anniversary of academician Zarifa Aliyeva. 21 research and review papers included in this issue were carefully selected by the editorial board and reviewed.

**Academician Irada Huseynova**  
*Vice President of ANAS,*  
*Deputy editor-in-chief of the journal*

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## **An Outstanding Ophthalmologist-Scientist**

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The 100<sup>th</sup> anniversary of the birth of the outstanding scientist, founder of the Azerbaijan Scientific School of Ophthalmology, academician Zarifa Aliyeva is widely celebrated in our country. The Azerbaijan National Academy of Sciences celebrates the jubilee of academician Zarifa Aliyeva due to her great services in the field of medicine development, as well as her valuable scientific contributions to the development of Azerbaijani medical science, at the level of the celebration of science in modern times.

Academician Zarifa Aliyeva lived an honorable and meaningful life. She was raised in an excellent educational school, in the family of Aziz Aliyev, a prominent statesman and scientist of Azerbaijan, and received higher education at the Azerbaijan Medical Institute named after Nariman Narimanov. Zarifa Aliyeva, who started working as a practical ophthalmologist, was also engaged in scientific activities, defended PhD and doctoral dissertations on current topics, and rose to the level of an academician of the Azerbaijan National Academy of Sciences (Fig. 1). She was recognized and accepted as an outstanding scientist who brought innovations to medical science throughout the former Soviet Union and beyond. Academician Zarifa Aliyeva's authority was multifaceted and covered various fields of medical science, the medical profession, and social activities. In each of the areas she was engaged in, she showed high professionalism and an example of true citizenship belonging to the people of Azerbaijan.

First of all, it should be noted that Azerbaijan's medical science has specialized mainly in the fields of treatment, therapy, and surgery for many years. In our country, both medical science and treatment work in areas such as neurology, eye diseases, oncology, and others have begun to develop since the sixties-seventies of

the XX century. In particular, ophthalmology was one of the lagging fields during this period. Thanks to the effective scientific and practical activity of the outstanding scientist, academician Zarifa Aliyeva, Azerbaijan has become one of the main scientific and medical centers developed from a republic that provided primary medical treatments.

Thanks to the efficient and tireless work of Zarifa Aliyeva as a doctor, the flow of patients forced to go from Azerbaijan to other allied republics due to eye diseases was stopped. Zarifa Aliyeva also prevented the flow from the region to the capital for the treatment of widespread eye diseases, including trachoma. Under her leadership, a team of doctors specializing in trachoma went to the regions of Azerbaijan and provided necessary assistance to patients on the spot. The team of doctors headed by Zarifa Aliyeva showed a real example of an organized fight against trachoma in Ganja city, Alibayramli (now Shirvan), and Salyan regions. The treatment work of her medical team fighting trachoma in Baku city, Baku villages, the Absheron region, and other regions was highly appreciated by thousands of people.



**Fig. 1.** Zarifa Aliyeva (second from the left in the 2nd row) with her colleagues, 1951 year

The treatment method developed and conducted by Zarifa Aliyeva, whose effectiveness was proven both theoretically and practically, played an important role in eradicating trachoma as a social disease.

Besides, academician Zarifa Aliyeva created a special laboratory for the treatment and research of eye diseases in the industrial enterprises of Baku for the first time, and in this way, she removed the barrier between the field of production and medical science. Thus, Zarifa khanim Aliyeva managed to gain the name and reputation of a real people's doctor with her selfless efforts.

Proximity to people and life enabled Zarifa khanim to write works of real scientific and practical importance based on the results of her experience in the field of eye diseases. Zarifa Aliyeva is the founder of Azerbaijani ophthalmology, which was established on the basis of experience reflecting the realities of the country in the science of eye diseases in Azerbaijan, rooted in general and abstract theoretical information. The generalized theoretical propositions in her research were not based on ready-made scientific schemes, but on the basis of scientific conclusions arising from experience and real observations. Zarifa Aliyeva's PhD dissertation, dedicated to trachoma diseases, is a scientifically based medical map of eye diseases in Azerbaijan. Besides, Zarifa Aliyeva's doctoral dissertation on "The condition of the visual organ of employees of some enterprises of the Azerbaijan chemical industry" defended with great success is highly valued as the first perfect and systematic scientific work dedicated to this problem in the former Soviet Union.

Academician Zarifa Aliyeva is recognized as a scientist who created a new field of ophthalmology - the scientific direction of occupational diseases in the former Soviet Union and beyond (Fig. 2). Academician Zarifa Aliyeva's valuable scientific works dedicated to occupational diseases are theoretical and practical training that is an example of true professionalism in the science of ophthalmology. Based on her experiences in production facilities where eye

diseases are more common, such as the chemical industry, she studied the secrets of occupational diseases in the Baku Tire Plant, through the treatment-diagnostic laboratory organized by the chemical-technological institutions of our country, and performed successful treatment based on scientific diagnostics. The academician discovered important regularities in the effect of low-intensity harmful substances and some physical factors on the visual organ in production.

It should be noted that the doctor's dissertation, which is an excellent scientific work written on the basis of the unity of experimental materials and scientific-theoretical analyses, is also dedicated to occupational diseases, the important results of this work have been repeatedly confirmed in practice.

These innovations are reflected in her books and monographs such as "Occupational Pathology of the Organ of Vision", "Prevention of Occupational Diseases in the Iodine Industry", "Ophthalmic pathology during chronic iodine intoxication", and "Occupational Pathology of the Eye in Tire Production".



**Fig. 2.** At the conference of Ophthalmologists of the USSR, October 18, 1977

The application of the scientific achievements of Zarifa Aliyeva in the field of viral diseases of the eye led to important progress



in the differential diagnosis and treatment of many diseases. Books and tutorials written by the scientist in this field such as "Herpetic eye disease", "Acute viral conjunctivitis", etc. are important sources of knowledge for students and young doctors.

Zarifa Aliyeva learned the possibilities of preventing and controlling eye injuries. She analyzed the existing methodological approaches to the surgical treatment of the consequences of eye damage and gave a number of useful recommendations for their improvement.

Zarifa khanim paid attention to the diagnosis and treatment of some malignant tumors of the eye, and at the same time studied the features of the clinical course of ocular melanoma. Her monographic works on dactriology - "Physiology of tear drainage", "Modern methods of surgical treatment of tear discharge", and "Preventive surgery of tear ducts" have become deskbooks not only for ophthalmologists but also for physiologists. With these works, the scientist laid the foundation of Azerbaijani lachrymology.

Rare monographic works written with her co-authorship "Therapeutic ophthalmology", "Basics of iridodiagnostics", "Actual problems of ophthalmology", "Tuberculosis of the organ of vision" are still of great interest to practicing ophthalmologists, teachers and students. It should be noted that the worldwide books on iridodiagnostics were first written by or with the participation of academician Zarifa Aliyeva.

A significant part of Zarifa Aliyeva's successful scientific path was connected with the Academician Abdulla Garayev Institute of Physiology of the Academy of Sciences of the Azerbaijan SSR. In 1979, under the initiative and direct leadership of Zarifa khanim, the laboratory of "Physiology of the visual organ and occupational pathology" was established in that institute for the first time in the USSR.

Academician Zarifa Aliyeva received the highest award of the Academy of Medical Sciences of the USSR in the field of ophthalmology in 1981 - the award named after M.I.Averbakh for the successful results obtained from the above-mentioned studies and published in her monographs (especially for the "Occupational pathology of the eye in tire production") (Fig. 3). It is remarkable that Zarifa

Aliyeva was the first among female specialists in the former USSR who received this award.

Zarifa Aliyeva studied the effects of toxic substances and some physical factors on the functional state of the retina, free radical processes, and the level of neurotransmitters. The results of those studies arouse great interest among specialists even today.



**Fig. 3.** Zarifa Aliyeva was awarded the Averbakh Prize, February 12, 1981

The study of the age-related changes of the organ of vision and the vision analyzer also occupied a special place in the field of scientific interest of Zarifa Aliyeva. Zarifa Aliyeva's book "Age-related changes of the eye and the path of the optic nerve: morpho-histochemical studies" marked the beginning of ophthalmological gerontology.

The observations and conclusions of Zarifa Aliyeva on heredity issues in eye diseases are also some of the important innovations for the medical world.

Thanks to the services of academician Zarifa Aliyeva, Azerbaijani science in the field of ophthalmology was recognized as one of the main centers. Academician Zarifa Aliyeva raised

Azerbaijani ophthalmology to the level of Moscow and Petersburg schools that gained fame in the former USSR. Large scientific forums dedicated to the actual problems of eye diseases held in Baku, meetings and discussions of well-known world ophthalmologists at scientific events of the international level organized in Azerbaijan are real embodiments of the great reputation gained by academician Zarifa Aliyeva in the ophthalmology.

In the seventies of the 20th century, the laboratory created by the Azerbaijan Academy of Sciences at the Institute of Physiology named after Abdulla Garayev and the treatment-diagnostic laboratories for rare eye diseases established by the Baku Tire Plant and the Household Air Conditioners Plant became research centers that had no analogs in the world medical science. The transfer of research on eye diseases from the Ministry of Health to the Academy of Sciences and industrial enterprises is connected not only in Azerbaijan but also in the Soviet Union in a broad sense with the name of academician Zarifa Aliyeva. Thus, in 1979, the scientific analysis of the results obtained in the laboratory at the Institute of Physiology brought academic weight to the field of ophthalmology, which is somewhat experimental in nature. By organizing the treatment of eye diseases in the regions of Azerbaijan, creating opportunities for treatment and diagnostics in industrial enterprises, bringing the topic of medicine to the academic scientific environment, and by multifaceted scientific and practical activities in those directions, academician Zarifa Aliyeva showed that she was a true ally of the outstanding statesman Heydar Aliyev, who fought resolutely for the development of the country as the head of the Azerbaijan Soviet Republic in those years.

Zarifa Aliyeva, who faithfully served her profession, showed an example of loyalty at every step of her family life and in the society of Azerbaijan. Zarifa Aliyeva was a real example of being the loyal lifelong friend and worthy ally of Heydar Aliyev, a world-famous prominent statesman. Zarifa Aliyeva, along with an incomparable Father, the National Leader Heydar Aliyev who gave her child, the President of the Republic of Azerbaijan, Ilham Aliyev, one of the world leaders, to our country, became an example

of the greatness and unattainability of being a real Mother.

Contemporaries describe her as a caring person who showed simplicity, politeness, respect, and sincerity in relation to people. The book "High conviction: medical ethics, the purity of the doctor's heart and thoughts" published in 2003 based on her articles, not only revives the image of Zarifa khanim as a real doctor but also draws attention to her calls to future doctors.

The scientific community of our country remembers Honored Scientist, academician Zarifa Aliyeva as a well-known public figure with great respect. The work she did through the Peace Protection Committee, the "Bilik" Society, the Scientific Society of Ophthalmologists, and the events she implemented, still bear the indestructible traces of her multifaceted scientific and social activities.

The services of academician Zarifa Aliyeva, who had extensive scientific and social activities, were highly appreciated. Academician Zarifa Aliyeva was awarded the title of Honored Scientist of Azerbaijan and the Order of the Red Banner of Labour.

The name of Zarifa Aliyeva, an outstanding ophthalmologist scientist and well-known public figure, has been immortalized in Azerbaijan. Today, the National Ophthalmological Center of the Ministry of Health of the Republic of Azerbaijan, named after academician Zarifa Aliyeva, is recognized as one of the prestigious medical institutions and one of the main centers of medical science in our country. An award named after academician Zarifa Aliyeva was established at the Azerbaijan National Academy of Sciences. One of the biggest streets in Baku and a school-lyceum are named after Zarifa Aliyeva. Today, the steps taken forward by the science of eye diseases in the Independent Republic of Azerbaijan are echoes of the ongoing scientific ideas of academician Zarifa Aliyeva, the founder of the National Scientific School of Ophthalmology.

The Decree of the President of the Republic of Azerbaijan Ilham Aliyev "On the celebration of the 100th anniversary of Academician Zarifa Aliyeva" dated November 3, 2022, is another expression of the great care shown at the state level to the education of new generations in the

example of academician Zarifa Aliyeva and the continuation of progressive scientific ideas in medical science.

The ideas of prominent scientist and well-known public figure, Zarifa Aliyeva, selfless work in the field of Heydar Aliyev's path and women's movement are successfully continued by the First Vice-President of the Republic of Azerbaijan, Mehriban Aliyeva.

A unique artistic image of academician Zarifa Aliyeva was created in Azerbaijani literature and art. Academician Zarifa Aliyeva's statue called "Elegy" in Alley of Honor, authored by People's Artist Omar Eldarov, is one of the masterpieces of Azerbaijani sculptural art. A perfect artistic image of Zarifa Aliyeva was created in the poems of the People Poets of Azerbaijan, Suleyman Rustam, Bakhtiyar Vahabzadeh, Mirvarid Dilbazi, Fikret Goja, and others.

National Leader of the Azerbaijani people, Heydar Aliyev, highly appreciated the multifaceted activities, scientific services and moral values of Zarifa Aliyeva, his companion in life and ally in the struggle: "Since my youth, my life has been related to government work. I have dedicated my whole life to this... I think that my family situation played a big role in my efficient and successful work on this path. I was happy that I had a wife like Zarifa khanim and that she created a very high moral environment in my family... She fulfilled this duty with honor, loyalty, and great skill (Fig. 4).

"Zarifa khanim was a great scientist. When we got married, she had already been on the path of science... Her scientific activity is known. She was a very talented, very kind, very simple person. I can talk a lot about her, today I bow in front of the grave of Zarifa Khanim for the survival of my family, for reaching these days, for the education of my children, and for her irreplaceable role in my life".

The President of our country, Ilham Aliyev, also pointed out that the multifaceted services of the outstanding scientist and public figure, academician Zarifa Aliyeva is an example for generations: "Zarifa Aliyeva was a great doctor, a great scientist.

She made a valuable contribution to the

Azerbaijan School of Ophthalmology, she was very active in the training of young personnel.

As a scientist, Zarifa khanim was able to reach great heights. Her works and scientific monographs do not lose their relevance even today.



**Fig. 4.** Academician Zarifa Aliyeva with her husband Heydar Aliyev, The National Leader of the Azerbaijani people

For every person, his parents, his mother are dear and sacred... I am very happy that I had a mother like Zarifa khanim.

The bright image of Zarifa khanim is always in our hearts. Those who know her and those who don't know her, and those who read about her in books and articles, I think, always keep her bright image in their hearts. Zarifa khanim was a great doctor, a great scientist, and a great person. Her bright memory will always live in the hearts of those who knew her."

An outstanding scientist, academician Zarifa Aliyeva, the founder of the national science of ophthalmology, the lifelong friend and ally of the outstanding world-class statesman, Heydar Aliyev, Mother of the President of the Republic of Azerbaijan Ilham Aliyev, one of the outstanding leaders of the 21st century, has become a legend and continues to live in the hearts and memories of our people.

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## **Nanomedicine for ophthalmology: potential therapeutic approaches**

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**The study of nanotechnology has ushered in a new era in medical science in recent years. The term ‘nano’ refers to the nanoscale particle spectrum that includes cellular and molecular structures and is usually between 10 and 1,000 nm in size. Nanoparticles have several advantages over conventional ophthalmic medications including abilities of smart drug targeting; extended drug release time; have reduced toxic effects; and use of gene therapy. After a quick introduction to nanoparticle classification, we’ll concentrate on the potential uses of various nanoparticles in the treatment and detection of various retinal pathologies and present cutting-edge nano-derived methodologies.**

**Keywords:** *Nanoparticles, ocular disease, drug delivery, medicine*

### **INTRODUCTION**

The basis for various medical uses of nanomaterials was established by the creation of materials, structures, processes, and equipment on the scale of cellular and molecular structures and in the range of 1-1000 nanometers. Nanomedicine is a branch of science that uses nanotechnology to diagnose and treat various illnesses. It offers important insights that are advancing modern healthcare. The development of nanotechnology, in particular, looks set to significantly accelerate progress in ophthalmology.

To treat a wide variety of retinal pathologies including retinal degenerations, retinopathy, Retinitis pigmentosa and uveitis, researchers have developed nanomedical agents in varied shapes, including hydrogels, liposomes, dendrimers, nanoemulsions and nanospheres (Sharma and Sharma, 2021). The permeability of therapeutic molecules as well as advancements in their

biodistribution and bioavailability has all been thoroughly investigated in recent years to address the problematic accessibility of different medications to the corneal and aqueous barriers as well as the inner and exterior blood-retinal and blood-ocular barriers. Drug targeting, regulated release, and penetration can all be improved with nanoparticles.

Moreover, it has been demonstrated in numerous studies that the use of nanoparticles increases the therapeutic efficacy of medications for ocular diseases. The use of some of these therapeutic nanoparticles for treating ocular diseases has been addressed in the sections that follow, along with a summary of their applications and a list of important factors to consider.

### **Nanomedicine**

By manipulating nanomaterials, the science of nanotechnology studies, creates, operates and



uses functional materials, tools and systems (1–100 nm). Nanotechnology is being used more and more in the diagnosis, treatment and management of different diseases. Nanomedicine is the name of this novel scientific discipline. The use of nanotechnology in ophthalmology has advanced along with advances in health and surgery. To maximize drug bioavailability, increase the time spent in contact with the eye and lessen the need for eye removal, novel eye nano-systems with various shapes and properties have been developed (Chaudhary and Mishra, 2022). Numerous nano-systems have been used in the management of numerous eye disorders. For the treatment of xerophthalmia, biodegradable subconjunctival implants, nanoparticle-filled contact lenses with acetazolamide, diclofenac eye-release nanocolloid systems based on hydrogel, polymer nanocolloid systems for inflammatory diseases and nanostructured lipid transporters for managing drug delivery in ocular infections are just a few examples.

For the delivery of ophthalmic drugs, various nanoscale materials have been created with particular properties. Numerous nanosystems exist, such as dry eye syndrome-specific nano-based subconjunctival implants, nanomicelle-based polymers, glaucoma-targeted nanoparticle-loaded contact and hydrogel-based polymers. Additionally, numerous varied kinds of nanoparticles are being extensively researched for the sustainable and nontoxic delivery of various medicines into the ocular system (Romanowski et al., 2016). Regardless of all the advances in nano-based drug delivery systems, most of the nanosystems listed above are still at the experimental stage

Nanomicelles are the most popular methods for delivering ophthalmic medications to the anterior and posterior eye regions. Amphoteric compounds can quickly and easily create self-assembling nanomicelles. Nanomicelles could be considered to be safer than other types of nanomaterials because fewer auxiliary materials are required in their production. Due to the hydrophobic core, hydrophobic agents easily dissolve and result in a clear aqueous formulation when ready for administration to the posterior and anterior segments of the eye.

For instance, nanomicelle-based

dexamethasone that was delivered to the anterior segment of the eye demonstrated greater bioavailability than the suspension of dexamethasone (Swaminathan, Vavia, et al., 2013). Additionally, therapeutic agents are easily delivered to the eye's posterior region by stable nanomicelles (Terreni et al., 2021). For the treatment of various ocular diseases, nanomicelles containing various medications, such as rapamycin, have been created recently (Wei et al., 2018).

One of the most preferable dendrimers for ocular agents' delivery is poly(amidoamine) (PAMAM) (Qin et al., 2020). Due to suitable biodistribution and adaptable structure in different ocular segments, dendrimers have proven to be one of the most useful drug delivery systems at the nanoscale.

It has been claimed that encapsulating fluoroquinolones like nadifloxacin and prulifloxacin within PAMAM dendrimers increase the antibacterial potency of the drugs relative to free drugs (Kamaledin, 2017).

Dendrimers may be applied as eye topical drops that can sustain the delivery of antimicrobial agents on the cornea's surface without affecting visual creation. PAMAM dendrimers have also been successfully used in rabbit ocular systems to deliver glucosamine, tropicamide, and pilocarpine nitrate. One of the concerns about the selective delivery of dendrimers to ganglion cells of the retina is toxicity and inflammation.

### **Delivery of nano-drug from retinal-blood barrier**

Water and small, water-soluble compounds can cross the inner BRB (iBRB) via paracellular transport, which is controlled by the dynamic opening and closing of complex junctional protein complexes that connect and seal two adjacent retinal microvascular endothelial cells RMECs. These complexes are typically smaller than 3 nm in radius and around 500 Da. Several functional classes of junctions, including tight junctions, adherens junctions and gap junctions make up these protein units.

Collectively, they control Microvascular endothelial cells (EC) growth inhibition and cell-to-cell adhesion, preserving cell polarity and

survival, and eventually regulating paracellular permeability. EC connections commonly entangle with one another to generate even more complicated junctional proteins, in contrast to epithelial cells, which typically have each set of their junctions as independent entities. For example, in RMECs, tight junctions frequently form higher structural protein complexes with adherens junctions and gap junctions, which is consistent with their role in arbitrarily limiting paracellular transport.

Certain medications, especially those with greater molecular weights, are unable to reach the retina in sufficient amounts because of the blood-retinal barrier's reduced permeability (Wang and Pang, 2023). The blood-retinal barrier works as a discriminating blocker between the circulatory and nervous systems assisting in preserving equilibrium throughout the retina. The interior dynamic structure of the blood-retinal barrier is organized by astrocytes, pericytes, endothelial cells and the outer blood-retinal barrier is the tight junction shaped by the retina's pigment epithelium cells (Wang and Pang, 2023).

Gold nanoparticles with a diameter of nearly twenty nm have been demonstrated to penetrate through the blood-retinal barrier and distributed throughout the retina's layers with minimal (if any) negative impacts on the survival of astrocytes and retinal endothelial cells (Masse et al., 2019).

Earlier research revealed that there is no place in the retina where 100 nm particles can be found, indicating the preferential impact of NPs' size when passing the blood-retinal barrier. The fact that these NPs have no toxic effects on the retinal structures or functional abnormalities was also further supported by histological investigations. It's important to note that surface chemistry can have an impact on how Nanoparticles are distributed. Positively charged nanoparticles can bind to the nearby anionic vitreous elements.

Anionic nanoparticles, on the other hand, are discovered to diffuse through the vitreous and enter the layers of the retina. Furthermore, there is no conclusive proof that the administration of gold nanoparticles has altered the expression or structure of any characteristics of biological molecules. These findings imply that gold nanoparticles are a viable alternative and have excellent *in vivo* applicability for the blood-retinal

barrier's delivery of drugs (Lohia et al., 2022).

### **Nano-based therapeutic strategies**

Because of immediate exposure of the cornea to the environment, it is vulnerable to a variety of diseases. The general circulation and the body's immune system are practically cut off from the cornea, making it easily available. In gene therapy, the transport of the desired genes to the cornea or nearby tissues is the prime option for gene therapy.

Better sustained delivery and transfection efficiencies provided by nanoparticles-based gene therapy are made possible by the improved uptake in cells, endosomal crossing and carriage capability to the nucleus. Corneal gene therapy has been effectively used in recent preclinical studies to stop herpetic stromal keratitis, neovascularization and rejection of cornea in animal models, nanoparticle intrastromal injection is a suitable technique for ocular gene therapy (Di Iorio et al., 2019).

For example, an anti-VEGF cassette expressing a short hairpin RNA with PLGA nanoparticles injected intrastromal inside of plasmid to treat corneal neovascularization. As a result of its immunosuppressive impact, anti-VEGF factors were also tried for delivery via subconjunctival injections to prevent murine cornea transplant rejection (Swetledge et al., 2021).

Keratitis, conjunctivitis and uveitis are the diseases that correlate to the inflammation of the uveal tissues, conjunctiva and cornea respectively. Nanoparticles have a greater bioavailability, longer residence time and longer duration of effect making them a potential treatment for uveitis. For instance, immunologic responses to nanoparticles containing betamethasone phosphate in rats with experimental autoimmune uveoretinitis were inhibited.

Accordingly, nanoparticles coated with tamoxifen and encapsulating betamethasone have shown anti-inflammatory benefits in instances of uveitis. The peptide is conjugated with the poly(ethylene glycol) (PEG)-coated nanoparticles for ocular administration. Nanoparticles coated with PEG for tamoxifen delivery showed the prohibitory impact on inflammation in experimental autoimmune uveoretinitis rats

(Bhuwane et al., 2022). These findings back up the earlier assertion that therapeutic agents enclosed in nanoparticles have greater bioavailability and efficacy. Fungal keratitis in rabbits has been treated with chitosan hydrogels, which have been revealed to improve maintenance time. Encapsulated nanoparticles also enhanced therapeutic effects, prolonged antimicrobial activity in contact lenses and showed sustained release in keratitis models (Chang et al., 2022).

Any damage to the cornea's cutaneous tissue will significantly impair eyesight. Suturing the eye is a common clinical procedure for corneal wound healing but it comes with several risks including infections, astigmatism, corneal scarring and postoperative cataracts. To lessen the drawbacks, various adhesives and polymer glues have been used but almost none of them have produced perfect results. Highly branched nanostructured dendrimer-based hydrogels were able to support wound healing quickly without scarring or inflammation because of their carefully controlled crosslink networks (Wang et al., 2021).

Additionally, crosslinker modifications altered adhesive degradation time which allows for exact control of the time it takes for wounds to heal. Fresh blood vessels develop inside the normally avascular corneal tissue as a result of the pathological condition known as corneal neovascularization, which is typically accompanied by inflammation, infection and traumatic or degenerative illnesses. Different treatments for corneal neovascularization aim to stop angiogenesis by blocking angiogenic factors (Shen et al., 2022). According to research, PLGA nanoparticles containing short hairpin RNA-containing plasmid are extra efficient against VEGF than bare plasmid (Zhang et al., 2010). Additionally, copolymer and plasmid DNA conjugated in nanomicelles that produce soluble VEGF receptor-1 was applied in gene therapy methods to block the signaling pathway of angiogenesis (Kanazawa et al., 2012).

Choroidal neovascularization (CNV), caused by the expansion of vessels choroid under the epithelium of the retina, causes hemorrhage and scarring. Nanomedicines are founded effective in inhibiting additional angiogenesis, similar to how corneal neovascularization is treated.

Dexamethasone acetate is shown to discharge under controlled conditions when enclosed in PLGA nanoparticles, which results in preventing impacts on CNV (Xu et al., 2007).

It has been suggested that poly (lactic acid/poly (ethylene oxide)) nanoparticles significantly suppress CNV and have a longer effect in the form of encapsulation (Heald et al., 2002). Using PLGA/chitosan nanoparticles, the proteolytic plasminogen kringle 5 plasmid enclosed and injected intravitreally (Abd et al., 2018).

The effectiveness of nanoparticles in inhibiting VEGF production has also been demonstrated by the use of encapsulation of small interfering RNA in PEGylated liposome protamine hyaluronic acid nanoparticles. Additionally, previous works showed that anti-VEGF plasmids enclosed in PLGA NPs can effectively reduce CNV through targeted delivery. By using the proper nanocapsules for targeted drug administration, anti-angiogenic medications are no longer inaccessible to the retina and subretinal spaces. Additionally, polyion complex micelles with 10 nm were used to deliver drugs for the successful treatment of CNV (Wang et al., 2009).

Another study has proposed using photodynamic therapy to treat neovascular disease using supramolecular nanocarriers loaded dendritic photosensitizers. The structure prevents the core sensitizer from aggregating which triggers a photochemical reaction that obstructs CNVs notably effectively while causing the least amount of unfavorable phototoxicity.

Elevated intraocular pressure (IOP) is a common symptom of glaucoma (ganglion cells of the retina gradually degenerate). Glaucoma is one of the major global causes of blindness. In the treatment of glaucoma, the goal is to decrease the pressure on the eyes either increasing or reducing aqueous humor production. By quantum dots monitoring and observing after injection into the ocular system, it may be possible to determine how the lymphatic of the eyes contributes to the drainage of liquids from the eye (Tam et al., 2011).

Timolol and brimonidine are two prevalent therapeutic drugs that have been delivered to the eye using various nanoscale structures with

improved sustained delivery and bioavailability. Similarly, by encapsulating and administering thermal shock proteins or glial cell line-derived neurotrophic factor, it is possible to regenerate damaged neuronal cells (Bigdeli et al., 2023).

### **Nanotechnology for Retinal Diseases Diagnosis**

Recently nanoparticles have been created as agents to boost imaging contrast in techniques including CT, X-ray and fluoroscopy. The amphiphilic cyanine dye indocyanine green (ICG) was approved by Food and Drug Administration for therapeutic use in 1954 (Haritoglou et al., 2012). ICG's limitations on the detection of retinal disorders including its reduced hydrolytic stability and little photo restrict ICG's potential usage (Haritoglou et al., 2012). It appears that one way to get around these limitations is to incorporate ICG into nanoparticle systems. To increase contrast, ICG enclosed in nanoparticles can be applied for imaging including photoacoustic and near-infrared (Haritoglou et al., 2012).

Since they can absorb NIR radiation gold nanoparticles have attracted attention as potential contrasting agents. This allows for discriminating visualization with little noise in the background. OCT imaging has improved thanks to gold nanoparticles. Previous work showed injecting Au NPs into mice's retinas to enhance contrast and significantly improve the clarity of scanned lesions (Hainfeld et al., 2006).

Au NPs were used to enhance the monitoring of transplanted photoreceptor precursors (PRPs), and after a month there were no negative side effects (Betzer et al., 2020). Chain-like Au nanoparticles were introduced that increased the OCT signal by up to 176% (Paulus et al., 2020). Peptide-functionalized silicon nanoparticles (SiNPs) were created to label angiogenesis and inhibit neovascularization having a dual impact (Dougherty et al., 2015).

Human embryonic stem cell (hESC) or induced pluripotent stem cell (iPSC)-derived RPE cell transplantation has demonstrated revolutionary results in eye illnesses (Singh et al., 2019). To maximize the functional advantages of cell therapy deep learning of the biodistribution and survival of transplanted cells is required. Magnetic nanoparticles primarily iron oxide

nanoparticles have the ability for 3 months labeling in magnetic resonance imaging (MRI) and this labeling has no impact on the precursors' capacity to differentiate, proliferate or stay viable (Giannaccini et al., 2017).

The stable magnetic properties and characteristics of carbon materials of the carbonized CM and CMT NPs allowed the nanoparticles to accomplish imaging simultaneously. Magnetic nanoparticles can deliver medications to the body through a variety of methods including intravenous infusion and topical application and they can be guided to particular body locations by magnetic fields. In one research magnetic nanoparticles and nerve growth factors were covalently coupled and controlled magnetic fields were used to transport the magnetic nanoparticle complexes to the retina (Giannaccini et al., 2017).

Poly (3,4-ethylenedioxythiophene) (PEDOT)/ gold nanocomposites have proven to be a reliable and accurate method for VEGF concentration measurement. To increase the binding of PEDOT to antibodies, PEDOT/gold nanocomposites were created. They discovered that over a range resistance of the charge transfer remained linearly linked with the concentration of the analyte's VEGF. The level of the retina's VEGF can be determined in this manner to increase the detection precision and exactly regulate the frequency and dosage of injections of anti-VEGF medications. Both of cost and the adverse effects of an overdose of anti-VEGF medications can be significantly reduced with accurate VEGF concentration measurement (Kim Jr et al., 2019).

### **Nanomedicine and toxicity**

The biocompatibility of NPs should be taken into consideration for ophthalmic therapeutics application, and biomedical administration of nanoparticles shouldn't result in genotoxicity, cytotoxicity, or any type of immunological reaction. The retina which contains numerous neuronal cells may be a possible target of the neurotoxicity of nanoparticles. In turn, the possible ability of nanoparticles to penetrate the blood-brain barrier by increased distribution in the layers of the retina could make NPs more toxic by

increasing their toxicity. Different toxicology tests should be conducted in conjunction with novel forthcoming carriers taking into account the potential side effects of nanoparticles (Khiev et al., 2021).

Multiple investigations have looked into the potential neuronal toxicity of nanoparticles. One of these processes with the most support is reactive oxygen species overproduction. For example, titanium dioxide (TiO<sub>2</sub>) nanoparticles perturbed the electricity activation in a network of neurons by causing intracellular reactive oxygen species production in glial and neurons (Wu and Tang, 2018). Additionally, after being exposed to silica NPs, microglial cells displayed phagocytic behavior and took them up, which increased the generation of reactive nitrogen species and intracellular ROS (Wu and Tang, 2018). Furthermore, the epithelium of retina pigments and cells of the epithelial lens have shown increased cytotoxicity and phototoxicity after receiving hydroxylated fullerene nanoparticles (Wielgus et al., 2010).

### **Nanomedicine and regenerative ophthalmology**

There are two areas of tissue engineering research. Additive tissue engineering, of them, substitutes cells or tissue attempts to replace missing tissue by growing new tissue. The other, called arrestive tissue engineering, seeks to halt unnatural development. Depending on the target, medicinal nanodelivery can have either an additive or an arrestive effect on the environment. The distribution and pace of healing can be manipulated by altering the environment at the nanoscale. Together, there are a huge number of procedures that could benefit from nano-based engineering in retinal diseases such as tests to determine whether retinal ganglion cells are still viable, cell transplantation of endothelial cornea and cell repair of retina ganglions the nanofiber-based scaffold construction and gene therapy to prevent neovascularization in intraocular tissue (Hunt et al., 2018).

In recent years a framework for delivering ocular therapeutics has been created. Particularly nanosystems have been developed for the transport of hydrophilic and lipophilic medications. For instance, polynucleotides on the

polysaccharide chitosan meet the needs of the topical ocular pathway (Hunt et al., 2018).

One significant issue in neuromedicine - the inability of nerves to reinstate neural tasks - has prompted researchers to investigate whether nanotools and nanomaterials can be applied. The creation of novel nano-bio-based scaffolds that act as ducts and promote regrowth is one potential solution. Another is the application of new nanoscale surgical tools for functional axon repair and surgical micro-splicing (Karamichos, 2015).

The use of novel tissue scaffold materials and architectural designs has improved regeneration, and straight restoration of nerves in individual axons and neurons has been made possible thanks to nanoscale technologies. Because of the use of nanotechnology in ophthalmology, new approaches to treating ocular diseases have been developed that can get past ocular obstacles and have a longer-lasting effect on the target tissues. For example, using eco-friendly scaffolds to transport progenitor cells of the retina for restoring the injured ocular system is a promising treatment for eye diseases.

### **CONCLUSION**

In conclusion, nanomedicine can help with gene therapy, increase bioavailability, exact delivery, constant release and offer novel diagnostic and therapeutic methods nanoparticles are an effective instrument opening up novel possibilities for the identification and management of the majority of ophthalmological pathologies.

There are still many problems to be resolved, though, because most NP research is still in the trial stage and only a small number of them are used in clinical settings. For instance, some nanoparticles including silver, zinc sulfide and titanium dioxide, have dose/size-related toxicity that restricts their usage in ophthalmology. It is critical to be mindful of the risks posed by nanoparticles including cytotoxicity to the eyes. In conclusion, more studies will improve the development of nanoparticles and the retina is a promising application area for nanomaterials.

### **Declaration of competing interest**



The authors declare no competing interests.

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## On the issue of occupational pathology in the eyes of workers of the modern petroleum industry of Azerbaijan

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The constant increase of the capacity of facilities used in the petroleum industry, their modernization as a whole necessitates a detailed study of the possible adverse effects of certain production factors on the organism of workers, including their ophthalmological status. The purpose of this research was to study the frequency of occurrence and the nature of possible changes in eye among workers of the modern petroleum industry. In total, 1852 employees from 13 enterprises of the State Oil Company of Azerbaijan Republic (SOCAR) were covered by a comprehensive ophthalmological examination in 2017-2019, taking into account age, work experience, severity of working conditions and exposure to harmful chemicals. A survey was conducted to identify the presence of complaints related to vision, chronic diseases, and contact during the work process with harmful chemicals. The degree of occupational causation of the identified diseases was determined based on the calculation of the absolute and relative risk, as well as the etiological fraction. It was found that 30% of workplaces belong to the high hazard class C (harmful working conditions), the most numerous were a group of people aged 50 to 59 years, the average age was  $48 \pm 2.3$  years; the average work experience of employees was  $19.3 \pm 1.7$  years. Of all respondents, 46.5% (861 people) had direct contact with harmful chemicals and petroleum in the process of work. The state of general ocular pathology was established in workers employed in the modern petroleum industry, 918 people (49.6% of all examined) had pathology in both eyes. In the structure of ophthalmopathology, the following nosologies were the most common: conjunctival and corneal diseases (AR=39%), various neoplasms of the eye and its adnexa (AR=26%) and refractive errors (AR=24%). The high level of etiological proportion and relative risk for conjunctival and corneal diseases (assuming  $2 < RR < 3.2$ , EF=33-50%), as well as for various eye neoplasms (assuming  $3.2 < RR < 5$ , EF=51-66%), make it possible to attribute these nosologies to work-related.

**Keywords:** Occupational eye pathology, absolute risk, relative risk, etiological fraction, petroleum production

### ABBREVIATIONS

SOCAR – State Oil Company of Azerbaijan Republic  
NCO – The National Center of Ophthalmology named after Academician Z.Aliyeva  
HCH – harmful chemicals

AR – absolute risk  
RR – relative risk  
EF – etiological fraction  
OU – both eyes  
OD – right eye  
OS – left eye  
PA - Production Association

## **INTRODUCTION**

The petroleum and petrochemical industry in Azerbaijan occupies a leading position and remains an important branch of the country's economy today (Askarova et al., 2012; Akhundova, 1998). Occupational pathology of the eyes has always been the focus of scientific research in our country and was most deeply studied by Academician Z.Aliyeva and her colleagues (1980, 1983, 1985, 1988). However, the constant increase in the capacity of facilities used in the petroleum industry, their modernization, in general, dictate the need for a detailed study of the possible adverse effects of some production factors on the organism of workers, including their ophthalmological status (WHO, 2016) Many publications state that some areas of the petroleum sector are still potentially dangerous for the health of workers (Janabayev et al., 2019, Rustamov, 2012, Blagun et al., 2018). In addition, the study of the causes and prevalence of eye diseases, the development of measures for their prevention, timely detection, reduction and treatment is one of the urgent tasks of medical science and healthcare. Publications in the scientific literature of the country on the assessment of individual occupational risks in a complex volume in the petrochemical industry are rare and fragmentary (Bagirov, 2004, Kasimov, 2017, Rustamov, 2012). Based on the above, early detection, investigation of the nature of changes, the study of eye pathology in workers in the modern petroleum industry, including depending on the harmfulness of working conditions, work experience and age, will allow an objective comprehensive assessment of their ophthalmological status.

The purpose of this research was to study the frequency of occurrence and the nature of possible changes in the eye among workers of the modern petroleum industry.

## **MATERIALS AND METHODS**

This cross-sectional study included an

analysis of the peculiarities of working conditions in the modern petroleum industry, a comprehensive assessment of the state of eye health of workers for 2017-2019 at the enterprises of the State Oil Company of Azerbaijan Republic (SOCAR). In total, the study covered 1852 employees from 13 SOCAR enterprises who agreed to the examination and questioning on a voluntary basis. The assessment of the ophthalmological status of workers employed in the modern petroleum industry was carried out using the methods of a comprehensive ophthalmological examination described by E.M.Kasimov (2017), such as visometry (using Huvitz Chart Projector CCP-3100 (HUVITZ Co, LTD, South Korea), biomicroscopy of the anterior segment of the eye (using slit lamp TOMEY TSL-5000, Tomey, Japan and portable slit lamp Reichert, Japan), measurement of intraocular pressure (applanate tonometer FT-1000 (TOMEY, Japan), refractometry (automatic keratorefractometer RC-5000 (TOMEY, Japan), ophthalmoscopy of the fundus (slit lamp TOMEY TSL-5000 (Tomey, Japan) using lenses (Ocular High Mag 78D, Ocular Instruments Inc., USA), etc. During assessing the ophthalmological status, a general clinical study was conducted at the workplace using a mobile clinic operating under the National Center of Ophthalmology named after academician Z.Aliyeva (NCO). The International Statistical Classification of Diseases and Related Health Problems 10<sup>th</sup> revision (ICD-X) was used for grouping by nosological groups. A survey was also conducted using the developed questionnaire, including, questions about complaints about vision, the presence of chronic diseases, or previous surgical interventions on the eyes, the presence of contact (direct or indirect) with harmful chemicals in the production process.

Age groups were subdivided on the basis of requirements and approaches to cross-sectional studies in industries ("18-29 years old", "30-45 years old", "46-59 years old", "60 years and older") (Obukhova et al., 2016; Petri, 2009); work experience is divided into three groups ("less than 5 years", "from 6 to 15 years", "over 16 years"). The severity of labour was assessed according to the HESCME principle (Health, Environment and



Social Capital Management in Enterprises) (Simon et al, 2012) and divided into three groups - "class A" - optimal conditions, "class B" - acceptable conditions and "class C" - harmful working conditions.

The degree of industrial conditionality of the detected diseases and health disorders of workers, depending on the work experience and working conditions, as well as on the presence of contact with harmful chemicals, was carried out based on the calculation of relative risk (RR relative risk, units). The relative risk values were estimated according to the methodological recommendations of Pavlovich T.P. et al. (2021) according to the formula:

$$RR = \frac{RF +}{RF -}$$

RF<sup>+</sup> - the risk of developing the disease in the presence of a risk factor,

RF<sup>-</sup> - the risk of developing the disease in the absence of a risk factor.

Etiological proportion, or the proportion of additional risk (EF, etiological fraction, units) - an indicator of the proportion of cases of eye disease and its adnexa under the exposure of the assessed risk factor in the total number of cases of detected diseases is calculated according to the formula (Pavlovich et al., 2021):

$$EF = \frac{AR}{RF +} * 100\%$$

AR<sup>-</sup> - the difference between RF<sup>+</sup> and RF<sup>-</sup>

RF<sup>+</sup> - the risk of developing the disease in the presence of a risk factor.

Absolute risk (AR) is the proportion of working with identified ophthalmopathologies from the total volume of the group. AR was calculated for both exposed (exposed to the factor – contact with harmful chemicals) and non-exposed persons. AR expresses the risk of developing the disease in the presence (or

absence) of the factor and corresponds to the prevalence of diseases.

According to the manual on occupational health risk for workers (2004), as well as the reports of N.F.Izmerov and I.V.Bukhtiyarov (2018), and all ophthalmopathologies detected during the study were classified as general, occupational conditioned and occupational diseases (Table 1). The relative risk values were evaluated according to the parameters recommended in Table 2.

## RESULTS AND DISCUSSION

The study of working conditions showed that 55.5% of workplaces in the enterprises belong to class A (optimal working conditions). However, the picture has changed for enterprises; for example, modern petroleum industry enterprises with good working conditions include departments (Bibi-Heybat, the head office of “SOCAR”, department of Petroleum and Gas Production named after N.Narimanov, PA «Azpetrol» and Educational and Methodological Department of Certification "SOCAR"). On average, 30% of workplaces belong to the high-hazard class C (harmful working conditions) (mainly "Petroleum rocks" and "Siyazan-petroleum").

The distribution of employees by age showed that the most numerous (41.5%) was a group of people aged 50 to 59 years. The average age of employees was 48 years (min=19; max=67; Me=50; Mo=56).

In our study, it was found that the most widely represented group with over 16 years of work experience (57.1% of all surveyed). Moreover, this trend is not observed for all surveyed enterprises.

**Table 1.** Assessment of the degree of causal-effect relationship of health disorders with work (according to epidemiological studies) (by P2.2.1766-03 Минздрав России, М., 2004)

0<RR<1	1<RR<1.5	1.5<RR<2	2<RR<3.2	3.2<RR<5	RR<5
EF=0	EF< 33%	EF=33-50%	EF=51-66%	EF=67-80%	EF=81-100%
Null	Small	Medium	High	Very high	Almost full
General diseases		Occupational conditioned disease		Occupational diseases	

**Table 2.** Estimation of relative risk values

RR	Interpretation
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<b>RR&lt;1</b>	The risk of getting sick in exposed persons is lower than in those who were not affected by the studied factor, therefore, this factor probably has a beneficial effect on health
<b>RR= 1</b>	There is no connection between the studied factor and the disease
<b>RR&gt;1</b>	The risk of getting sick in the presence of the studied factor is higher than in its absence, therefore this factor is damaging, i.e. a risk factor and leads to illness

So, for example, in such organizations as the “Siyazan-Petroleum” petroleum and gas production Plant, the Department of Petroleum and Gas Production named after Amirov and the Bibi-Heybat Petroleum and Gas Production Department had a high percentage of highly-qualified employees and amounted to 90.7, 76.8 and 69.0%, respectively. The total average work experience of the surveyed employees is 19.3±1.7 years. Thus, the study found that at most of the surveyed petroleum facilities, the overwhelming majority were specialists with high work experience.

The study of the opinion of workers in the modern petroleum industry showed that 46.5% (861 people) of all respondents have direct contact with harmful chemicals and petroleum in the process of work. At the same time, 30.5% of respondents noted the presence of indirect contact with harmful substances. Of all the respondents, 23% noted the absence of contact with harmful chemicals. The results of ranking the 10 main frequency ranks of groups of harmful chemicals to which workers of the modern oil industry are exposed during professional contact showed that most often - in 244 cases (56.1±4.8% of all indicated groups of HCH) workers in modern oil production have to come into contact with oil and oil products. The second place is given to gas and various gas condensates (30.3±3.4%). Only 3 respondents indicated contact with heavy metals during work which amounted to 0.7±0.01% of all these groups of harmful substances. It has been established that in class A production conditions, contact with harmful chemicals is completely absent. In class B production conditions, contact with various aromatic hydrocarbons, salts, alkalis, acids and sulfur compounds accounted for 30% of positive responses each. Thus, the possible exposure to harmful chemicals is still present in enterprises despite the modernization of the studied industries. In addition, as many native and foreign authors point out (Alieva, 1985; Akhundova, 1998; Blagun et al., 2018), office work, work outdoors, indoors with insufficient

illumination indirectly can also be provoking factors in the formation of ocular pathology. According to our data, in the production group of class A, out of 398 detected cases, ocular diseases in 341 (88%) were observed in the form of isolated and 55 (12%) - in comorbidity. The studied pathology of the eye occurs 2 times more often (RR=1.7, CI 0.79-0.91, p<0.005) among workers employed in hazardous working conditions than those employed in optimal conditions of severity.

The calculation of extensive indicators revealed that among all examined workers, 307 (33.4±4.1%) workers who had contact with HCH during the production process had various diseases of the eye. In 124 (13.3±0.9%) examined patients despite the presence of HCH exposure no eye pathology was found.

Among the workers of the modern petroleum industry the frequency of practically healthy according to self-assessment data, was 1,739 people (93.9% of all surveyed). In the structure of morbidity from the anamnesis of the remaining 113 surveyed workers of the modern petroleum industry presence of diabetes mellitus was most often noted – in 68 people (60.2%) and arterial hypertension – in 32 (28.3%). 7% of the surveyed indicated the presence of various allergic conditions. Our studies have established the state of general eye pathology in workers employed in the modern petroleum industry (Table 3). Thus, 918 people (49.6% of all examined) had pathology in both eyes, of which 83% were isolated and 17% were combined.

Table 3 shows that 91 examined patients had pathology of the right eye of which 97.8% were isolated and 2.2% were combined. In 121 people pathology of the left eye was revealed: 99.2% – isolated and 0.8% – combined. In this regard, it was interesting for us to study the structure and features of pathology in both eyes.

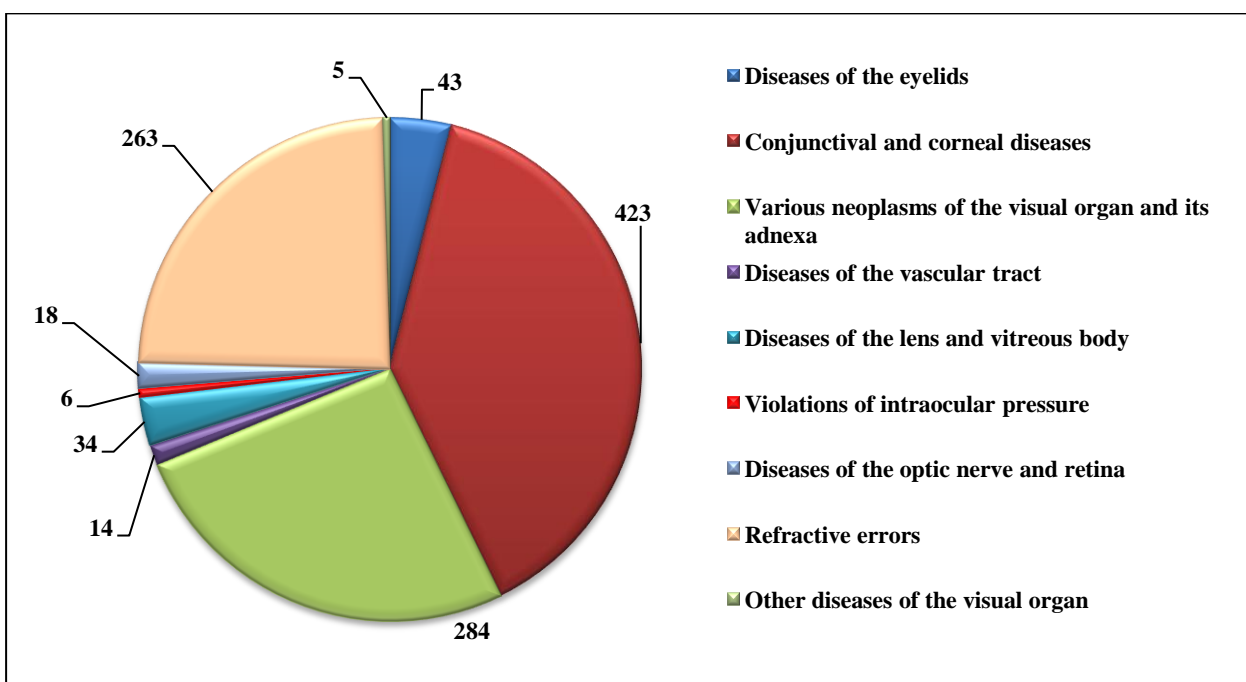
The structure of the general isolated and combined pathology of both eyes in workers of the modern petroleum industry is shown in Figure.

Figure clearly demonstrates a wide range of pathology of the eye in workers of the modern petroleum industry. Based on our obtained data, the following nosologies have the highest proportion in the structure of ophthalmological morbidity among the examined: conjunctival and corneal diseases (absolute risk is 39%), various

neoplasms of the eye and its adnexa (AR=26%) and refractive errors (AR=24%), and statistically reliably traced the frequency of various eye neoplasms depends on exposure to harmful chemicals ( $\chi^2=5.7, p<0.05$ ) and on the severity of labor ( $\chi^2=4.9, p<0.05$ ).

**Table 3.** Revealed pathology of eyes in workers of the modern petroleum industry

Pathology of the eyes		Examined workers (N=1852)	
		abs.number	%
OU	Not identified	934 50.4	
	Isolated	918	762 83
	Combined		156 17
OD	Not identified	1761 95.1	
	Isolated	91	89 97.8
	Combined		2 2.2
OS	Not identified	1731 93.5	
	Isolated	121	120 99.2
	Combined		1 0.8



**Fig.** The share distribution of the general pathology of both eyes among workers of the modern petroleum industry.

The studies also found that despite minor changes in the proportion of the main leading ophthalmological pathologies depending on the work experience and the age of the examined, the absolute risk of combined pathologies increased with the increase in the work experience of those

who worked reaching a maximum in the high experience of examined (AR=9.8%) and in the distribution by age groups – in the group "over 60 years" (AR=21.6%).

Among ophthalmopathologies, a statistically significant dependence of detection rate on work

experience was found ( $p < 0.005$ ). Thus, the frequency of detecting pathologies of the eye is 2.1 times higher for workers with more than 16 years of work experience compared with workers with 6-15 years of work experience ( $RR=1.4$ ,  $CI$  0.59-0.83,  $p < 0.005$ ).

Thus, our studies once again confirmed the fact that despite minor changes in the proportion of the main leading ophthalmological pathologies depending on the work experience, the percentage of comorbidities increased with the work experience of those working at modern petrochemical production enterprises, reaching a maximum in the highly trained group of examined (21.6%).

According to the manual on occupational health risk for workers (Guidance on occupational health risk assessment for workers, 2004), based on the calculations, the degree of causal-effect relationship between the influence of harmful substances in the process of work and the occurrence of various pathologies of the eye was estimated by us as high and very high for eye neoplasms (provided  $3.2 < RR < 5$ ,  $EF=51-66\%$ ) that allows us to consider the studied nosologies as work-related diseases for the petroleum industry. Value of the relative risk for the occurrence of refractive errors equal to one ( $RR=1$ , assuming  $EF < 33\%$ ) shows the absence of a causal-effect relationship with both work experience and dependence on contact with HCH. In general, the application of risk analysis of certain production factors in ophthalmology will allow us to solve a number of important tasks in the future such as developing a mechanism and strategy for various regulatory measures to reduce it; obtaining quantitative characteristics of potential and real damage to health from the impact of adverse production factors; promote the establishment of more reliable safe levels and hygienic standards and others.

## CONCLUSIONS

1. At modern petroleum production, the absolute risk of such eye pathologies as conjunctival and corneal diseases ( $AR=39\%$ ), refractive errors ( $AR=24\%$ ) and various neoplasms of the eye and its adnexa ( $AR=24\%$ ) was the

highest.

2. The high level of etiological fraction and relative risk for conjunctival and corneal diseases as well as for various eye neoplasms make it possible to attribute the studied nosologies to occupational eye pathologies.

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## **Electrophysiological study of effects of basolateral Amygdala on the function of visual system structures**

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**Epilepsy is one of the most spread neurodegenerative diseases. This disease occurs in people of all ages from infancy to old age and can have lethal effects as well. The present work explores the electrical activity in the visual system structures, specifically in the visual cortex, lateral geniculate body, colliculus superior and retina in penicillin-induced amygdala epilepsy. Experiments were carried out on awake, non-anesthetized immobilized rabbits. The development dynamics of epileptic activity were observed in all visual system structures. Electroencephalogram and analysis of evoked potentials in the studied structures reaffirm the idea of direct connections between the visual cortex and the amygdala. This is evidenced by the generation of short-latency responses in the visual cortex and other visual system structures. For the first time, under the conditions of experimental epilepsy, it was shown that the epileptic activity is spread along the centrifugal pathways from the basolateral amygdala to neuronal elements of the primary visual cortex, lateral geniculate body, colliculus superior and ganglionic layer of the retina.**

**Keywords:** *Electroencephalogram, amygdala epilepsy, visual cortex, lateral geniculate body, superior colliculus*

### **INTRODUCTION**

According to the latest statistics epilepsy occurs in only 1% of the world's population, but due to its specific gravity ranks third among neurological diseases and shows a clear upward trend in recent years. Affecting more than 70 million people worldwide is one of the most common serious brain diseases (Thijs et al., 2019). Despite the progress made in the study of this problem, the study of epileptogenesis in new aspects on the one side, and the creation and use of technologies, on the other side require a more precise study with the new achievements of fundamental sciences. Recently, the basolateral amygdala function attracts the attention of a significant number of researchers (Panakhova et al., 2022; Mahmood et al., 2023).

In literary sources, there are different

systematics of epilepsy. However, the most interesting is the systematics created due to the localization of the focus in the brain. So, the most widespread is its temporal form, which at this time, the pathological focus develops in the temporal structures (hippocampus, amygdala) belonging to the limbic system of the brain (Falco-Walter, 2020; Pitkanen et al., 1998). These structures are involved in the regulation of various behavioral and cognitive processes; in pathological conditions, especially during the onset of epileptic activity regulation of the functioning mechanisms of this system is disturbed. The basolateral amygdala (BLA) has an important role in the initiation and propagation of epileptic seizures. Correlates of cognitive functions of the brain as known that there are rhythmic processes in different frequency ranges. During epileptogenesis, the rhythmic activity of

the brain is disturbed.

Under the influence of harmful factors, foci of convulsive activity can occur as a result of morphological and functional changes in the brain. Activity synchrony may increase for some regions, for others, it may decrease. Thus, during epileptogenesis, dysfunction of the rhythmic activity of the brain occurs. At present time to understand the mechanisms of epileptogenesis and to study the brain's activity in the general and epileptic processes the most convenient method is the use of electroencephalogram (EEG) analysis. EEG allows the evaluation of the activity in different parts of the brain, as well as obtaining certain information about epileptic activity (Koutroumanidis et al., 2017).

## **MATERIALS AND METHODS**

In the experiments, we used adult rabbits of the "chinchilla" breed weighing 2.5-3 kg. Animals raised in a vivarium were kept in special cells with free access to food and water before and after the research. Before starting research kept in a dark chamber for 7-10 minutes, so that the photosensitivity levels of the photoreceptors are the same.

Electrodes were placed on structures (colliculus superior - CS, lateral geniculate body-LGD, visual cortex-VC, basolateral amygdala-BLA) according to the coordinates of the stereotaxis atlas (Blinkov et al., 1973). The diameter of used electrodes for cortical structures was 0.5 mm, for subcortical structures - 0.1-0.15 mm. ERG from the retina was recorded using contact lenses.

Registration of electrical activity brain structures was carried out with a multichannel

encephalography "Neuron-Spectrum-5". The retina photostimulation was carried out using a flash lamp of a photostimulator located at a 25-30 cm distance from the animal eye. An experimental model of epilepsy was created by injecting penicillin sodium (300 U in 10  $\mu$ l of distilled water) through a cannula placed in a BLA.

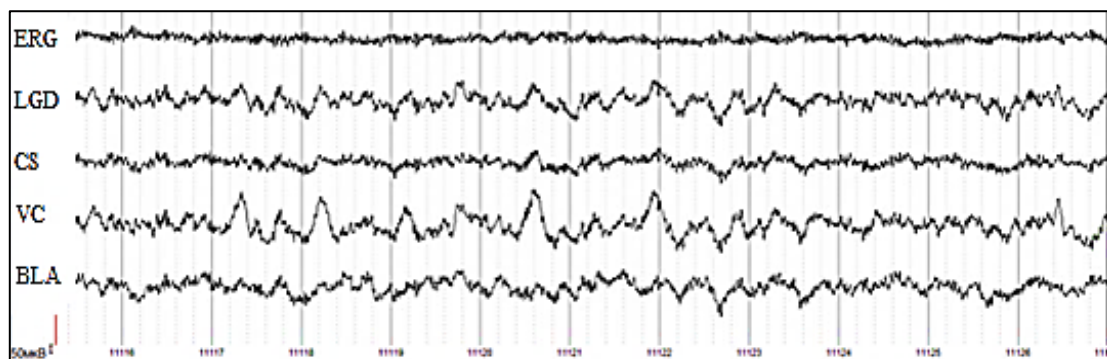
For checking electrodes at the end of each experiment animals were preliminarily coagulated, then embolized with air, the brain was removed from the skull and placed in a 10% formalin solution, after making frontal incisions.

## **RESULTS AND DISCUSSION**

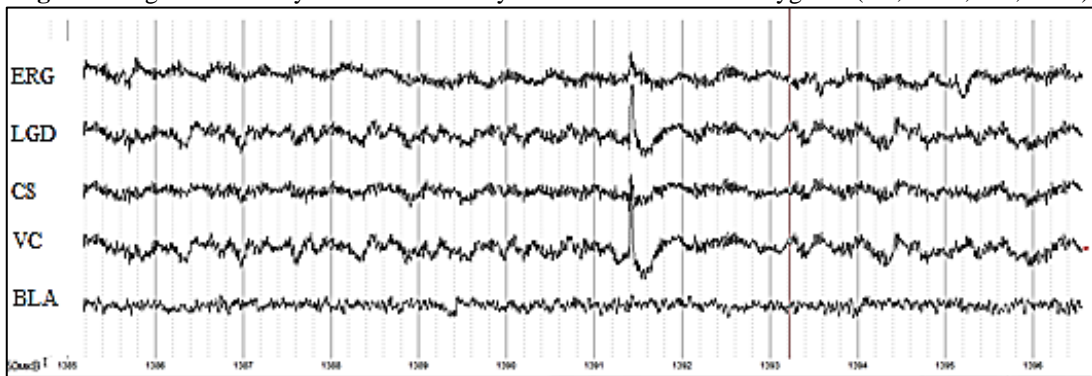
Electrophysiological research was carried out in several stages. First, background activity was recorded in the studied structures of animals (EEG, EP-evoked potential) ( Fig. 1.)

The results obtained revealed that a high degree of correlation between the activity of the studied structures in healthy animals indicates their close functional relationship. This is confirmed by the available information about the presence of anatomical relationships between them. At the next stage of research, penicillin is injected into the amygdala through a cannula placed in it. After the registration of the brain electrical activity of the animal, it was placed in a sound and lightproof chamber.

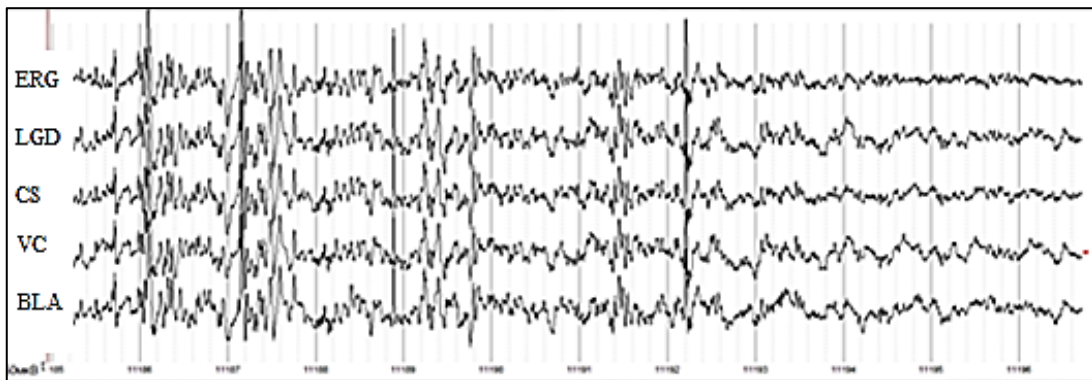
As a result of the research, it was found that the introduction of penicillin led to the development of prolonged epileptic activity. In 15-20 minutes after the injection, epileptiform discharges are observed in all structures (Fig. 2). The epileptiform activity involves all brain structures. Then the duration of the attacks is lengthened.



**Fig. 1.** Background activity of the visual analyzer structures and the amygdala (CS, LGD, VC, BLA)



**A** - The first single spike was registered on the recording



**B** - Epileptic activity spreading to all visual structures

**Fig. 2.** Amygdala epileptogenesis.

Penicillin caused the appearance of generalized spikes in all visual structures, accompanied by massive myoclonic convulsions. Sometimes there were epileptic seizures, which quickly turned into myoclonic convulsions. Such changes in the EEG were recorded within 3-4 hours. Within an hour, the convulsions reach their peak, after which certain dynamics of epileptiform waves are formed. So, over time the attacks begin to alternate.

In our research, we recorded ERQ from the retina as well as EP from the cortical and subcortical structures.

At this time, reactions to light are formed both in the retina and in the amygdala. This fact confirms that the centrifugal pathway continues from the retina to the amygdala.

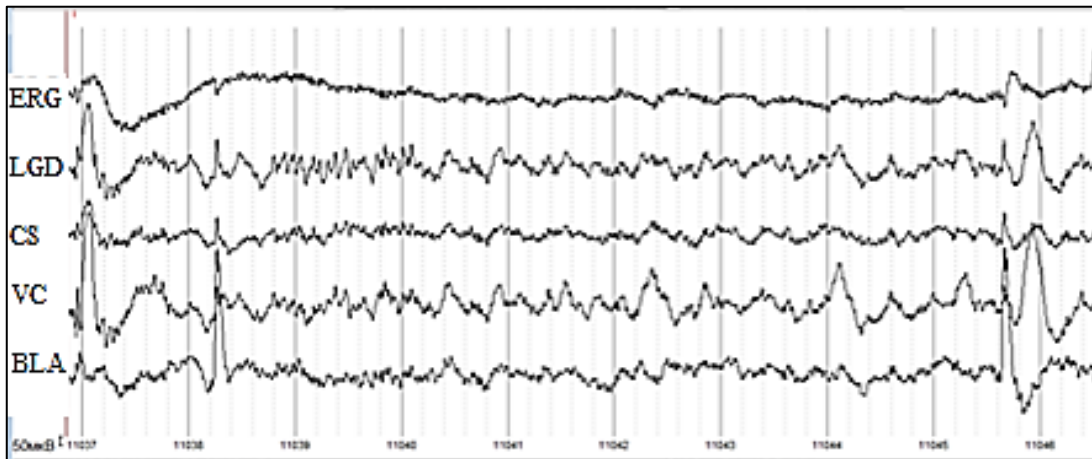
The number of alternating epileptic seizures begins to decrease and the activity stops 2.5 hours after the injection of the penicillin into the

amygdala. In addition, during epileptogenesis in the amygdala spontaneous responses are generated, that extend to the retina (Fig. 3). These responses in the amygdala resembled EP, although no external stimulus was used.

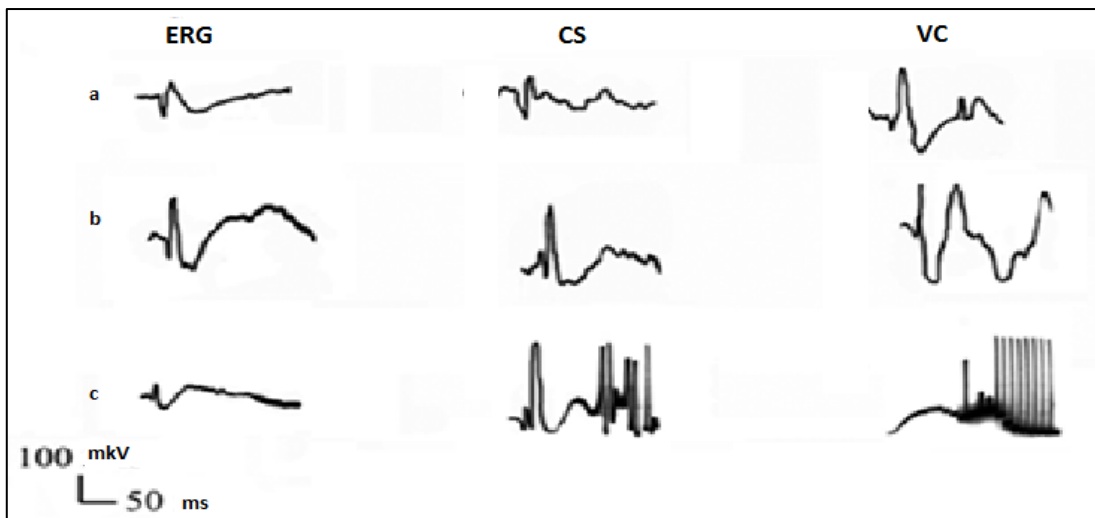
Registration of EEG from several brain structures allows you to study the time sequence of the involvement of the studied structures in the pathological process. EEG analysis showed that epileptic activity first occurs in the amygdala, then in the VC, CS, and LGD and also affects retinal activity. Most likely, manifestations of such a sequence of activity distribution between structures are associated with the presence of unilateral and bilateral morphofunctional relationships between these structures. The duration of experimental amygdalar epilepsy caused by the administration of penicillin into the amygdala was 3-3.5 hours.

In electrophysiological research along with

the EEG, we also recorded EP and ERG (Fig. 4).



**Fig. 3.** Spontaneous activity originating in the amygdala and spreading to other structures.



**Fig. 4.** Comparative analysis of spike characteristics of visual system structures: a-control, b-effect of penicillin instillation into the amygdala, the c-centrifugal spread of epileptiform activity to retina's distal elements.

After the instillation of penicillin into the amygdala, significant changes occur in the retina and visual cortex evoked potentials. From a clinical point of view, it is accompanied by a high level of arousal in the amygdala, the appearance of seizure activity, breath shortness and difficulty breathing. From an electrographic point of view, epileptic spike activity is recorded not only in the cerebral cortex EP and even on an electroretinogram. Interictal spikes, as an indicator of epileptic activity, occur first in the visual cortex, then after 10-12 ms are visible in the retina. There is a sharp increase in the

amplitude parameters of the retina's c-wave, which is not involved in the transmission of information to the centers of vision. From the results obtained, it can be said that epileptic foci caused by penicillin in the amygdala have a stimulating effect on the central subcortical and peripheral parts of the visual system.

According to literature sources, it is known that discharges cause the death of GABAergic cells in the hippocampus, septum (up to 40%), amygdala (44-75%) and piriformis cortex (46%). (Cendes et al., 1993).

Changes in the brain structure activity of

several begin to be synchronized during experimental epileptogenesis. The activity that begins in the amygdala extends to the brain's visual structures and the retina. The initiation of paroxysmal activity in the amygdala confirms the information that, along with the hippocampus, it can be the center of epileptic genesis and cause convulsive activity due to the presence of a powerful glutamatergic system in it (Mc Namara, 1994).

Thus, in the studied structures, the processes of excitation begin to predominate over the processes of inhibition, which is expressed in an increase in the frequency of activity on the EEG. In addition, the death of GABA-ergic cells weakens the inhibitory control of pyramidal cell activity, projected into different parts of the basal brain, and contributes to the generalization of convulsive activity (Salamon et al., 2005).

Thus, the electrophysiological results obtained are evidence of a direct connection between the basolateral amygdala and the primary area of the visual cortex (area 17). This is evidenced by the appearance of epileptic discharges (ictal and interictal). First, epileptic spikes appeared in the visual cortex, then similar spikes (ictal and interictal) were observed in the retina. The first results were obtained indicating that under the conditions of creating experimental epilepsy, the fact of spread along the centrifugal pathways from the basolateral part of the amygdala to the neuronal elements of the VC, CS, LGD was revealed. American scientists J.L.Freese and D.G.Amaral using electron and confocal microscopes, investigate the synaptic organization of these projections by injecting anterograde tracers into the amygdaloid complex of Macaca fascicularis monkeys and examining labeled boutons in areas TE and V1. The authors conclude from these observations that the amygdaloid complex (magnocellular part of the basal nucleus) provides excitatory input to areas TE and V1 that primarily influence spiny, probably pyramidal neurons in these cortices (Jennifer, 2006).

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## Changes in corneal endothelium and risk factors for surgical treatment of cataracts in the presence of pseudoexfoliation syndrome

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**Characteristic of pseudoexfoliative syndrome (PEX) corneal changes in the form of keratopathy which affects all layers of the cornea as the process progresses is a risk factor in cataract surgery especially in countries with a large elderly population. In cataract surgery, the corneal endothelium deserves increased attention because cells do not recover from their damage (Schlotzer-Schrehardt et al., 1993; Zheng, 2013). We studied corneal endothelial changes in 58 patients (58 eyes) with PEX from the perspective of the progression of the syndrome, risk factors of cataract surgery. From the standpoint of the risk of potential complications of cataract surgery dystrophic symptoms complex of eye structures, the degree of development of PEX (3 stages) analyses of indicators of morphological changes in the corneal endothelium revealed significant differences in indicators depending on the degree of PEX progression. From the standpoint of cataract surgery in the eyes with PEX, there is a tendency for changes in the morphological parameters of the corneal endothelium with progressing syndrome.**

**Keywords:** Pseudoexfoliation syndrome (PEX), corneal endothelium, cataract surgery, risk factors, stage of PEX, open-angle glaucoma (OAG), pseudoexfoliative material (PEM)

### INTRODUCTION

Currently, there is a steady trend toward increasing life expectancy and aging of the population. While in many countries people over 60 years old (or 841 million) account for 20% of the population, by 2050 according to the UN, this figure will increase 2.5-3 times (up to 2 billion people or more) (Chan, 2015; <http://www.who.int>). According to the WHO, the majority of the 1.3 billion global population with visual impairment are over 60 years of age, whereas cataracts and glaucoma are the main causes of blindness (<http://www.who.int/ru/news-room/fact-sheets/detail/blindness-and-visual-impairment>; Zhao et al., 2015).

Pseudoexfoliation syndrome (PEX) is among the age-related changes and leads to a high incidence of cataracts and glaucoma worldwide.

As a systemic dystrophic process, PEX is differed by characteristic deposits of pseudoexfoliative material (PEM) not just inside the eye but orbit, skin, internal organs and has a close link to cardiovascular pathology (Schlötzer-Schrehardt and Naumann, 2006; Wirostko et al., 2018; Yildirim et al., 2017; Chung et al., 2018; Siordia et al., 2016; Zikou et al., 2018).

PEX remains a mystery in the field of ophthalmology which is confirmed by the numerous publications of scientists from different countries on various aspects of the problem (Detorakis et al., 2021).

Nevertheless, cataract surgery is one of the most common procedures worldwide, surgery in patients with PEX is still very challenging and possesses an increased risk of complications, such as corneal decompensation, contractile capsular syndrome, dislocation of the capsular-bag

complex which are known to be based on the ligamentous failure and reduced tolerance to mechanical (surgical) trauma (Namazova et al., 2016; Baig et al., 2021; Shingleton et al., 2017; Tekin et al., 2019; Vanags et al., 2020).

Among the structures that are subject to the most careful care and increased attention during cataract surgery is corneal endothelium, since these cells do not recover after their damage. The corneal changes in the form of keratopathy are characteristic of PEX. While PEX progresses, it affects all the layers of the cornea causing an increased incidence of bullous keratopathy, especially in countries with a large elderly population, such as Japan (Schlotzer-Schrehardt et al., 1993; Zheng, 2013).

Some studies emphasize the relationship between changes in the corneal endothelium and the severity of changes in PEX (Aoki et al., 2020; Palko et al., 2017; Yüksel, 2016; Demircan et al., 2015). At the same time the assessment of the degree (stage) of PEX from the standpoint of the surgical risk for potential complications remains ambiguous (Naumann and Schlotzer-Schrehardt, 2000; Inazumi et al., 2002; Takhchidi et al., 2010).

Thus, it is extremely important in eyes with signs of a potential danger of corneal endothelium decompensation, to prevent possible changes by choosing the optimal surgical technique. At the same time, the results of many studies of corneal endothelium have found a statistically significant lower density of corneal endothelium in the eyes of patients with PEX compared with healthy subjects, although the cornea guttata not found in patients with PEX. Available data on the presence of changes in open-angle glaucoma (OAG) and PEX are mixed (Bozkurt et al., 2015; Palko et al., 2017; Yüksel et al., 2016; Demircan et al., 2015).

Perhaps this is due to the characteristics of the instruments used, the size, the ethnicity, age and other factors that can contribute (Palko et al., 2017; Yüksel et al., 2016).

All these factors to a certain extent create difficulties in assessing the process, predicting and choosing optimal treatment tactics. Thus, given that the problem remains within the discussions, assumptions, the purpose of this study was chosen to elaborate on optimal tactics

to approach those kinds of pathologies.

The purpose of the current work was to study changes in the corneal endothelium in the eyes of patients with pseudoexfoliation syndrome from the point of view of the syndrome progression and risk factors for cataract surgery.

## **MATERIALS AND METHODS**

The results of the examination of 58 patients aged from 56 to 86 years (mean age  $72.2 \pm 0.9$ ), 26 men (44.8%), 32 women (55.2%) hospitalized for cataract surgery. Criteria for inclusion in the study: patients with cataracts and concomitant PEX confirmed by two-stage biomicroscopy and ultrasonic biomicroscopy (UBM). Among them, 46 (79.3%) patients with PEX had intraocular pressure (IOP) within the normal range, 12 (20.7%) had open-angle pseudoexfoliative glaucoma (OPXG). The groups were equal in gender and age. All patients underwent all necessary ophthalmological examinations.

The ophthalmic examination consisted of visual acuity check (uncorrected, corrected), 2-stage biomicroscopy (undilated, tonometry and dilated) gonioscopy, ultrasound biomicroscopy (UBM-plus, Accutome, USA), biometry (IOL - Master, Zeiss, FRG), specular microscopy (Tomey EM-3000, Japan).

Results of anterior segment biomicroscopy in patients with PEX were analyzed according to the criteria of the potential risk of cataract surgery complications (including inflammatory ones). Evaluation criteria in UBM were usual landmarks: relief of surface profile, the reflectivity of the investigated structure, spatial and quantitative parameters of the interrelation of anatomical structures. Axial longitudinal, tangential slices (scanning variants), color reproduction were used (Pavlin et al., 1998; Inazumi et al., 2002).

The corneal endothelium studies by specular microscopy before surgery in patients with varying degrees of surgical risk, varying degrees of structural changes total in 58 eyes of patients with PEX.

Statistical processing: one-dimensional statistics methods with calculations of the Kruskal-Wallis and Mann-Whitney tests.

The research protocol has been approved by



the ethical committee of the National Ophthalmology Centre named after acad. Zarifa Aliyeva. Informed consent was obtained from all patients.

## RESULTS AND DISCUSSION

Based on the risk factors significant for cataract surgery 3 types of different criteria were identified. The aforementioned criteria were based on the severity of dystrophic changes in the eye structures and deposits of the PEM within them.

In the first variant, it was established in 11 (18.97%) eyes with a distinctive feature as the preservation of the pupil's reaction to light and mydriatics; I stage PEX). At the same time, the destruction of the pigmented border of the pupillary edge (partial) in the control group was present only in 14 (35%) eyes. In 20 (43.48%) eyes dystrophic component of PEX was more pronounced: smoothness of the iris pattern, moderate pigmentation of the surface was accompanied by changes in the pigmented border of the pupillary edge manifesting as partial disappearance and more often as a complete destruction indicating a more significant change (II stage of PEX). Deposits of PEM along the edge of the pupil were more pronounced, dystrophic changes in the stroma were especially evident in the trabecular and mixed type of iris structure, rather than spongy. Pseudoexfoliative deposits were present on the corneal endothelium of 10 (50%) eyes. There was a decrease in pupil response to mydriatics but no significant changes were observed in the zonules of crystalline fibers. A decrease in pupillary reactions was more often caused by the development of iridocapsular synechia. At the same time, the presence of iridocapsular planar synechia was explained by increased extravasation from the vessels of the iris, appearance of proteins unusual for it in the anterior chamber (Podgornaya et al., 1988).

In 15 (32.61%) eyes with PEX the dystrophic component was the most significant (stage III PEX). Leaching of the pigmented border up to its absence was combined with changes in the pigment layer of the iris in a number of cases - up

to translucence and exposure of the stromal vessels. Significance was acquired by changes in the Zinn ligament. If during biomicroscopy there was a deepening of the anterior chamber or tremulation of the lens or its subluxation, then UBM revealed changes in the fibers of Zinn's ligament.

The study results of the anterior segment of patients from the point of view of surgical risk for potential complications of cataract surgery the severity of the dystrophic symptom complex, where three degrees of development of PEX identified were analyzed with indicators of morphological changes in the cornea during mirror microscopy.

Results of corneal endothelium studies by specular microscopy before cataract surgery in patients with varying degrees of surgical risk, varying degrees of structural changes total in 58 eyes of patients with PEX were reflected in Table 1.

When comparing and evaluating the arithmetic mean values of the morphological parameters of the corneal endothelium a number of significant differences in indicators were established between grades I and II of PEX, grades II and III of PEX, grades I and III of PEX, including changes in the eyes with concomitant glaucoma.

The average density of corneal endothelium with variations in values (min 2743 cells/mm<sup>2</sup>, max 3460 cells/mm<sup>2</sup>) in patients with PEX in the 1 degree was 2871.6 ± 57.3 cells/mm<sup>2</sup>, II degree - 2388.6 ± 55.0 cells/mm<sup>2</sup> (min 188.5 ± 11.5 cell/mm<sup>2</sup>, max 687.0 ± 53.6 cell/mm<sup>2</sup>); PEX III degree - 1867.1 ± 95.6 cells/mm<sup>2</sup> (min 474.0 ± 51.5 cells/mm<sup>2</sup>, max 1228.1 ± 120.7 cells / mm<sup>2</sup>).

Comparison of morphological changes at different degrees of development of PEX, namely, with PEX of the I PEX of the II degree PEX, revealed that the cell density (CD) in PEX of the I degree in comparison with PEX of the II degree was 1.2 times greater value (p<0.05); cell polymorphism index (AVG) in PEX grade 1 was significantly lower by 1.2 times compared to PEX grade II (p<0.05) degree had a 1.3 times significantly lower value in comparison with the II degree PEX (p<0.05).

**Table 1.** The results of corneal endothelium studies by specular microscopy before surgery with varying degrees in patients with PEX

The degree (stage) of PEX	N	Corneal thickness	Min	Max	AVG	CD	SD	CV	HEX
PEX I stage	11	0.526±0.046	177.9±11.5	687.0±53.6	349.5±7.0	2871.6±57.3	105.0±11.4	30.0±2.8	59.9±5.6
PEX II stage	20	0.521±0.010	188.5±11.5	836.8±34.4	423.2±10.5	2388.6±55.0	134.5±7.4	32.0±1.7	54.8±4.2
PEX III stage	15	0.525 ±0.014	474.0±51.5	1836.5±158.9	943.6±85.9	1181.2±97.7	315.5±39.6	35.2±4.5	40.3±9.4
PEX+glaucoma	12	0.522±0.006	202.3 ±30.1	1228.1±120.7	563.6±51.0	1867.1±95.6	214.4±28.2	37.3±2.8	84.1±13.9
<b>Total</b>	58	0.523 ±0.006	263.2±22.3	1147.9±76.7	572.8±38.9	2060.0±89.0	192.2±16.1	33.5 ±1.5	58.1±4.5

Among non-significant differences, the coefficient of variation (CV) in PEX of the 1st degree in comparison with the indicator of PEX of the II degree had a 1.1-fold lower value ( $p>0.05$ ), during the recovery of the corneal endothelium its value is less than 33 and it is considered normal. An increased coefficient of variation is often considered an early sign of disease as it is a marker of corneal endothelium cell remodeling.

The percentages of hexagonal cells (HEX) did not have significant differences. However, with the first degree PEX it was 1.1 times more than in the II PEX ( $p>0.05$ ), degree of PEX was greater than 1.0 in comparison with the II degree of PEX ( $p>0.05$ ). Differences between the II and III stages of PEX led to significant differences in indicators: CD at stage II PEX was 2 times more ( $p<0.05$ ); AVG at grade II PEX was 2.2 times less ( $p<0.05$ ), SD at grade II PEX was 2.3 times lower ( $p<0.05$ ).

Among the non-significant differences there were: corneal thickness 1.0 time greater at the II stage ( $p>0.05$ ), the CV variation coefficient at the II degree of PEX was 1.1 times less ( $p>0.05$ ), the percentage of HEX at grade II PEX 1.4 times higher ( $p>0.05$ ). Differences between the 1st and the 3rd stages of PEX were studied and they showed: the cell density (CD) in PEX of the 1st degree was 2.4 times higher ( $p<0.05$ ); the index of cell polymorphism was 2.7 times lower in PEX grade 1 compared with PEX grade 3 ( $p<0.05$ ). The standard deviation (SD) of the mean cell area in PEX grade 1 was 3 times less ( $p<0.05$ ).

Among the non-significant differences: the corneal thickness index was 1.0 time greater in PEX grade 1 ( $p>0.05$ ), CV in grade 1 PEX was 1.2 times lower ( $p>0.05$ ), hexagonal cells percentage HEX in PEX grade 1 was 1.5 times higher ( $p>0.05$ ).

Differences in corneal endothelium were assessed in eyes with normal ophthalmotonus at

grade I PEX with eyes where glaucoma also occurred. Significant differences included: CD was 1.5 times higher ( $p<0.05$ ); AVG was 1.6 times less ( $p<0.05$ ); SD was 2 times less ( $p<0.05$ ). Among the non-significant differences was the index of corneal thickness, which at grade 1 was 1.0 times greater ( $p>0.05$ ); CV was 1.2 times less ( $p>0.05$ ), the percentage of HEX was 1.4 times less ( $p>0.05$ ).

Differences in indicators were assessed in the II degree of PEX with eyes with glaucoma, cataracts against the background of patients with PEX. Among the significant differences in grade II PEX without glaucoma compared to eyes with glaucoma, CD was 1.3 times higher ( $p<0.05$ ); AVG was 1.3 times less ( $p<0.05$ ); SD is 1.6 times less ( $p<0.05$ ).

Among the non-significant differences the AVG index at grade II compared with the eyes with glaucoma the HEX index was 1.5 times lower ( $p>0.05$ ); CV 1.2 times less ( $p>0.05$ ); corneal thickness was 1.0 times less ( $p>0.05$ ).

Differences in indicators were evaluated for grade III PEX in eyes with glaucoma. Among the significant differences CD was 1.6 times less ( $p<0.05$ ); SD was greater than 1.5 times ( $p<0.05$ ); CV is 1.1 times less ( $p>0.05$ ); AVG was 1.7 times greater ( $p<0.05$ ).

Among non-significant differences, the percentage of HEX was 2.1 times higher ( $p>0.05$ ); corneal thickness was 1.0 times higher ( $p>0.05$ ). In terms of corneal thickness, it was not possible to identify significant correlations with any indicators.

The CD indicator for PEX for the 1st grade was 1.2 times greater than for the 2nd grade, and 2 times greater than for the 3rd grade, respectively ( $p<0.05$ ); and the difference between the 1st and the 3rd CD was greater by 2.4 times ( $p<0.05$ ).

Comparing the CD in patients with glaucomatous changes in PEX the 1st grade was

greater by 1.5 times ( $p < 0.05$ ) and by 1.3 times greater ( $p < 0.05$ ) compared with the 3rd grade PEX.

Comparing polymorphism of the endothelial cells (AVG) in PEX, the 1st grade was 1.2 times less than in PEX the II grade and 2.2 times less than in PEX the III grade and between the 1st and the 3rd grade - 2.7 times less. In all cases the  $p$ -value was less than 0.05.

Standard deviation (SD) was for PEX 1 in 1.3 times less than in PEX II, PEX II and PEX III 2.3 times less and PEX 1 and PEX III 3 times less. In all cases, the  $p$ -value was less than 0.05.

Results for the difference in patients with PEX the II grade in glaucomatous eyes and cataracts compared without glaucoma demonstrated CD 1.3 times greater ( $p < 0.05$ ) and for AVG parameters 1.3 times less ( $p < 0.05$ ) in patients with glaucoma. When it comes to SD values, PEX 1 to PEX II grade was 1.5 times greater ( $p < 0.05$ ), PEX II to PEX III grade was 1.1 greater ( $p < 0.05$ ) and PEX 1 to PEX III was 1.7 times greater ( $p < 0.05$ ).

It is known that PEX affects almost all layers of the cornea, causing the development of specific slowly progressive keratopathy and also causes concomitant disorders of the tear film and ocular surface in its uneven thickening as well as the capture of melanin by endotheliocytes (Naumann, and Schlötzer-Schrehardt, 2000; Zheng et al., 2011; Potemkin et al., 2017).

In addition, patients with PEX showed a decrease in density as well as an increase in the level of polymegethism and polymorphism of the corneal endothelium (Zheng et al., 2011, 2013; Aoki, 2020). According to the results of a number of studies, patients with pseudoexfoliative glaucoma have an even more pronounced degree of morphological changes in the endothelial layer of the cornea than patients with PEX (Yüksel et al., 2016; Sarowa et al., 2016).

The CD indicator in patients with PEX of the 1st degree in comparison with PEX of the II degree had a 1.2 times higher value ( $p < 0.05$ ); at grade II PEX was 2 times greater than grade III PEX ( $p < 0.05$ ). The difference between the I and the III stages of PEX showed that the cell density in PEX of the I degree was 2.4 times more than the III stage ( $p < 0.05$ ).

In comparison with glaucoma, the CD

indicator in eyes with PEX grade I PEX was 1.5 times higher ( $p < 0.05$ ); at the 2nd degree PEX was 1.3 times higher ( $p < 0.05$ ).

The indicator of cell polymorphism (AVG) in PEX of the I degree had a significantly lower value by 1.2 times in comparison with PEX of the II degree ( $p < 0.05$ ); at the II degree PEX was 2.2 times less than the III ( $p < 0.05$ ) and with PEX of the 1st degree in comparison with PEX of the III degree, it was 2.7 times less ( $p < 0.05$ ).

The standard deviation of the mean cell area (SD) within the framework of the study in PEX of the 1st degree was 1.3 times lower than in the II degree of PEX ( $p < 0.05$ ); in the II degree of PEX it was 2.3 times less than the 3-rd ( $p < 0.05$ ) and with PEX of the 1st degree it was 3 times less than the III grade PEX ( $p < 0.05$ ).

Differences in indicators were assessed in the II degree of PEX with eyes with glaucoma, cataracts in patients with PEX. Among the significant differences in grade II PEX without glaucoma compared to eyes with glaucoma CD was 1.3 times higher ( $p < 0.05$ ); AVG was 1.3 times less ( $p < 0.05$ ). Among the significant differences, SD is greater than 1.5 times ( $p < 0.05$ ); CV is 1.1 times less ( $p > 0.05$ ); AVG was 1.7 times greater ( $p < 0.05$ ).

In our study, there was a progressive decrease in the density of the corneal endothelium at different degrees of the progression of PEX.

Previous studies confirm the hypothesis that a decrease in corneal endothelium can be associated with both the progression of PEX manifestations and the development of the glaucoma process and these factors can act independently of each other (Beletskaya et al., 2018; Yüksel, 2016; Sarowa et al., 2016).

Polymegethism reflects the presence of cells of different sizes among the studied cell population. With age, there is a physiological slight increase in the coefficient of variation of cells. At the same time the level of polymegethism in the norm should not exceed 30 % (Galgauskas et al., 2013; Duman et al., 2016).

A number of authors note the relationship between changes in the corneal endothelium and the severity of changes in PEX. Results showed a significant decrease in the densities of the corneal endothelial cells in PEX eyes and their fellow eyes. In addition, the clear confocal images

allowed us to detect pleomorphisms and polymegathisms of the endothelial cells. These results have shed light on the pathogenesis of decreased corneal sensitivity in eyes with PEX syndrome (Zheng, 2011, 2013; Inoue et al., 2003; Naumann and Schlötzer-Schrehardt, 2000; Aoki et al., 2020). At the same time, it is important to emphasize the lack of a unified approach in assessing the degree (stage) of PEX development. Thus, classifications reflecting the severity differ both in the number of stages of the development of the syndrome (two to five stages) and in the choice of evaluation criteria, approaches (morphostructural changes, changes with UBM scores, etc.) (Nizankowska, 2001; Naumann and Schlötzer-Schrehardt, 2000; Inazumi et al., 2002; Takhchidi et al., 2010; Aoki et al., 2020).

We have to agree with the opinion that changes in the density of endothelial cells of the cornea before cataract surgery in eyes with PEX can definitely be considered as prognostic indicators (criteria) of increased risk of corneal endothelium decompensation after surgery (Quiroga et al., 2010).

In summary, thus the progress of PEX is accompanied by the corneal changes by the increase of the corneal decompensation risk during cataract surgery.

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## Hypoxia-induced apoptosis of eyeball cells

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**We aimed to study the effect of acute hypoxic hypoxia and acute hypobaric hypoxia on eye tissue cells in adult rats. In each experiment with acute hypoxic hypoxia and acute hypobaric hypoxia, 12 (4 animals in each group, 24 rats in total) male Wistar rats (24 eyes in each experiment, 48 eyes in total) were used, divided into 3 groups: Group I - intact control, group II - 1 hour after hypoxia, group III - 3 hours after hypoxia. Identification of apoptotic cells in the tissues of the eye was performed by the TUNEL method on frozen eye sections with additional staining with nuclear fluorescent dye Hoechst 33342. The localization and intensity of fluorescent emission in damaged cells were analyzed under a fluorescent microscope using Image J software. When exposed to acute hypobaric hypoxia, selective primary apoptotic damage to the cells of the conjunctiva and the anterior epithelium of the cornea was found. But under conditions of simulated acute hypoxic hypoxia, apoptotic damage to the conjunctiva, corneal epithelium, choroid and photoreceptor layer of the retina was observed. Cells of different parts of the eye of adult rats are characterized by different sensitivity to apoptotic damage in acute hypoxia modeled in this study.**

**Keywords:** Acute hypoxia, eye, conjunctiva, apoptosis

### INTRODUCTION

Hypoxia plays an important role in the pathogenesis of dry eye syndrome, hereditary, dystrophic, ischemic, inflammatory, infectious and other diseases of the eyeball (Blasiak et al., 2014; Chao et al., 2013). During hypoxia, the conditions for maintaining normal metabolism and functioning of cells are violated, which can lead to their death. In many diseases of the human eye: glaucoma, cataract, diabetic retinopathy, retinal dystrophy, cell death is observed by the apoptosis mechanism (Ermilov and Maxonin, 2011; Slepova et al., 2012; Servellati et al., 2014; Ishibashi, 2012; Kaur et al., 2008; Ozaki et al., 2012; Saccà and Izzotti, 2008).

Experimental studies of the effect of hypoxia on apoptotic cell death were carried out on individual tissues of the eye, under *in vitro* conditions. Thus, in cultures of purified rat retinal ganglion cells, as well as corneal keratocytes, it has been shown that hypoxia induces apoptosis in these cells (Unterlauff et al., 2014; Yang et al., 2013).

We aimed to study the effect of acute hypoxic hypoxia and acute hypobaric hypoxia on the tissues of the eye of adult rats in various experimental models of hypoxia *in vivo*.

### MATERIALS AND METHODS

The work was performed on 24 mature male



Wistar rats, aged 3-4 months. In each experiment, 12 rats (24 eyes) were used: group I - intact control (4 rats); Group II - after hypoxia in 1 hour (4 rats); Group III (4 rats) - after hypoxia after 3 hours. Control animals (4 rats) were not exposed to hypoxia. In the experimental groups, the eyes of the animals were examined 1 and 3 hours after hypoxia.

**Experimental modeling of acute hypoxia.** In the experimental group, the animals were subjected to a single exposure to acute hypoxic hypoxia. Hypoxia was achieved by replacing the air with nitrogen from a hermetic chamber with a volume of 0.12 m<sup>3</sup>, where experimental animals were placed, for 7-10 minutes, until convulsions occurred. The eyes of animals from the experimental group were analyzed three hours after hypoxic exposure. The control group of animals not subjected to hypoxia was kept at room temperature. Animals from both groups were taken out of the experiment by anesthesia in ether, after which the eyes of rats from both groups were enucleated, their histological examination was carried out, and the distribution of apoptotic cells in the tissues of the eye was also analyzed.

**Experimental modeling of acute hypobaric hypoxia.** In the experimental group, the animals were subjected to a single action of acute hypoxia, which was achieved by pumping out air for 1 min until the pressure chamber reached a pressure of 180 mm Hg. Under these conditions, the rats were kept for 3 min before the onset of convulsions. The results of the experiments were recorded 3 hours after hypoxia. Animals were withdrawn from the experiment by intraperitoneal injection of chloral hydrate (Riedel-de-Haen, Germany) followed by euthanasia with ether vapor until the animals recovered from anesthesia. The eyes of experimental and control rats were enucleated.

The experiments were carried out in accordance with the Rules for the Keeping and Use of Laboratory Animals and the provisions of the European Convention for the Protection of Animals Used for Experimental and Other Scientific Purposes. To detect apoptosis in eye tissues, the traditional TUNEL method (Terminal deoxynucleotidyl transferase – mediated deoxyuridine triphosphate (UTP) – nick end – labeling) was used using the Dead End

Fluorometric TUNEL System reagent kit (Promega Corporation, USA).

**Histological examination.** The material for light microscopy was processed by conventional histological methods. For histological analysis, the eyes of rats were fixed in Bouin's fluid, embedded in paraffin, and used for sectioning according to the standard protocol (Sennlaub et al., 2002). Sections 7 µm thick were glued onto adhesive-coated slides (Silane-Prep Slides, Sigma) and after deparaffinization, the sections were stained with hematoxylin and eosin. The preparations were examined under a Leica light microscope (Germany).

**Material preparation and DNA labeling reaction according to the TUNEL method.** The eyes were fixed in 4% neutral formalin prepared in 0.1 M phosphate buffer (pH 7.4) for 4 h. Then the samples were washed in phosphate buffer, three changes of phosphate buffer with 5% sucrose, three changes of phosphate buffer with 10% sucrose, then 20% sucrose (in each solution for 15 minutes) and left overnight in phosphate buffer with 20% sucrose at 4°C. After freezing the eyes in a special medium (Tissue-Tec OST, Leica, Germany), using a cryostat (Leica M1900, Germany), transverse sections of the eyeball were obtained and selected for analysis. The slice thickness was 12 µm.

Fragmented DNA was labeled by the TUNEL method according to the manufacturer's protocol. Before carrying out the enzymatic reaction, the sections were washed in 0.1 M PBS, fixed in 4% paraformaldehyde for 5 minutes, then washed from the fixative in 0.1 M PBS three times for 5 minutes. The reaction was carried out for an hour at a temperature of 37°C, then the reaction was stopped by washing the sections in a 2x SSC solution. To confirm the specificity of the reaction, a standard control reaction was also performed in the absence of the rTdT enzyme. Cell nuclei were stained with Hoechst 33342 diluted in 0.1 M PBS (1:1000, Leica, Germany) for two minutes. After staining, the sections were washed in several shifts with 0.1 M PBS, for 15 minutes in each solution, and placed in a special medium for preparations with a fluorescent label - Vectashield (Vector, USA).

**Microscopy and computer image analysis.** The localization of the fluorescent glow and its



intensity in the cells of the eye tissues were analyzed using a Leica DM RXA2 fluorescent microscope (Germany), with the image transferred to a computer console equipped with the Leica for Windows program. No fluorescent emission was observed in the control preparations. The images were processed using the Image J computer program.

## RESULTS AND DISCUSSION

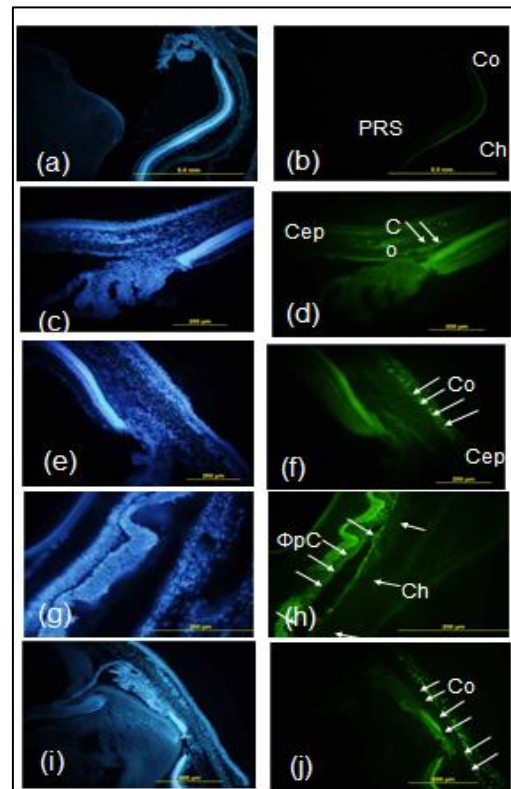
Viewing the selected transverse sections to identify apoptotic cells in the tissues of the eye clearly showed that under conditions of acute hypoxic hypoxia, the primary lesion occurs in all layers of the conjunctiva, in the anterior corneal epithelium, in the own choroid, and also in the photoreceptor layer of the retina (Fig. e, f; g, h; i, j). In dynamics, there is an increase in cell damage by apoptosis, i.e. 3 hours after hypoxia, a more intense glow of damaged cells is noted (Fig. i, j) than after 1 hour (Fig. e, f.)

It has been shown that the simulated conditions of hypoxia cause a pronounced reaction of the cells of the conjunctiva, in the anterior epithelium of the cornea and in the photoreceptor layer of the retina, which leads to apoptotic death of a significant part of them. Staining with DNA dye Hoechst 33342 confirms the localization of apoptosis in the cell nuclei. In other tissues of the eye - the lens, iris, ciliary body - apoptotic cells were absent in this type of lesion. The same picture was observed in all studied eyes.

In the studied tissues of the eye of animals from the control group (without exposure to hypoxia), only single apoptotic cells were found in the conjunctiva and in the anterior epithelium of the cornea. In the control group of animals, there were no apoptotic cells in the lens, iris, ciliary body, and retina (Fig. c, d). In the control preparations, which served as a negative control to confirm the specificity of the reaction in the experiment, no labeled cells were observed (Fig. a,b).

Thus, during acute hypoxic hypoxia under the conditions modeled in this work, causes an intense process of DNA fragmentation and apoptosis in the cells of the tissues of the anterior surface of the eye, the own choroid, and the photoreceptor layer of the retina.

In acute hypobaric hypoxia, we found the same type, selective localization of cells with damaged DNA in the anterior corneal epithelium and in the conjunctiva. These cells were subjected to apoptosis, which was confirmed by staining eye sections with the fluorescent DNA-binding dye Hoechst 33342. In dynamics, there is an increase in cell damage by apoptosis, i.e., 3 hours after hypoxia, a more intense glow of damaged cells is noted than after 1 hour.



**Fig.** Apoptotic cells in rat eye tissues in normal conditions and after experimental hypoxia. Arrows show TUNEL-positive cells in the conjunctiva and corneal epithelium. Nuclei were stained with Hoechst 33342. Intact negative control: (a, b); intact control conjunctiva (c, d); fixation after a single hypoxia: after 1 hour the conjunctiva (e, f), fixation after a single hypoxia after 1 hour the retina (g, h); fixation after 3 hours, conjunctiva (i, j). Cep – corneal epithelium; Co – conjunctiva; PRS, photoreceptor layer of the retina; Co – choroid (choroid). Scale: 200 (a–f), 500  $\mu$ m (g–h).

In the studied tissues of the eye of animals from the control group (without exposure to hypoxia), only single apoptotic cells were found. In the lens, iris, ciliary body, choroid and retina,

apoptotic cells were absent, both in the experimental and control groups. In the control preparations, which served as a negative control to confirm the specificity of the reaction in the experiment, no labeled cells were observed.

Acute hypoxia under the conditions modeled in this work causes an intense process of DNA fragmentation and apoptosis in the cells of the tissues of the anterior surface of the eye - the conjunctiva and the anterior corneal epithelium. Unlike acute hypobaric hypoxia, hypoxic hypoxia also causes apoptosis of the own choroid and retina. Acute hypoxia in the conditions of these experiments in the lens, iris, ciliary body does not occur apoptotic changes in cells, i.e. they remain undamaged. Thus, the cells of different parts of the eye of adult rats are characterized by different sensitivity to hypoxia modeled in this study.

The results obtained by us open up further prospects for experimental studies of the mechanisms of eye tissue pathology under conditions of hypoxia of various genesis. Given the role of apoptosis in the pathogenesis of pathologies of the surface of the eye and retina, it is possible to study the fundamental mechanisms of the effectiveness of certain drugs in the treatment of eye diseases using these experimental models.

## CONCLUSIONS

1. Acute hypobaric hypoxia causes an intense process of DNA fragmentation and apoptosis only in the cells of the tissues of the anterior surface of the eye - the conjunctiva and the anterior epithelium of the cornea.
2. Acute hypoxic hypoxia causes an intense process of DNA fragmentation and apoptosis in the cells of the tissues of the anterior surface of the eye - the conjunctiva and the anterior epithelium of the cornea, choroid and retina.

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## **Lacrimal activity and immunological performance in children with enterovirus uveitis**

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The control group consisted of 50 healthy people. The study was conducted among 150 children aged from 3 months to 14 years old with uveitis caused by enterovirus uveitis (EVU) in the population of Azerbaijan. They were divided into three groups depending on the etiology of the disease. The first group included 82 children with viral eye diseases; the second group included 42 children with viral-bacterial diseases; the third group included 26 children with viral-allergic eye diseases. One of the factors for the alternative activation of the complement system is considered to be the increase in the level of immunoglobulins of class A that leads to increased vascular permeability and the development of edema, which in turn has chemotoxic and devastating effects. In children with allergic eye changes, the IgE level increases on average by 2.2 times ( $p<0.05$ ), which indicates an increase in the chemotaxis of eosinophils with eye age. As shown by the survey data, when comparing with healthy children, there is a 3.2-fold increase in the index of intoxication and aggravation of the inflammatory process in 26 sick children ( $p<0.05$ ), while in 42 children allergy index is on average 2 times higher, increases are observed ( $p<0.05$ ). At this time, the rise in the leukocyte intoxication index is associated with a decrease in the percentage of nuclear white blood cells and a decrease in the number of lymphocytes. The formation of young and immature forms of neutrophils in the blood of examined children with pronounced eye allergies indicates the strain of compensatory processes providing detoxification. Analysis of the data found that the tear concentration and fibrinogen levels in both groups of patients differed from and between control values indicating an increase in local hemostatic potential. At this time the dynamics of the coagulation activity of lacrimal liquid and the amount of fibrinogen in this pathology indicate the presence of hemocirculatory disorders in the retinal vessels and choroidea, which indicates the importance of the study of hemostasis in inflammatory and allergic processes.

*Keywords: Enteroviral uveitis, etiology, immune status, enteroviral infection, coagulation activity, lacrimal fluid, children*

### **INTRODUCTION**

Children's health is a global health priority. Visual protection, prevention and treatment of eye diseases and injuries, prevention of blindness and impairment, reduction of disability are considered as one of the main links in the struggle for children's health, which is of great socio-economic importance.

In modern times, a dominant increase in

allergic diseases among children is spread. In Western countries, the number of persons suffering from allergic diseases is on average 20% of the total population, in some regions reaching 40-50% (Heydarova, 2014; Mammadova, 2015). In this case, 80-90% of all allergies are diagnosed with eye lesions.

Over the last 30 years, new, severe forms of enterovirus infection have appeared - acute hemorrhagic conjunctivitis caused by enterovirus

70 and polio-like diseases caused by enterovirus 71, enterovirus infection manifesting via uveitis, retinal diseases caused by the enterovirus Koksacki B4 (Xiao et al., 2014; Jacob, 2014). The emergence of relatively new etiological factors underlying pathological conjunctival changes plays a major role in changing and increasing the structure of the disease. In the medical literature, a lot of research is devoted to the role of various allergens in eye diseases. There are also national features of the clinical course of allergic eye diseases in children, the study of which is of great practical importance. Tears consist of a poly-component metabolic active biological system in which various metabolic, immunological, regulatory, protective and many biochemical processes are actively developed (Schwartzman, 2016).

The purpose of the study was to learn the coagulation activity of tears and immunological indicators in children with enterovirus uveitis.

## MATERIALS AND METHODS

The study was conducted among 150 children aged 3 months to 14 years old with uveitis caused by an enterovirus infection in the population of Azerbaijan. The control group consisted of 50 healthy people.

All children were examined for hematological indicators, for which they calculated: leukocyte intoxication index (LII), allergy index (AI) - this was done by G.N.Chistyakov et al. (2005). We have studied determining the ratio of cells to the total amount in the blood (Mammadova, 2015). The concentration of C-reactive protein was studied by the IFA method, using the sets of the company «Monolind» and «Immunodiagnosics», presented by the company «BioKsimMc». Immunoglobulin concentrations of classes G, M and A were studied by immune analysis (IFA). The sets of companies «NIMAN», «Monolind» and

«Immundagnostic» (Russia) presented by BioKsimmak company were used and the results were expressed in BV/ml. The state of the local hemostatic potential was determined on the basis of a study of the coagulatory activity of the tear, which was estimated on the basis of the difference in the time of the clot formation with and without the addition of observed tears to the serum of donor blood (Schwartzman, 2016). The quantity of fibrinogen was determined by means of sets «Nemostat». The statistical processing of the obtained results was carried out by determining the mathematical mean number (M) and the error index (t) according to the known method of variation statistics.

## RESULTS AND DISCUSSION

Children with EWN were divided into three groups depending on the etiology of the disease. The first group included 82 children with viral eye diseases; the second group included 42 children with viral-bacterial diseases; and the third group included 26 children with viral-allergic eye diseases. The control group consisted of 50 healthy children under the age of 14 years old without clinical-functional and laboratory signs of viral and allergic eye diseases. The criteria for exclusion in all groups were: taking antihistamines and hormonal drugs. All children were given standard ophthalmological examinations. The tears were collected from the lower conjunctival arch of the eye into a dry, sealed vial of 0.5 ml through a microcannula for examination.

One of the factors for the alternative activation of the complement system is considered to be the increase in the level of immunoglobulins of class A that leads to increased vascular permeability and the development of edema which in turn has chemotoxic and devastating effects (Table 1).

**Table 1.** Tear rates (SG) in patients with allergic eye diseases

Parameters	Control group (n=50)	1 <sup>st</sup> group (n=82)	2 <sup>nd</sup> group (n=42)	3 <sup>rd</sup> group (n=26)
sIgA, g/l	0.32 ±0.01	0.14±0.01*	0.19±0.01*	0.27±0.01*
IgA amount, g/l	1.04±0.12	1.21±0.04	1.32±0.07	0.72±0.06
IgM amount, g/l	0.018±0.01	0.015±0.05	0.017±0.04	0.042±0.02*
IgG amount, g/l	0.28±0.02	0.42±0.04*	0.51±0.03*	0.81±0.08*
IgE amount, BV/ml	12.6±1.04	18.7±1.14*	19.4±1.12*	26.8±2.14

**Note:** \*-valid differences from the control group ( $p < 0.05$ )

**Table 2.** Tear rates in patients with viral-allergic eye uveitis

Parameters	Control group (n=50)	1st group (n=82)	2nd group (n=42)	3rd group (n=26)
Leukocyte intoxication index	0.36 ±0.02	1.16±0.02*	1.22±0.08*	0.08±0.02*
Allergization index	0.96±0.11	0.52±0.06	0.71±0.06	1.65±0.24
S-reactive protein, µg/ml	5.48±0.76	112.5±12.6	94.6±11.9	55.8±4.96*
Coagulation activity (sec)	32.75±2.34	65.72±4.28*	0.51±0.03*	0.81±0.08*
Fibrinogen (ng/dl)	237.8±9.12	256.8±8.25*	262.2±9.3*	327.4±12.6

**Note:** \*-valid differences from the control group

In children with allergic eye diseases associated with infectious changes, secretory immunoglobulin A decreased on average by 24.0% ( $p < 0.05$ ), while in the group with allergic changes the level of sIgA in the eyes was 21.0% ( $p < 0.05$ ). In this type of reaction, children who have been diagnosed with eye infection may develop antibodies to the eye tissue, mainly Ig and IgM class antibodies. Analysis of the obtained survey data shows an increase in the level of antibodies M-class 2.3 times and antibodies G-class 3.6 times ( $p < 0.05$ ).

These antibodies are called precipitators because of their ability to form sediment when combined with the corresponding antigen. An acute type of allergic reaction is associated with the formation of IgE class antibodies - they are fixed in barrier cells and histamine, heparin, etc. create conditions for secretion. The increase in eosinophils in both groups was probably determined by the presence of histamines and Heparinoids in these cells - they neutralize biogenic amines and heparin. In turn, it should be noted that histamine is chemotoxic.

The acute hypersensitivity reaction during their activation and degranulation causes the release of biologically active mediators from granule barrier cells into the conjunctiva due to the high level of IgE class antibodies. In children with allergic eye changes, the IgE level increases on average by 2.2 times ( $p < 0.05$ ), which indicates an increase in the chemotaxis of eosinophils with eye age. However, among the children examined with eye infections, the study exceeded the baseline by 43 percent ( $p < 0.05$ ). Using hematologic indicators, we decided to approach the understanding of some aspects of etiopathogenesis of allergic eye diseases in

children and conditionally divided the affected children into 2 groups: with infectious changes and with allergic changes.

As shown by the survey data (Table 2), when comparing with healthy children, there is a 3.2-fold increase in the index of intoxication and aggravation of the inflammatory process in 26 sick children ( $p < 0.05$ ), while in 42 children, IA is on average 2 times higher, increases are observed ( $p < 0.05$ ).

At this time the rise in LII is associated with a decrease in the percentage of nuclear white blood cells and a decrease in the number of lymphocytes. The formation of young and immature forms of neutrophils in the blood of examined children with pronounced eye allergies indicates the strain of compensatory processes providing detoxification. In children with infectious changes, the opposite clinical picture was observed, as in children with infectious changes, LII indicators increased by 3.3 times compared with the benchmarks ( $p < 0.05$ ) and 17.3 times compared with children who have got allergic eyes ( $p < 0.01$ ). In the 1-st group of children inflammatory process activation was also assumed by the amount of S-reactive protein - its level was 30 times higher than the initial value in the group with infectious changes while the level of S-reactive protein in the group associated with allergic changes only 10 times higher than the reference group ( $p < 0.05$ ). So, in the eye age, we proved the nature of allergic eye diseases associated with infection and allergies by studying the integral hematological indicators and the level of S-reactive protein.

As it is known, local hemostatic potentials play an important role in this pathology. Therefore, we decided to assess the

informativeness of the study of tear-coagulation activity in this pathology. Thus, the coagulation activity of the tears increased reliably depending on the indicators of the reference group ( $p < 0.05$ ). In children with infectious changes, these indicators were significantly reduced and differed from those with allergic changes ( $82.6 \pm 7.01$  against  $62.8 \pm 4.5$ ,  $p < 0.05$ ). The amount of fibrinogen tended to increase in both groups compared to the control group ( $p < 0.05$ ). The highest rates were recorded in children with allergic changes ( $264.6 \pm 8.51$  ng/dl versus  $331.2 \pm 11.02$  ng/dl  $p < 0.05$ ).

## CONCLUSIONS

1. Analysis of the data found that the tear concentration and fibrinogen levels in both groups of patients differed from and between control values, indicating an increase in local hemostatic potential. At this time, the dynamics of the coagulation activity of GH and the amount of fibrinogen in this pathology indicate the presence of hemocirculatory disorders in the retinal vessels and choroidea, which indicates the importance of the study of hemostasis in inflammatory and allergic processes.

2. In children with allergic eye diseases associated with allergic changes, there is a noticeable increase in the amount of the allergenic index and IgE against the background of a decrease in the level of S-reactive protein and the amount of IgM and G per eye age. Based on the examination of hematologic indicators in children

with infectious changes, an allergic lesion of slow-type hypersensitivity eyes was found, which was accompanied by an increase in LII, S-reactive protein and IgG in the eye age.

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## **Evaluation of retinal changes in diabetic retinopathy by optical coherence tomography**

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**The importance of optical coherence tomography (OCT) examination in the evaluation of the retina in the neoproliferation stage of diabetic retinopathy (DR) has been studied with the conducted research. In the present study, while fulfilling the goal set for 2020-2022 according to clinical and laboratory criteria, 100 patients with diabetic retinopathy (200 eyes) (average age 50-70) were examined. The control group consisted of 50 patients (100 eyes) with diabetes but diabetic retinopathy was not yet detected. For many years, fluorescence angiography was considered the gold standard in the diagnosis of diabetic retinopathy. In modern ophthalmology, an examination method called optical coherence tomography is used to perform a cross-sectional image of the retina. The results of the conducted research allow the detection of changes in the thickness of the macular part of the retina in the early stage of OCT diabetic retinopathy. It has been determined that the initial damage due to diabetes is observed in most cases between 6-10 years, which depends on the hyperglycemic index in many cases. In the diagnosis of DR, OCT examination plays an important role in the selection of treatment tactics, it facilitates the measurement of macular thickening, the detection of diffuse and cystic macular edema, and the detection of vitreoretinal tractions.**

**Keywords:** *Diabetic retinopathy, optical coherence tomography, diabetes, retina, diabetic macular edema*

### **INTRODUCTION**

There are approximately 347 million people with diabetes mellitus (DM) in the world (Varma et al., 2014). By 2030, the worldwide prevalence of DM is predicted to reach 430 million patients (Korobelnik et al., 2014). Diabetic retinopathy (DR) is characterized by diabetic microangiopathy, retinal microaneurysms, capillary non-perfusion and ischemia (Adhi et al., 2013; Hwang et al., 2016; Hwang et al., 2015; Ishibazawa et al., 2015). It can cause a number of complications such as diabetic macular edema (DME) and diabetic macular ischemia (DMI) (Varma et al., 2014; Elman et al., 2010; Agemy et al., 2015; Conrath et al., 2015). is considered to be a serious process. Although fluorescence

angiography (FA) is considered the gold standard in the diagnosis of DR, the fact that the examination method is invasive has got contraindications and the duration of the examination is about 10-15 minutes which limits its use.

Optical coherence tomography (OCT) was first used to visualize the eyeball more than 20 years ago and still remains an indispensable diagnostic method in ophthalmology. With the help of OCT, it turned out to be possible to non-invasively obtain optical sections of tissues with a higher resolution than when using other visualization methods. Currently, OCT allows refinement of the structure of the tissue or its pathology at the level of 1-15 microns, which is more accurate than ultrasound examination, MRI,



or CT (Ishibazawa et al., 2015). Currently, OCT is successfully used for screening, monitoring and diagnosis of eyeball diseases, as well as for conducting scientific research (Hwang et al., 2015; Ishibazawa et al., 2015; Agemy et al., 2015). According to the studies of various authors, modern optical coherence tomography is undoubtedly the "gold standard" in the study of the fundus; it has great diagnostic possibilities and prospects for development, which will allow the detection of diseases at subclinical stages in the future (Puliafito et al., 1995).

The purpose of this research was to clarify the role of OCT examination in the evaluation of the retina in the neoproliferation stage of diabetic retinopathy.

## MATERIALS AND METHODS

Our observational study was conducted at the Teaching Surgery Clinic of Azerbaijan Medical University. In the present study, 100 diabetic retinopathy patients (200 eyes) (average age between 50-70) were examined while fulfilling the goal set in 2020-2022 according to clinical and laboratory criteria. Patients in the stage of neoproliferation were involved in the study. After biomicroscopy, visometry, tonometry, ultrasound examination, ophthalmoscopy the patients were sent for OCT examination. Retinal thickness measurements were performed on an OCT device. The control group includes 50 patients (100 eyes) with diabetes but diabetic retinopathy has not yet been detected. The examination was performed on the RS 330 Nidek OCT device (Japan) for 4-5 minutes (two eyes).

## RESULTS AND DISCUSSION

In our observational study, we analyzed the dependence of the dynamic changes in different areas of the retina on the duration of diabetes (Table 1).

As can be seen from the table, according to the state of illness with diabetes mellitus, primary damage is observed in most cases between 6-10 years, which depends on the hyperglycemic index in many cases.

According to our results, there was no noticeable change between the control group and the main group. In the control group, the thickness of the foveola corresponded to its anatomical thickness of 120-200 microns. Foveal thickening was observed in 4 patients. According to some scientists, although no morphological changes are observed in the OCT protocol, the thickening of the foveola may be a sign of early edema (Korobelnik et al., 2014; Hwang et al., 2016). In addition, vitreoretinal traction was observed in 2 patients in the main group.

**Table 1.** Determination of the duration of diabetes mellitus in the main group

Duration of being sick with diabetes mellitus	Main group	
	Number of patients	Number of eyes
0-1	-	-
2-5	10	20
6-8	25	50
8-10	65	130
<b>Total</b>	100	200

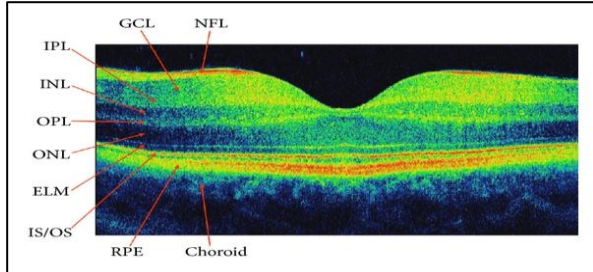
**Table 2.** Thickness of different areas of the retina in the observed groups

Observed zone	Control group, n=50	Main group, n=100
Foveola	146.74±18.35	160.04±21.07
Fovea	200.02±20.09	185.75±19.70
Temporal inner part	250.45±14.75	259.31±21.56
Temporal outer part	214.12±12.14	216.56±15.19
Upper inner part	268.75±16.03	265.98±12.04
Upper outer part	218.54±11.30	233.45±23.34
Nasal inner part	265.02±19.09	259.00±15.08
Nasal outer part	248.07± 13.00	246.05±21.00
Lower outer part	224.76±12.16	260.13±17.09

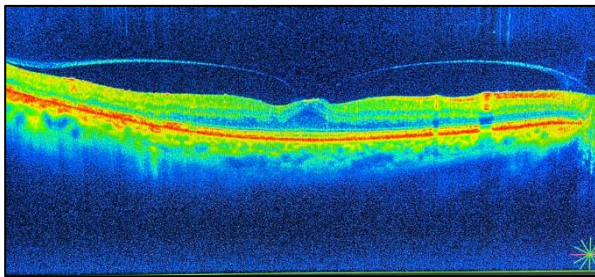
In all patients, the condition of diabetes continued for different periods of time. At the same time, the thickening in different parts of the retina was different in these patients. The changes in the retina are observed not only in the macular part, but also around the optic disc.

The results of the study allow OCT to detect changes in the thickness of the macular part of the retina at an early stage during diabetic retinopathy. Also, OCT is an indispensable examination method for determining the direction of the treatment of DR and monitoring the treatment results. OCT helps to monitor the volume of DME and intraretinal damage (Fig. 1,

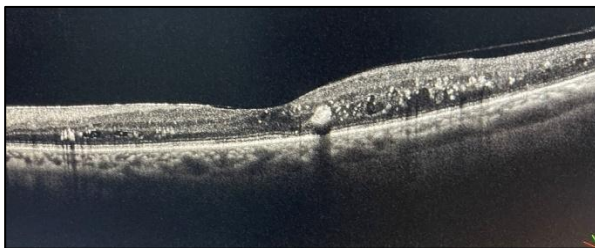
2, 3, 4). OCT examination remains the main method for early detection of macular edema, selection of treatment of diabetic maculopathy and monitoring of results.



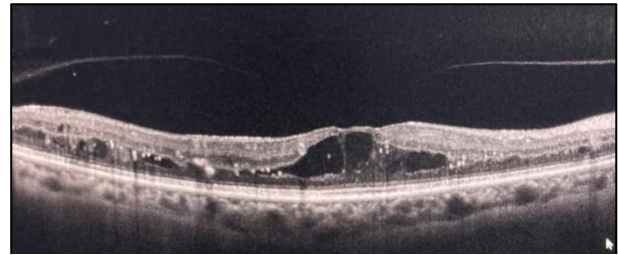
**Fig. 1.** Cross-sectional view of the retina of the eye with OCT. NFL: nerve fiber layer; GCL: ganglion cell layer; IPL: inner plexiform layer; INL: inner nuclear layer; OPL: outer plexiform layer; ONL: outer nuclear layer; ELM: outer limiting membrane; IS/OS: intra-node and outer photoreceptor segments; RPE: retinal pigment epithelium.



**Fig. 2.** OCT examination: Image of posterior vitreoretinal traction in a patient with diabetic retinopathy.



**Fig. 3.** OCT examination records the relative thickening of the macular part in the retina of the eye and traces of exudates in a patient with diabetic retinopathy.



**Fig. 4.** OCT examination: observation of vitreoretinal traction with macular edema on the retina in a patient with diabetic retinopathy.

## CONCLUSION

OCT provides micrometer-level imaging that closely approximates the histological layers of the retina. One of the advantages of OCT is that the examination is non-invasive, there is no need for additional preparation before the examination, and the examination period is short. In patients with diabetic retinopathy, OCT quantifies retinal edema, which can be successfully used as an objective method of monitoring macular thickening before and after therapy. At the same time, OCT is also very useful for vitreous evaluation. It is useful in detecting clinically undiagnosed vitreoretinal traction. OCT should be the number one choice of ophthalmologists to detect diabetic macular edema and monitor treatment progress from focal/grid laser and anti-VEGF therapies and select the next treatment step.

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## **Development of occupational ophthalmology: from dry eye syndrome to artificial tears**

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**In this short review, we convey the history of occupational ophthalmology in the former Soviet Union that has been developed under the supervision of academician Zarifa Aliyeva. Research initiated by acad. Z. Aliyeva has provided the foundation for the new discipline of “occupational ophthalmology”. Occupational ophthalmology, as a part of occupational medicine, has a long history of development. The first studies and implementation of occupational ophthalmology in the world started in about the fourth decade of the twentieth century. Particular aspects of occupational ophthalmology depend on the specifics of the particular industry. Here we describe dry eye syndrome that has been diagnosed in millions of people worldwide. Dry eye syndrome is described as one of the consequences of certain occupations, particularly prolonged work with a computer. The structure of the ocular surface of the human eye is described for a better understanding of the consequences of a prolonged dry eye condition. Experimental data suggest that tears play the principal role in maintaining and protecting ocular surface health.**

**Keywords:** *Occupational ophthalmology, human cornea structure, tear film, dry eye syndrome*

### **History of occupational ophthalmology.**

For a long time, not much attention was paid to the specificities of human eye diseases developed in various professional activities. The term “occupational ophthalmology” was first coined by Dr. Kuhn in 1946 (Kuhn, 1946). Various environmental conditions associated with the particular workplace (occupation) were recognized as risk factors for different diseases. The eye problems developed in various areas of the industry are multifactorial and very complicated. This situation set the foundation for the development of a new academic discipline—occupational ophthalmology. It was immediately clear that industry-eye physician collaboration is pivotal for sustained progress in preventing eye injuries.

In the former Soviet Union acad. Zarifa Aliyeva first recognized the importance of the development of occupational ophthalmology. For the first time, in 1978 she established a laboratory

of “occupational pathology of visual organ” in the Baku plant of domestic air conditioners. The workers exposed to styrene and tetrachlorethylene vapors were identified and a specific therapy was developed for them. This collaboration with industry was very important in creating safe and healthy workplace conditions for the workers of the Baku plant of domestic air conditioners. Academician Z. Aliyeva not only treated the worker exposed to toxic environments but also optimized the workplace to prevent health risk conditions. Thus, a new discipline of “occupational ophthalmology” was founded by acad. Z. Aliyeva. Now, “occupational ophthalmology” is part of “occupational medicine” that covers almost all aspects of human health issues.

Acad. Z. Aliyeva was also among the first ophthalmologist who realized the importance of tears to keep the eye surface healthy. Her fundamental book “Physiology of tear secretion”

published in 1983 still has a great scientific value (Sultanov and Aliyeva, 1983). The anatomy and physiology of the main and accessory lacrimal glands are described in great detail. Of course, last 40 years a lot of progress has been made in this direction. But the main idea still highlights the importance of healthy tears to prevent many eye surface diseases including dry eye syndrome.

#### Origin of dry eye syndrome.

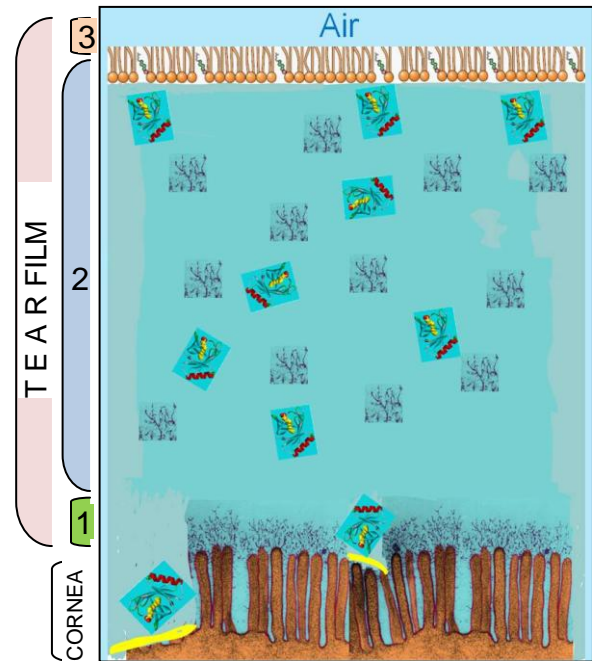
In this review, we present a summary of an up-to-date understanding of human tears compositions and functions to keep the ocular surface in healthy conditions. Special emphasis will be given to dry eye conditions that can be the result of various disorders.

It is widely accepted to describe human tear film with a layered structure (Fig. 1). The main functions of the tears are to keep the cornea wet (lubricated), prevent various infections, and provide nutrition to the cornea that lacks blood vessels (Sridhar, 2018). The average diameter and thickness of the human cornea are ~ 11 mm and 0.6 mm, respectively. The outermost part of the cornea is composed of epithelial cells that have about 50 mm thickness. The cornea epithelial cells are constantly renewed and have a lifespan of about 7-10 days. Epithelial cells are connected to the stroma via Bowman's layer (Fig. 2). The epithelial cells of the normal cornea have a five-layered structure (DelMonte and Kim, 2011) and are classified into three types of cells: basal layer (the innermost layer), wing cells and superficial cells (the outermost layer) (Fig. 3).

During the epithelial cell progression, the basal cell layer moves toward the upper part while transforming into a wing and then superficial cells. Superficial cells develop microvilli that produce transmembrane mucins, such as MUC1, MUC4, and MUC16 (Gipson, 2004; Govindarajan, Gipson, 2010). This cell-attached mucin layer keeps the cornea surface wet, and lubricated and provides a mechanical barrier to pathogens and other exogenous particles. The corneal stroma is transparent and its central part lacks blood vessels. Transparency of the stroma results from the particular organization of its constituents, stromal fibers and extracellular matrix (ECM).

Corneal fibrils of the stroma are mainly composed of collagen Type I. A much smaller

amount of collagen Type VI and XII are also found in the stroma (Sridhar, 2018). Endothelial cells form a single layer and positioned innermost part of the cornea (Figure 3).



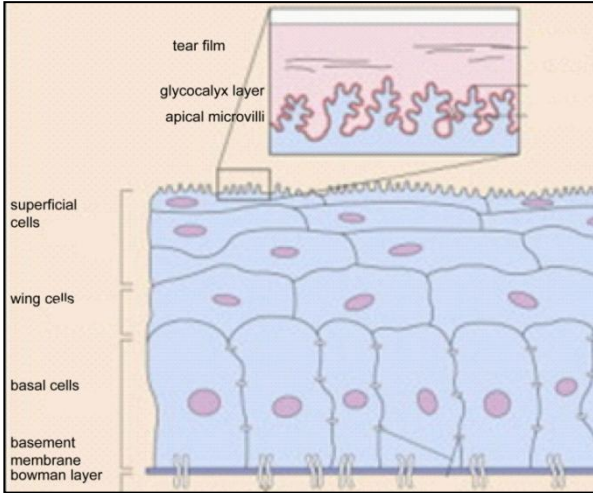
**Fig. 1.** Tear film of the human ocular surface. Microprojections from the corneal surface are microvilli. Transmembrane mucins (1), like MUC1, MUC4, and MUC16, cover the apical surface of the microvilli and are considered the innermost 1st layer. The aqueous layer (2) of the tears is made of electrolytes, various types of peptides and proteins, and soluble mucins, such as MUC5 and MUC7. The outermost layer (3) of the tear film is the lipid layer composed of various phospholipids, fatty acids, cholesteryl esters, triglycerides, etc.

The main function of the endothelial layer is to pump water out of the cornea to keep its normal hydration (Waring et al., 1982). Aquaporins, water channel proteins of the endothelium, provide pump functions (Verkman et al., 2008). A damaged endothelial layer does not sufficiently pump water out of the cornea and, therefore, the cornea may swell resulting in a disease called bullous keratopathy.

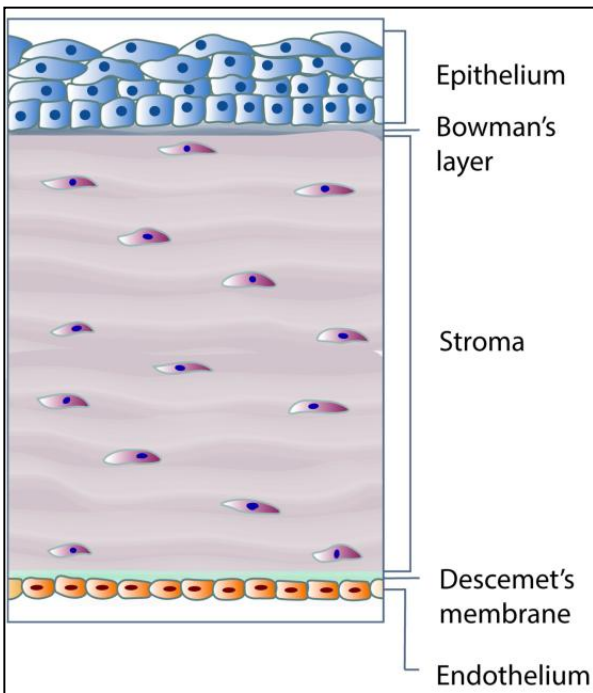
The thickness of the stroma that provides mechanical properties of the cornea is also essential to vision. In some conditions, a stroma is getting thinner and the cornea gradually bulges outward to take a cone shape. This disorder is



called keratoconus and causes blurred vision. The cornea surface is covered by tear film which is made of three layers (Fig. 1).



**Fig. 2.** Details of the epithelial layer of the human cornea.



**Fig. 3.** Structure of human cornea.

As mentioned above, the innermost layer of the tear film is the mucin layer composed of transmembrane mucins (MUC1, MUC4 and MUC16) located at glycocalyx (Gipson, 2004). The next layer of the tear film is an aqueous layer.

The aqueous layer of the tear film is positioned between the mucin and lipid layers. The aqueous layer is mainly formed by the secretion of the lacrimal gland.

The aqueous layer is composed of various electrolytes, proteins, peptides, hormones, etc. Up to 150 different extracellular proteins and peptides have been detected in the tear film. In addition, proteins from degraded cells have also been detected. Lactoferrin, Tear lipocalin, and Lysozyme composed up to 90% of protein content. The total protein concentration in the tear film is about 5-6 mg/ml. The aqueous layer contains water-soluble mucins, such as MUC 5 and MUC 7, produced from conjunctival goblet cells (Hodges et al., 2013). Lactoferrin and Lysozyme are the major sources of antimicrobial activity in the tears (McDermott et al., 2011). The ability to bind free iron confers antimicrobial function to Lactoferrin. Removal of free iron from the media diminishes the availability of iron necessary for microbial growth. Lysozyme uses a different way to provide antimicrobial activity to tears. Lysozyme has a high capacity to attack the bacterial cell wall that provides antimicrobial activity (Flanagan et al., 2009; Gasymov et al., 1999). Tear lipocalin (TL), which comprises about 33% of total proteins is a major lipid-binding protein in tears. TL binds to a variety of lipids, such as fatty acids of different hydrocarbon chain lengths, phospholipids, glycolipids, cholesterol, etc. (Glasgow et al., 1995; Fullard et al., 1991). The promiscuous binding of tear lipocalin is well explained by the three-dimensional solution structure of TL determined by *Site-Directed Tryptophan Fluorescence* (Gasymov et al., 2001). It has been shown that the smaller-sized hydrophobic side chains positioned in the hydrophobic binding site of TL are the main determinant for promiscuous binding. TL shows multifunctional properties, among which are antimicrobial activity, cysteine proteinase inhibition, endonuclease activity, and retinol transport in tears (Yusifov et al., 2008; Gasymov et al., 2002).

The outermost layer of the tear film is the lipid layer secreted by the meibomian glands of the eyelids. The major function of the lipid layer is believed to prevent evaporation of the aqueous layer. The excessive evaporation observed in

some meibomian gland disorders results in corneal drying. This condition leads to epithelial erosions. It has been shown that the interaction of TL with the lipid layer stabilizes this layer to prevent its disruption. This results in an increased tear break-up time which is one of the stability parameters for the tear film.

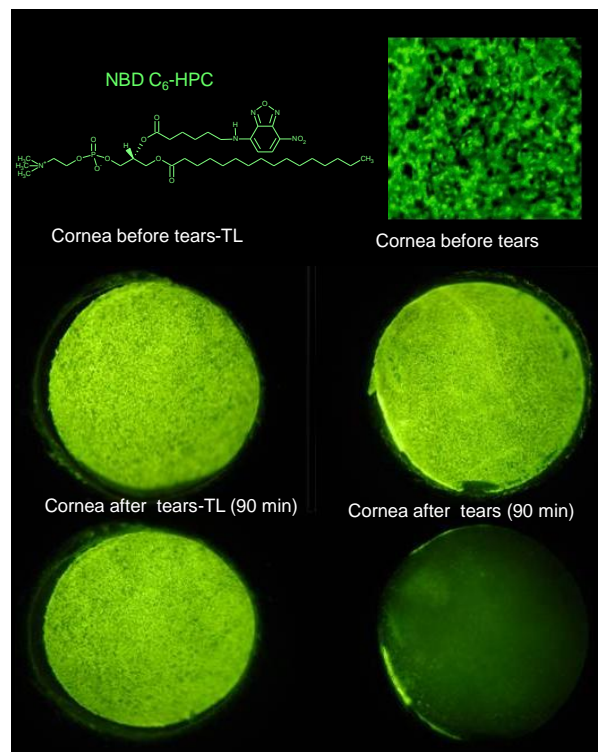
Each layer of the tear film plays an important role to keep the cornea healthy and malfunction of any component may result in dry eye conditions. If not treated on time, dry eye conditions may result in blindness. Below we will provide a brief discussion of how the failure of any of these components leads to dry eye conditions in different ways. The membrane-associated mucin plays a pivotal role in keeping the cornea wet and tear film stable over the cornea. It has been shown that the ectodomain of the mucins acts as a barrier function to prevent bacterial adhesion. This domain also keeps the surface of the cornea wet. Enzymatic ectodomain shedding of membrane mucins at a normal rate is important for many biological functions. However, in some disorders, enhanced ectodomain shedding resulting from hyper enzyme activity creates naked epithelial cells that are prone to lipid contamination (Fig. 1).

**Tear Lipocalin is the principal lipid-scavenging protein in human tears.**

In normal conditions, TL is acting as a scavenger of the lipid from the epithelial cell surface (Glasgow et al., 1999; Gasymov et al., 2005). However, TL does not have enough capacity to remove lipids from the contaminated epithelial surface in excessive lipid binding events. As it is clear from this discussion, TL is a very important protein to keep the epithelial cells clean from lipids and prevent cornea surface drying. The experiments performed on the human cornea identify TL as a lipid scavenger from the cornea surface (Fig. 4). The cornea contaminated with the fluorescent phospholipids is seen as a dotted fluorescence surface under a fluorescent microscope. Incubation of the cornea with tears for 90 minutes almost fully removes the phospholipids from the cornea surface. However, removal is not evident when the contaminated cornea was incubated with the reconstituted tears where TL is removed. This experiment indicates that among many proteins, TL is the principal protein to keep the cornea surface free from

lipids.

TL concentration was significantly lower in some dry eye patients indicating its importance for cornea health. Dry eye conditions may have resulted from meibomian dysfunction, even from the absence of pivotal lipids like phospholipids. The stability of the lipid layer is also provided by the interaction of TL with the lipid layer. Thus, proper functioning of all three layers is important to keep the cornea in a healthy condition.



**Fig. 4.** The figure was modified from the work published in O.K.Gasymov et al., Tear Lipocalin: Evidence for a scavenging function to remove lipids from the human corneal surface, *Investig. Ophthalmol. Vis. Sci.*, 2005, 46, 3589-3596.

One can get dry eye syndrome for various reasons that we have tried to describe above. Artificial tears of different origins are the main treatment options for dry eye diseases. Currently, there are no single best artificial tears that are suitable for all patients with dry eye syndromes. Artificial tears formulated with various polymeric materials, such as polyethylene glycol, carboxymethylcellulose/carmellose sodium, hydroxypropyl methylcellulose, etc have recently been critically reviewed (Semp et al., 2023).

Artificial tears with high concentrations of liposomes have been shown to be very effective to treat patients with dry eye disease with evaporative nature.

Effective artificial tears have also been formulated with protein components. Serum eye drops are an effective treatment for dry eye patients. The major protein component of the blood serum is albumin, which is a fatty acid-binding protein (Oktiadewiand Putra, 2023). It is possible that albumin as tear lipocalin, which is the major fatty acid binding protein in tears, may have properties of lipid scavenging from the corneal surface. On the other hand, albumin has high surface activity and may stabilize the superficial layer of the tears.

## CONCLUSION

It should be noted that proper blinking is very important to keep the cornea lubricated. It is essential for contact lens wears. Today it is very difficult to imagine our life without computers. Spending much time in front of the computer, one should regularly exercise blinking, which is a treatment option for some people with dry eye syndrome. Various types of artificial tears are available to treat dry eye conditions. Finally, pharmaceutical industries have realized the importance of proteins to keep the cornea healthy. Artificial tears with essential tear proteins are now formulated and available in pharmacy stores. Although somewhat expensive; we believe that artificial tears with proteins should be the first choice for dry eye patients.

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## **A new approach to the treatment of optic nerve atrophy as an inflammatory disease**

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**The aim of the study was to conduct an antibacterial treatment of optic nerve atrophy against the microflora of the conjunctiva of the eye (microbiota) in small doses. The treatment was performed in 65 patients with optic nerve atrophy. The age of patients ranged from 12 to 75 years. The patients underwent general ophthalmological studies before and after the treatment. A special place was occupied by the perimetry before and after the treatment. Before the treatment, a bacteriological analysis of the conjunctiva of the eye was determined in patients to establish a nature of the microbial population (microbiota). Then, considering this population (microbiota) a specific drug was selected. The drug was taken in such a minimum concentration in order not to "kill" the existing microflora but only to weaken it. When this dose was exceeded no improvement in visual indices has been observed. This drug was administered subcutaneously. The effectiveness of the therapy was determined 8 and 30 days and 3 months after the end of the treatment; then every 3 months. Thus, in 100% of cases, there was an improvement in the visual field and visual acuity in all patients. Today there is no treatment for optic neuropathy worldwide. The antibacterial treatment of optic nerve atrophy implemented by us gives an effect in almost all cases. This treatment is based on creating a balance between the microbiota of the conjunctiva of the eyes and the antibodies surrounding them. The use of this method can significantly reduce the number of blind people in the world from optic nerve atrophy.**

**Keywords:** *treatment of optic nerve atrophy, optic nerve atrophy, optic neuropathy*

### **INTRODUCTION**

Optic nerve atrophy is a polyetiological disease leading to low vision, blindness, and disability. A common cause of optic nerve atrophy is traumatic neuropathy. The main treatment is the administration of corticosteroids. If vision does not appear within 48 hours with corticosteroid treatment, then this is a poor prognostic sign. Also, a risk factor for vision loss is the presence of blood in the posterior ethmoid cells and loss of consciousness (Carta et al., 2003).

Indirect traumatic optic neuropathy refers to damage to the optic nerve resulting from blows or concussions to the head. The mechanism of damage is not clear. There are no protocols to

prevent, mitigate and treat this disease. Injuries occur in sports, or on vehicles, from the ballistic impact caused by an explosion in war (Eric et al., 2016).

Some authors describe chronic relapsing steroid-responsive inflammatory optic neuropathy. This form of neuropathy is characterized by a strong decrease in vision and the presence of pain in the head during exacerbations of the (Saini and Khurana, 2010) process.

With optic neuropathies, a violation of colour vision occurs, boundaries of the visual field narrow, visual acuity decreased.

The use of digital imaging of the optic nerve and optical coherence tomography has revolutionized the diagnosis of (Biousse and

Newman, 2016) optic neuropathies.

A minor effect in the treatment of chronic optic nerve atrophy occurs with the use of corticosteroids (Yu-Wai-Man and Griffiths, 2011). Literature data of the last 15 years show that the effectiveness of corticosteroids in the treatment of optic neuropathy is low (Levin et al., 1999). It is therefore clinically reasonable to decide to treat or not, on an individual patient basis (Stunkel and Van Stavern, 2018).

No clear benefit was found for either corticosteroid therapy or optic canal decompression surgery. The number of patients studied was sufficient to rule out major effects in the treatment groups, although clinically relevant effects in specific subgroups could have been missed.

These results and the existing literature provide sufficient evidence to conclude that neither corticosteroids, nor optic canal surgery should be considered a standard of care for patients with traumatic optic neuropathy (Wu et al., 2008). Therefore, there is no real cure or treatment for optic nerve atrophy (optic neuropathy).

The studies conducted by me earlier (Hajiev, 2014) showed that any inflammatory process is based on an imbalance between the microbial population and the antibodies surrounding them.

The purpose of the study was to conduct a specific antibacterial treatment of optic nerve atrophy in small doses taking into account the identified microflora of the conjunctiva of the eye.

## **MATERIALS AND METHODS**

The examined group included 75 patients with optic nerve atrophy. 19 of them had an optic nerve atrophy associated with traumatic brain injury; 2 patients were with a background of multiple sclerosis; 9 patients were after neurosurgical operations; and the etiology of the remaining 45 was unknown. Hereditary atrophy of the optic nerve, secondary atrophy associated with edema of the optic nerve were not included in our examination. Age ranged from 12 to 75 years. 38 of them were women, 37 were men. Most of them have been unsuccessfully treated elsewhere.

Patients underwent determination of perimetry, biomicroscopy, tonometry, ophthalmoscopy. As the main indicator of the treatment effectiveness, we used visometry and perimetry. Perimetry was carried out on the automated perimeter 'Medmont M700' company 'Medmont Pty Ltd' (Australia).

Before the treatment, a bacteriological analysis of the conjunctiva of the eye was performed in patients to establish the nature of the microbial population. Then, considering this population (microbiota), a specific drug was selected. This drug was taken in such a minimum concentration in order not to "kill" the existing microflora but only to weaken it. When the indicated dose was exceeded, no improvement in visual parameters was observed. The drug was administered subcutaneously. The effectiveness of the therapy was determined 8 and 30 days after the end of treatment; then every 3 months.

The treatment was carried out with antibacterial agents taking into account the sensitivity of the microflora of conjunctiva of the eyeball. Antibacterial injections were injected subcutaneously into the arm. At the same time, patients were advised to exclude alcoholic beverages, products containing vinegar, ketchup, pickles and fermented milk products from the diet.

We noticed that if patients simultaneously consumed microbial fermentation products at the same time as treatment, then there was no improvement.

## **RESULTS AND DISCUSSION**

Patients were re-examined after treatment on the 8th day, a month later, and 3 months later. It should be noted that improvement occurred already on the 8th day of treatment. This was manifested in a significant expansion of the field of view.

Moreover, 10 patients noticed an improvement in vision, which was expressed in an improvement in visual acuity, improved orientation and an increase in the amount of light. In the rest, visual acuity increased significantly after a month and after 3 months.

A month after the treatment, subjective

improvement in vision was noted by almost all patients. When analyzing computer static perimetry parameters in patients before and after treatment of optic nerve atrophy, a significant positive trend was revealed in the form of a decrease in sensitivity depression in terms of area and intensity.

Moreover, these dynamics improved from the end of the treatment to 3 months or more. Thus, in 100% of cases, there was an improvement in visual field and visual acuity in all patients.

It should be noted that in 3 patients after two years there was a deterioration in vision. In two patients, this was due to covid infection. One patient after cataract extraction surgery. Antibiotics are used after cataract extraction. It is the use of antibiotics after treatment of optic nerve atrophy that can lead to relapse. Repeated treatment resulted in improved vision.

*Example 1.* Patient A.E., 24 years old. Complaints about the lack of vision in the right eye and constantly deteriorating left eye. Visual acuity of the right eye is 0 (zero), left - 0.3 is not correct.

In 1997, a tumor was found in Moscow that filled the sphenoid sinus, cells of the ethmoid bone affecting the platform of the sphenoid bone,

the Sella turcica, the upper and middle third of the clivus (tumor of the base of the skull).

In the same year, a successful operation was performed to remove this tumor. The postoperative period proceeded without complications.

Then radiation therapy was performed and the disease completely disappeared. But afterwards, the vision of the left eye gradually began to deteriorate. The patient was repeatedly treated in Moscow and in other clinics of the country (The Russian Federation is meant).

But the vision continued to deteriorate. A bacteriological analysis was taken from the patient's eye and injections were made in accordance with the sensitivity to an antibacterial drug. Already on the 4th day, the patient noticed an improvement in visual acuity. The improvement continued for the next 3 days. The field of vision expanded dramatically.

*Example 2.* Patient M.A., 16 years old, noticed a deterioration of his/her vision about a year ago. The vision gradually worsened. The vision of both eyes is 0.2. The boundaries of the view field are sharply narrowed.

After the treatment the visual acuity of the right eye in a month became 0.4, the left 0.5 is not correct.

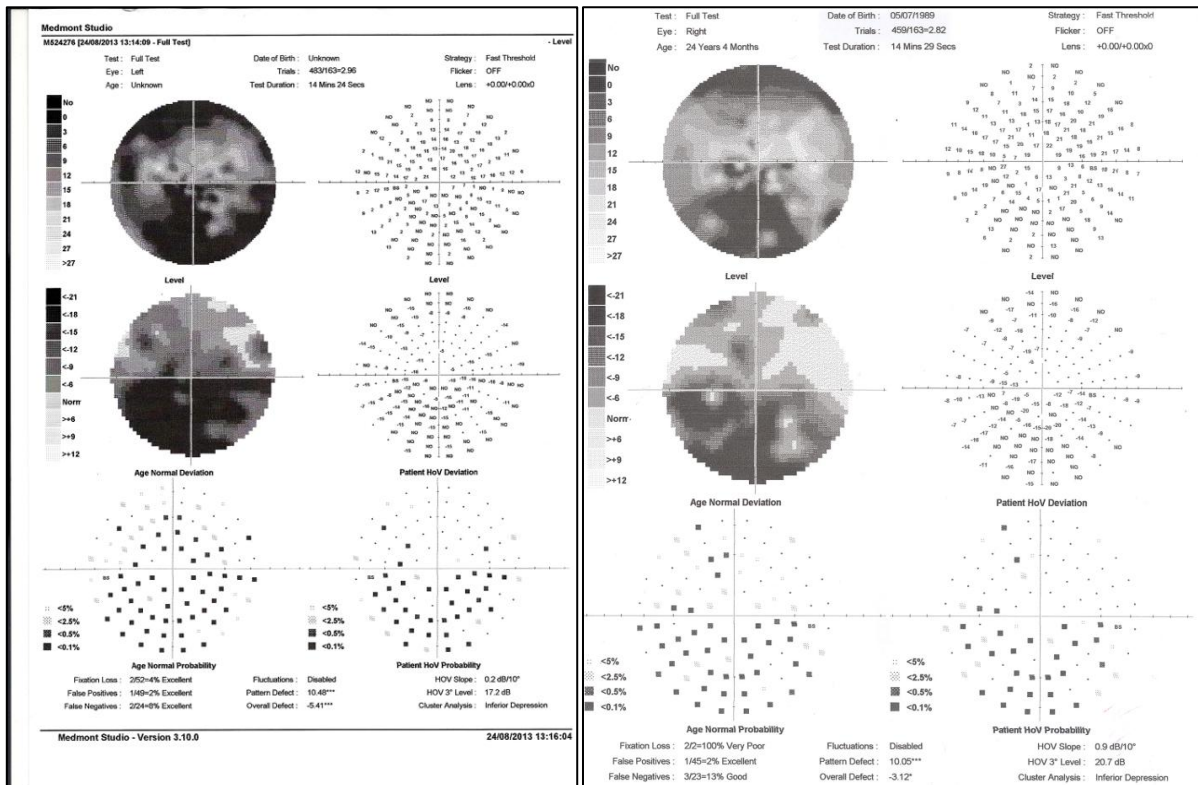


Fig. 1. Perimetry of the left eye of the patient A. E. before and 2 weeks after treatment.

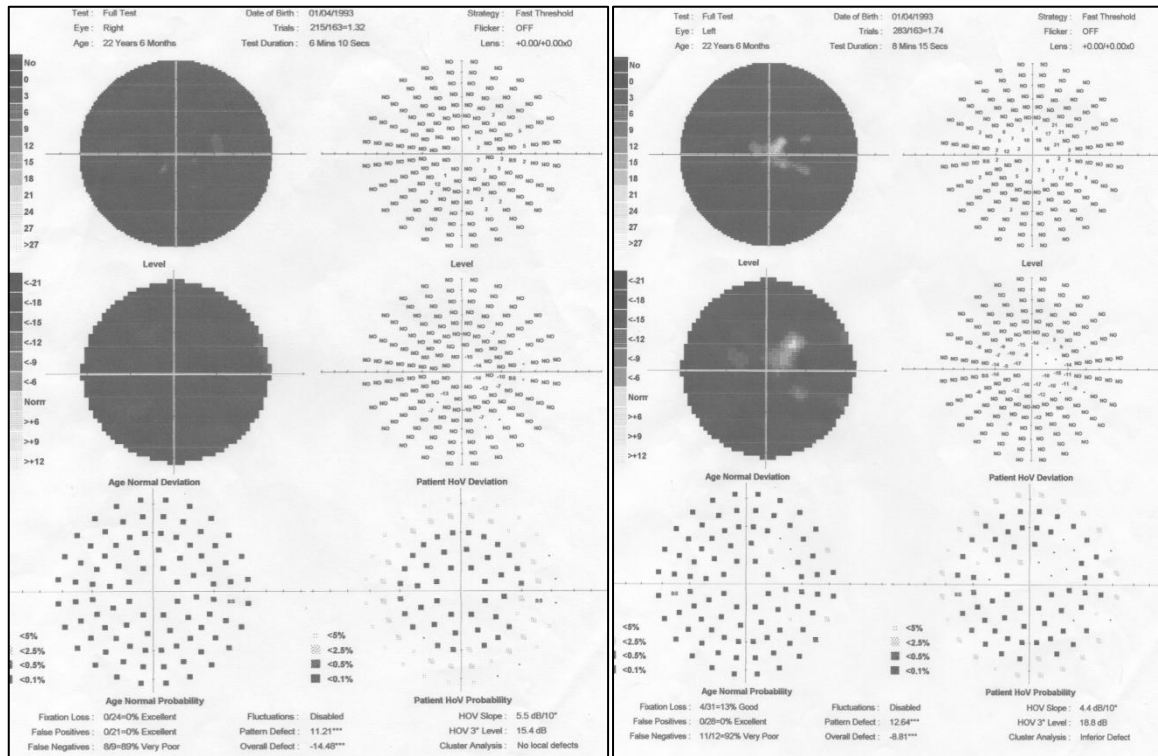
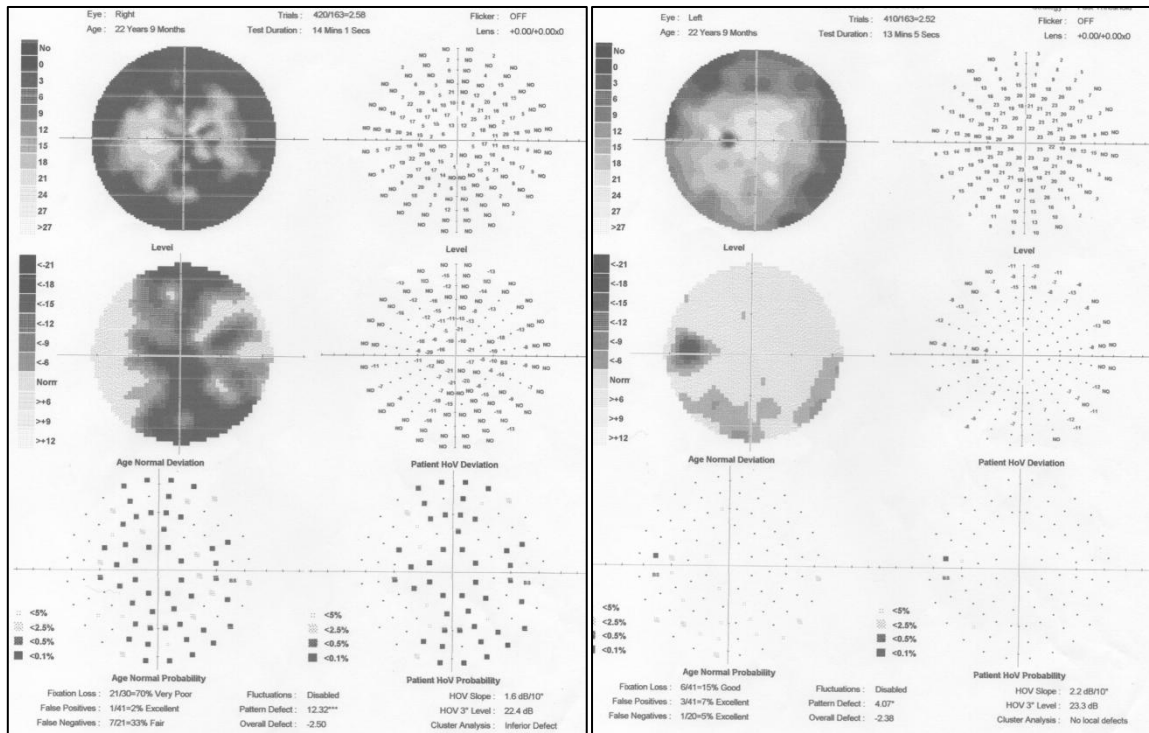


Fig. 2. Perimetry of the right eye of the patient M.A. before treatment and one month after treatment.



**Fig. 3.** Field of vision of the left eye of the patient Z.A. before and after 3 months of treatment.

*Example 3.* Patient Z.A., aged 16, contacted us in 2012. A diagnosis of optic nerve atrophy was made but they refused treatment. treated elsewhere. After repeated appeals to us, the visual acuity of the right eye was almost not determined as 0.01, the left eye was 0.1 (not correct). The field of vision is significantly narrowed.

Antibacterial treatment was carried out according to the method described by us. The vision began to improve already on the 4th day after the start of treatment. A re-examination was carried out after 8 days, a month, and 3 months.

After 3 months visual acuity of the right eye was 0.1, the left eye was 0.6. Fields of view were greatly expanded.

Thus, antibacterial treatment is aimed at weakening the microbial population of the conjunctiva of the eye. Similar studies were carried out by us earlier in the treatment of chronic prostatitis using the hemagglutination reaction (Haciyev 2004, 2014). These studies show that improvement in the condition of patients with chronic prostatitis when using antibacterial drugs was accompanied by an increase in the titer

between the patient's serum and antigen (isolated from urine). Apparently, similar changes occur during the treatment of atrophy (neuritis) of the optic nerve.

## CONCLUSION

Antibacterial-specific treatment of optic nerve atrophy directed against the microbiota of conjunctiva of the eye stops the progression of the disease and significantly improves visual functions.

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## **The dynamics of the volume and structure of demand of the population for high-tech ophthalmological care**

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**To assess the demand of the population for high technological ophthalmologic care. Materials from the Clinic of the Azerbaijan Medical University have been used. Indications for high-tech ophthalmological care were determined by the method of expert evaluation of highly qualified ophthalmologists. The demand of patients of the clinic for high-tech ophthalmological care was  $9.5\pm 0.4\%$  (95% confidence interval 8.7-10.3%). During the 2018-2022 years, the volume of the demand increased from  $5.6\pm 0.7$  to  $14.1\pm 1.2\%$  ( $p=0.01$ ). The demand of contingent patients with eye pathologies for high-tech ophthalmological care dynamically grows mainly due to the need for endoviral surgery.**

**Keywords:** *Eye diseases, volume of demand, structure dynamics, high-tech ophthalmological care*

### **INTRODUCTION**

Wide opportunities for the diagnosis and treatment of diseases in all fields of medicine, especially in ophthalmology have been created depending on scientific and technical progress. A new level of specialized ophthalmological care – high-tech ophthalmological care (HTOC) system is one of the perspective fields of healthcare (Bass and Sherman 2004; Mets et al., 2012; Kraus et al., 2021). HTOC is developed especially during the last 20 years in former Soviet states. In the Russian Federation, expensive medical services are attributed to high-tech medical services and people are not fully provided with these services. That's why high technological medical services are provided on basis of quotas set at the expense of the health insurance account. The social and economic situation in regions of the Russian Federation extremely differs from each other and the planned volumes and structures of HTOC are different (Tereshchenko et al., 2018; Tsipyashchuk et al., 2017). Nearly 70% of HTOC provided to the population in the region Kaluga of the Russian Federation consists of transpupillary,

micro-invasive energetic optic-reconstructive surgery related to vitreoretinal pathologies (Tereshchenko et al., 2018). Most of the HTOC (44.6%) in the Saratov region of the country is related to cataract phacoemulsification surgery resulting in intraocular lens implantation. In this case, the share of vitreoretinal surgery in HTOC is 13.9 (Tsipyashchuk et al., 2017). The World Health Organization recommends to all countries prepare strategic plans for the development of HTOC (World Health Organization, 2022). In most countries' material, technical and personnel potential for HTOC is created on basis of Medical Universities and Scientific-Research Institutes (Egorov, 2019; Egorov, 2019). The optimal conditions for HTOC in the state healthcare system of the Republic of Azerbaijan are available in the National Ophthalmological Center named after Z.Aliyeva and in the Surgical Clinic of the Medical University. The experience of these institutions allows us to scientifically justify the medical and organizational problems of HTOC.

The purpose of the study was to assess the demand of the population for high technological ophthalmologic care.

## MATERIALS AND METHODS

The scientific research work was conducted in the Department of Ophthalmology of the Teaching-Surgery Clinic of the Azerbaijan Medical University. The patient undergoing surgical treatment at the clinic was an observation object of the study. The object of observation of the society as a whole was formed by a randomized method by the selection of one among every five numbers of the outpatient directory. According to the nosologic forms creating the need for HTOC (complicated glaucoma; diseases of the cornea, crystal and vitreous body; proliferative diabetic retinopathy combined with eye diseases: retinal detachment with tearing; complicated cataract; complicated eye injuries; Complicated corneal ulcer; corneal clouding and scarring; complications of previously performed optical-reconstructive operations) groups with the similar number of patients (100 patients in each group) have been detected. The number of patients in each group ensures that the margin error of expected results (tm) is less than 5%.

The need of patients for HTOC was detected by an expert assessment of experienced doctors of the clinic. The statistical processing of the results was done by statistical methods of qualitative features with the help of the "data analysis"

envelope of the Excel program on a personal computer. Differences between groups were assessed by the  $\chi^2$  test (Sabirziyanova et al., 2021).

## RESULTS AND DISCUSSION

The achieved results on the demand of the population for HTOC connected with eye diseases are given in Table 1.

As seen in the table,  $9.5 \pm 0.4\%$  of patients who applied to the Ophthalmology Department of the Medical University for Surgical Treatment (95% confidence interval 8.7-10.3%) need HTOC. The volume on demand ranges within the narrow interval depending on nosologic form: low rate demand ( $2.4 \pm 0.7\%$ ; 95% confidence interval 1.1-3.7%) is characteristic of chorioretinal inflammation, retinoschisis, retinal cyst, occlusion of retinal vessels, proliferative retinopathy, diseases of the vitreous body that occur together with degeneration of the posterior arc and macula. The high level of demand on HTOC ( $15.6 \pm 1.6\%$ ; 95% confidence interval 12.4-18.8%) was detected among patients with diagnosed crystal, vitreous body diseases, secondary glaucoma, a patient diagnosed with proliferative diabetic retinopathy with macular damage.

**Table 1.** The frequency of HTOC demand depending on the nosological form of eye diseases

Nosological forms causing HTOC demand	N	n	The frequency of HTOC demand %	95% confidence interval	
Glaucoma is complicated by high intraocular pressure	500	32	6.4±1.1	4.2 – 8.5	
Associated with chorioretinal inflammation, retinoschisis, retinal cyst, retinal vessel occlusion, proliferative retinopathy, posterior arc and macular degeneration, vitreous hemorrhage	crystal diseases	500	41	8.2±1.2	5.8 – 10.6
	corneal diseases	500	18	3.6±0.8	2.0 – 5.2
	vitreous diseases	500	12	2.4±0.7	1.1 – 3.7
Crystal and vitreous diseases, secondary glaucoma, proliferative diabetic retinopathy with macular compensation	500	78	15.6±1.6	12.4 – 18.8	
Retinal tears are complicated by diseases of the cornea, lens, retina, and vessels	500	61	12.2±1.5	9.3 – 15.1	
Cataracts complicated by glaucoma, vitreous, retinal and choroidal diseases	500	70	14.0±1.6	11.0 – 17.0	
Crystal and vitreous diseases, ophthalmological hypertension, eye injuries complicated by orbital fracture	500	27	5.4±1.0	3.4 – 7.4	
Corneal clouding and scarring	500	62	12.4±1.5	9.5 – 15.3	
Complications of previously performed optical reconstructive operations	500	74	14.8±1.6	11.7 – 17.9	
Total	5000	475	9.5±0.4	8.7 – 10.3	

*Note:* N- the number of expert assessments; n – patients who need HTOC;

**Table 2.** Dynamics of the frequency of HTOC demand during 2018-2022 years (%)

Nosological forms causing HTOC demand	2018	2019	2020	2021	2022	P≤	
Glaucoma complicated by increased intraocular pressure	2±1.4	4±1.9	7±2.6	9±2.9	10±3.1	0.05	
Associated with chorioretinal inflammation, retinoschisis, retinal cyst, retinal vessel occlusion, proliferative retinopathy, posterior arc and macular degeneration, vitreous hemorrhage	crystal diseases	5±2.1	6±2.3	9±2.9	9±2.9	12±3.3	0.05
	corneal diseases	2±1.4	3±1.7	4±1.9	4±1.9	5±2.1	0.08
	vitreous diseases	2±1.4	2±1.4	2±1.4	3±1.7	3±1.7	0.12
Crystal and vitreous diseases, secondary glaucoma, proliferative diabetic retinopathy with macular compensation	11±3.1	14±3.5	16±3.7	17±3.8	20±4.3	0.01	
Retinal tears are complicated by diseases of the cornea, lens, retina, and vessels	6±2.3	8±2.7	11±3.2	16±3.8	20±4.3	0.01	
Cataracts complicated by glaucoma, vitreous, retinal and choroidal diseases	8±2.7	10±3.0	13±3.4	18±4.0	21±4.3	0.01	
Crystal and vitreous diseases, ophthalmological hypertension, eye injuries complicated by orbital fracture	3±1.7	4±1.9	5±2.1	7±2.6	8±2.7	0.06	
Corneal clouding and scarring	7±2.5	8±2.7	9±2.8	17±3.9	21±4.4	0.01	
Complications of previously performed optical reconstructive operations	10±3.0	12±3.2	14±3.5	17±3.9	21±4.3	0.05	
Total	5.6±0.7	7.1±0.8	9±0.9	11.7±1.1	14.1±1.2	0.01	

High frequency of demand on HTOC is characteristic for patients applied with diagnosed complications of previously performed optical-reconstructive operations (14.8±1.6%; confidence interval 11.7-17.9%), cataract complicated by glaucoma, diseases of the vitreous body, retina and blood vessels (14.0±1.6%; 95% confidence interval 11.0-17.0%).

HTOC demand is on a high level among patients with diagnosed retinal detachment with tearing alongside diseases of the cornea, lens, retina and choroid (12.2±1.5%; 95% confidence interval 9.3-15.1%), corneal clouding and scarring (12.4±1.5%; confidence interval 9.5-15.3%).

The demand for HTOC of patients with crystal pathologies (8.2±1.2%; 95% confidence interval 5.8-10.6%) that appear together with chorioretinal inflammation, retinoschisis, retinal cyst, retinal vessel occlusion, proliferative retinopathy, posterior arc and macular degeneration, iris hemorrhage is more for 2-3 times in comparison with patients with corneal diseases (3.6±0.8%; 95% confidence interval 2.0-5.2%) and vitreous body diseases (2.4±0.7%; 95% confidence interval).

Thus, the HTOC demand is not stable and undergoes a significant change depending on the nosological forms of eye diseases, their comorbidity (polymorbidity) degree.

Information about the frequency of HTOC

demand of patients applied to the clinic during the 2018-2019 years is given in Table 2.

HTOC demand of patients during 5 years of observation increased from 5.6±0.7% (95% confidence interval 4.2–7.0%) to 14.1±1.2% (95% confidence interval 11.7-16.5%) (p=0.01). The increasing trend is linear, the approximation of the regression equation describing the dynamics ( $y=2.16x+3.02$ ; x-calendar years) is very high (determination coefficient  $R^2=0.9881$ ). A similar trend was typical for common variants of all distinguished nosological forms. The frequency increase rate of the demand for HTOC during the five-year observation period (the ratio of the indicators of 2022 and 2018) is mostly typical for patients diagnosed with complicated glaucoma (5 times), corneal clouding and scarring (3 times). A relatively low rate of increase was observed according to HTOC frequency related to cornea diseases within chorioretinal vessel occlusion, proliferative retinopathy, posterior arc and macular degeneration, vitreous hemorrhage (2.0±1.4% in 2018, 5±2.1% in 2022; p=0.08).

Thus, the demand of the patients applied to the clinic for treatment during 2018-2022 years for HTOC changes with different dynamics depending on the nosological form and variants of polymorbidity, growth rate is typical for all pathologies.

Based on the recommendations of experts to

meet the demand for HTOC special weights of the necessary operations are arranged correspondingly: treatment of vitreoretinal pathologies with transpupillary, energetic optical-reconstructive microinvasive, 23-25G endovitreol surgery (44.2%), glaucoma (6.7%) and corneal diseases (14.7%) with microinvasive energetic optical reconstructive and laser surgery complex treatment, other operations (34.4%).

There is limited information about HTOC in the literature. 23-27G of transpupillary, energetic optical-reconstructive microinvasive endovitreol surgery treated vitreoretinal pathologies (69.66%) occupied the first place among the types of HTOC provided in the Kaluga Branch of the Russian Eye Microsurgery complex, the specific share of other surgeries was between 0.22-11.34% (Tereshchenko, 2018). In the clinic of Saratov Medical University, the specific share of these types of surgeries was 78.5 and 21.5%, respectively (Tsipyashchuk et al., 2017). In the Khabarovsk branch (Egorov 2019), these indicators were 44.2 and 55.8%. As can be seen, the division of the population demand for HTOC according to the type of surgical operations is different.

The volume of the demand for HTOC tends to increase according to the results of all studies (Tereshchenko, 2018; Tsipyashchuk et al., 2017; Egorov 2019; Egorov, 2019; Glantz 1999). The volume of HTOC provided to the population during 2016-2021 years increased in Tatarstan 1.7 times (Sabirziyanova, 2021), 3/5 times in Khabarovsk during 2006-2010 years (Egorov, 2019), and 2.5 times during 2018-2022 in our observations. The general trend in these results is clearly observed dynamic growth of population demand.

So, the obtained information about the demand of the population for HTOC generally agrees with the information given in the literature, although they have different characteristics according to their quantitative characteristics (structure and dynamics of demand-forming pathologies and necessary surgical operations).

## CONCLUSIONS

1.  $9.5 \pm 0.4\%$  of patients who should undergo surgical treatment of eye diseases need high-

tech ophthalmological care.

2. The frequency of demand for high-tech ophthalmological care depends on nosological forms of eye diseases:  $15.6 \pm 1.6\%$  of patients with chorioretinal inflammation, retinoschisis and retinal cyst, retinal vessel occlusion, proliferative retinopathy, proliferative diabetic retinopathies co-occurring with vitreous diseases co-occurring with degeneration of the posterior arc and macular need high-tech ophthalmological care
3. The specific share of those demanding high-tech ophthalmological care among patients requiring surgical treatment for eye diseases during the 2018-2020 years increased from  $5.6 \pm 0.7\%$  to  $14.1 \pm 1.2\%$ .

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## The application of regenerative medicine in ophthalmology

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**The causes of blindness vary widely around the world including the degeneration of particular groups of retinal cells, such as retinal pigment epithelium (RPE), photoreceptors, and retinal ganglion cells. These cells could be generated ex vivo from progenitor stem cells thanks to advancements in retinal regenerative medicine over the past ten years. Here, we discuss the development of cell replacement using novel technologies, to restore eyesight in degenerative diseases. We go over the state of advanced preclinical research for other cell kinds as well as human clinical trials for RPE transplantation. We also discuss the developments in using endogenous progenitor cells to fix retinal degeneration in situ. Last but not least, we give a high-level summary of the development of prosthetic ocular vision restoration using advanced photovoltaic devices, opsin-based gene therapy, and small-molecule photoswitches.**

**Keywords:** *Tissue engineering, Ocular disease, regenerative medicine, retinal disease*

### INTRODUCTION

Our eyes, which serve as the openings to the outside world, give us data regarding our surroundings every 20 milliseconds, enabling us to recognize objects, motions, and a wide range of color tones. The retina, a layer of nervous tissue that includes above 100 million rod and 6 million cone photoreceptors in humans, is in charge of perceiving and integrating sensory data (Rehman et al., 2018).

The retina has two extra neuronal layers that are located below the photoreceptor layer. One of these layers contains horizontal cells (HCs), bipolar cells (BCs), and amacrine cells (ACs), and the other contains displaced ACs and retinal ganglion cells (RGCs). The axonal and dendritic events of the neighboring neurons create two plexiform layers in between those layers, creating a special environment for the establishment of horizontal and vertical synaptic networks through the tissue (Mahabadi et al., 2021).

Retinal cytoarchitecture depends on Mueller glial cells due to the lack of an extracellular matrix, which is similar to other areas of the central nervous system (MGs). In addition to providing structural support, 2 MGs are crucial for maintaining a healthy balance of neurotransmitters, trophic factors, and compounds because they extend complex cellular pathways from the inner to the outer limiting membrane (ILM/OLM) and across both plexiform layers (Liu et al., 2021).

Considering the enormous structural intricacy, retinal development is highly regulated and evolutionary conserved. The retinal pigment epithelium (RPE) and the neural retina are two distinct regions that form upon the stimulation of the eye field. In the latter, the unique layering of the retina is shaped in an inside-out manner by the growth, differentiation, and interkinetic nuclear migration of retinal progenitor cells (RPCs) (Cheng et al., 2022).

All subgroups of retinal neurons and MGs are created during this process from a single RPC community, but astrocytes and microglia present

in the mature retina are not created from RPCs. RGCs are created first during neurogenesis, followed by ACs and HCs, and then cone photoreceptors. Along with MG cells, rod photoreceptors, and BCs are produced last and, in some species, only reach full maturity after delivery.

Interestingly, whereas neurogenesis only persists for limited hours in lower animals, it can last for days or even weeks in rodents, pigs, primates, and humans (Amrein et al., 2011). In higher animals, the end of retinogenesis occurs simultaneously with the full reduction in RPCs. On the other hand, RPCs are limited to the ciliary in lower vertebrates (Amrein et al., 2011).

From this moment on, only lower vertebrates undergo one of three processes for intrinsic regeneration following the deterioration of retinal neurons, glia, or RPE: Mueller glial cells can either: (1) reenter the cell cycle; (2) reactivate growth within RPC in the CMZ; or (3) dedifferentiate from RPEs into RPCs (Willardsen et al., 2014).

The ability of MGs to regenerate in higher vertebrates appears to be diminished, and despite recent research showing that MG-specific overexpression of ASCL1 and histone deacetylase inhibition can restore this ability in young mice, the retina of higher vertebrates is still inherently prone to disease and damage (Fan et al., 2016).

Neurodegenerative ailments, including glaucoma and age-related macular degeneration (AMD), are caused by extra stress or insult in addition to the continuous, age-associated loss of neurons (Bhattacharyya et al., 2022).

According to the National Eye Institute, by 2050, there will be twice as many people suffering from neurodegenerative diseases, necessitating more suitable care (Meleth et al., 2011). By 2020, 196 million people are expected to be affected by AMD alone, which has an 8.7% worldwide prevalence (45-85 years old) (Wang, 2020). Glaucoma is expected to influence 76 million individuals globally. Both circumstances' symptoms can presently be controlled for long years, but the causative neurodegeneration that causes the progressive loss of vision is not possible to be stopped (Smith, 2017).

Cell/tissue replacement has recently emerged as an apparent method to stop damage progression

and restore formerly lost vision, thanks to developments in stem cell biology. The earliest clinical trials in the area of cell-based treatment have been conducted as a result of ongoing partnerships between basic scientists and active ophthalmologists, with further clinical studies in the upcoming years. In the current paper, we will highlight the advancements and successes of regenerative medicine.

## **Biomaterials for regenerative ophthalmology**

### **Collagen**

The most prevalent element of extracellular tissue is collagen (ECM). For the best transparency, refractive, and mechanical properties, corneal collagen is extremely organized, and its fibrils are grouped with regular spacing and alignment. Majumdar et al. (2018) measured the cornea's power, strength, and elastic elasticity (Majumdar et al., 2018). Therefore, cross-linked collagen scaffolds that imitate the natural corneal stroma have been applied a lot in corneal regenerative medicine, along with collagen and other collagen-based strategies (Goodarzi et al., 2019).

Utilizing collagen, common tissue engineering techniques use hydrogels, sponges, films, and enhanced substrates. A conventional method based on solubilized collagen is the use of collagen hydrogels. These platforms are not sufficiently sturdy, though. Some cross-linking substances may break down in living things and cause cytotoxicity (Spoerl et al., 2007). In contrast, the microstructures found in collagen sponges and films are better in the context of physiochemical characteristics.

Gels constructed from fibrillar collagen are known as collagen hydrogels. To create firm, rigid shapes, crosslinking is required for all collagen hydrogels (Liu et al., 2019). DC/N-hydroxysuccinimide (NHS)-cross-linked recombinant human collagen (RHC) type III scaffolds were the first to be implanted in patients by Fagerholm et al. (2014). This led to the regeneration of corneal epithelial with the generation of subepithelial nerves and the proliferation of stromal cells in scaffolds. Additionally, 24-month follow-up tests confirmed that all patients' ocular implants remained stable



and avascular and that their tear film had returned.

A permeable collagen fibril network makes up collagen sponges. Collagen sponge production makes it easier to regulate porosity and strength. As collagen sponges, fibrillar sponges, and type I collagen have both been utilized. The primary benefit of bovine collagen type I is that it is porous, allowing corneal stromal cells to enter corneal scaffolds and enhance scaffold remodeling in animal models. Compared to collagen hydrogels, collagen sponges have a considerably greater light transmission. Additionally, they are preferred for stromal matrix repair over collagen hydrogels.

### **Gelatin**

Gelatin is made of collagen after it is hydrolyzed. It is mainly used in three different areas for the engineering of regenerative eye tissue: bioadhesives, cell-sheet carriers, and structural scaffolds (Rose et al., 2014). Gelatin has recently been used in regenerative eye tissue engineering. Gelatin is primarily used as cell-sheet carriers and structural supports in implantable materials. For the repair of optic tissue, 3D printing technologies in terms of gelatin-based 3D tissue models are advancing quickly.

Animal collagens can be used to make gelatin. Additionally, it can be produced as type A gelatin using alkaline pre-treatment and type B gelatin by acidic pre-treatment (Chancharern et al., 2016).

The ocular and limbal epithelium uses gelatin. In a rabbit model, the use of a membrane made of gelatin, HA, and carboxymethyl chitosan (CMCTS) as an epithelial transplantation scaffold led to successful ocular wound healing. Research might provide a novel outlook in light of the pressing need for the creation of an ideal scaffold for corneal regeneration.

The use of gelatin in the ocular stroma. The primary difficulty in producing a fibrous matrix from a single, robust, and entirely transparent structure is sparsely populating it with the proper nerve fibers and dormant keratocytes. Glutaraldehyde cross-linked gelatin can promote fibroblast adhesion and ECM deposition in vivo. It is also being implanted into the corneal stromal pocket of rabbits (Angunawela et al., 2012). Gelatin/ascorbic acid cryogels as keratocyte

carriers were found to be biocompatible, stable, and cytoprotective constructs (He, Wang et al., 2021).

The ocular endothelium uses gelatin. The possibility of a gelatin carrier for endothelial sheet. In this study, the intraocular pressure (IOP) and corneal thickness of the human corneal endothelial cell-gelatin implantation were found to be comparable to those of the healthy cornea. For the transport of cultured human corneal endothelial cells into the eye's anterior chamber, in another study heparin-modified gelatin scaffolds were developed (Niu et al., 2014). These scaffolds, which have been altered to ingest and then release growth factors, might work well as cadaveric corneal transplant substitutes.

### **Chitosan**

Chitosan (CS), a naturally occurring, biodegradable linear polymer with a variety of biological activities, plentiful sources, and generally stable physicochemical characteristics, is frequently used in biomedical applications. CS-based delivery materials provide significant benefits over traditional drug delivery systems in combating ocular problems (Mahmoud et al., 2011) (both local and systemic) because of their unique physiological barriers.

Glucosamine and N-acetylglucosamine molecules make up the CS family of linear polysaccharides, which are connected by (1-4) glycosidic links. Chitin which is typically used in its partial deacetylation form is a natural polysaccharide (Heustis et al., 2012). The cell walls of fungi and the carapace of crustaceans like prawns and crabs are both rich in chitin. Chitin from natural sources must first be freed from proteins and minerals. The key elements influencing the characteristics of CS are its average molecular weight and degree of deacetylation, which typically ranges from 66% to 95%. (3.8–20 kD).

Although CS has low alkalinity, it has high hydrophilicity, is water-insoluble, and is stable in both neutral and alkaline conditions. The amino groups of CS are protonated in diluted aqueous acidic solutions, which aids in the substance's breakdown. As a solvent, an acetic acid buffer between 1% and 3% is typically used. It has been possible to alter CS chemically through a number of processes, including carboxymethylation,

reductive amination with phosphorylcholine glyceraldehyde, carboxyethylation, sulfation, N- or O-acylation, and quaternization.

Strong plasticity in CS allows it to be transformed into a variety of shapes, including filaments, gels, microspheres/microcapsules, and micro/nanoparticles. Additionally, it has a lot of functional groups, like free hydroxyl or amino groups, in its construction as well as surface charges that allow it to variably absorb or encapsulate medications with various properties. Additionally, the addition of CS has no impact on the physicochemical characteristics of medicines. CS has an outstanding biological adhesion, coagulation capability, and immune-inducing function in ocular mucosa due to its powerful biological adhesion and instantaneous intercellular permeability (Sun et al., 2022).

Controlled drug release is made possible by CS nanoparticles, which enhance drug durability, solubility, and effectiveness while lowering toxicity. The drug release from CS nanoparticles is controlled by several processes, including drug diffusion, polymer swelling, drug diffusion through the polymeric matrix, and polymer erosion or degradation. Drug diffusion from the polymer surface and polymer swelling, which forms pores, are thought to be responsible for the early liberation of drugs from CS nanoparticles. Additionally, due to their stability, CS nanoparticles display pH-dependent drug release.

CS nanoparticle-related ocular drug delivery can substantially enhance the bioavailability of drugs in the eye when compared to conventional delivery methods (Janagam et al., 2017). Additionally, pH-responsive, thermosensitive, and ion-sensitive CS-based hydrogels are possible. One instance is the management of ocular hypertension, a major glaucoma risk factor (for additional instances, see the section on smart biomaterials). The drawbacks of traditional ocular therapies include their quick clearance and low ocular bioavailability. Antiglaucoma medications must therefore be used frequently and over an extended period, which leads to the formation of local side effects and non-adherence - one of the main reasons why treatments fail.

### **Hyaluronan (HA)**

HA as an unbranched polysaccharide

macromolecule, contains repeats of N-acetyl glucosamine and glucuronic acid. HA is the most abundant component of the ECM weighing in the range of 0.1 to >2 million Da. HA is the key element of diverse cellular processes such as wound repair, regeneration, matrix organization, and signaling cascades.

Furthermore, it has exceptional physicochemical features, such as high biocompatibility, biodegradability, mucoadhesive, and viscoelasticity.

This has led to the development of exogenous HA as an excellent drug delivery system.

Chen et al. developed a HA hydrogel scaffold-based xeno-free culture method for the ex vivo cultivation of human cornea epithelial stem cells (Chen et al., 2017) in which there is no need for allogenic or heterogenic biological products, such as transmissible diseases, tumorigenesis, the acceleration of immunologic rejection, or biological diversity (e.g., fetal bovine serum, human amniotic membrane, and murine feeder cells). Using this innovative culture method, a native-like corneal comparable construct with proliferative potential is available. In another study gelatin, carboxymethyl CS, and HA were used to create the best scaffold for growing main rabbit CEpCs.

In corneal alkali-burned rabbits, the biodegradable, and transparent, composite membrane for CEpC binding and growth was successfully implanted. In place of high-quality donor cornea transplantation, HA-based hydrogels crosslinked with hydrazine were used for the therapeutic delivery of adipose stem cells to regenerate injured corneal stromal tissues (Koivusalo et al., 2018).

Despite HA's benefits as a substance for scaffolds, using HA scaffolds has led to some unfavorable effects. The exterior of HA scaffolds might take up different body proteins and trigger inflammatory reactions, which in turn cause denaturation. Furthermore, due to HA's big molecular size, inflammation brought on by HA scaffolds cannot be eliminated by macrophage phagocytosis. As a result, HA scaffolds are rarely used in regions with increased blood flow (e.g., retina and choroid). It is necessary to conduct additional research to expand their utility.

The anti-inflammatory properties of HA, along with its favorable biocompatibility and biodegradability, make it a popular choice for slow-release liquid chemical delivery and nanotherapy. In order to stop excessive fibroblast proliferation and scarring after glaucoma filtration surgery, Shao et al. (2011) suggested a HA film as a vehicle for the slow release of LDL-MMC-CS nanoparticles at the subconjunctival filtering site (Shao et al., 2011). Similarly to this, Huang et al. (2018) showed how to successfully treat DED in rabbits by reducing inflammation using gelatin-epigallocatechin gallate nanoparticles and HA eye drops (Huang et al., 2018).

### **Products of platelets**

Platelet procoagulants contain significant amounts of cytokines and growth factors that are necessary for tissue regeneration and can stop blood loss at the location of a vessel injury. Platelet derivatives are described as preparations produced from autologous or allogeneic platelets that have a greater platelet concentration than was seen at baseline. Platelet gel, platelet-rich plasma, platelet-rich fibrin, and platelet eye drops, are the made products. To encourage and hasten tissue repair, RM practitioners frequently use these preparations. The structure, composition, growth factors, and cytokine concentrations of these compounds vary.

The implementation of blood and its derivatives in ophthalmology is not novel (Giannaccare et al., 2020). In the past, using blood was items on the surface of the eye were mentioned in the Ebers Papyrus, which dates back 3,000 years. Initially, autologous serum eye drops (SEDs) were used to treat patients with DED, and a mobile ocular perfusion pump was applied to administer autologous serum or plasma to the ocular surface of the eyes injured with chemical burns (Giannaccare et al., 2020).

The concept of concentrating and using platelets as a treatment opportunity has steadily gained ground as the research in platelet physiology and pathology has advanced. In reality, a fibrin gel with and without platelets was first presented as a biomaterial with hemostatic and adhesive possessions in the early 1990s (Peng et al., 2021). Platelet-rich preparations were intended to take the place of blood clots as the

original justification for platelet goods. Large quantities of proteins and growth factors are secreted by activated platelets. This local environment promotes tissue regeneration mechanisms.

The typical method for making platelet variants involves drawing blood and centrifuging it. Although there are numerous preparations available, none have yet received widespread acceptance. The release and effectiveness of PDGF may undoubtedly be impacted by variations in platelet preparation techniques (Reigstad et al., 2005).

The conditions for centrifugation, the quantity and quality of platelets, exogenous platelet preactivation, the break between injections and the quantity of treatments required are some of their manufacturing factors. Centrifugation is a crucial factor that significantly affects quality and differs greatly in terms of duration and speed. High centrifugal forces during preparation are able to stimulate platelets, reducing platelet activity and function. Additionally, there is debate over the ideal platelet percentage.

The present method for making platelet-rich plasma entails collecting blood in an acid, citrate, and glucose solution and centrifuging it. To produce platelet pellets and platelet-poor plasma, a second, quicker centrifugation is performed on platelet-rich plasma (PRP) (PPP). Thrombin, the most effective platelet activator, causes PRP to produce growth factors. There is a significant risk of coagulopathy when bovine thrombin is administered in clinical settings because it can rarely cause advances in the production of antibodies to coagulation factor V, factor XI, and human thrombin.

The decision between autologous and allogeneic platelet derivatives is crucial in therapeutic practice. The use of autologous platelet derivatives eludes the ethical consequences related to the risk of exposing patients to allogeneic blood constituents in the absence of a pathogen inactivation process, especially in nations with high infection rates and restricted donor testing. Contamination during gathering and processing poses the lone infection risk connected to autologous products (De Pascale et al., 2015). Additionally, patients might find

autologous goods more agreeable. Individual biologic variations are another drawback of allogeneic platelet derivatives made from healthy donor blood via standardized methods used at blood transfusion services.

### **Stem cells**

Stem cells can self-renew and can differentiate into different kinds of cells. These cells can experience limitless self-renewal, exist in an unspecialized state, and are able to differentiate into various cell types, which are their three defining characteristics. Stem cells can be categorized into embryonic stem cells (ESCs), neonatal stem cells, and adult stem cells depending on the source (ASCs). The embryo-derived stem cells, including ESCs, amniotic stem cells, and umbilical tissue-derived stem cells, are the subject of the following part. These pluripotent cells are a very hopeful source for future treatment options for various eye diseases.

### **Gene therapy**

Voretigene Neparvovec, which contains the RPE65 photoisomerase gene delivered to the retina through an adeno-associated virus (AAV)55, was the first viral gene therapy authorized for any neurologic condition. The US FDA authorized this biopharmaceutical in 2017. The congenital blinding illness 'Leber congenital amaurosis' RPE65-deficient variant is treated with subretinally administered virus injections (LCA). In controlled clinical trials, the treatment restored functional vision in young patients with this condition, demonstrating the viability of ocular gene therapy. Nevertheless, this particular form of gene therapy is focused on a single gene.

Replacement treatments are difficult because retinitis pigmentosa has been linked to more than 60 genes. Intraocular gene therapy may have extra drawbacks, such as persistent degeneration in the face of the treatment. However, this strategy is still hopeful for some inherited retinal illnesses, whether it be through replacement or immune system activation in reaction to the viral vector 58. Utilizing viral-based gene therapy is an alternative method for expressing trophic factors that indicate proliferation and survival and whose expression might leisurely the degenerative procedure.

Such a strategy has been shown to be successful in animal models for some trophic

factors, including ciliary neurotrophic factor (CNTF), pigment epithelial-derived factor, brain-derived neurotrophic factor, and rod-derived cone viability factor, though anatomy preservation is typically more important than visual function preservation (Hojo et al., 2004).

In a different method of gene therapy for vision restoration known as optogenetics, phototransducing opsins, which turn light into electrical impulses, are inserted into the bipolar or retinal ganglion cells that are still present in outer retinal degeneration using viral vectors. Seven transmembrane proteins that gate cation or anion channels in response to light exposure, derived from microbes, are packaged in AAV under either a general promoter or a promoter unique to retinal ganglion cells.

There are currently at minimum 3 clinical studies that use channelopsins delivery through gene therapy. Since channelopsins are less light-sensitive than natural human opsins, they must be expressed widely using the best gene therapy vectors. This approach holds great potential for vision restoration, but it also carries the theoretical danger of immune reactions to foreign proteins.

It has been demonstrated that a comparable strategy utilizing the human G-protein-coupled receptors rhodopsin or cone opsin can restore visual function in animals suffering from outer retinal degeneration. Due to G-protein amplification, mammalian opsins are more sensitive than channelopsins and, as natural proteins, do not trigger immune reactions. Clinical studies involving humans are being developed for this strategy.

Animal models of outer retinal degeneration have also demonstrated the effectiveness of eyesight restoration using azobenzene-based photoswitches. Through photoisomerization of an azobenzene moiety that is covalently linked, voltage-gated potassium channel-blocking drugs are made active in light using this method (Bregestovski et al., 2019). These substances make retinal ganglion cells immediately photosensitive after intravitreal injection (Bregestovski et al., 2019). This strategy prevents the irreversibility of gene therapy but calls for multiple sessions.

In recent years, numerous basic challenges in

cell production and retinal disease modeling that stood in the way of the clinic have been overcome. The present push in the arena is towards clinical translation, critical assessment of functional outcome measures, and the integration of combinational treatments. The most challenging fundamental problems, such as synaptogenesis and retinal rewiring, may be approached by considering developmental theories.

While the experience garnered from RPE is presently influencing the clinical translation of retinal cell replacement, overall critical advancements that would transform the replacement of retinal neurons from a laboratory model to a clinically relevant treatment are still missing. The results of cell transplantation into animal models of retinal disease have generated a lot of excitement, but the next stages towards human translation must be taken carefully. Planning and carrying out clinical trials must be based on the all-out possible inclusion of objective metrics for safety, efficacy, and mechanism of action in light of the identification of artifacts like cytoplasmic transfer and the ongoing quest to separate neuroprotective effects from functional improvements attributed to real cell replacement.

#### Declaration of competing interest

The authors declare no financial interests or personal relationships.

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## **Surgery of blepharoptosis with concomitant periorbital changes in the elderly population**

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**Blepharoptosis is an abnormally low-located upper eyelid that can cover the visual axis and cause narrowing of the visual field and in extreme cases, also impacts negatively the visual acuity. Quite frequently it goes hand by hand with dermatochalasis which is defined as extra skin on the upper eyelid that also has a similar impact on visual function. Age is considered to be a risk factor for both of these entities and in an increasingly aging population, the number of patients is calculated to be on the rise. We evaluated the patients who suffered from one or both of these conditions. To determine the frequency and optimal surgical treatment of acquired blepharoptosis in patients with age-related periorbital changes (dermatochalasis) in the elderly group of patients, 31 patients aged 56 to 86 years (mean 68±8 years), men 5 (16 %), and women 26 (84%) were examined. One-stage minimally invasive surgical correction of blepharoptosis, supplemented by upper blepharoplasty to eliminate dermatochalasis should be based on the individual characteristics of the patient, his pathology. It is advisable to consider the simultaneous correction of blepharoptosis and dermatochalasis as the most optimal, since it provides both functional improvement and cosmetic satisfaction for the patient, restoration of the quality of life.**

**Keywords:** *Blepharoptosis, dermatochalasis, levator resection, upper blepharoplasty, ageing population, quality of life*

### **INTRODUCTION**

The demographic trends of the planet indicate increasing life expectancy, the older population will almost double from 2005 to 2050, and in 2025 the population over 60 years old will be 1.2 billion. Ensuring a decent quality of life for older people is one of the main missions of a developed social society (United Nations WPP, 2012). Acquired blepharoptosis is one of the reasons for visual function deprivation in an aging population (Anderson, 1985). Eyelid surgeries hold the 4th position in the list of the most frequent plastic surgeries, indicating the high frequency and demand for adnexal surgery of the eye (American Society of Plastic Surgery, 2018).

Diabetes is one of the growing pathologies among adults and it is also considered to be a risk factor for droopiness of the eyelid (Moon, 2015).

Another factor predisposing patients to develop BP (blepharoptosis) is various types of ophthalmic surgeries, which is on the rise due to the increased availability of ophthalmic care in nations (Koh, 2017).

The main complaint of patients attending the ophthalmologist is droopiness of the eyelid that leads to a decreased visual field, chin-up position, and headaches due to constant overreaction of frontal muscle. The goal of BP surgery is to lift a drooping eyelid, eliminate discomfort, and improve quality of life, which is a logical criterion for evaluating the outcome of treatment (Clauser et al., 2006).

Surgical correction of senile BP is widely discussed, both by ophthalmologists and plastic surgeons, where the evaluation criterion undoubtedly remains the functional and cosmetic satisfaction of the patient. Two indications for the

surgery are functional and cosmetic. Purely functional indications are based on the elimination of droopiness of the eyelid with the main goal to ameliorate decreased visual field and open up the visual axis, which is performed by different surgical approaches (internal and external techniques) with their advantages, disadvantages, complications, and numerous modifications of known methods (Putterman et al., 1975).

The second trend in the research includes surgical correction of BP in combination with aesthetic blepharoplasty which addresses not just functional, but also cosmetical issues. The most frequent pathology that goes hand in hand with ptosis is dermatochalasis (abundant skin of the upper eyelid) which is addressed by additional upper blepharoplasty (Bhattacharjee et al., 2017).

What are the arguments for an ophthalmologist in favor of expanding the scope of surgery for the correction of senile BP with a one-step correction of concomitant changes in the periorbital region? It is well documented that dermatochalasis is one of the most frequent pathologies in patients above 45 years of age and is as frequent as 16% in this age group (Damasceno RW et al., 2015). Simultaneous correction of BP and dermatochalasis of the upper eyelid can significantly improve vision, ameliorating functional disturbances, and as a result improving the quality of life.

Senile BP is associated with stretching and weakening of the attachments of the aponeurosis of the levator palpebrae muscle from the upper eyelid tarsus. Age-related topographic and anatomical changes include a progressive decrease in elasticity and firmness of the skin, weakening of connection with the underlying tissues, in particular, with the hypotonic (atonic) orbicular oculi muscle of the eyelid. Muscle fibers become thin, fragmented, transverse striation also becomes altered. The skin of the periorbital region undergoes involutional changes as well and in conjunction with other structures of the upper eyelid, it plays a major role in the development of dermatochalasis. Changes include the mechanisms of compensation of the eyelids and eyebrows, the volume depletion of the fat pads of the upper eyelid, orbit and eyebrows along with bone resorption are contributing factors to aging (Koh et al., 2005). It is worthwhile to mention that

all these changes accompanied by BP play a role in visual field constriction leading to a decrease in the quality of vision and cause additional functional and cosmetic difficulties for the patient (Bellinvia et al., 2014).

Despite the huge number of publications on the surgical treatment of BP and dermatochalasis (upper blepharoplasty), it is not well described the adequate tactic with simultaneous intervention to improve condition simultaneously in these both pathologies.

Taking into account an increased number of visits of older patients, the problem of blepharoplasty for ophthalmologists remains relevant. From the point of view of the choice of surgical tactics, optimal approaches for correcting BP and periorbital region changes to achieve a functionally as well as cosmetically acceptable result remains a significant issue for both patient and treating physician.

The aim of this study was to determine the optimal surgical treatment of acquired blepharoptosis in patients with age-related periorbital changes (dermatochalasis) in the elderly group of patients.

## **MATERIALS AND METHODS**

In total 31 patients aged 56 to 86 years (mean age  $68 \pm 8$  years), complaining of the drooping upper eyelid (eyelids) were included in the study, among them 5 men (16%) and 26 women (84%). All cases of ptosis of the upper eyelid were acquired. Examination of patients included a thorough collection of anamnestic data in order to determine the nature of ptosis, somatic factors that can provoke its development.

After general ophthalmological examination, additional measurements of the eyelids were undertaken and consisted of the levator function of the upper eyelid; the distance from the ciliary edge of the upper eyelid to the central corneal reflex (MRD 1) and the distance from the palpebral fold to the central corneal reflex (CCRD).

The degree of ptosis was assessed according to Finsterer (Finsterer, 2003).

Among the studies, evaluating the "Bell phenomenon", the presence of lagophthalmos is to

prevent postoperative complications. Tests for myasthenia gravis were determined: a fatigue test and a cold test. Changes in the upper eyelid are suggestive of dermatochalasis.

Methods of surgical correction of BP and upper blepharoplasty were used.

Blepharoplasty of the upper eyelid in dermatochalasis was performed before correction of the BP. The operation included marking excess skin followed by infiltration with 2% lidocaine anesthetic, surgical excision with subsequent correction of the BP, which was performed by the traditional method of resection of the aponeurosis according to Anderson (Anderson, 1979) and the method of conjunctival-mullerectomy according to A.Putterman and M.J.Urist (using a Karl Ilg & Co., St. Charles clamp) (Putterman et al., 1975). In the first variant, to determine the symmetry and adequate vertical position of the eyelid, the assessment of intraoperative correction was carried out in a sitting position under local anesthesia. In the second variant, a 4 mm resection nomogram was used for each millimeter of ptosis.

Photographing patients before and after the operation was a necessary step for assessing changes, obtaining the patient's consent for surgery. Informed consent was obtained from all patients. Examinations of patients and the study itself were carried out according to the principles set out in the Guidelines for the requirements (provisions) of the Declaration of Helsinki (and its amendments).

## RESULTS AND DISCUSSION

As shown by the preoperative examination, senile BP was unilateral only in 4 (12.1%) cases, and bilateral (87.9%) in 27 cases. For establishing the surgical approach patient complaints, degree of BP, and changes in the periorbital region were of decisive importance.

When examining the upper eyelids, attention was focused on dermatochalasis (with or without excess medial fat pad).

In total, BP was observed without dermatochalasis in 10 patients (32%); combined BP and dermatochalasis in 12 patients (39%).

The choice of surgery was based on if

patients had just ptosis or dermatochalasis as well, in the first group it was enough to correct the BP, in the second group BP surgery was performed simultaneously with blepharoplasty of the upper eyelids. In the first group for BP repair posterior approach was utilized (Putterman). Taking into account that upper blepharoplasty and levator resection surgery share the same surgical approach (transcutaneous), BP repair in the second group was achieved by levator aponeurosis resection. The surgeries performed have been demonstrated in Table 1.

**Table 1.** Surgery types performed

Aging changes	Surgery	BP bilateral/unilateral	Patients
BP	CM	7/3	10(32%)
BP+DCh	LR	20/1	21(68%)

Complications during the surgical treatment were not observed.

A rate of under-correction was observed in 8 (26%) cases, out of which in 5 (16%) cases there was insufficient correction of the BP (resection of the aponeurosis), in 2 (6%) cases there was excess skin of the upper eyelid in the second group. As it is known, levator resection surgery does not always have a predictable outcome, and the success rate varies from 70% to 95% (Putterman and Urist, 1975).

After the surgical treatment, all patients were under dynamic observation. Postoperative studies in the period 6 months to a year showed improvement in all parameters (Table 2).

**Table 2.** Measured data before and after the procedures

Groups	MRD1 before/after	CCRD before/after
Group 1	0.98 (±0.97)	7.05 (±1.94)
	2.51 (±0.70)	5.53 (±1.63)
	<i>p</i> -value < 0.05	<i>p</i> -value < 0.05
Group 2	1.12 (±0.42)	5.16 (±1.59)
	2.89(±0.37)	7.30 (±0.71)
	<i>p</i> -value < 0.05	<i>p</i> -value < 0.05

MRD1 - margin reflex distance

CCRD - crease corneal reflex distance

The results of the study confirmed that the method of conjunctivo-mullerectomy for the isolated correction of BP is preferable due to the greater predictability of the results compared to

anterior levator resection (Frueh BR et al., 2004). The important factor in choosing a treatment is a clear understanding of the result that the patient expects. The result expected by the patient does not always coincide with the required volume of intervention planned by the surgeon. Thus, out of 31 patients who were involved in the study due to complaints of drooping eyelids, only in 10 (32%) patients, isolated correction of the BP could provide the patient with the desired result. Worthwhile mentioning that in 21 (78%) patients the BP surgery alone was not enough to achieve patient satisfaction since contributing factor was not just BP, but dermatochalasis as well, which is almost twice as much as in BP only group.

While choosing surgery to be performed one should agree that in the elderly group of patients, the degree of ptosis is not the only factor to be considered (Ben Simon et al., 2005). Simultaneous surgical interventions have been previously presented in the literature and discussed in terms of their advantages. Among them, emphasis was placed on reducing the operation time (compared to if the operations were performed separately), the financial burden (which is related both to the cost of the operation and which may fall either on the patient or the national budget) as well as the number visits to the clinic that almost double up in case if surgeries performed on separate visits. The latter is even more complicated if the patient lives far from the hospital. The benefits of single-stage interventions have been discussed and also presented in terms of greater efficiency especially in the field of periorbital surgery (Askeroglu et al., 2019). Simultaneous correction of BP and dermatochalasis of the upper eyelid provides even greater elimination of functional complaints that are bothersome for the patient. Bilateral surgery has a positive impact on the improvement of vision, peripheral visual field, especially on an attempt to gaze up and also improves the quality of life (An SH. et al., 2016). In blepharoplasty, optimal removal of excess eyelid skin after measuring it at rest and after stretching provides the desired result in most cases (Kashkouli et al., 2017). In our study among the additional arguments in favor of combined surgery, there were patients in whom only the BP was removed without the removal of the excess skin from the

upper eyelid (group 1). When lifting the eyelid, the crease of the upper eyelid falls lower than preoperatively due to a shortening of the distance between a lash line and an eyebrow (Karlin et al., 2020). This fact was reflected in the 1-st group of patients where the ciliary margin was raised from 0.98 mm to 2.51 mm, the fold was lowered from 7.05 to 5.53 mm. The drooping of the fold, in turn, leads to an even greater overhang, thereby affecting the deterioration of the visual field and a less attractive cosmetic result (Ho et al., 2011).

It should be agreed that the adoption of surgical decisions includes, in general, taking into account individual age-related changes, the optimal tactics for their elimination with the choice of less traumatic surgical approaches. During the surgical correction of the BP, preference is given to the classic technique of the muscle that lifts the upper eyelid (levator) or its aponeurosis, as more physiological (Martin et al., 2015). Thus, the optimal tactics for planning BP surgery in older people involve a proper examination algorithm, a cumulative assessment of all existing age-related changes in the eyelids which determines the choice of the surgical technique, more often techniques that can provide a cosmetic and functional effect. Predicting a patient-satisfying result is often not limited only to the correction of the BP, but includes the simultaneous elimination of age-related changes that affect the outcome, especially in women.

## **CONCLUSION**

The optimal surgical technique should be based on the individual characteristics of the patient and his pathology and should be minimally invasive. It is reasonable to consider simultaneous surgery for the correction of BP and dermatochalasis in older people since it provides both functional improvement and cosmetic satisfaction for the patient in order to restore the patient's quality of life.

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## **Subtle mechanisms of the visual cascade: searching for critical amino acid residues in signal transduction of the visual receptor rhodopsin**

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**Rhodopsin is the first G protein-coupled receptor (GPCR) whose three-dimensional structure has been resolved using X-ray crystallography. The crystal structure of rhodopsin revealed the molecular mechanism of photoreception and signal transduction in the visual system. Although several other GPCR crystal structures have been reported over the past two decades, the structure of rhodopsin remains an important model for understanding the structural and functional properties of other GPCRs. This review summarizes the structural properties and photoactivation of rhodopsin and details the contact points between rhodopsin and visual G-protein transducin during the initiation of visual signaling.**

**Keywords:** *Visual transduction, GPCRs, transducin, crystal structure*

### **INTRODUCTION**

The human visual system is astonishing for the amount and quality of information it provides about the world surrounding it. A cursory glance is enough to describe the location, size, shape, color and composition of objects, and if the objects are moving, their direction and speed.

The organ of vision - the eye, consists of a fluid-filled sphere surrounded by three layers of tissue. Only the retina, the innermost layer of the eye, has neurons that are sensitive to light and capable of transmitting visual signals to central targets. The tissue layer immediately adjacent to it consists of three different but continuous structures, which together form the uveal tract.

The most anterior part of the uveal tract is the iris. It has two sets of muscles whose opposing movements allow it to adjust pupil size under the control of the nervous system. The sclera is the outer layer of tissue in the eye and is made up of

tough white fibrous tissue. At the front of the eye, this opaque outer layer becomes the cornea, a special transparent tissue. Outside the cornea, light rays pass through two different fluid media before entering the retina. In the anterior chamber, behind the cornea and in front of the lens is the aqueous humor, a clear, watery fluid that supplies both of these structures with nutrients (Sultanov and Alieva, 1983). Aqueous humor is formed by ciliary processes in the posterior chamber.

The retina or neural part of the eye is in fact, a part of the central nervous system. The retina is formed of the inner wall of the optic cup, and the pigment epithelium of the retina is formed of the outer wall (Purves et al., 2004).

Photoreceptor cells in the human retina are divided into two types - rod cells and cone cells which are the primary receptors of visual stimulation. While cones perform colour vision, rod cells can be stimulated by a wide range of wavelengths of light starting with a dim light such

as moonlight. Photoreceptor cells signal (synapse) with overlapping layers of interneurons innervated by different combinations of photoreceptor cells (Nakanishi, 2000). All these signals are processed and transmitted through the visual thalamus to the part of the brain called the visual cortex, where they are interpreted. Signal reception and its five-step processing are carried out by a number of protein and non-protein components included in a signal transmission chain, and this process is called "Phototransduction activation" (Fig. 1). In step 1 of this process, the light is absorbed in the eye and activates rhodopsin through a conformational change from cis-retinal to trans-retinal in the rod disc membrane (Fig. 2), and it dissociates from the opsin molecule, which opens transducin binding sites on the opsin protein. In step 2, the opsin rebinds to transducin molecules and GDP release catalyzes transducin activation by binding cytoplasmic GTP to it. In step 3, the GTP-bound subunit then removes the PDE alpha subunit and activates it. In Step 4 the activated PDE hydrolyzes cGMP to GMP. In step 5, the decrease in cGMP levels causes the closing of gated ion channels, which prevents the influx of  $\text{Na}^+$  and  $\text{Ca}^{2+}$ , thereby depolarizing the cell.

Rod cells sense light through a light-sensitive GPCR called rhodopsin. Rhodopsin is composed of an opsin protein with a visual GPCR structure and a light-absorbing pigment called retinal covalently attached to it (Hofmann et al., 2009; Nakanishi, 2000). Found only in rod cells, rhodopsin is located in the ~1500 flattened membrane discs that form the outer segment of

these rod-shaped cells (Fig. 2). A single human rod cell contains about  $4 \times 10^7$  molecules of rhodopsin. A trimeric G protein (Gt) called transducin that binds to rhodopsin has a  $G_\alpha$  subunit called  $G_{\alpha t}$ , like rhodopsin,  $G_{\alpha t}$  is found only in rod cells.

Rhodopsin (R) differs from other GPCRs in that ligand binding does not activate the receptor. Conversely, the absorption of a photon of light by the retina coupled to the receptor is the activating signal (Calvert et al, 2006; Hofmann et al., 2009). Upon absorption of a photon, the retinal moiety of rhodopsin is immediately converted from the cis isomeric form (known as 11-cis-retinal) to the all-trans isomeric form, causing a conformational change in the opsin protein (Fig. 2). This is equivalent to the activation of a conformational change by ligand binding in other G protein-coupled receptors, which allows rhodopsin to bind to the  $G_{\alpha t}$  subunit of its G protein-coupled transducer, causing GDP to be replaced by GTP in its  $G_\alpha$  subunit. The resulting activated rhodopsin, denoted as  $R^*$ , is unstable because its covalent bonds with retinal are spontaneously broken. Since retinal-free opsin cannot find the transducer, the initiation of visual signal transduction is broken at this point. Free all-trans-retinal is converted back to 11-cis-retinal in the dark through a series of steps involving enzymes in the rod cells and adjacent retinal pigment epithelium cells (Smith, 2010). The resulting 11-cis-retinal moves to the rod cells, where it combines with opsin again to form rhodopsin, and completes the visual cycle.



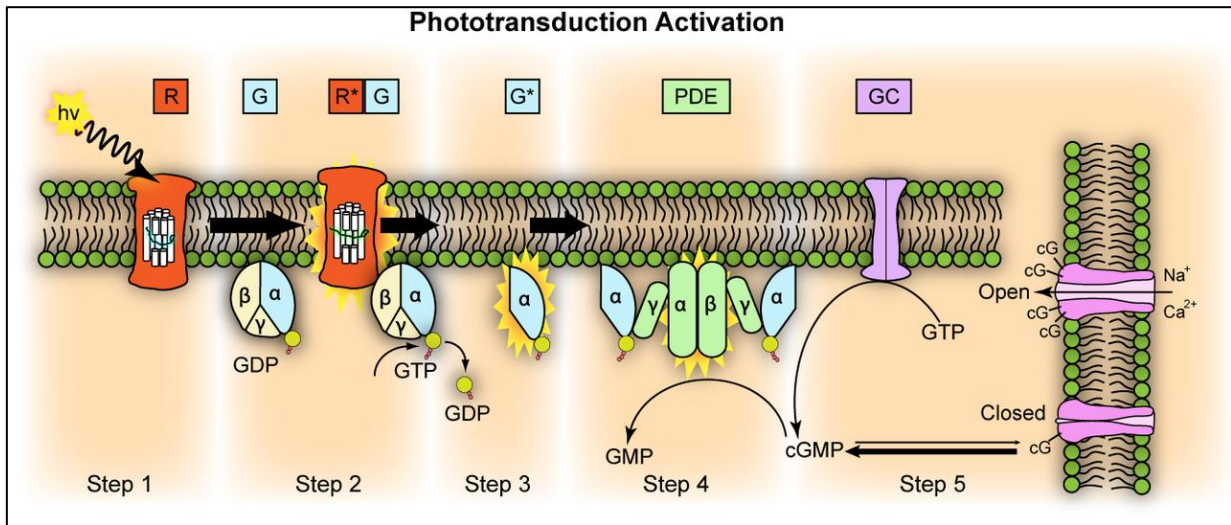


Fig. 1. Initiation of phototransduction of the visual cascade in vertebrates.

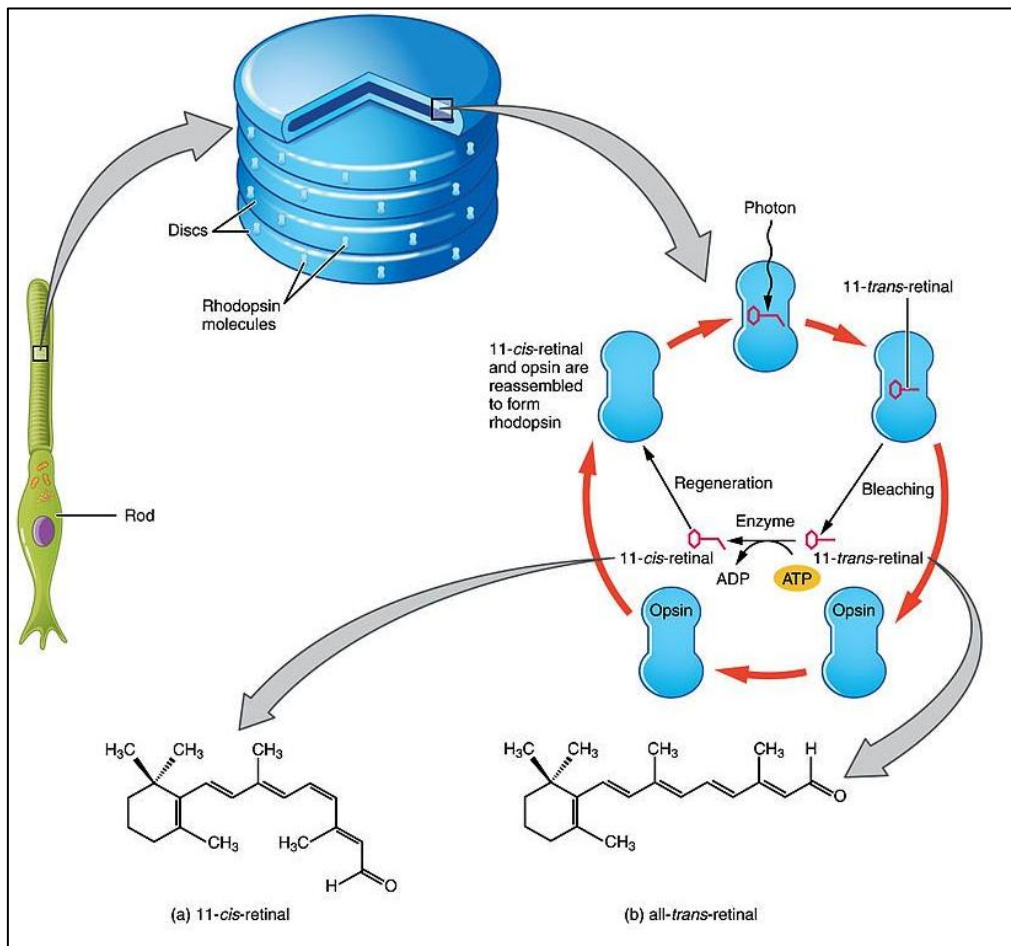


Fig. 2. Retinal isomerization and rhodopsin activation cycle.

**Structure of rhodopsin**

Rhodopsin is the first GPCR to have its

crystal structure obtained (Palchewski et al., 2000). To obtain the crystal structure, bovine

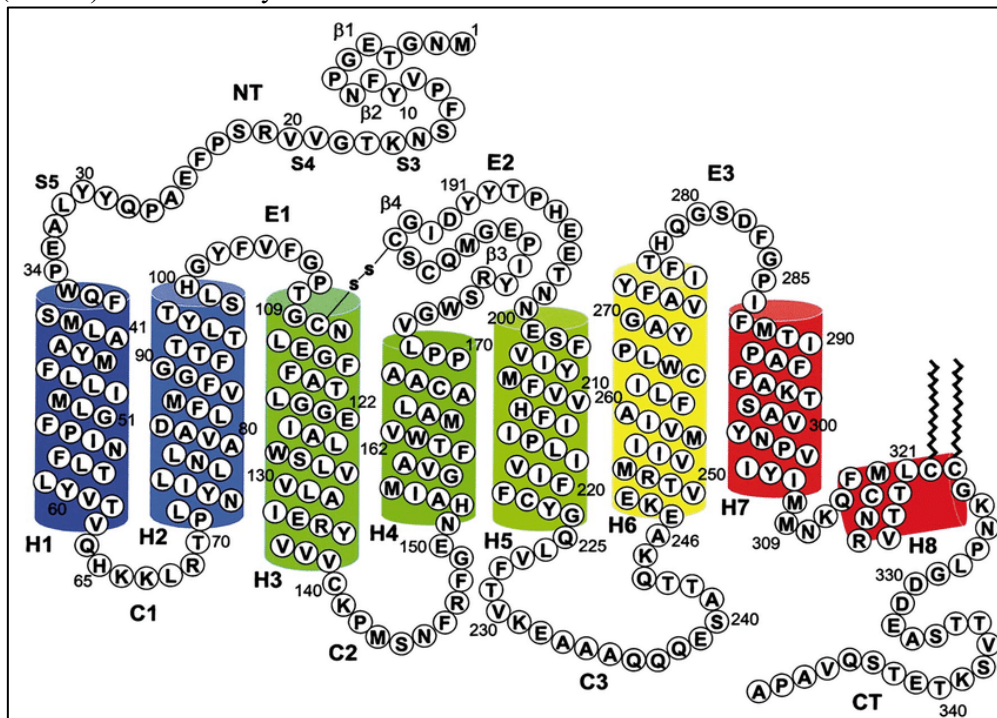
rhodopsin was purified by separating it from the outer segmental membranes of rod cells and crystallized from nonyl-thiol-glucoside detergent solution to which amphiphilic heptane 1,2,3-triol was added (Okada et al., 1998; 2000).

Rhodopsin, an integral membrane protein, is composed of three topological domains: an extracellular surface domain, a membrane-embedded transmembrane domain, and an intracellular surface domain (Figure 3). Since rhodopsin is located in the disc membranes of the rod outer segment (Fig. 2), the extracellular domain is often called the intradiscal domain. The amino terminal of the protein is in the extracellular part and the carboxyl terminal is in the intracellular part. The membrane-bound domain consists of seven transmembrane segments (H1-H7) dominated by an  $\alpha$ -helix. The

helical segments form a compact bundle and contain a retinal (RET) binding site (Menon et al., 2001).

In addition to the NT segment, the extracellular surface domain has three extracellular interhelical loops: E1 loop of 101–106 amino acids and connects H1 and H2, E2 loop of 174–199 amino acids and connects H4 and H5, E3 loop consists of amino acids residues 278-285 and connects H6 and H7.

Using site-directed mutagenesis, it has been shown that the extracellular loops and NT of bovine rhodopsin are required for the proper folding of the receptor which plays important roles in cell processing, chromophore binding (Doi et al., 1990) and in cell trafficking (Borjighin and Nathans, 1994).



**Fig. 3.** Secondary structure of rhodopsin. Regional schematic diagram of transmembrane helices, intradiscal regions and cytoplasmic regions. The position of each residue is depicted in a single-letter code.

Rhodopsin is known to be glycosylated at Asn-2 and Asn-15 residues of NT. Non-glycosylated rhodopsin has been shown to be defective in the light-dependent activation of Gt (Kaushal et al., 1994).

Two conserved cysteine residues in the extracellular domain, Cys-110 and Cys-187, have been shown to be important for the folding of the

opsin protein (Karnik et al., 1988). Elegant studies by site-directed mutagenesis have shown that these two residues are involved in the formation of disulfide bonds (Karnik and Khorana, 1990). It appears that this disulfide bond stabilizes the ground-state structure of the chromophore-binding pocket.

### ***Extracellular surface domain of rhodopsin***

The extracellular surface domain is composed of an amino-terminal tail (NT) and three interhelical loops (E1, E2, and E3). The extracellular domain contains significant secondary structural elements and several intra- and inter-domain interactions. The NT extends from the amino terminus to Pro-34 and consists of a distorted strand labeled as  $\beta$ 1,  $\beta$ 2, S3, S4, and S5 (Figure 3).

### ***The membrane-embedded domain of rhodopsin***

The crystal structure of rhodopsin shows that 194 of the 348 amino acid residues of bovine rhodopsin make up its seven membrane-embedded (H1-H7) segments (Menon et al., 2001). These are H1 (residues 35-64), H2 (71-100), H3 (107-139), H4 (151-173), H5 (200-225), H6 (247-277), and H7 (286-306). The crystal structure shows that this domain has multiple bends and disordered structures in individual transmembrane segments, but the overall secondary structure is  $\alpha$ -helical (Menon et al., 2001).

The chromophore moiety of rhodopsin binds to retinal (RET) in its chromophore-binding pocket. RET is a derivative of vitamin A1 and consists of 20 carbon atoms. The RET chromophore bound to the rhodopsin crystal structure is in the 6-s-cis, 11-cis 12-s-trans conformation (Menon et al., 2001). The RET chromophore binding site is located in the membrane-embedded part of the receptor. All seven transmembrane segments and part of the extracellular domain are involved in the interaction of the opsin protein with the chromophore. The chromophore is located closer to the extracellular side of the transmembrane domain than to the intracellular side. Chromophore polyene of C6 and C11 runs almost parallel to H3. This transmembrane segment provides most of the amino acid side chains involved in the chromophore-binding pocket: Glu-113, Gly-114, Ala-117, Thr-118, Gly-120, and Gly-121. The extracellular-facing polyene chain of the receptor is covered by  $\beta$ 4-sheet amino acid residues (Ser-186 to Gly-121) of the E2 loop.

### ***Cytoplasmic domain of rhodopsin***

The cytoplasmic domain of rhodopsin

consists of three cytoplasmic loops and a carboxyl terminal tail: C1 (amino acids 65-70), C2 (140-150), C3 (226-246), and CT (307-348). CT is divided into two structural domains. C4 extends from Ile-307 to Gly-324 at the end of H7, just beyond vicinal Cys residues (Cys-322 and Cys-323), which are post-translationally palmitoylated (Menon et al., 2001). The remaining part of CT extends from Lys-325 to Ala-348 at the carboxyl terminus of rhodopsin. A characteristic feature of the C4 loop is that it forms an  $\alpha$ -helix, denoted as H8 (Fig. 3). H8 lies almost perpendicularly on H7.

The C1 loop exhibits a rigid conformation involving the basic residues His-65, Lys-66, Lys-67, and Arg-69. Lys-66 and Arg-69 side chains are facing the membrane bilayer. His-69 is located close to the C4 loop within H8. And Lys-67 interacts with CT located approximately parallel to C1. C2 resembles an L-shaped structure when viewed from a viewpoint parallel to the putative membrane plane. A short  $\beta$ -barrel structure extends from Met-143 to Phe-146. C2 and C3 lie at approximately the same level on the cytoplasmic border of the receptor.

The main characteristic of C4 is that it has an  $\alpha$ -helical structure, designated H8 as shown above. H8 binds to H7 by the tripeptide Met-309/Asn-311/Lys-311 and extends perpendicularly to it.

### ***Rhodopsin residues responsible for interaction with G-protein transducin***

Transduction and amplification of the signal from rhodopsin ( $R^*$ ) to  $G_t$  occurs as a result of specific interactions between the cytoplasmic surface domain residues of  $R^*$  and at least two regions of  $G_t$  – the C-terminus of the  $\alpha$ -subunit and the C-terminus of the  $\gamma$ -subunit (Garcia, et al., 1995; Helmreich and Hoffman, 1996, Kisselev, 1999).

The most studied receptor-G protein interactions have been between bovine rhodopsin and bovine  $G_t$  which can be easily isolated and purified or expressed recombinantly. Extensive and accurate biochemical and biophysical analyzes of  $R^*$ - $G_t$  interactions were performed by mutagenesis of cytoplasmic domains of bovine rhodopsin.

Initial mapping of the  $G_t$ -recognizing

rhodopsin residues was performed in the late 1980s using site-directed antipeptide antibodies (Weiss et al., 1987). These antibodies were generated specifically for the putative cytoplasmic domain of rhodopsin. Studies have shown that the potential G<sub>i</sub>-recognizing residues of bovine rhodopsin are located in cytoplasmic loops 3, 4 and the C-terminus. Ala-scanning mutational studies were able to detect several transducin-recognizing rhodopsin residues only in the C3 loop (Shi et al., 1995).

A More extensive mapping of G<sub>t</sub>-recognizing rhodopsin residues was performed using a pre-chemically activated cross-linking reagent N-succinimidyl 3-(2-pyridyldithio) propionate examining the contact sites that interact between light-activated rhodopsin and transducing (Itoh et al., 2001). In these studies, cross-linking with T $\alpha$  was demonstrated for rhodopsin mutants K141C, S240C and K248C. The major site of cross-linking in transducin is within the Leu-19-Arg-28 peptide sequence in the N-terminal region of T $\alpha$ . Both the N and C termini of T $\alpha$  have been shown to be closer to the third cytoplasmic loop of rhodopsin in the Rho-T complex, and cytoplasmic loop 2 residue Lys-141 and cytoplasmic loop 3 residues, Ser-240 and Lys-248 are critical residues for transducin recognition.

Complete mapping of transducin-recognizing rhodopsin residues was carried out in N. Artemyev's laboratory (Natochin et al., 2003). In this study, a gain-of-function mutational approach was used to identify rhodopsin residues critical for G<sub>i</sub> activation. The target regions of rhodopsin for mutagenesis included the C4 loop, the non-helical parts of the C2 and C3-loops. Three mutant opsins with residues 140–148 in loop C2, 229–244 in loop C3, and 310–320 in loop C4 (helix H8) were substituted with poly-Ala sequences of equivalent length and used as templates for mutagenesis. In each of the templates, Ala residues were replaced by original rhodopsin residues. Template mutants with poly-Ala substitutions in the C2 and C3 loops produced 500-nm-long absorbing pigments but failed to activate transducin. In each of the templates, back-replacement of the Ala residues with the original rhodopsin residues (recovery of function) Cys140/Lys141 and Arg147/Phe148 residues in

the C2 loop significantly (~50%) restored rhodopsin/transducin binding. In the C3 loop, residues Thr229/Val230 and Ser240/Thr242/Thr243/Gln244 completely restored G<sub>t</sub> activation. These studies revealed the role of Asn310/Lys311 residues in 11-cis-retinal binding and Phe313 and Met317 residues in G<sub>t</sub> activation in the C4 template mutant.

Recent advances in structural biology have made it possible to obtain high-resolution structures of protein complexes of GPCR-G proteins, including cryoelectron microscopy structures of 4.5 Å engineered Rho-bound G-protein (Kang et al., 2018) and dominant-negative G-protein mutants (Liang et al., 2018; Draper-Joyce et al., 2018; Garci'a-Nafri'a et al., 2018; Kang et al., 2018). Use of binding partners such as nanobodies (Rasmussen et al., 2011; Zhang et al., 2017; Liang et al., 2017, 2018) or antibody fragments (Koehl et al., 2018; Kang et al., 2018; Krishna Kumar et al., 2019) has revolutionized the analysis of the G-protein-rhodopsin complex. A cryo-EM structural image of a fully functional and signal-transmitting Rho-G<sub>t</sub> complex is presented in the presence and absence of engineered nanobodies that do not interfere with G-protein activation (Gao et al., 2019). These studies showed that light-activated Rho forms extensive contacts with G<sub>t</sub>, resulting in an interface area of 1042 Å<sup>2</sup>. Formation of this interface is provided by the  $\alpha$ 5 helix,  $\alpha$ 4- $\beta$ 6 loops,  $\beta$ 2- $\beta$ 3 loops, and the  $\alpha$ N- $\beta$ 1 eGat loop in the G protein, as well as the H3, H5, H6 transmembrane helices, intracellular loop 2 (ICL2) and H8 of rhodopsin.

Thus, studies using many different approaches and methods have revealed the important role of certain residues in the three cytoplasmic loops of rhodopsin (C2, C3 and C4), especially those adjacent to the H3, H5, H6 transmembrane helices and the intracellular H8, in the formation of the complex of rhodopsin with transducin and initiation of phototransduction via transducin activation.

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## **Effects of nucleus rapher on the spectral character of electrical activity in the structures of the vision analyzer**

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**The present work is devoted to the study of neurophysiological mechanisms of participation of the nucleus rapher in the plastic properties of the visual analyzer in the correction of dysfunction caused by retinal dystrophy. To achieve this goal, we used the method of spectral analysis of the potentials of the electroencephalogram in the central structures of the vision analyzer. First of all, a comparative analysis of the spectral characteristics of the EEG potentials at the studied structures was carried out during the background activity of the brain. The results of the studies performed showed that one of the characteristic features of the EEG in the structures of the visual analyzer is that the maximum level of the spectrum is mainly concentrated in the delta and theta frequency ranges of the EEG. After exposure to electrical stimulation of one of the nuclei of the 5-HT-ergic system, the polymodality of spectral properties in the visual cortex (VC) and colliculus superior (CS) is disturbed. The main maximum level of the spectra after stimulation of nR the EEG waves shift to higher frequency regions (4-5 Hz and 9-10 Hz). As a result of dysfunction, the amplitude of the main peak of EEG spectral features in the delta frequency range decreases and the second maximum level of the spectrogram decreases in the range of theta. Similar changes are observed in the spectra of EEG potentials in the subcortical structures of the visual analyzer. In the case of experimental retinal dystrophy, this corresponds to an increase the generation of potentials of the EEG at the delta range after exposure to electrical stimulation of the 5-HT-ergic system.**

**Keywords:** *EEG, nucleus rapher, serotonin, experimental dystrophy*

### **INTRODUCTION**

One of the actual problems of modern neurophysiology is the plasticity of nervous processes, more precisely, it is the ability to adapt through optimal structural-functional reconstruction of the nervous system (Kharchenko and Telnova, 2017). The monoaminergic (MA-ergic) neuromodulatory system of the brain has been recognized as one of the important components of the endogenous mechanisms of plasticity regulation. To date, a large number of experimental materials have been collected in the literature on the characterization of cellular and systemic mechanisms of the participation of MA-ergic neurotransmission in the regulation of

neuronal plasticity and interneuronal connections (Sinakevitch et al., 2018a, 2018b). It is clear that the morphological features of the organization of MA-ergic neurotransmission, based on the phenomenon of the spatial organization of nervous processes, indicate the need to study their role in the mechanisms of plasticity (Freitas, 2008).

The results of experimental studies prove that dynamic changes occurring at different levels of the visual analyzer system are not only attributed to morphofunctional features of its cellular organization. They are also associated with the activation of various nonspecific regulatory centers of the brain (Yokogawa and Hannan et al., 2012; Miryusifova et al., 2015). That is why the study of the mechanisms and patterns of



participation of neuromodulatory centers in the functional regulation of the visual analyzer has become in recent years one of the main problems of modern neurophysiology and neuropathology. There is information in the literature about the modulating effect of nR electrical stimulation on the spectral characteristics of slow potentials in the structures of the sensory systems of the brain (Cransac et al., 1998). In addition to morphological studies, there are a large number of other studies on the role of 5HT-ergic systems in the processing of visual, auditory and gustatory information. Based on the collected experimental data, it was found that nR has both inhibitory and excitatory effects on neocortical neurons. It is believed that the multiple complex effects of 5HT on information processing in the visual cortex are associated with the effect on different types of pyramidal interneuron receptors in the fifth layer of the visual cortex. This last result is explained by the change in the amplitudes of various potentials observed due to spontaneous inhibition of postsynaptic potentials (Xiang and Prince, 2003). Based on these data, it is assumed that an increase in the spectral power of slow waves after nR electrical stimulation can be considered as a physiological equivalent of the activating effect of the 5HT-ergic system at the level of excitability of cortical neurons (Zhang and Towns, 2002). 5HT has also been found to limit the occurrence of long-term synaptic plasticity in the second and third layers of the visual cortex (Jang et al., 2012). The modern literature contains interesting data on the involvement of 5-HT-ergic neurons in the regulation of pathological processes in the CNS. In particular, it has been found that nR can be involved in the mechanisms of sleep disorders, depressive disorders, neurodegenerative diseases, and psychopathological conditions such as anxiety and depression (Son et al., 2012).

## **MATERIALS AND METHODS**

The research has been conducted in the condition of chronic experiments on awakened mature rabbits weighing 2.5-3.5kg. All electrophysiological studies were conducted humanely in accordance with generally accepted international principles of the European

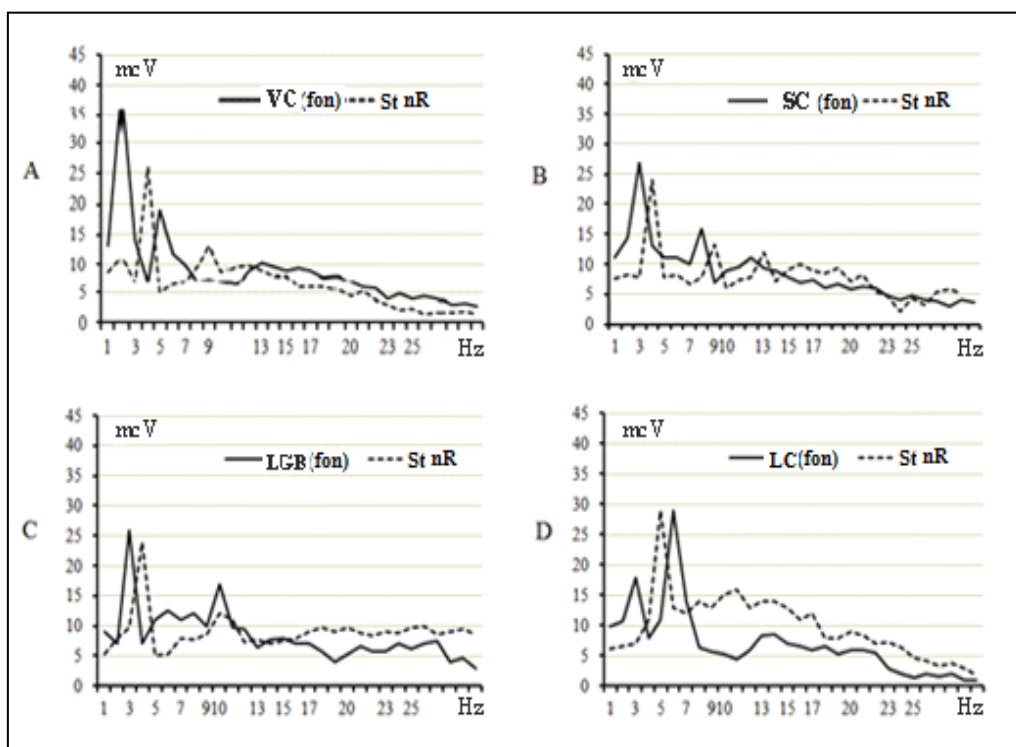
Convention on Experimental Animals (2010/63/EU). Scalping and implantation of macroelectrodes were performed under Nembutal anesthesia (35 mg/kg) and local anesthesia (0.5% novocaine solution) along the coordinates of the stereotactic atlas were used to register biopotentials. Potentials of the visual cortex (VC), superior colliculus (SC), lateral geniculate body (LGB), nucleus raphersdorsalis (nR) were registered. In the experiments, the EEG recording lasted from several minutes to several hours. For analysis, we selected artifact-free 30-second segments EEG activity of animals with precisely positioned electrodes. Analyses of EEG have been conducted using the software "Brainsys" (Russia) in the frequency diapason 0.5-45.0 Hz. 2.0% monoiodoacetic acid (MIAA) has been used to induce experimental retinal dystrophy. For bipolar electrical stimulation of the neuromodulatory center (nR), a laboratory electrical stimulator ESL-2 and the following stimulation parameters were used: amplitude 3.0-5.0 V, frequency 150-200 Hz and the duration of a single impulse is 0.4 ms, the duration of one session of electrical stimulation is 4-5 minutes. The spectral composition of the EEG was analyzed using the fast Fourier transform method. The reliability of intragroup and intergroup comparisons of EEG indicators was determined using the ANOVA program package.

## **RESULTS AND DISCUSSION**

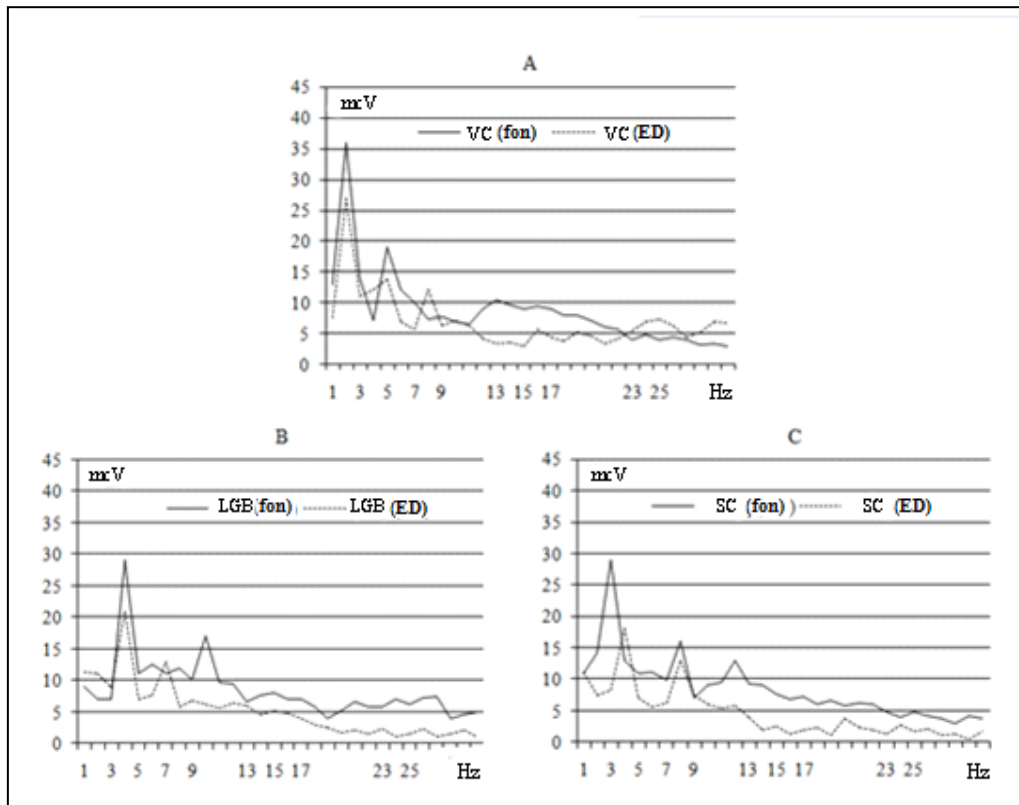
The research was carried out in several stages. First of all, we carried out a comparative analysis of the spectral characteristics of the EEG potentials of the studied structures during background brain activity. For this, we registered background EEG in the studied structures. Although at first view they are very similar to each other, as can be seen from the results of the correlation-spectral analysis, there are significant differences in their spectral composition. First of all, it should be noted that the maximum level of spectral properties of the total potentials of the analyzer structures, which have insignificant differences, is mainly concentrated in the delta and theta ranges of EEG frequencies. Thus, characteristic signs of maximum amplitudes distribution of primary

visual potentials spectra of the cerebral cortex at frequencies of 2–3 and 5–6 Hz are usually accompanied by a predominance of EEG delta rhythms. A similar figure is also observed in the distribution of peak values of spectral features amplitude for the LGB potentials. However, the maximum levels of the spectral characteristics are changed and fluctuate between 4-5 Hz and 9-10 Hz. Results of further experimental studies have shown that a somewhat different pattern of the frequency distribution of the dominant EEG waves is observed in the background of EEG spectra after exposure to electrical stimulation of the 5-HT-ergic system of the brain (Fig. 1). The results of the analysis showed that in the current experimental situation, in the VC and SC subcortical structures of the vision analyzer at the stage following the effect of electrical stimulation of the nuclei of the 5-HT-ergic system of the brain, the polymodality of the spectral properties is (amplitude spectra have many peaks) disturbed. With background activity in the VC, the first peak of the spectral composition of the EEG potentials

fluctuates within 2–3 Hz, and the second peak, within 6–7 Hz and at the same time, the main maximum level of the spectra after stimulation nR is located in a relatively intense range, shifting to a higher frequency (4–5 Hz and 9–10 Hz) region of EEG waves (Fig. 1A, B). Considering, that distribution of the maximum amplitude levels of EEG potentials during background activity in the SC in the frequency range of 3-4 Hz and 8-9 Hz indicates the formation of a bimodal spectrum pattern and at this time, after exposure to electrical stimulation of nR neurons, it was observed that the first peak of the amplitude parameters in this structure was transformed into a high component part, starting from the frequency range of 4-5Hz and even the second and the third peaks. This, in turn, leads to the formation of a polymodal image of the EEG potentials amplitude parameters. From the results of the spectral analysis of the studies performed, it can be seen that after exposure to electrical stimulation of nR neurons, a slightly different pattern of the distribution is observed (LGB and LC).



**Fig. 1.** Effect of nR electrical stimulation on the background spectral character of the EEG of the visual analyzer structures. A-VC, B-SC, C-LGB, D-LC.



**Fig. 2.** Consequences of experimental retinal dystrophy change in the EEG of the central structures spectral nature of visual analyzer.

In these structures, under the influence of nR, it is important to transform the bimodal form of the spectra into a monomodal one, expressed by the peak values of the spectrum amplitudes in the range of 4-5 Hz (Fig. 1 C, D).

The formation of experimental dystrophy (ED) affects not only the amplitude parameters of retinal responses but also to some extent affects the spectral character of the EEG potentials of brain structures (Fig. 2).

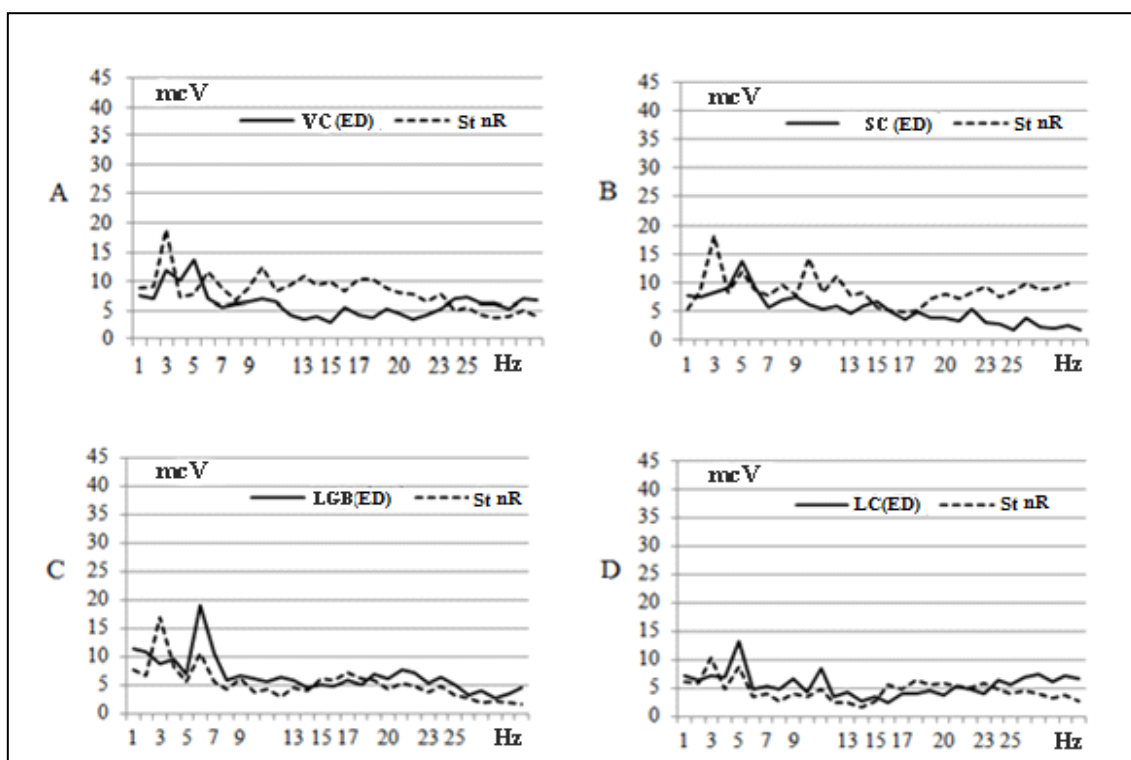
As it can be seen from the spectral character shown in the figure at the level of the primary visual cortex as a result of dysfunction around the analyzer, the amplitude of the main peak of the EEG spectral features in the delta frequency range decreases and the second maximum level of the spectrogram decreases in the theta frequency range. Similar changes are observed in the spectra of EEG potentials of the subcortical structures of the visual analyzer.

Corresponding changes are characteristic especially for the LGB potentials and also for a

noticeable decrease in the amplitude of the main peak of the spectral character and a shift of a significant secondary peak to a lower frequency range of the EEG. It is natural to expect that some changes will be found at the SC level as well. Indeed, analogous changes in dysfunction conditions of the analyzer of peripheral vision are distinctly observed on spectrograms of SC.

At the same time, during the formation of pathology in the analyzer it is necessary to pay special attention to the appearance of relatively less noticeable changes in the level of general EEG potentials of neuromodulating centers. While the amplitude spectrum of nR EEG potentials remains unchanged, for LC potentials in this case, it is clearly seen that the level of the maximum of the main spectral feature shifts somewhat and changes its position.

In the case of experimental retinal dystrophy, the effect obtained after exposure to electrical stimulation of the 5-HT-ergic system was different (Fig. 3).



**Fig. 3.** In the background of experimental retinal dystrophy the effects of nR stimulation on the spectral character of EEG in the structures of the vision analyzer. A-VC, B-SC, C-LGB, D-LC.

Under this condition, the observed changes in the spectra of EEG waves have a similar characteristic and mainly correspond to the increase in the generation of potentials in the delta range of EEG waves. This sign is clearly observed mainly at the level of visual cortex potentials of the brain.

It is clear from the presented spectrograms that formed under conditions of experimental dystrophy and the maximum level expressed in the range of 2-3 Hz is kept in the same frequency interval due to the effect of nR. Similar changes are observed in the spectral character of EEG potentials at the level of SC and LGB. In this case, despite the formation of a clearly noticeable level of the spectral amplitude peak in the interval of 3-4 Hz, the level of LC potentials is somewhat weakly expressed (Fig. 3 D).

Besides, the increase in the level of synchronization of EEG potentials, first of all, should be seen as an increase in the possibility of information interaction between brain structures in different experimental situations. The process that forms the basis of the characteristics of the

amplitude regulation of EEG waves against the background of retinal pathology was observed in our experimental studies. Under the influence of electrical stimulation of the 5HT-ergic system of the visual analyzer of the brain, accompanying changes in the pathology of the retina of an eye and parameters of electrical activity not only in the retina itself, but it is also reflected to some extent in the electrogenesis of the central structures of the visual analyzer.

By choosing an artificially created pathology in the visual sensor system as an experimental model addition, special attention was paid to the 5-HT-ergic neuromodulation systems of the brain. The choice of dystrophy model of vision analyzer in experimental studies is not random. Thus, the studied 5HT-ergic neuron terminals it is represented in a wide form at the level of the visual analyzer almost from the periphery to the central structures and neurotransmitters play an important role in the transmission of signals from the retina to the visual cortex. First of all, what was revealed in our research it should be especially noted that the spectral characteristics of potentials with small

differences in the vision analyzer are mainly concentrated in delta and theta frequencies of the EEG. Differences mainly belong to the amplitude of the spectral character peak values of EEG. It is interesting that under the influence of 5-HT-ergic systems in the background subcortical structures (VC and SC) the maxima of the spectra shift towards higher EEG frequencies (9-10 Hz), while the spectra of LGB and LC potentials in the range of 4-5 Hz, it acquires a monomodal characteristic with clearly expressed amplitudes peak values of the spectra. The obtained results showed that the activation of 5-HT-ergic neurotransmission increases the synchronization of EEG potentials in LC. Before and after ED the fact of reestablishing relationships between the results of the spectral analysis and the studied structures of the vision analyzer this method gives reason to say that the analysis of regulatory processes is one of the important indicators. However, it should also be noted that an increase in the level of synchronization of EEG waves should be considered primarily as an increase in the probability of information interaction between the structures of the vision analyzer in different experimental situations.

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## Clinical and immunological features of cataract surgery in uveitis

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There is much information about successful cataract surgery for different uveitis in the literature. However, the frequency of postoperative inflammation in uveitic cataract surgery even at the present stage of microsurgery remains quite high. The aim of this study was to evaluate the clinical and immunological features of the surgical treatment of uveitic cataracts. The study group of 52 patients (74 eyes) with uveitic cataracts (male - 29, female - 23) has been chosen on the basis of the clinical material of the National Centre of Ophthalmology named after Academician Zarifa Aliyeva. The examination complex was presented by ophthalmologic and immunological methods before and after the surgery. In all patients, phacoemulsification with intraocular lens implantation was used in order to remove cataracts against the background of the disease remission and no recurrence within 3 months prior to surgery. Despite the high professional level of surgeons and careful preoperative preparation, there were minor intraoperative and postoperative complications the structure of which depended on the initial severity of the operated eyes. Shortly after the surgery inflammation of varying severity was reported in 32 eyes (43.3%) and in the late period - in 28 eyes (37.8%). In patients with developed postoperative inflammation prior to the surgery there was observed a significant increase in the average immunoregulatory index (CD4+/CD8+), complement component C3 ( $1753 \pm 10.01 \text{ mg/L}$ ), immunoglobulin A ( $563.8 \pm 4.7 \text{ mg/dl}$ ), immunoglobulin G ( $2028 \pm 7.4 \text{ mg/dl}$ ) and antinuclear antibody ( $1.8 \pm 0.11$ ) in serum relative to the control index ( $p < 0.05$ ). Careful preoperative and postoperative control of the activity of the inflammatory process, early detection of postoperative complications, complete clinical examination of the posterior segment of the eye are the key to the successful treatment of uveitic cataracts. An immunological survey before surgery may serve as an additional method of assessment to predict the risk of postoperative exudative inflammatory reaction.

**Keywords:** Uveitis, cataract, inflammation, survey, immune response

### ABBREVIATIONS

UC - uveitic cataract	CRP - C-reactive protein
OCT - optical coherence tomography	ANA – antinuclear antibody
IOL- intraocular lens	IgA – immunoglobulin A
BCVA - best corrected visual acuity	IgM – immunoglobulin M
IOP - intraocular pressure	IgA – immunoglobulin A
CME - cystoid macular edema	IgG – immunoglobulin G
CMT - central macular thickness	IgE – immunoglobulin E
	IRI - immunoregulatory index
	LLC - limited liability company

## **INTRODUCTION**

Cataracts in uveitis can develop as a result of a direct inflammatory process of the uvea as well as long-term use of corticosteroids (Aliyeva and Shulpina, 2011; Gasimov et al., 2023; Agayeva et al., 2020). Pathological changes in UC are characterized not only by local anatomical and morphological processes, but also by systemic disorders, which determine the features of clinical and surgical management of such patients (Choi et al., 2022; Curnow et al., 2004). The incidence of cataracts in uveitis varies from 8% to 78% depending on the type of uveitis (Sen et al., 2016). Opacification often develops in the posterior layers of the lens with gradual progression but may also be diffuse. There are a lot of data in the literature on the successful surgical treatment of cataracts in various uveitis (Shilovskikh, Safonova, 2015; Llop and Papaliadis, 2018). But the frequency of postoperative inflammation in UC surgery even at the current level of development of microsurgery is relatively high (Chiu et al., 2017). The main postulates of success in UC surgery are careful preoperative and postoperative control of the activity of the inflammatory process, impeccable surgical technique, compliance with the requirements for biocompatibility and design of IOL, effective and early detection and treatment of postoperative complications, and a complete clinical examination of the posterior segment of the eye. The etiological diagnosis of uveitis is of paramount importance in planning a surgical strategy and further management of such patients. The complexity of diagnosis and choice of treatment tactics in many cases are complicated by the polyetiological nature of uveitis, the complexity of their pathogenesis, and the frequent combination of infectious and non-infectious factors (Balta et al., 2018).

The main importance in the pathogenesis of uveitis is given to violations of the immunological response. Uveitis refers to immune-mediated inflammatory diseases characterized by impaired regulation of immunity, chronic inflammation, and tissue damage (Minkus et al., 2021). Therefore, the formation of an immune response in the postoperative period of UC surgery occurs with the participation of all components of immune-active cellular structures.

The purpose of this research was to evaluate the clinical and immunological features of the surgical treatment of UC.

## **MATERIALS AND METHODS**

The study group consisted of 52 patients (74 eyes) with UC (men - 29, women - 23); it was selected by blind sampling based on the clinical material of the National Center of Ophthalmology named after Academician Zarifa Aliyeva. The examination complex was presented by methods of ophthalmological and laboratory examination.

Ophthalmological examination: anamnesis survey, determination of visual acuity (using Huvitz Chart Projector CCP-3100 (HUVITZ Co, LTD, South Korea), refractometry (automatic keratorefractometer RC-5000, TOMEY, Japan), biomicroscopy of the anterior segment of the eye (slit lamp TOMEY TSL-5000, TOMEY, Japan and portable slit lamp Reichert, Japan), tonometry (applanation tonometer FT-1000, TOMEY, Japan), ophthalmoscopy of the fundus (slit lamp TOMEY TSL-5000, TOMEY, Japan) using lenses (Ocular High Mag 78D, Ocular Instruments Inc., USA), ultrasound examination of the eye (ophthalmic ultrasound scanner E-Z Scan AB5500, SONOMED, USA), specular microscopy (specular microscope EM-3000, TOMEY, Japan), OCT (Cirrus optic coherence tomography HD-OCT 5000, Carl Zeiss Meditec AG, Germany).

Laboratory methods: general blood analysis, determination of blood glucose, CRP, rheumatoid factor, antistreptolysin, fibrinogen, coagulogram, serological reactions to various infections and viruses, assessment of immune status. When assessing the immune system (factors of cellular and humoral immunity) the level of serum IgA, IgM, IgG, IgE, ANA, complement component C3 and the presence of ANA were calculated. Cellular immunity included the determination of the relative content in the blood of lymphocyte subpopulations expressing surface markers CD4+, CD8+ by staining cells in an immunofluorescent test using a set of monoclonal antibodies (LLC "Sorbent", Russia, No. 9398-331-13180653-02). Monoclonal antibodies manufactured by LLC "Sorbent" were tested within the framework of the 5th (USA, 1993), 6th (Japan, 1996) and 7th



(Great Britain, 2000) International Human Leukocyte Differentiation Antigens workshops. Phenotyping of stained cells was performed using an AXIO Scope A1 Carl Zeiss fluorescent microscope (Germany). The factors of humoral immunity were determined by the Mancini immunodiffusion method. Benchmarks were determined by examining 50 voluntarily apparently healthy representatives of the appropriate age.

All subjects were warned about the ongoing scientific study and gave informed consent to participate in it (protocol No. 25 of the Ethics Committee of the Azerbaijan Medical University). A comprehensive laboratory examination was carried out for several purposes: to determine the etiology of the disease, to study the presence of allergic sensitization and associated viral infection, to assess the state of the immune status during treatment and the risk of postoperative inflammation, and to determine the timing of the next examination.

In all 52 patients with UC (74 eyes), the clouded lens was removed by phacoemulsification with IOL implantation against the background of disease remission and no recurrence of uveitis within 3 months before surgery. Patients were observed in dynamics before and after surgery (1-3 days, 1 month, 6 months, 1 year after surgery). Depending on the etiology of uveitis patients received specific general and local treatment (anti-inflammatory, desensitizing, anti-infective, etc.) before and after surgery. The duration of the uveitis in all patients ranged from 6 months to 5 years. If necessary, patients were consulted by narrow specialists (rheumatologist, phthysiatrician, endocrinologist, cardiologist, dentist, neuropathologist, etc.).

The results obtained were processed by the method of variation statistics with the calculation of arithmetic means (M), minimum (min) and maximum (max) values of the sample. The reliability of the obtained results was assessed using Student's t-test.

## RESULTS AND DISCUSSION

Age of the examined patients ranged from 20 to 60 years: 20-30 years old - 7 patients (13.5%);

31-40 years old - 14 (26.9%); 41-50 years old - 12 (23.1%); 51 - 60 years - 19 (36.5%).

Fig. 1 presents the results of the etiological diagnosis of uveitis, against which a cataract developed in the examined patients. Most often, UC was observed in reactive arthritis (23%), ankylosing spondyloarthritis (16.2%), rheumatoid arthritis (14.9%), in general - 54.1%. The most frequent spread of UC among these patients is explained by the mutual influence of both the inflammatory process itself and the use of steroids in the complex systemic therapy of the underlying disease which is the etiological cause of uveitis.

As a result of the analysis of the incidence of cataract development depending on the duration and frequency of relapses of uveitis, predominant clouding of the lens was recorded in the first five years of the disease in the vast majority of patients with a history of 5-8 exacerbations of the inflammatory process of the choroid (38 patients, 73.1%). The development of UC in both eyes was diagnosed in 22 patients (42.3%).

According to the international classification of uveitis by localization (Standardization of Uveitis Nomenclature (SUN)), the development of UC was registered against the background of 4 types of uveitis: anterior - in 31 (59.6%), intermediate- in 2 (3.8%), posterior - in 8 (15.4%) and panuveitis - in 14 (26.9%) (Fig. 2). As it can be seen, the most common clouding of the lens developed in anterior uveitis (59.6%).

The mean value of BCVA before surgery was  $0.1 \pm 0.09$  (pr.l.certae to 0.4). The average value of IOP is 17 mm Hg (9 to 24.5 mm Hg). Given the preoperative clinical features of operated eyes with UC (Table 1), ophthalmic surgeons had to resort to additional surgical procedures (Table 2). The main condition for this is the desire to minimize surgical trauma to reduce postoperative inflammation risk. Table 1 shows biomicroscopic signs of changes associated with UC in the examined group. As can be seen from the biomicroscopic examination results, ophthalmic surgeons had to face the following local status features most often during the operation: iris dystrophy of varying severity (71.6%), posterior synechiae (66.2%) and pupil rigidity (41.9%). It should be noted that in the majority of patients (45 patients - 86.5%) more than two of the above signs were observed

simultaneously in one eye which was one of the main reasons for the need for a thorough approach

to the upcoming cataract surgery and the development of postoperative inflammation.

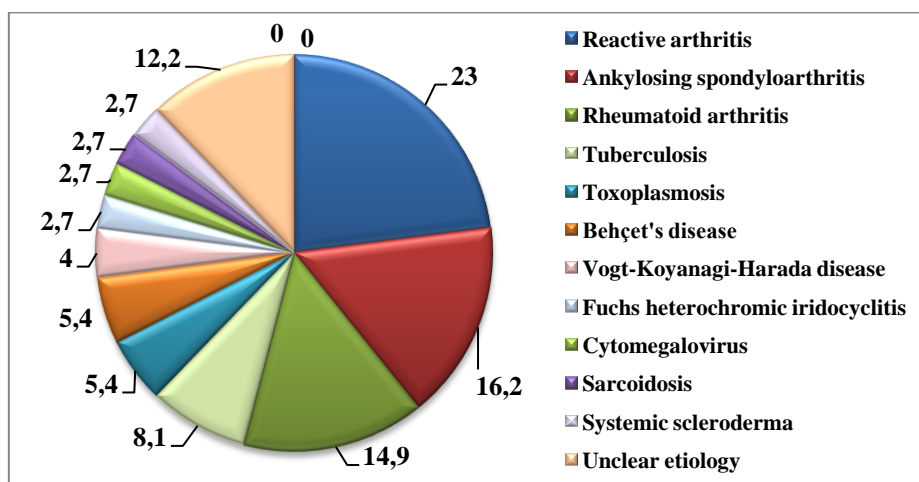


Fig. 1. Distribution of patients with UC by etiological feature (in %).

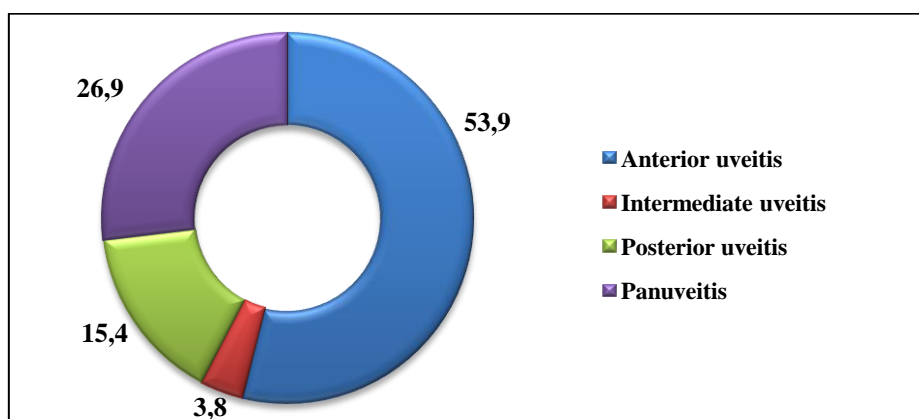


Fig. 2. Distribution of patients with UC by the anatomical feature of uveitis (in %).

Considering that the density of cataracts is the leading factor determining the energy parameters of phacoemulsification and the time of ultrasound exposure which in turn also has a certain effect on the degree of postoperative inflammatory reaction. Table 3 shows the distribution of operated 74 eyes with UC in the examined group according to the degree of density of the nucleus of the clouded lens according to L. Buratto.

Despite the high professional level of operating surgeons and the necessary preoperative preparation of patients with UC, there were also minor intraoperative complications the structure of which depended on the initial severity of the

operated eyes: hemorrhage in the anterior chamber - 4 eyes (5.4%), damage to the pupillary edge of the iris - 1 eye (1.4%), rupture of the posterior capsule and prolapse vitreous body - 2 eyes (2.7%).

An increase in visual results was noted in all 52 patients. The dynamics of postoperative BCVA are presented in Table 4. BCVA below 0.2 in 16 eyes (21.6%) in the early postoperative period was due to the initial presence of the consequences of uveitis affecting functional parameters: ribbon-like corneal degeneration (6 eyes); secondary glaucoma (5 eyes) and CME (5 eyes). BCVA in the range of 0.2 – 0.5 after a year (40 eyes – 54.1%) was caused by secondary

cataract (10 eyes); recurrence of uveitis with proliferative changes in the vitreous (22 eyes); CME (8 eyes). BCVA above 0.5 was registered in

22.9% of cases of operations performed (17 eyes) after a year.

**Table 1.** Features of the local status of patients with UC before surgery

Clinical features	Absolute number of eyes	Relative amount (%)
Ribbon-like corneal degeneration	8	10.8
Anterior synechiae	7	13
Posterior synechiae	49	66.2
Pupil rigidity	31	41.9
Shallow anterior chamber	24	32.4
Iris dystrophy	53	71.6
Secondary glaucoma	10	13.5
Degree I-II lens subluxation	14	18.9
Destruction of the vitreous body	18	24.3
Epiretinal membrane	8	10.8

**Table 2.** The frequency of additional surgical procedures in UC surgery

Surgical manipulation	Absolute number of eyes	Relative amount (%)
Staining of the anterior capsule	65	87.8
Pupil dilation	29	39.2
Separation of synechiae	56	75.7
Application of iris retractors	17	22.9
Iridoplasty	7	9.5
Basal iridectomy	5	6.7

**Table 3.** Distribution of patients with UC according to the degree of density of the lens nucleus (according to L.Buratto)

The degree of density of the lens nucleus	Absolute number of eyes	Relative amount (%)
2	21	28.4
3	44	59.4
4	9	12.2
<b>Total</b>	<b>74</b>	<b>100</b>

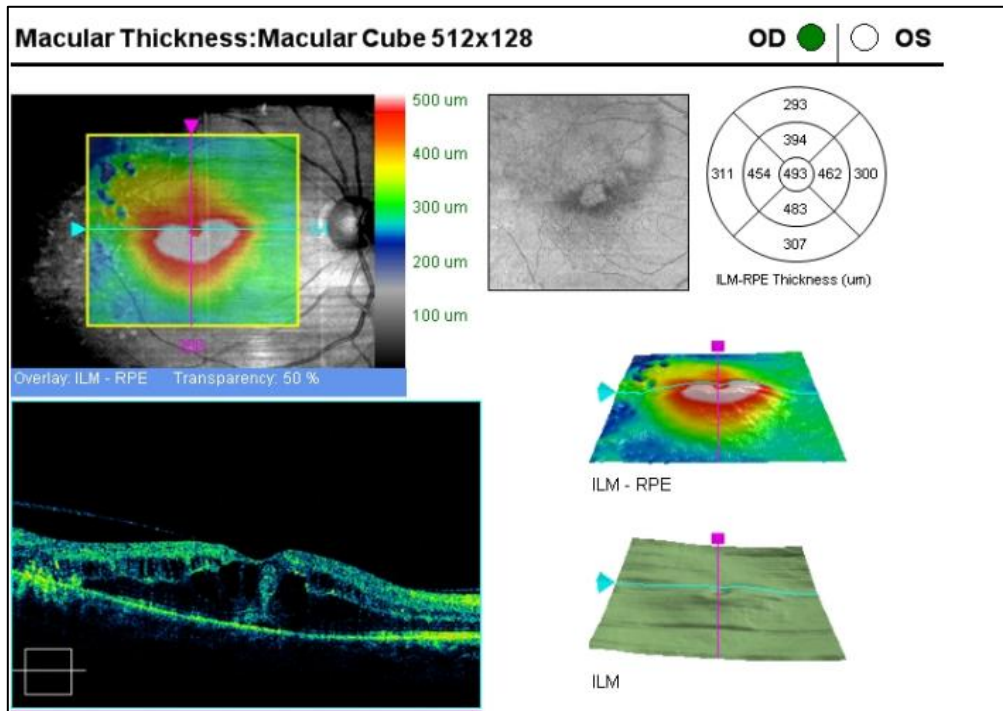
**Table 4.** Dynamics of BCVA after uveal cataract surgery (absolute number of eyes, %)

BCVA	1-3 days after surgery	A month later	In a year
<0.2	16 (21.6%)	10 (13.5%)	17 (22.9%)
0.2 – 0.5	39 (52.7%)	37 (50%)	40 (54.2%)
>0.5	19 (25.7%)	27 (36.5%)	17 (22.9%)
<b>Total</b>	<b>74 (100%)</b>		

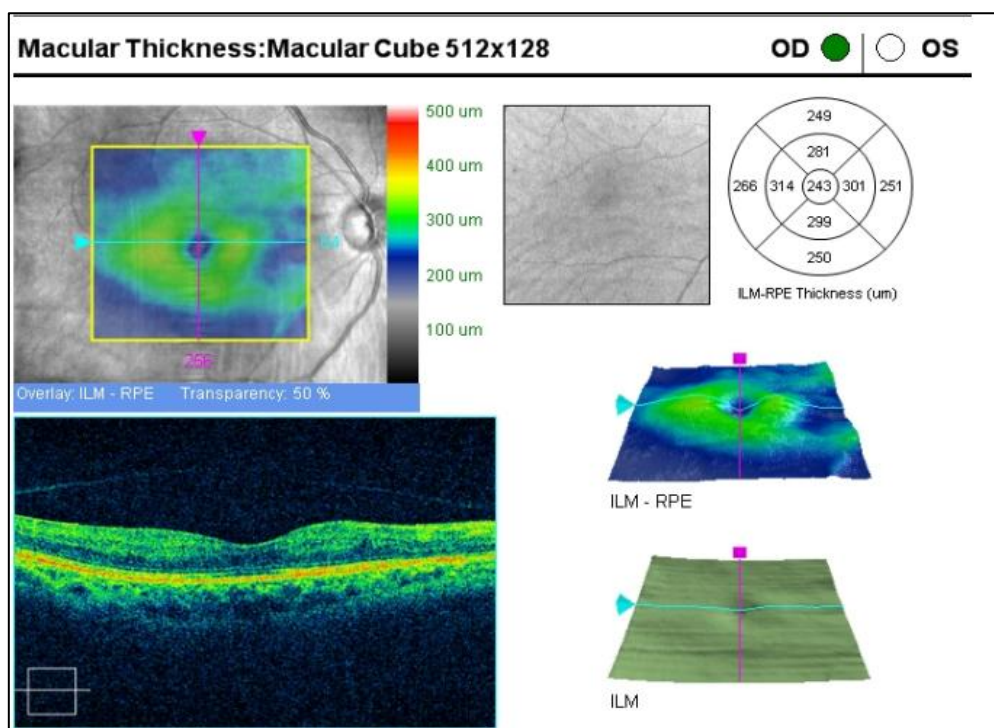
**Table 5.** Results of evaluation of the frequency of postoperative complications after UC surgery

Postoperative complication	Absolute number of eyes	Relative amount (%)
<b>Early postoperative complications</b>		
Degree 1 Tyndall effect	14	18.9
Degree 2 Tyndall effect	3	4.1
Precipitates on the IOL	8	10.8
Posterior synechiae, vitreous effusion	7	9.5
Increase in IOP	5	6.8

Late postoperative complications		
Recurrence of uveitis	28	37.8
CME	8	10.8
Secondary cataract	10	13.5
Secondary glaucoma	2	2.7



**Image 1.** CME before treatment in a patient B.M. with UC, CMT =493 um (OCT, Cirrus optic coherence tomography HD-OCT 5000, Carl Zeiss Meditec AG).



**Image 2.** CME after treatment in a patient B.M. with UC, CMT =243 um (OCT, Cirrus optic coherence tomography HD-OCT 5000, Carl Zeiss Meditec AG).

Results of the analysis of the frequency of early and late postoperative complications are presented in Table 5. As can be seen from Table 5, postoperative complications were caused by the inflammatory reaction of the operated eye to surgery and were all stopped by the appointment of appropriate anti-inflammatory therapy. In the early postoperative period, complications of inflammatory etiology of varying severity were registered in 37 eyes in 16 patients (30.8%). The most common of these was an inflammatory reaction with the degree 1 Tyndall effect in 14 eyes out of 74 operated (18.9%). The most common late postoperative complication was a recurrence of uveitis in 28 eyes (37.8%). Recurrence of uveitis, which developed within a year after surgery, was stopped by the appointment of specific immunosuppressive therapy in 6 patients (12 eyes) with ankylosing spondylarthritis; 3 patients (6 eyes) with Behcet's disease; 3 patients (6 eyes) with rheumatoid arthritis; 2 patients (4 eyes) with Vogt-Koyanagi-Harada disease. Even before surgery, all patients with pathological changes in the macula (epiretinal membrane with traction

component in 8 eyes) in the late postoperative period had the development of CME (8 eyes - 10.8%) (Image 1). Restoration of visual functions and the thickness of the macula in 6 cases were noted after conservative anti-inflammatory therapy; in 2 cases were noted after intravitreal steroid injection (Image 2).

Secondary cataract in 10 eyes was resolved by YAG-laser capsulotomy on the background of anti-inflammatory therapy. In secondary glaucoma, 2 patients (2 eyes) were prescribed topical hypotensive therapy followed by laser iridectomy. One of them due to the absence of a persistent hypotensive effect of the treatment underwent surgery with implantation of glaucoma drainage.

The clinical examination was carried out along with the analysis of the results of the laboratory immunological examination. It was interesting for us to present comparatively the results of an immunological study conducted in dynamics in 32 patients without postoperative complications (Table 6) and in 20 patients with postoperative inflammation of varying severity (Table 7). In patients without the development of inflammation in the postoperative period, there

were no significant changes in the average values of immunological parameters of the immune response. Some factors of humoral immunity (IRI=CD4+/CD8+, IgM, IgG, IgA, complement

component C3) were higher than the control indicators but this difference had no special significance ( $p>0.05$ ).

**Table 6.** Results of a comparative immunological survey of patients without postoperative complications (n=32)

Immunology factor	Control	Unit	Before surgery, M±m	3 days after surgery, M±m
CD4+/CD8+	1.2 - 2.5	-	2.37±0.11	2.45±0.09
Complement component C3	1032 - 1495	mg/l	1234±14.2	1376±17.7
IgM	60 - 280	mg/dl	221.8±9.7	234.9±10.4
IgA	90 - 450	mg/dl	464±11.3	469±10.9
IgG	800 - 1800	mg/dl	1981±12.8	2017±12.7
IgE	0-100	IU/ml	34±7.8	45±9.9
ANA (IgG)	≤1,0 - negative; >1,0 - positive	-	0.8±0.02	0.9±0.07
CRP	0-6	mg/l	6 ±0.02	6 ±0.01

**Table 7.** Results of a comparative immunological survey of patients with postoperative complications (n=20)

Immunology factor	Control	Unit	Before surgery, M±m	3 days after surgery, M±m
CD4+/CD8+	1.2 - 2.5	-	2.89±0.23	3.17±0.01*
Complement component C3	1032 - 1495	mg/l	1753±10.01*	2069±9.31*
IgM	60 - 280	mg/dl	219.4±8.2	263.7±7.1
IgA	90 - 450	mg/dl	563.8±10.7*	589.8±11.4*
IgG	800 - 1800	mg/dl	2028±13.4*	2154±12.8*
IgE	0-100	IU/ml	43±6.8	45±10.9
ANA (IgG)	≤1.0 - negative; >1.0 - positive	-	1.8±0.11*	2.1±0.09*
CRP	0-6	mg/l	6 ±0.01	6 ±0.03

*Note:* statistical significance of differences with control indicators: \* -  $p < 0.05$

IRI is the ratio of the number of CD4+ T-helpers and CD8+ T-suppressors in peripheral blood (CD4+/CD8+). According to the results of a comparative immunological examination in patients with postoperative inflammation, there was a statistically significant increase in the mean values of the IRI, the complement component C3, IgA, IgG, and ANA in blood serum relative to the control parameters ( $p<0.05$ ) even before surgery. After surgery on days 1-3, these indicators still remain elevated. An increase in IRI is noted against the background of a decrease in CD8+T-killers and an increase in CD4+ T-helpers which can contribute to the strengthening of autoimmune processes in turn. The complement component C3 is also a protein of the acute phase of inflammation and participates in the activation of the complement system both in the classical (acquired immunity) and in an alternative way (innate immunity). IgA are glycoproteins that are synthesized mainly by plasma cells of the mucous membranes in response to local exposure to antigens and are an important factor in the local

protection of the mucous membranes. They enhance the phagocytosis of antigens by activating complement via an alternative pathway. IgG is the main type of blood and intercellular fluid antibodies; therefore, it participates in the control of infection throughout the body by binding to a variety of pathogens. The level of IgG in the blood is considered an indicator of the activation of an individual's immune system against a certain pathogen. IgM is normally first secreted during the humoral response of the immune system to the primary contact of the body with the antigen and are indicators of an acute infectious process. ANA is a family of autoantibodies that bind to nucleic acids and their associated proteins. ANA is detected in more than 90% of patients with diffuse connective tissue diseases, therefore this immunological test was of great diagnostic importance in the etiological diagnosis of uveitic pathology. CRP is a protein in the acute phase of inflammation. Neither before, nor after the operation average values of IgM and CRP were elevated in these patients which was an

immuno-biochemical confirmation of the absence of an acute process and clinical activity of systemic inflammation in the operated patients. The results obtained indicate that even against the background of clinical well-being in patients with UC, deviations of some systemic immunological parameters (CD4+, CD8+, IgG, IgA, complement component C3, ANA) are possible. These disorders are mainly indicators of unfavorable autoimmune sensitization, and as the studies show they can serve as harbingers of the development of undesirable immune responses and an indication for strengthening preoperative preventive anti-inflammatory training with an emphasis on immunosuppressive treatment.

Many sources of modern ophthalmological literature agree with our results of a more frequent spread of cataracts in anterior uveitis (59.6%) (Choi et al., 2022; Balta et al., 2018). Also in the literature, many authors point to the multifactorial etiological structure of the pathogenesis of cataracts in uveitis: the effect of autoimmune inflammation increased permeability of the hemato-ophthalmic barrier, the role of free radical oxidation as well as corticosteroids used for a long time in the treatment of uveitis (Chiu et al., 2017; Macarie and Macarie, 2018). In our study, the incidence of uveitis in autoimmune diseases was higher in patients taking systemic steroids (54.1%) (Drozdova, 2012). Many leading ophthalmic surgeons of the world agree with the conjugation of uveitic cataract surgery with certain technical difficulties. Thus, in this study during the phacoemulsification of cataracts, additional surgical measures were applied to achieve a highly functional result: the elimination of synechiae (75.7%), the use of iris retractors (22.9%), iridoplasty (9.5%). But additional manipulations during surgery, along with anatomical and physiological features of the UC are a risk of postoperative inflammation with the development of early and late postoperative complications such as pupil overgrowth, iris bombage, glaucoma, vitreous fibrosis, retinal detachment and eye subatropia (Sen et al., 2016; Llop and Papaliadis, 2018).

In this study, the most frequent complication in the early and late postoperative period was postoperative inflammation of varying severity (37.8%). Also, UC is often combined with the

pathology of the posterior segment of the eye: vitreous opacity, proliferative vitreoretinopathy with the formation of traction retinal detachment, CME, detachment of the ciliary body and hypotension of the eye in connection with which vitreoretinal interventions are often required. In this study, CME that occurred in 10,8% of surgical cases was successfully cured by anti-inflammatory therapy (Juthani et al., 2017).

But it is necessary to note a certain aspect of this study, the novelty of which is the main purpose of its conduct. In all patients with UC, the operation was performed in the remission stage of uveitis. At the same time in patients with the development of inflammation in the postoperative period even before surgery statistically significant deviations of the average values of some immunological parameters (CD4+, CD8+, IgG, IgA, complement component C3, ANA) were noted with normal indicators of the activity of the inflammatory process (CRP, IgM).

Thus, cataract surgery for uveitis requires careful monitoring of the activity of the inflammatory process before and after surgery. Despite the stage of uveitis remission, depending on the indicators of the immunological survey of a patient with UC before surgery, it is possible to outline a certain algorithm of preoperative immunosuppressive treatment to minimize postoperative inflammation risk.

## CONCLUSIONS

1. Cataract development was most often noted in anterior uveitis (59.6%).
2. The mutual influence of the inflammatory process and the systemic use of steroids in the complex therapy of uveitis contributed to a more frequent spread of uveitic cataracts in autoimmune diseases (54.1%).
3. Patients with uveitic cataracts are at high risk of developing postoperative inflammation in the early and late postoperative periods. Immunological surveys in the preoperative period during cataract surgery in patients with uveitis can serve as an additional method for predicting postoperative inflammation risk.



## STATEMENTS

The author declares no conflict of interest. This article and the research underlying it would not have been possible without the exceptional support of the staff of the National Center of Ophthalmology named after Academician Zarifa Aliyeva. I accept the terms of the publication.

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## The effect of atropine with different concentration (0.025% and 0.01%) on myopia programming

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In recent decades, myopia is becoming an epidemic. This is proven by a steady increase in the number of this type of refractive error around the world. One of the commonly used drugs for myopia is 1% atropine. However, frequent use of this drug has several side effects. The purpose of this study is to examine the effectiveness of 0.025% and 0.01% atropine compared with placebo for a year. The study involved 62 (124 eyes) children and adolescents between 4 and 14 years old with myopia of at least -1.0 and astigmatism up to 2.5 diopters. Considering the progression of the controlled spherical equivalent and the increase in the axial length of the eye in a year, 0.01% atropine was the most effective of all the applied concentrations. The pupil size in photopic and mesopic conditions increased and the accommodative amplitude decreased by  $1.71 \pm 2.61D$ ,  $0.28 \pm 3.4D$  and  $0.32 \pm 2.91D$  in the groups 0.025%, 0.01% atropine and placebo respectively ( $p \leq 0.01$ ).

**Keywords:** Accommodative amplitude, atropine, myopia, spherical equivalent

### INTRODUCTION

In recent decades, myopia is becoming an epidemic. It is evidenced by the steady increase in the number of people with this type of refractive disorder around the world. B.A.Holden et al. (2016) suggest that by 2050 4,758 million people will have myopia, of whom 938 million will have high myopia (Sun et al., 2012).

An actual method of pharmaceutical control of myopia progression in children and adolescents is the use of antimuscarinic ophthalmic drugs, which are used in routine practice to dilate the pupil. Antimuscarinic ophthalmic drugs include Atropine (non-selective M-anticholinergic blocker) and Pirenzepine (selective M1-anticholinergic blocker, affecting mainly the ciliary body and having a minimal dilating effect on the pupil).

The first attempts to use atropine to prevent the progression of myopia were associated with the hypothesis of the excessive tension of the accommodation apparatus as the main cause of

the development of this type of ametropia. In some contradiction with this hypothesis is the revealed fact of the effectiveness of atropine in preventing the development of myopia in an experiment on chickens, in which accommodation is mediated by n-cholinergic receptors (McBrien et al., 1993). In recent years, the possible stabilizing effect of atropine on the course of myopia has been associated with changes in the posterior segment of the eye.

The body of evidence shows that dopamine is one of the retinal neurotransmitters involved in the signaling cascade that controls eye growth (Feldkaemper and Schaeffel, 2013). For example, McBrien et al found that dopamine levels were reduced in the eyes of myopic deprivation chickens and mammals (McBrien et al., 2001). N.Schwahn et al. established that atropine administered intravitreally increased dopamine release from the retina in myopic deprivation chicks (Schwahn et al., 2006). In addition to dopamine, nitric oxide (NO) may play a role in the mechanism of atropine's effect on the

progression of myopia (Carr and Stell, 2016).

A series of studies on chickens revealed that of the many m-cholinergic receptor antagonists (including non-selective ones), only atropine, pirenzepine and oxyphenonium influenced the progression of deprivation myopia (Carr and Stell, 2016; Luft et al., 2003). Based on this, it has been suggested that the effect of atropine on preventing the development of myopia may not be associated with an effect on m-cholinergic receptors (Carr and Stell, 2016). It should be noted that chicken receptors have a different affinity for drugs as the mechanism of action has been studied only on mammalian receptors. Despite the data above, the question of the pharmacological mechanism of action of atropine on myopia's progression, which is unrelated to the effect on the accommodative apparatus of the eye, remains open and requires further study.

In turn, 1% atropine, which is widely used in preventing the development of myopia, has a number of negative effects on the eye. This includes photophobia due to persistent mydriasis and reduced ability to work at close range due to cycloplegia. The use of 1% atropine in both eyes might be photochromic, multifocal lenses and complications are possible (e.g. dryness and itching in the eyes, dry mouth and throat, constipation, redness and itching of the skin, difficulty urinating). Such side effects are not observed when using atropine at lower concentrations.

The purpose of the study was to investigate the efficacy of atropine at concentrations of 0.025% and 0.01% compared with placebo over one year.

## MATERIALS AND METHODS

Sixty-two (124 eyes) children and adolescents aged from 4 to 14 years with myopia of at least -1.0 and astigmatism of up to 2.5 diopters participated in the study. Thirty-five girls (70 eyes, 56.4%), 27 boys (54 eyes, 43.6%); Twenty-nine (58 eyes, 46.8%) participants were diagnosed with a mild degree, 33 people (66 eyes, 53.2%) had an average degree of myopia. Subjects received 0.025% and 0.01% atropine or placebo eye drops. Drops were prescribed 1 time

at night in both eyes for a period of 1 year. We studied: cycloplegic refraction (Plusoptix apparatus), the axial length of the eye (SonomedPacScan 300), accommodation amplitude, pupil diameter and maximum corrected vision. In addition, visual acuity was studied, tonometry (Canon Full Auto Tonometer TX-F), ophthalmoscopy tests were performed. Studies were conducted at the beginning and then 2 weeks, 4 months, 8 months and 12 months into the study. The main results were changes in sphere equivalent, eye length and the difference between groups. Statistical processing was performed in Statistics 20.

## RESULTS

Pupil size under photopic and mesopic conditions was increased by  $0.69 \pm 0.8$  mm and  $0.43 \pm 0.61$  mm, respectively, in the 0.025% atropine group, by  $0.59 \pm 0.9$  mm and  $0.25 \pm 0.56$  mm in the 0.01% atropine group and in the placebo group, respectively,  $0.15 \pm 1.09$  mm and  $0.3 \pm 0.55$  mm ( $p \leq 0.01$ ).

After 1 year, the changes in the spherical equivalent averaged  $-0.59 \pm 0.55D$ ,  $-0.54 \pm 0.61$  and  $-0.81 \pm 0.57D$ , respectively, in the groups ( $p \leq 0.01$ ) (Table 1), with the corresponding mean increase in axial length equal to  $+0.3 \pm 0.25$  mm,  $0.27 \pm 0.2$  mm,  $0.39 \pm 0.29$  mm and  $0.51 \pm 0.22$  mm ( $p \leq 0.01$ ).

**Table 1.** Average change in spherical equivalent (-) (D)

	2 weeks	4 months	8 months	1 year
<b>0.025% Atropine</b>	0.60±0.50	0.58±0.55	0.61±0.51	0.59±0.55
<b>0.01% atropine</b>	0.59±0.55	0.57±0.59	0.60±0.55	0.54±0.61
<b>placebo</b>	0.98±0.56	0.91±0.54	0.90±0.55	0.81±0.57

The accommodative amplitude was less by  $1.71 \pm 2.61D$ ,  $0.28 \pm 3.4D$  and  $0.32 \pm 2.91D$ , respectively, in the groups (0.025%, 0.01% atropine and placebo) ( $p \leq 0.01$ ). The pressure was constant in all groups.

## CONCLUSIONS

On the basis of the conducted research, the following conclusions can be drawn:

1. In groups of 0.25 and 0.01% atropine, a slowdown in the progression of myopia was observed in proportion to the concentration of the drug.
2. All concentrations were well tolerated without side effects that impair the quality of life associated with vision.
3. Of all the applied concentrations 0.01% atropine was the most effective, given the progression of controlled spherical equivalent and increase in axial length of the eye over a year.

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## **Endemic saffron (*Crocus sativus* L.): A promising therapeutic agent for retinal dystrophies**

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**A comprehensive approach was applied to study the mechanism underlying saffron stigmas extract therapeutic effect on the retina by employing biochemical, pharmacological and electrophysiological methods. It was found that saffron extract inhibits free radical processes in the retina and reduces the accumulation of lipid peroxidation products. The activity of key "protective" enzymes such as catalase, superoxide dismutase and glutathione peroxidase was preserved while the activity of transport ATPases was increased as well. In addition, the study demonstrated better preservation of retinal electrogenesis and its restoration following parabulbar injection of a 0.5% saffron extract solution in animals with experimental dystrophy.**

**Keywords:** *Saffron, free radical processes experimental dystrophy of retina, antioxidant enzymes, transport ATPases, electroretinogram, antioxidant*

### **INTRODUCTION**

One of the most significant challenges modern pharmacology faces is identifying and studying plant-derived bioactive substances (phytochemicals) to have potential in disease treatment and health promotion.

In the past century, advances in chemical technology have hastened progress in synthetic drug discovery and development. However, the last decade has renewed interest among biologists, pharmacologists and physicians in plant-based medicines. The side effects resulting from the combined action of some synthetic drugs and the quick drug resistance development are challenging issues that make searching for effective pharmaceutical compounds from natural sources even more relevant.

In this regard, saffron (*Crocus sativus* L.) is of significant interest in practical medicine. There are 12 species of saffron in the Caucasus, 5 species of which grow wild in Azerbaijan and a species has been introduced into culture, where it is used as a spice, a coloring agent and in

traditional medicine. The medicinal properties of saffron have been known since ancient times, and it was believed that saffron cures more than 100 diseases (Karomatov et al., 2018; Charles et al., 2013; Lim et al., 2014; Pandita, 2021). Recently, scientists from different countries have been studying the beneficial effects of saffron on a scientific basis, relying on empirical information (Abdullaev, 2003; Abdullaev et al., 2004; Butnariu et al., 2022; Bukhari et al., 2022).

Despite clinical trials being conducted in Azerbaijan to study the therapeutic effects of saffron water extract on various eye diseases, there is as yet insufficient data on its pharmacological, physiological, and biochemical activity (Nesrullaeva et al., 2001a; Nesrullaeva et al., 2001b; Nesrullaeva et al., 2002a; Nesrullaeva et al., 2002b).

The purpose of this work was to elucidate the possible mechanisms behind the therapeutic effects of saffron stigma extract in experimental ocular pathology.

To achieve this goal, on the model of experimental dystrophy of the retina the intensity

of lipid peroxidation (LPO), changes in the activity of antioxidant enzymes (SOD, CAT, and GPx), transport ATPases in photoreceptor cells and the electroretinogram (ERG) were performed after saffron extract administration.

## **MATERIALS AND METHODS**

The study was conducted on rabbits kept under standard vivarium conditions. The experimental animals were divided into the following groups (each consisting of 4-5 animals): 1) an intact control, which included intact animals and served as background measurements; 2) a control group, which included animals in which experimental retinal dystrophy was modeled, but the saffron extract was not administered; 3) experimental group, which included animals with experimental retinal dystrophy to which saffron extract was administered.

Experimental retinal dystrophy (pigmentary retinitis) of moderate severity was induced by a single intravenous injection with a 4% solution of monoiodoacetic acid (MIA) at a dose of 0.5 ml per 1 kg body weight of the animal (23-25 mg/kg).

The saffron used in the study was grown in the village of Bilgyah in the Apsheron Peninsula. Saffron extract was administered to the animals in the experimental group as a 0.5% aqueous solution (at a dose of 5 mg/kg) by parabolbar injection for 20 days. The control group received a physiological saline solution alone.

The subject of the study was the retina of the eyes of the experimental animals. The material was collected on the 5th, 10th, 15th, and 20th days of the experiment after the decapitation of the animals.

Animals of all three groups were subjected to decapitation. All experimental procedures were carried out in accordance with international and institutional regulations and guidelines relating to the use of animals for scientific purposes (Directive 2010/63/EU of the European Parliament and of the Council of the European Union on the protection of animals used for scientific purposes, 2012) and complied with the rules for the use of animals in the study of the eye and vision, developed by the Association for Research in Vision and Ophthalmology (ARVO),

as well (Zhiqing et al., 2008).

The intensity of lipid peroxidation was judged by the level of primary product - hydroperoxide (HP) and the secondary product malondialdehyde (MDA), which were determined by the method of T.Asakawa, S.Matsushita (Asakawa et al., 1980). The enzymatic activity of superoxide dismutase (SOD) in the retina was determined by the method of C.Beauchamp, J.Fridovich (Beauchamp et al., 1971), catalase activity was determined using the method of H.U.Bergmeyer (Bergmeyer, 1956) and glutathione peroxidase activity was determined by the method of D.Paglia, W.Valentine (Paglia et al., 1967).

The sum of the activities of Na, K and Mg-ATPase in retinal tissues was determined by the method of S.L.Bonting in Sobota modifications (Bonting et al., 1964). Electrophysiological studies were conducted as described in the study (Abdullaev et al., 1975).

The difference between the means of groups was compared using Student's t-test.

## **RESULTS AND DISCUSSION**

Research studies have demonstrated that experimental dystrophy is accompanied by a significant accumulation of lipid peroxidation products in the retina. Administration of a 0.5% aqueous solution of saffron extract via parabolbar injection to the animals in the experimental group for 20 days prevented the intensity of lipid peroxidation processes.

On the 15th day of saffron extract administration, the level of glutathione peroxidase (GP) in the rabbit retina tissue decreased to  $2.3 \pm 1.2$  relative units (RU), while the level of malondialdehyde (MDA) remained stable at  $2.1 \pm 0.5$  nmol/mg protein.

By the 20th day, the GP content in the rabbit retina had decreased to  $2.0 \pm 0.6$  RU, which was statistically significant compared to the control group ( $7.1 \pm 2.3$  nmol/mg protein,  $p < 0.05$ ). The MDA level had also decreased to almost intact control levels, reaching  $1.7 \pm 0.6$  nmol/mg protein (Table 1).

It is worth noting that a low level of free radical-mediated lipid peroxidation process activity is necessary for normal life to occur. Cells



possess a multi-component antioxidant defense system to prevent lipid peroxidation reactions from to be going out of control.

**Table 1.** Dynamics of changes in hydroperoxides content in the retina of animals with experimental dystrophy under saffron extract administration ( $M \pm m$ ,  $n=5$ )

Intact control	Day of experiment	Control group (experimental dystrophy of the retina)	Experimental group (experimental dystrophy + saffron extract)
2,45±0,12	5 <sup>th</sup> day	3.4±1.0	2.8±0.8
	10 <sup>th</sup> day	5.6±1.8*	3.6±1.2°
	15 <sup>th</sup> day	4.3±1.3*	2.3±0.6°
	20 <sup>th</sup> day	7.1±2.3**	2.0±0.6°°

**Note:** Significant difference: \* $p < 0.05$ , \*\* $p < 0.01$  - compared to intact control; ° $p < 0.05$ , °° $p < 0.01$  - compared to the control group.

Meanwhile, in the course of experimental retinal dystrophy, the variation of peroxidation processes in the photoreceptor cell should influence the functioning of its antioxidant system (AOS). To prove that we have studied the dynamics of the activity of antioxidant enzymes - catalase (CAT), superoxide dismutase (SOD), and glutathione peroxidase (GPX) in the retina during

experimental moderate dystrophy and saffron extract administration.

Analysis of the experimental data obtained in this series of experiments showed that a slight decrease in CAT and SOD activity was observed in the control group animals from the first days of the experiment. On later observation there was a tendency for an increase in enzyme activity but for catalase, it was statistically insignificant (Table 2).

This, in turn, can be caused by the imbalance or damage in the systems responsible for maintaining - lipid peroxidation reactions at a low, steady-state level in the cells. Saffron extract reactivates the studied antioxidant reserves in the retina. Thus, the activity of SOD doubles on the 5th day of observation, increases by 1.5 times on the 10th day of the experiment, and by 2.2 times on the 15th day of observation. By the 20th day of administration of the saffron extract, the activity of SOD increased by 3.2 times, almost reaching intact levels. A similar trend is observed when analyzing the activity indicators of GPx, which correlates with the data on the nature of changes in thiol metabolism in the retina under the influence of saffron extract in experimental toxic dystrophy (Table 2).

**Table 2.** Effect of saffron extract on the activity of antioxidant defense system in the retina behind moderate-severity experimental toxic retinal dystrophy ( $M \pm m$ ,  $n=5$ )

Indicators	Intact control	Control group (experimental dystrophy of the retina)	Experimental group (experimental dystrophy + saffron extract)
5th day			
Catalase, units/mg protein	209.6±1.2	199.3±0.22	201.4±0.24
Superoxide dismutase (SOD), units/mg protein	192.0±17.3	81.0±7.4***	160.0±15.5 *°°°
Glutathione peroxidase (GPO), nmol of NADPH per mg protein per min	23.64±0.19	18.85±0.45*	18.92±0.12°
10th day			
Catalase, units/mg protein	209.6±1.2	200.0±0.2	205.2±0.22*
Superoxide dismutase (SOD), units/mg protein	192.0±17.3	97.0±9.5***	143.0±13.4*°°
Glutathione peroxidase (GPO), nmol of NADPH per mg protein per min	23.64±0.19	17.49±0.54***	22.84±0.19°°°
15th day			
Catalase, units/mg protein	209.6±1.2	198.6±0.18	208.4±0.24°°°
Superoxide dismutase (SOD), units/mg protein	192.0±17.3	78.0±6.2***	174.0±16.5°°°
Glutathione peroxidase (GPO), nmol of NADPH per mg protein per min	23.64±0.19	14.16±0.57***	25.18±0.48°°°
20th day			
Catalase, units/mg protein	209.6±1.2	198.0±0.14	209.5±0.2
Superoxide dismutase (SOD), units/mg protein	192.0±17.3	65.0±4.7***	204.0±19.2°°°
Glutathione peroxidase (GPO), nmol of NADPH per mg protein per min	23.64±0.19	10.18±0.48***	27.63±0.25°°°

**Note:** Significant difference: \* $p < 0.05$ , \*\* $p < 0.01$  - compared to intact control; ° $p < 0.05$ , °° $p < 0.01$  - compared to control group

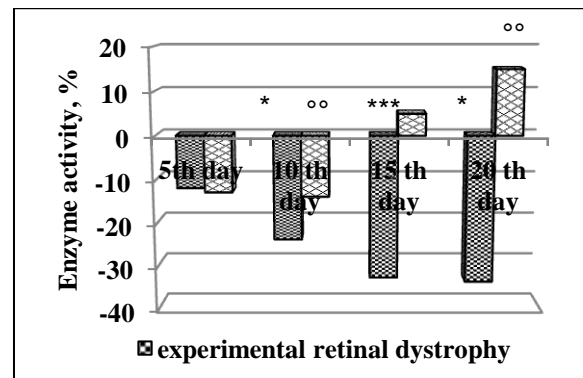
*Endemic saffron (Crocus sativus L.): A promising therapeutic agent for retinal dystrophies*

It is known that ion movements in the outer segments of retinal photoreceptor cells, particularly rods, constitute the basis of photoreceptor functioning. Some authors assign a special role in the primary processes of photoreceptor cell excitation and the generation of electrical potential to the ATPase systems of the retina, which provide a directed flow of potential-forming ions (Bonting et al., 1964). Thus, the processes of generating and propagating bioelectrical potentials in photoreceptor cells are closely related to the functioning of energy-dependent ion transport ATPases fixed on plasma membranes.

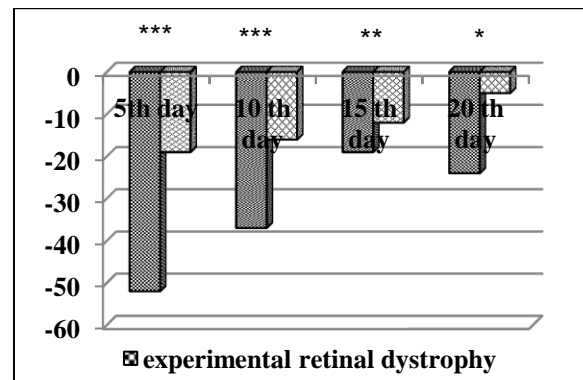
Studies on rabbits have shown that the toxic effects of MIDA lead to a suppression of the activity of transport enzymes in the rabbit retina, although the sensitivity of enzymes to toxic exposure varied: on the 10th day of the experiment, the activity of Na<sup>+</sup>, K<sup>+</sup>-ATPase decreased by 39%, while the activity of Mg<sup>2+</sup>-ATPase decreased by 10%, compared to intact control data. On the 20th day of the experiment, the activity of Na<sup>+</sup>, K<sup>+</sup>-ATPase in the examined eye structure decreased by an additional 10%. As it is seen in Fig. 1, retrobulbar injection of saffron extract contributed to the preservation of the activity of transport ATPases in the rabbit retina. Thus, on the 5th day of the experiment, the activity of Na<sup>+</sup>, K<sup>+</sup>-ATPase was 19% lower than the corresponding value in the intact control (the difference is statistically significant p<0.01), and 5% higher than the value in the control group. In turn, the activity of Mg<sup>2+</sup>-ATPase decreased by 33%.

Starting from the 10th day of the experiment the activity of transport enzymes remained stable and continuously increased throughout all the subsequent observation periods (see: Fig. 1 and Fig. 2).

Electrophysiological methods, such as electroretinography, play an important role in the differential diagnosis of eye diseases. These methods are valuable tools in the clinical-physiological arsenal that enables an objective and more precise assessment of the functional status of various parts of the visual system to be one of the leading methods in the objectification of the functional state of the retina (Abdullaev et al., 1975).

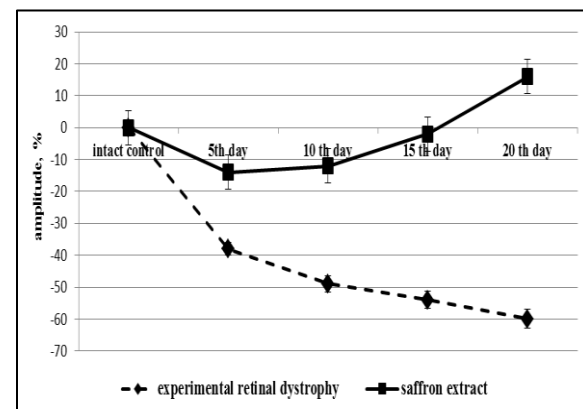


**Fig. 1.** Effect of saffron extract on changes in the activity of Na<sup>+</sup>, K<sup>+</sup>-ATPase (μmol Pi/mg protein/h) in the retina of animals with moderate-severity experimental toxic retinal dystrophy.

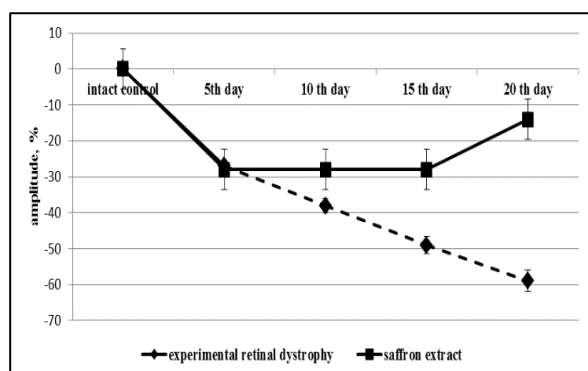


**Note:** Significant differences: \*p<0.05, \*\* p<0.01 - compared to intact control; ° p<0.05, °° p<0.01 - compared to control group.

**Fig. 2.** Changes in the activity of Mg<sup>2+</sup>-ATPase (μmol Pi/mg protein/h) in the retina of animals with moderate-severity experimental toxic retinal dystrophy.



**Fig. 3.** Dynamics of the a-wave of the electroretinogram (ERG) upon parabolbar administration of saffron in animals with moderate-severity experimental toxic retinal dystrophy.



**Fig. 4.** Dynamics of the b-wave of the electroretinogram (ERG) upon parabolbar administration of saffron in animals with moderate-severity experimental toxic retinal dystrophy.

During dynamic observation of the control group animals, simultaneous depression of the amplitude parameters of all components of the ERG was observed; its nature objectively reflected the topography of the damage, which can be considered as a result of the initial stage of the visual act being affected. Analysis of the data suggests that the depression of the amplitude of the a- and b-waves of the ERG in experimental medium-severity dystrophy can be attributed to the toxic effects of MIDA; it leads to an increase in the level of lipid peroxidation products occurring in the retina behind the development of pigmentary retinitis.

A moderate depression of retinal functional activity was detected after parabolbar injection of saffron extract in the experimental group of animals. The stabilization of the a-wave has maintained (remained) until the end of the experiment (on the 20th day after injection of the saffron extract), as shown in Figure 3. Similarly, better dynamics of the b-wave were found in the ERG under saffron extract treatment (Fig. 4).

The analysis of the electrical responses of the retina to a single light stimulus also revealed an increase in the c-wave of the electroretinogram under saffron extract administration. The available literature presents interesting evidence that the c-wave of the ERG cannot be registered without normal physical and biochemical relationships between the pigment epithelium and the outer segments of the photoreceptors, without disc renewal,

photochemical transformations of visual pigments and normal retinal nutrition.

## CONCLUSION

Therefore, registration of the local ERG c-wave in our experiments is objective evidence of the stimulating effect of saffron on the biochemical processes in the retina; it manifests in the normalization of metabolic processes and as a result, improves visual function and functional activity parameters.

In this regard, it is plausible to hypothesize that the relatively superior preservation of retinal electrogenesis and its subsequent recovery in the experimental group animals following the administration of saffron extract may be attributed to the neuroprotective and retinoprotective properties of saffron compounds on retinal function during the progression of moderate-severity experimental toxic retinal dystrophy.

In summary, it can be inferred that saffron exerts a normalizing and stimulating influence on certain biochemical processes within the retina. By mitigating oxidative stress, saffron prevents the suppression of transport ATPase enzymes and antioxidant defense enzyme activity within the organism promoting the normalization of metabolic processes and consequently improving the functional state of the retina.

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## Anti-vascular endothelial growth factors and cystoid macular edema after cataract surgery: When to use – case study

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Refractory PCME is a rare, but serious vision-threatening complication of cataract surgery. For this reason, this complication has to be managed timely and adequate. Non-steroidal and steroidal anti-inflammatory and anti-VEGF agents like bevacizumab are useful for refractory PCME. OCT has an important role in detecting PCME and measuring central macular thickness to control the treatment of PCME. In this case study, a patient is described after routine uncomplicated PACO surgery with refractory PCME at postop 4<sup>th</sup> week. Despite using topical NSAID+steroidal anti-inflammatory treatment there was no improvement in poor vision and CMT remained stable increased (811  $\mu\text{m}$ ). The vision was increased only after 2 weeks of i.v. bevacizumab injection, macular edema resolved and CMT decreased (280  $\mu\text{m}$ ) which was revealed by OCT. In conclusion, OCT-guided i.v. bevacizumab is a safe and well-tolerated therapeutic option for refractory PCME.

**Keywords:** Intra-vitreous bevacizumab, OCT, cataract surgery, cystoid macular edema

### INTRODUCTION

Pseudophakic cystoid macular edema (PCME) is one of the vision-threatening complications after cataract surgery (Copete et al., 2019). PKME, also known as Irvine-Gass syndrome, was first described in 1953 by S.R.Irvine (Irvine, 1953) and later by D.Gass (Gass, 1966). In general, as in cystoid macular edema (CME), PCME is a result of the accumulation of retinal fluid between the inner and outer nuclear layers of the retina and the formation of fluid-filled cystic spaces (Kanski, 2007).

The incidence of PCM varies from 1 to 30% (depending on factors such as surgical methods, intraoperative complications, surgeon's experience, presence of concomitant risk factors, etc.) (Copete et al., 2019; Grzybowski et al., 2016; Kodjikian et al., 2017). In the absence of risk factors and intraoperative complications, 1-2% (Grzybowski et al., 2016) are found. PCME after phacoemulsification of the cataract (PACO)

is less frequent compared to extracapsular cataract extraction (ECCE) and especially intracapsular extraction (Wetzig et al., 1979; Powe et al., 1994; Montes et al., 2003). Modern imaging methods such as Fundus Fluorescein Angiography (FFA) and Optical Coherence Tomography (OCT) and medical treatment (non-steroidal and anti-steroidal anti-inflammatory) have a special role in early detection, effective targeting and treatment of PCME (Kodjikian et al., 2017; Guo et al., 2015; Wittpenn et al., 2008; Ching et al., 2006). However, refractory CME remains on the agenda as it is difficult to respond to traditional treatment. Recently, it has been shown that anti-VEGF drugs are more effective in the treatment of refractory PCME than oral, subtenon and intravitreal (i/v) administrations of non-steroidal and steroidal anti-inflammatory drugs (Arevalo et al., 2009; Barone et al., 2009; Demirel et al., 2012; Akay et al., 2020; Spitzer et al., 2008). Measuring the central macular thickness (CMT) with OCT is a very important step in evaluating and guiding the effectiveness of treatment (Ching et al., 2006).



The aim of this work was to evaluate the clinical efficacy, safety and timing of i/v bevacizumab guided by OCT in the treatment of refractory PCM after uncomplicated PACO.

## MATERIALS AND METHODS

Clinical case: a patient with type 2 diabetes a long time visited our clinic with a complaint of poor vision in both eyes; initial diagnosis - cataract of both eyes.

During examination:

Vis OD=0.2 n/cor, Tn OD=16 mm.Hg.

Vis OS=0.04 n/cor, Tn OD=18 mm.Hg.

In both eyes, the fundus is not examined in detail. Due to the poor dilation of the pupil, diclofenac sodium was prescribed to the eye to be operated on, 1 drop 3 times a day for 3 days before surgery. FACO + intraocular lens implantation (IOL) surgery was performed in the patient's left eye. The operation and the postoperative (postop) period were uneventful.

Postop Day 1:

Vis OS=0.8, Tn OS=17 mm Hg

The cornea is transparent, the anterior chamber fluid is clear, and the pupil is alive.

Treatment: prednisolone acetate - 1 drop 6 times, moxifloxacin - 1 drop 6 times, diclofenac sodium - 1 drop 3 times.

Postop 3rd week:

Visual functions are fixed, fixed drops have been reduced.

However, in the 4<sup>th</sup> postoperative week, the patient returned with a complaint of a serious decrease in vision in the left eye.

During examination:

Vis OD=0.08 n/corrected, Tn OS=20 mm.Hg

The cornea is transparent, the anterior chamber fluid is clear, the vitreous is stable (US), the pupil's reaction to light is alive, the IOL is in a stable position inside the capsule bag.

Fundus examination: significant macular edema and loss of foveal reflex are noted.

OCT: intraretinal cysts, subretinal fluid accumulation and increased CMT (811  $\mu$ m) are noted (Fig. 1 and 2).

Prednisolone acetate - 1 drop 6 times, nepafenac - 1 drop - 4 times, oral diacarb 1 tab (0.5 mg) 1 time per day for 5 days, subtenon

triamcinolone acetate (0.5 mL) was injected.

2 weeks later: the vision in the left eye has improved slightly.

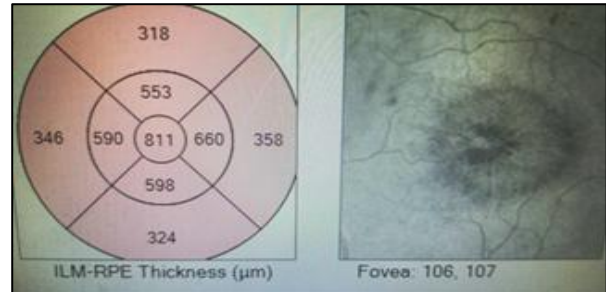


Fig. 1. OCT shows a prominent increase of CMT (811  $\mu$ m) due to the accumulation of subretinal fluid

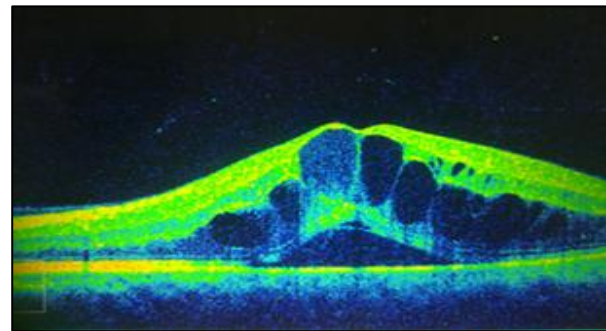


Fig. 2. OCT: Multiple fluid-filled intraretinal cystic spaces typical of PCME

Vis OS=0.2 n/cor, Tn OS=19 mm.Hg

However, since there was no significant change in CMT, close follow-up of the treatment was continued. Postop 8<sup>th</sup> week: visual acuity did not change and did not differ from the 6<sup>th</sup> week.

OCT - CMT slightly decreased (680 $\mu$ m), (Fig. 3 and 4). Bevacizumab (1.25 mg) was injected i.v. into the left eye.

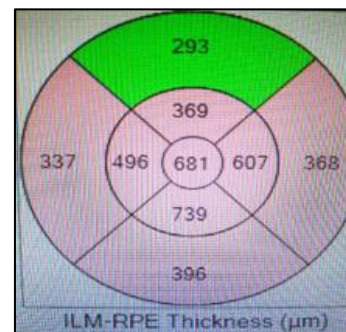


Fig. 3. OCT-postop 8th week: CMT continues to remain decreased (680 $\mu$ m)

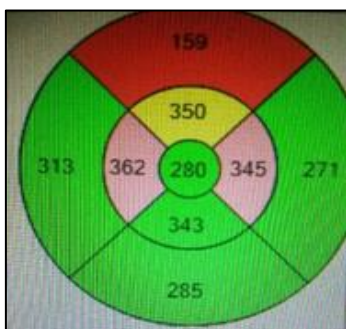


**Fig. 4.** OCT-postop 8th week: cystic spaces remain

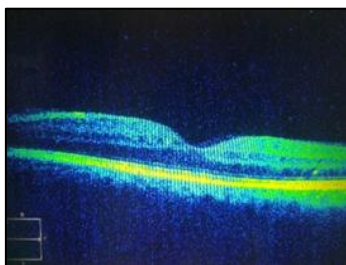
2 weeks after the injection:

Vis OS=0.7 n/cor, Tn OS=17 mm.Hg

OCT - CMT significantly decreased (280  $\mu$ m) (Fig. 5 and 6).



**Fig. 5.** 2 weeks after i/v injection: OCT confirms a significant reduction in CMT thickness



**Fig. 6.** 2 weeks after the injection: OCT shows the absorption of intraretinal fluid and loss of cystic spaces

Nepafenac was continued with 1 drop 3 times a day.

Postop 12<sup>th</sup> week:

Vis OS=0.9, Tn OS=16 mm.Hg, foveal reflex is clear, Amsler test-negative.

## RESULTS AND DISCUSSION

Irvine-Gass syndrome or PCME causes cystoid edema in the macula after cataract

surgery, causing vision loss (Copete et al., 2019; Sonmez et al., 2007). Although PCME can occur after uncomplicated cataract surgery (Grzybowski et al., 2016), more commonly PCME (especially refractory PCME) develops after complicated surgery due to anterior vitreous displacement and vitreous loss during surgery (Schepens et al., 1984), posterior capsule tear (Onal et al., 2004), and inflammation caused by contact of the IOL with the iris (Zaczek et al., 1998). It is thought that the disruption of internal and external blood-retinal barriers or the activation of the inflammatory cascade in all structures of the eye as a result of the inflammation caused by the increase of prostaglandins due to surgical trauma has an important role in the development of PCME (Xu et al., 2011; Zur et al., 2017). On the other hand, it is thought that the increase in metabolic activity in the RPE due to the effect of light entering into the eye during surgery leads to activation of the angiogenesis, which can lead to the development of PCME (Ueta et al., 2012). Factors such as diabetes, uveitis, epiretinal membrane (ERM), use of prostaglandin analogues, choroidal tumors and aging are known to play a role in the development of PCME also (Arcieri E.S. et al., 2005). Due to the high metabolic activity of the fovea, the mentioned pathological changes create the basis for the development of CME in the foveal zone, which in turn leads to an increase in CME and a decrease in visual acuity (Nagpal et al., 2001).

It should be noted that opinions on the treatment of PCME are controversial: although self-regression of PCME is possible in many cases, treatment is very important in other cases, especially in refractory PCME (Kodjikian et al., 2017). There is strong evidence of the effectiveness of anti-VEGFs along with non-steroidal and steroidal anti-inflammatory drugs in the treatment of refractory CME (Kodjikian et al., 2017; Kessel et al., 2014; Mitropoulos et al., 2015, Lin et al., 2018).

In the patient we report, refractory PCME was detected in the 4<sup>th</sup> postoperative week. In addition to a serious decrease in visual acuity, the loss of the foveal reflex, a prominent increase in the CMT in the OCT examination, and the appearance of fluid-filled cystic spaces between the layers of the retina, the typical clover leaf sign

(Rishi et al., 2013, Fig.7) in the FFA confirm Irvine-Gass syndrome.



**Fig. 7.** The clover leaf sign in FFA confirms the presence of fluid-filled cystic spaces between the layers of the retina

It should also be noted that the patient has long-term diabetes mellitus as a risk factor. Although the patient was given subtenon triamcinolone acetate injection and oral carbonic anhydrase inhibitor along with topical non-steroid and steroid drops, the visual acuity improved slightly, but the OCT image at week 8 postop was not encouraging: no decrease in CMT was noted. Prolonged elevation of CMT despite of treatment posed a serious threat to visual acuity as an indicator of refractory PCM. In order to eliminate this threat, as shown in the scientific literature (Kodjikian L. et al., 2017, Arevalo J.F. et al., 2009, Barone A. et al., 2009, Demirel S. et al., 2012), intravitreal bevacizumab was injected. 2 weeks after the injection, an increase in visual acuity to 0.7 was noted. Here we should especially mention the role of OCT. Thus, OCT is a method that allows clinical monitoring of macular edema by measuring macular thickness and detecting intraretinal cysts (Guo S. et al., 2015). Therefore, the correct decision - intravitreal bevacizumab injection - was made because OCT showed no decrease in CCT despite a subjective improvement in vision at the 8<sup>th</sup> week postoperatively.

Continued observation showed the complete recovery of the patient's visual acuity and foveal reflex, regression of the CCT to its normal size, absorption of macular edema.

## CONCLUSION

In conclusion, we should note that refractory PCMO is a serious vision-threatening complication that develops after cataract surgery. OCT-guided intravitreal bevacizumab injection in eliminating this complication resistant to medical treatment is effective and safe, leading to significant improvement of visual acuity, reduction of CMT, and absorption of macular edema.

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## Mycosis infections of the eye and some agents of their etiology

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**In some parts of the world, particularly in Asia, where they are the leading causes of blindness, ocular fungal infections are thought to be a substantial cause of considerable vision loss. The cornea is usually damaged by the inflammatory process, although it can also impact the orbit, eyelids, lacrimal apparatus, conjunctiva, sclera, and interior components of the eye. The diagnosis and treatment of ocular infections with a fungal aetiology continue to be difficult for ophthalmologists. Establishing a clinical diagnosis, isolating the fungus responsible for the infection, and providing effective local treatment, particularly in cases of corneal infections, are the key challenges. Negative predictive factors include insufficient initial treatment and delayed diagnosis brought on by a lack of suspicion of a fungal cause. The poor commercial availability of drug formulations and the poor penetration of antifungal medications into the eye tissue are also problems.**

**Keywords:** Ocular mycoses, fungal keratitis, antifungal drugs

Due to the rise in individuals with acquired immunosuppression brought on by prolonged use of immunosuppressive drugs, long-term broad-spectrum antibiotics, and AIDS, the frequency of ocular fungal infections has significantly grown over the past few decades (Kalkanci and Ozdek, 2011). The epidemiology of the disease is connected to the pathogenesis of eye infections. The most frequent cause of endogenous endophthalmitis is a species of *Candida*, and these infections typically occur in immunosuppressed patients who have a chronic systemic illness, septicemia that is related to it and being treated with broad-spectrum systemic antibiotics, intravenous hyperalimentation requiring a chronic indwelling catheter, or an organ transplant requiring immunosuppression (Lemley and Han, 2007). The second most frequent cause of endogenous fungal endophthalmitis is *Aspergillus species* (FE). Endophthalmitis has been linked to *Aspergillus flavus*, *A. fumigatus*, *A. niger*, *A. terreus*, *A. glaucus*, and *A. nidulans*. Other developing infections like *Fusarium*, *Penicillium*, *Pseudallescheria*, *Cryptococcus species*,

dimorphic fungi like *Histoplasma capsulatum*, *Blastomyces dermatitis*, *Sporothrix schenckii*, and *Coccidioides immitis* produced endogenous endophthalmitis have been implicated in a number of documented instances (Valluri and Moorthy, 2009). Therefore, any saprophytic fungus present in natural settings has the potential to infect the eye exogenously. *Candida species*, particularly in the postsurgical group, are the primary mycotic causes of exogenous endophthalmitis (Słowik et al., 2015), but *Fusarium species* were only discovered in the posttraumatic and postkeratitis patients. Exogenous endophthalmitis instances have also been linked to *Paecilomyces*, *Aspergillus*, *Acremonium*, *Exophiala*, *Pseudallescheria*, *Scytalidium*, *Sporothrix*, and *Penicillium species* (Rosenberg et al., 2006). Numerous and related to those causing fungal keratitis, fungi are the primary pathogens in posttraumatic endophthalmitis. *Exophiala jeanselmei*, *P. boydii*, *A. niger*, *Scytalidium dimidiatum*, *Helminthosporium spp.*, *S. schenckii*, and *Penicillium chrysogenum* are some of the reported organisms. The third clinical

manifestation of ocular fungal infections is keratomycosis, often known as fungal keratitis.

The use of both hard and soft extended-wear contacts is linked to bacterial infections, which are typically brought on by *Pseudomonas aeruginosa*. *Candida* species are virtually always to blame for bacterial infections and fungal keratitis. In agricultural labourers, fungal keratitis typically develops following contact with fungus-contaminated plant material. The majority of instances are brought on by filamentous soil saprophytes like *Zygomycetes*. Instances of ocular infections have been linked to fungi from over 56 genera. There have been reports of septate fungi such as *Epidermophyton floccosum*, *Scedosporium apiospermum* (Saracli et al., 2003), *Absidia* and *Rhizopus* species of *Zygomycetes* group, *Fusarium*, *Aspergillus*, *Curvularia*, *Acremonium*, and *Phialophora* species. Recently, the first account of *Carpoligna pleurothecii*-related fungal keratitis was described (Słowik et al., 2015). Although they are relatively uncommon in the industrialised world, keratitis caused by fungi is more common in many developing nations, particularly those that are tropical. Depending on the study's nation of origin, the percentage of fungal keratitis patients may range from 6 to 53 percent overall (Bharathi, 2007). In Asia, a prominent cause of blinding eye disease is fungus keratitis. The prevalence of fungal keratitis is still quite low in temperate regions like northern America and Britain.

The most frequent cause of fungal keratitis is corneal damage infected with plant matter. Wearing contacts has been linked to an increased risk of *Fusarium keratitis* since around 1980. There have been several epidemics of *Fusarium keratitis* among people who wear contact lenses in Singapore, Hong Kong, the United States, Puerto Rico, and the Caribbean. This specific type of storage solution encouraged the development of contaminating *Fusarium spp.* The orbit and its surrounding tissues, especially the paranasal sinuses, are particularly susceptible to invasive *Aspergillus* and *Zygomycete* infections (Fairley et al., 2000). Although *Aspergillus* can cause eye illness in healthy hosts as well, it is more invasive in immunocompromised hosts. Rhino-orbito-cerebral (ROC) zygomycolosis, an invasive zygomycolosis, is a severe consequence of diabetic

ketoacidosis and the use of immunosuppressive medications after organ donation. For both the initial assessment and for tracking disease development and response to treatment for sinoorbital illness, radiographic imaging of the orbit and paranasal sinuses is quite helpful.

Particularly in the developing countries, ocular fungal infections still play a significant role in causing ocular morbidity and vision loss (Kalkanci and Ozdek, 2011). Broad-spectrum antibiotic use, the rise in patients undergoing operations that cause immunosuppression, postoperative infection, trauma, and chronic corticosteroid use are all contributing factors to the rise in these infections over the past few decades (Tabbara, 2014). The anatomical location of the infection helps to classify ocular fungal infections. These infections can affect the front and posterior portions of the eye as well as the area surrounding the eye (ocular adnexa) (Sodhi et al., 2016; Ramirez-Soto and Bonifaz, 2022).

*Aspergillus*, *Candida spp.*, *Cryptococcus species*, and *Coccidioides spp.* are the most common pathogenic fungi of the eye, along with *Fusarium*, *Penicillium*, *Pseudallescheria*, and dimorphic fungi like *Histoplasma capsulatum*, *Blastomyces dermatitidis*, *Sporothrix spp.*, and *Coccidioides spp.* (*C. immitis*, *Aspergillus*, *Candida spp.*, *Cryptococcus* species, and *Coccidioides spp.* are the most common pathogenic fungi of the eye, along with *Fusarium*, *Penicillium*, *Pseudallescheria*, and dimorphic fungi like *Histoplasma capsulatum*, *Blastomyces dermatitidis*, *Sporothrix spp.*, and *Coccidioides spp.* (*C. immitis* and *C. posadasii*) (Khairallah and Attia, 2014). The non-specific clinical symptoms of ocular fungal infections might make diagnosis challenging. The use of laboratory and diagnostic tools, as well as the identification of the clinical symptoms of ocular fungal infections, have improved it recently (Khairallah and Attia, 2014). This has raised the prevalence of these disorders and the frequency of accurate diagnoses. As a result, it's critical to stay up to date on emerging breakthroughs in the diagnosis and treatment of infectious eye illnesses. In this context, articles describing novel discoveries and reviews on the epidemiology, diagnosis, and treatment of ocular fungal infections have been published in this Special Issue, with a focus on infections in the



ocular adnexa, endophthalmitis, keratitis, and ocular sporotrichosis in particular.

Eye fungus infections present a challenging set of clinical issues for both ophthalmologists and infectious disease specialists. Ocular fungal illness is rare, but it poses a major problem because it can lead to blindness from disorders like fungal keratitis or endophthalmitis (King et al., 2003). Four different types of fungal eye disorders have been identified: mycotic keratitis, an infection of the cornea that typically follows trauma; endogenous oculomycosis; extension oculomycosis; and miscellaneous infections, such as those of the tear ducts, eyelids, and conjunctiva (Shukla et al., 2008).

One of the most difficult types of microbial keratitis for the ophthalmologist to diagnose and treat is fungus keratitis. Human keratitis is caused by fungi that may look like mould or yeast. Because some cases of mould keratitis don't respond to extensive treatment, a therapeutic penetrating keratoplasty may be required (Uno, 2008). Geographical and climatic conditions play a significant role in determining the prevalence of specific infections. *Aspergillus* and *Fusarium* spp. are the most prevalent isolates in fungal keratitis, according to reports from throughout the world. Keratomycosis is most common in warm areas and coincides with seasonal increases in temperature and humidity. Due to the limited availability of infected material and the slow growth of a wide variety of fungi on commonly used culture media, the diagnosis of oculomycosis is frequently delayed (El-Sayed et al., 2010). This prompted the creation of culture-independent diagnostic procedures such as PCR, nucleic acid probes, immunological detection, fluorochromatic stains (CFW), and immunological detection (Gaudio et al., 2002).

## CONCLUSION

The fungi infections continue to be significant contributors to eye disorders. Fungal corneal infections are the main cause of blindness in Asia. The most typical pathogens inflicting corneal infections and eyeball mycoses are *Candida* spp., *Fusarium* spp., and *Aspergillus* spp. Immunodeficiency (such as AIDS), diabetes,

surgery, antibiotic and corticosteroid therapy, as well as drug addiction, are all linked to an increased risk of fungal infections. Early detection of the condition and prompt, intensive antifungal treatment have a substantial impact on how the illness develops and may lessen consequences, such as blindness.

According to the anatomical component of the eye affected by the disease, the epidemiology and etiological agents of ocular fungal infections were discussed. The most often isolated fungi in keratitis and fungal endophthalmitis are *Candida*, *Fusarium*, and *Aspergillus*. The identification of the fungus, which determines the clinical result and optimizes antifungal medication, requires the use of laboratory techniques. Antifungal treatment for this infection has been studied using systemic and intraocular applications. For prospective research intended to assess host characteristics in order to generate results equivalent to those obtained in humans, experimental models are essential. Animal models will enable the study of the aetiology of ocular infections despite the vast research that has been done on the effectiveness of various treatment options.

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## **Effect of complex therapy by acupuncture, retinalamin and OMK-1 on eye hemodynamics in children with retinitis pigmentosa**

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**The work concerns the study of retinitis pigmentosa (RP) – a hereditary generalized retinal dystrophy and complex therapy method via acupuncture (AP), endonasal electrophoresis with Retinalmin and OMK-1 instillations. The aim of this work was to assess the effect of complex therapy with the use of AP, endonasal electrophoresis with Retinalmin and OMK-1 instillations on the hemodynamics of the eye in children with retinitis pigmentosa based on the results of colour Doppler imaging (CDI). The study included 25 children (50 eyes) diagnosed with RP at the age of 7-14 years. Of the diagnosed, 10 patients were girls and 15 were boys. All patients underwent routine ophthalmic examination methods, electroretinography (ERG - general, macular, rhythmic, and cone). The state of the eye hemodynamics was determined by using the CDI of the vessels of the eyeball and retrobulbar space. The hemodynamic parameters (Vmax, Vmin, RI) of the ophthalmic artery (OA), central retinal artery (CRA), short posterior ciliary arteries (PSCA) were studied. Complex therapy of patients included AP, endonasal electrophoresis with Retinalamin, and OMK-1 instillations. 10 treatment sessions were carried out at the National Center of Ophthalmology within two weeks (5 sessions per week). Each AP session lasted 25 minutes and included the placement of acupuncture needles in a standard set of points on all patients located on the head, around the eyes, on the hands, feet, arms and legs. Endonasal electrophoresis was performed using Potok-1 apparatus equipped with 2 electrodes. Complex therapy with the use of AP, endonasal electrophoresis with Retinalamin and OMK 1 instillations is a minimally invasive method and has no negative impact. The positive therapeutic effect of our treatment method may pave the way for future research. Thus, complex therapy with the use of AP, endonasal electrophoresis with Retinalamin and OMK-1 instillations is a painless, affordable method, quite feasible in children and has a positive effect on visual functions and hemodynamics of the eye. The complex therapy implemented in our work can be used as a method of treating RP including in children.**

**Keywords:** *Retinitis pigmentosa (RP), colour Doppler imaging (CDI), acupuncture (AP), retinalamin, OMK-1*

### **INTRODUCTION**

Retinitis pigmentosa (RP, retinitis pigmentosa, retinal degeneration) is a hereditary disease of the retina that has a significant impact on the quality of life of patients. RP ranks first among all hereditary retinal pathologies

(Ayshegul, 2022).

This pathology refers to heterogeneous hereditary dystrophic changes in the retina, damaging photoreceptors, the frequency of which is 1:4000 (Verbakel, 2018).

One of the clinical signs of this disease is known to be vasoconstriction. However, the role

of vascular disorders in the development of the dystrophic process has not been practically proven. On the basis of single data on a systemic and local decrease in blood flow parameters, it is assumed that insufficient circulation of the retina can change its chemistry, leading to a violation of metabolism and the structure of cell membranes (Pozdeyeva, 2005). One of the modern and informative methods for studying eye hemodynamics is CDI. This method allows us to explore the orbital vessels of medium and small diameters. It is a non-invasive, painless method. This method is applicable to almost all age groups (Dimitrova, 2010; Steuer, 2005).

Despite the existing number of scientific studies devoted to the study of RP, this problem remains relevant today (Liu, 2022). Plenty of research is devoted to the study of the effectiveness of Retinalamin in dystrophic processes of the retina. Retinalamin is a polypeptide that belongs to the pharmacotherapeutic group - a stimulator of tissue repair. This medication has a stimulating effect on photoreceptors and cellular elements of the retina, improves the functional interaction of the pigment epithelium and the outer segments of photoreceptors, glial cells in dystrophic changes (Khavinson, 2014; Hasanova, 2008). In the literature, there are also works with the use of Omk-1 instillations, that have a neuroprotective effect in various eye pathologies. Citicoline being part of Omk-1 enhances the regeneration of damaged cell surfaces, maintains cellular integrity and prevents apoptotic and necrotic cell death by reducing the content of phospholipases (Matteucci, 2014; Pawel, 2016).

In the field of eye dystrophic diseases, works devoted to the use of AP are of interest. AP is one of the methods of reflexology characterized by the impact on certain points on the human body in various ways (acupuncture, cauterization, pressure), differing in strength, nature and duration. In ophthalmology, AP is used in the complex treatment of various eye diseases: glaucoma, dry eye syndrome, keratopathy, refractive error, myopia, optic nerve atrophy, initial cataract, macular degeneration, blepharoptosis and RP. AP has been used in Asian countries since ancient times (Japan, Korea, Egypt, etc.). AP found its application in Europe in

the 13th century. The development and practical use of AP by European medicine received a new impetus in the 1950s (Zainullina, 2020; Gavaa, 1992, 2013).

Patients with RP are interested in the use of additional or integrative therapies to slow down the progression of the disease since there is currently no pathogenetic treatment for this pathology (Liu, 2022). Therefore, it seems interesting to study the state of ocular blood flow in order to elucidate the role of the vascular factor in RP.

The aim of this research was to estimate the effect of complex therapy with the use of AP, endonasal electrophoresis with Retinalamin, and OMK-1 instillations on the hemodynamics of the eye in children with retinitis pigmentosa based on the results of CDI.

## **MATERIALS AND METHODS**

The study included 25 children (50 eyes) diagnosed with RP at the age of 7-14 years. Among them 10 patients were girls and 15 were boys. All patients underwent routine ophthalmic examination methods: visometry, tonometry, perimetry, ophthalmoscopy and fundus photography were performed (Fig. 1). To assess the functional activity of the retina, electroretinography (ERG - general, macular, rhythmic, and cone) was performed.



**Fig. 1.** Fundus photo of the RP patient

The state of the eye hemodynamics was determined using the CDI of the vessels of the eyeball and retrobulbar space. The study was carried out on the Nemio XG SSA-580A

ultrasonic diagnostic system (TOSHIBA) with a linear transducer with a frequency of 8 MHz to visualize blood flow in the ophthalmic artery (OA), central retinal artery (CRA), short posterior ciliary arteries (PSCA). The following hemodynamic velocity parameters were analyzed: maximum systolic blood flow velocity (Vmax), minimum diastolic blood flow velocity (Vmin), and resistance index (RI). The control group consisted of 50 practically healthy children aged 5-14 years. These children underwent CDI of eye vessels to determine the age- normative hemodynamic parameters.

Statistical data processing was carried out using the Excel-2007 program. The results obtained were processed by the method of variation statistics with the calculation of the arithmetic mean values of the sample and the statistical significance index p.

Complex therapy of patients included AP, endonasal electrophoresis with Retinalamin and OMK-1 instillations. 10 treatment sessions were carried out at the National Center of Ophthalmology within two weeks (5 sessions per week). Each AP session lasted 25 minutes and included the placement of acupuncture needles in a standard set of points on all patients located on the head, around the eyes, on the hands, feet, arms and legs (Fig. 2).

Endonasal electrophoresis was performed using Potok-1 apparatus equipped with 2 electrodes. The indifferent electrode was placed at the level of the cervical vertebrae and the active electrode was fixed on a turunda moistened with a Retinalamin solution and placed in the nasal passages. The duration of the procedure was 10-15 minutes. OMK-1 instillations were carried out

in the course of 4 months. Hemodynamic parameters were examined before therapy, as well as 3 months after treatment to evaluate the effectiveness.

## RESULTS AND DISCUSSION

Based on the results of CDI there was some decrease in OA speed parameters (Vmax and Vmin) in all patients before the therapy but these changes were not statistically significant. The indicator of peripheral resistance RI in this artery was statistically significantly lower (Table 1). Statistically significant changes in the speed indicators and resistance index were observed in CRA and PSCA.

3 months after complex therapy improvement of all arteries' hemodynamics was revealed as evidenced by the data of the CDI. Hemodynamic parameters were statistically significantly increased in CRA and PSCA. A statistically significant increase of RI in OA was observed (Table 1).

As for changes in visual acuity, there were subjective symptoms of improvement in all types of visual function in most cases (Table 2). As can be seen from Table 2, a minor improvement in all types of vision was observed in over 50% of patients.

From the available literature sources, there are works whose results are consistent with the results of our studies. Thus, the violation of blood flow in retinitis pigmentosa is confirmed by the authors G. Dimitrova and E. Steuer (Dimitrova, 2010; Steuer, 2005).

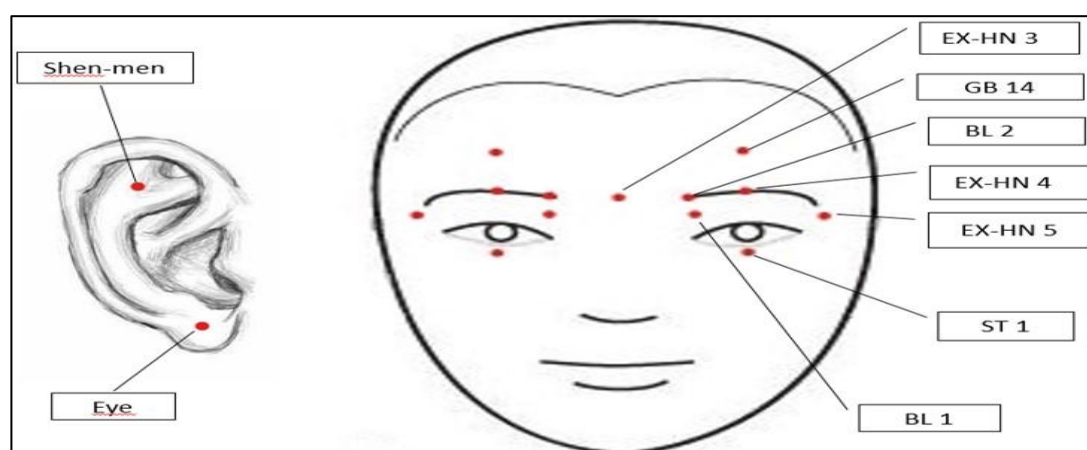


Fig. 2. Acupuncture points used on the patient (Ayshegul, 2022).

Table 1. CDI results

Arteries	Parameters	Before therapy	3 months after	Norm
OA	Vmax (cm/sec)	36.8±2.01	38.3±1.1	38.8±0.3
	Vmin (cm/sec)	8.91±0.8	9.07±0.06	9.19±0.14
	RI	0.75±0.01*	0.76±0.001#	0.76±0.003
CRA	Vmax (cm/sec)	9.8±0.13**	11.6±0.5###	12.8±0.1
	Vmin (cm/sec)	3.44±0.2*	3.57±0.06#	3.63±0.04
	RI	0.61±0.003***	0.67±0.02 ##	0.7±0.002
PSCA	Vmax (cm/sec)	7.2±0.5**	8.7±0.08 ###	9.1±0.1
	Vmin (cm/sec)	3.2±0.1*	3.4±0.1 ###	3.61±0.04
	RI	0.55±0.1**	0.59±0.02 ###	0.60±0.002

\* - p < 0.05; \*\* - p < 0.01; \*\*\*- p < 0.001 –statistically significant difference in relation to the norm

# - p < 0.05; ## - p < 0.01; ###- p < 0.001 – statistically significant difference in relation to the initial data

Table 2. Subjective feelings of patients with PR after complex therapy

Types of visual function	No improvement	Minor improvement	Moderate improvement	Significant improvement
Scotopic (night)	23.2%	62.7%	14.1%	–
Photopic (daytime)	9.1%	68.8%	22.1%	–
Central visual acuity	22.4%	61.8%	15.8%	–
Peripheral visual acuity	8.9%	53.2%	32.6%	5.3%

In a study by E. Steuer et al. (2005) the average value of Vmax in the CAS was reduced to 7.07±1.6 cm/sec, in the PCA - to 8.56±3.4 cm/sec. The authors also mention a decrease in vascular resistance in both vessels. G. Dimitrova et al. (Dimitrova, 2010) studied hemodynamic parameters in the CRA using CDI. The authors note a significant decrease in blood flow velocity, Vmax, and Vmin in this artery. Changes in the hemodynamic parameters in these studies are similar to the results of our work. But there are some differences in the level of indicators in

different arteries, which may be due to the age of the studied patients. In the available literature, only a few publications are devoted to the study of the state of eye hemodynamics in retinitis pigmentosa, and in pediatric patients, these studies are practically absent.

In a number of works with the use of AP in patients with RP, positive results are noted. Ayshegul Elbir et al. revealed signs of restoration of the function of optic nerve cells and an increase in the regenerative capacity of the CNS (Ayshegul, 2022). H. Xu et al. (2016) used the AP in 26

patients with retinitis pigmentosa and published data after 3 months of treatment in a 20-year study. At the end of the sessions, 2/3 of the patients noted an improvement in visual acuity and quality of life. H.Huang et al. created a protocol for a randomized controlled trial on this issue in 2021 (Huang, 2021). The authors concluded that this method improves local microcirculation of the eye and limits the pathological reaction associated with RP. F. Fereydouni et al. conducted a study involving 23 patients with RP and found a significant improvement in visual acuity after AP sessions (Fereydouni, 2017).

Also, in the literature of recent years, there are a number of works devoted to the study of the effect of Retinalamin and OMK-1 in various pathologies of the eye (Khavinson, 2014; Hasanova, 2008; Matteucci, 2014; Pawel, 2016; Trofimova, 2003). So, according to Hasanova and Belyaeva (Hasanova, 2008), the use of Retinalamin in experimental animals leads to a significant increase in the ERG b-wave compared to the control. So, with a moderate degree of retinal damage, the therapeutic effect of Retinalamin is detected already on the 15th day of observation, reaching its maximum by 35 days. The positive effect of Retinalamin is also observed in the case of severe damage of the retina in dystrophic processes of various origins (Khavinson, 2014; Trofimova, 2003). All these data prove the positive effect of Retinalamin on the retina. In the works of various authors with the study of the effect of OMK-1 eye drops, positive results were obtained (Matteucci, 2014; Pawel, 2016). So, A. Matteucci et al. prove the neuroprotective effect of OMK-1 on the basis of improved ERG, perimetry and optical coherence tomography in various degenerative retinal pathologies (Matteucci, 2014). In the work of G.Pawel et al., patients with primary open-angle glaucoma were studied. Positive perimetry and VEP results were obtained after the use of OMK-1 drop in these patients (Pawel, 2016).

## CONCLUSION

In a disease such as retinitis pigmentosa, possessing extremely limited treatment options it

is important to explore the potential of integrative therapy to improve visual function. Complex therapy with the use of AP, endonasal electrophoresis with Retinalamin and OMK-1 instillations is a minimally invasive method and has no negative impact. The positive therapeutic effect of our treatment method may pave the way for future research. Further research will explore the potential of the integrated treatment modality to improve ocular hemodynamics and visual function, thereby improving the quality of RP patients' life. While other promising therapies such as stem cells, gene therapy and pharmacological agents are still under development.

Thus, complex therapy with the use of AP, endonasal electrophoresis with Retinalamin and OMK-1 instillations is a painless, affordable method, quite feasible in children and has got a positive effect on visual functions and hemodynamics of an eye. The complex therapy used in our work can be recommended as a method of treating RP including in children.

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## **A patient with ulcerative colitis and central serous chorioretinopathy**

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**To report a case of a patient with ulcerative colitis and central serous retinopathy, which is a chronic ophthalmological condition that is frequently aggravated by corticosteroid treatment and may sometimes result in severe visual impairment. This case represents an interesting therapeutic dilemma pertaining to the treatment of ulcerative colitis exacerbation in a patient with this rare condition.**

**Keywords:** *Ulcerative colitis, chorioretinopathy, corticosteroids*

### **INTRODUCTION**

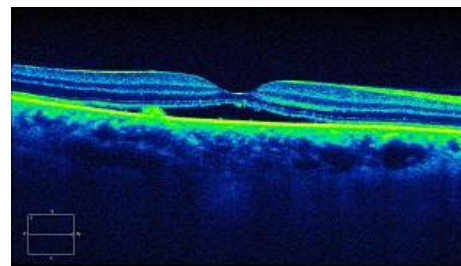
Ulcerative colitis (UC) is an inflammatory bowel disease limited to the mucosal layer of the colon. The disease course is often characterized by exacerbations requiring intensive anti-inflammatory treatment (Ilvio et al., 2011).

Central serous chorioretinopathy is a chorioretinal disease usually characterized by limited serous detachment of the neurosensory retina with a focal detachment of the affected retinal pigment epithelium that mostly affects young people and impairs central vision (Semeraro et al., 2019). This disease has a multifactorial and complex etiopathogenesis. Combining full ophthalmologic examination (anamnesis and clinical) and multimodal imaging methods will help us to reach an accurate and definitive diagnosis. The disease has features in the 25-51 age range, 40% of which show bilaterality and 80% of the patients have spontaneous regression. The probability of recurrence of the disease is around 50% in different series (Ersoz et al., 2018).

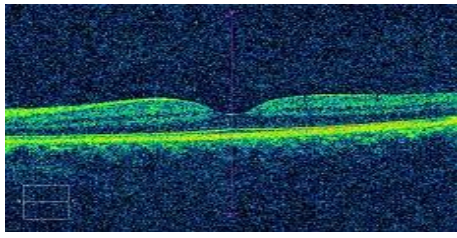
### **CASE REPORT**

A 35-year-old female patient was admitted to our outpatient clinic with the complaint of sudden

painless low vision in the right eye. It was learned from her history that she was treated with oral prednisolone due to the activation of ulcerative colitis. On examination, the best corrected visual acuity was 0.3 in the right eye and 1.0 in the left eye. Intraocular pressures of 12 and 15 mmHg in the right and left eyes. The anterior segment was normal in the slit-lamp examination. On fundus examination, punctate retinal pigmented epithelial detachment (PED) appearance with serous elevation in the macula and lower quadrant of the right eye; punctate PED appearance was detected in the left eye. Optical coherence tomography (OCT) of the patient revealed subretinal fluid and localized pigment epithelial detachment (PED) in the right macula and lower quadrant (Fig. 1), and localized PED in the left eye (Fig. 2) (Central macular thickness right: 309 microns; left: 267 microns).

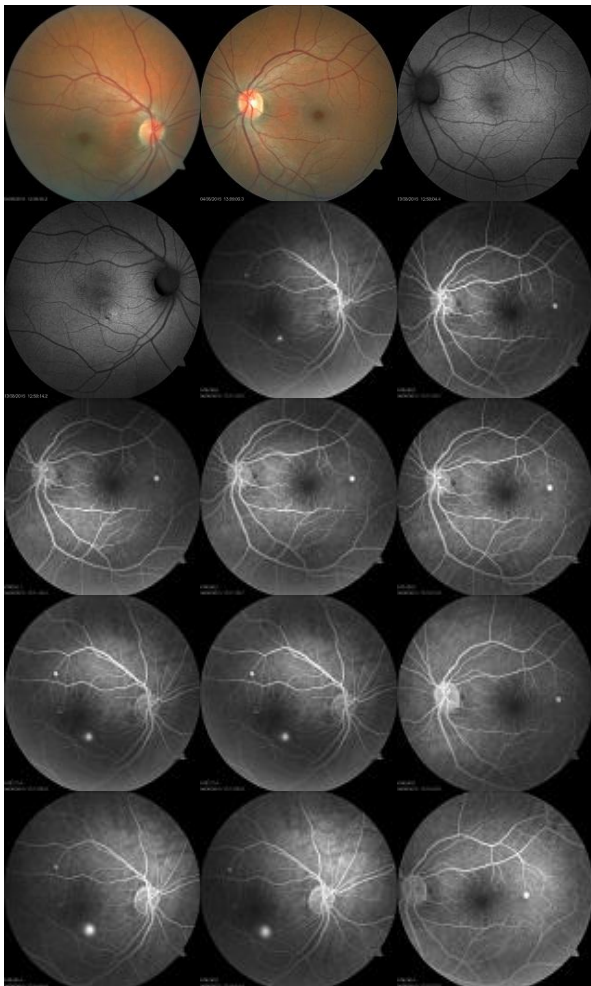


**Fig. 1.** The right macula and lower quadrant



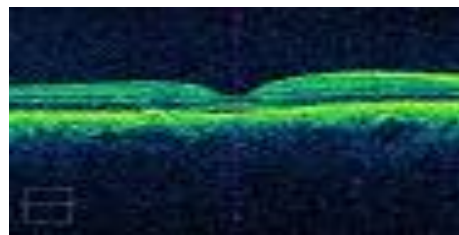
**Fig. 2.** Localized PED in the left eye

Fundus fluorescein angiography of the patient revealed hyperfluorescent leakage (inkblot) consistent with central serous chorioretinopathy (CSC) in the right eye and hyper fluorescence in areas matching the PED (Fig. 3).

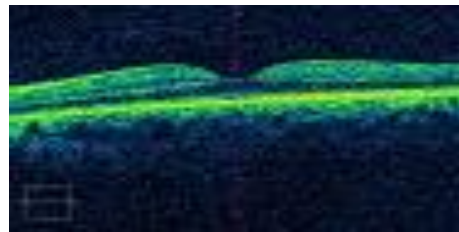


**Fig 3.** Fundus fluorescein angiography of the patient revealed hyperfluorescent leakage (inkblot) consistent with central serous chorioretinopathy (CSC) in the right eye

In consultation with gastroenterology, it was planned to reduce the steroid dose with the regression of activation. With the decrease in the steroid dose, it was observed that the subretinal fluid under the macula partially decreased and the subretinal fluid in the lower quadrant disappeared at the control after 3 weeks. On examination, the best corrected visual acuity was 0.3 in the right eye and 1.0 in the left eye. On OCT, the central macular thickness of the right eye was 242 microns (Fig. 4) and the central macular thickness of the left eye was 277 microns (Fig. 5).

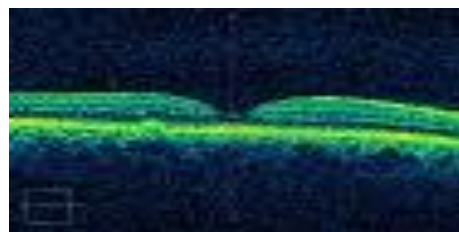


**Fig. 4.** The central macular thickness of the right eye was 242 microns

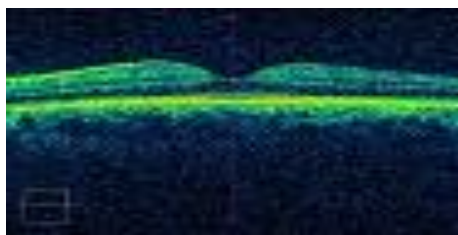


**Fig. 5.** The central macular thickness of the left eye was 277 microns

In the follow-up examination of the patient a month later, the best corrected visual acuity in the right eye was 0.5 and 1.0 in the left eye. Central macular thickness was measured as right: 227 (Fig. 6) left: 274 on OCT (Fig. 7). There was a punctuated PED on the right.



**Fig. 6.** Central macular thickness was measured as right: 227 on OCT



**Fig. 7.** Central macular thickness was measured as right: 274 on OCT

## DISCUSSION

The increase in the permeability of the choriocapillaris and the deterioration of the outer retinal layers play an important role in the pathophysiology of CSC. Although exogenous steroid use is among the risk factors. Carvalho-Recchia et al. (1999) and Bouzas et al. (1999) reported in their studies that the risk of CRC development increased with the use of systemic corticosteroids. It is not understood how corticosteroids impair choroidal circulation (Carvalho-Recchia et al., 1999; Bouzas et al., 1999). While spontaneous recovery occurs in 3-6 months in most patients, follow-up is appropriate in most cases. If possible, discontinuation of the exogenous steroids used by consulting the necessary departments and changing the lifestyle to reduce the possible stress may affect the prognosis positively. The use of drugs such as infliximab, anti-TNF and intravenous cyclosporine which are alternatives to steroid therapy is controversial because of their risks such as kidney toxicity and infectious diseases (Geyshis et al., 2013).

Although the disease is defined as inflammatory choroiditis in the literature and inflammation plays a major role in its pathogenesis, it has been reported that it is the only condition worsened with corticosteroids (Darulch et al., 2015). If the feeding of the figure receptors continues it is often possible to achieve an excellent visual prognosis either spontaneously or with treatment. In cases with prolonged disease and bullous serous retinal detachment, figure receptors stay away from the choriocapillaris which is the oxygen source and as a result, ischemia and loss of figure receptors develop. This process may result in the development of

choroidal neovascularization and retinal atrophy, loss of vision (Gemenetzi et al., 2010).

In diseases where steroid treatment is inevitable such as ulcerative colitis, the patient's current or past eye diseases should be questioned before the treatment is started. If visual symptoms are present, steroid treatment should be discontinued or alternative treatments should be applied, taking into account the risk-benefit ratio, by consulting an ophthalmologist (Samidh et al., 2011).

## CONCLUSION

Activation of ulcerative colitis and use of oral steroids may lead to CSC.

Due to the relationship between steroids and CSC, these patients experience a treatment dilemma, whether they are treated for the primary disease or CSC and they require a multidisciplinary approach.

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## The relationship between hospital employees' knowledge levels and behaviors about chemical risks and occupational safety and employee health culture

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This study was conducted to determine the relationship between the knowledge levels and behaviors of hospital employees about chemical risks, occupational safety and employee health. Determining the relationship between the factors will be useful for studies on this subject. It has been observed that the study on the relationship between chemical risks for hospital workers and occupational safety and employee health culture is limited. Therefore, it is important in terms of shedding light on future studies and contributing to the literature. Hospital staff, Doctor, Nurse/midwife/EMT, Health Technician/Technician, Cleaning Staff, Pharmacist/Pharmacy Worker, and Secretary were included in the study. The study was conducted with 251 people who agreed to participate in the study. In the study, a 9-question socio-demographic questionnaire, a 20-question chemical knowledge questionnaire created by the researchers and OHS culture scale consisting of 30 questions were used. The data were delivered to the employees via Google form. The collected data were evaluated with Anova correlation and regression analyses in SPSS. When the findings obtained in our study are examined; A statistically significant relationship was found between the level of education, occupation, place of work and exposure to chemical substances of the participants in the study and OSEHCS. As a result of the linear regression analysis of the factors affecting the OSEHCS score, it was concluded that there was a statistically significant difference in the OSEHCS score in those who worked in the profession, those who worked as a doctor and support staff, those who worked in intensive care and operating rooms, and those who did not experience chemical exposure. A relationship was found between the knowledge levels and behaviors of hospital staff about chemical risks and OSEHCS in terms of working year and occupation.

**Keywords:** Occupational safety and employee health (OSEH), hospital worker, chemical risks

### INTRODUCTION

Hospitals, which occupy the largest place in the health sector in our country, are faced with great dangers grouped as physical, ergonomic, chemical, biological and psychosocial risks. There are many chemical agents that can cause work accidents and occupational diseases in hospitals,

which are the largest workplaces in the health sector. With the development of the chemical industry, the diversity of chemicals continues to increase and it is known that there are approximately 5-7 million various chemicals in the world (Obenaus-Emler et al., 2019). A chemical substance is defined as all elements, compounds or mixtures that can be found in the

natural structure, during processing, as post-work waste or accidentally. In short, a chemical substance is the naming of substances in a solid, liquid or gaseous state that have a certain chemical formula or composition and are produced for a purpose. The American National Institute of Occupational Health and Safety reported that there are 24 types of biological and 25 types of chemical risks and hazards in hospitals (Meydanlıoğlu et al., 2019). In addition, it has been determined that there are 299 different chemical components in the form of dust, gas, steam and liquid that harm human health in the health sector (Solmaz et al., 2017). With the advancement of medicine, these chemicals have started to be used more and more in hospitals. Healthcare workers may be exposed to chemical risks and hazards such as disinfectants, chemicals used in sterilization such as formaldehyde, anesthetic gases, detergents, volatile chemicals, acid and base solvents and drugs. It is observed that health problems are gradually increasing due to occupational accidents and occupational diseases in the health sectors (10). In fact, every object in our environment has a chemical formula and component. Even water, natural gas used in homes, salt has a chemical formula. However, when chemicals are mentioned, substances that are produced for a specific purpose, that have a negative effect on human health, that must be stored and transported in specially designed packages, and that must be used under supervision by experts come to mind. The basic way of occupational safety and protection is to know the effects of chemical substances in the working environment. It is very important to create a culture of occupational safety in terms of protecting the health of employees, preventing disability and reducing deaths due to toxic causes. For the first time, the concept of occupational safety culture was mentioned in the evaluation report of the nuclear accident that took place in Chernobyl in 1986, organizational error, lack of design and neglect of employees (Wilburn et al., 2004). Security is the state in which people can live fearlessly and safely. Occupational health and safety culture, on the other hand, is defined as the culture of occupational health and safety, on the other hand, the beliefs that belong to the individual or the whole that determine the style,

duties and responsibilities of the institution, and fields of action in line with occupational health and safety (Aslanhan et al., 2006). According to the International Labor Organization (ILO), it has been concluded that 4 workers per minute and an average of 6300 workers a day die due to work accidents or occupational diseases (Özkan et al., 2006). In the world, an average of 317 million occupational accidents occur annually and it is reported that 2.5 million of them result in loss of life. The occurrence of such a high rate of occupational accidents and loss of life shows that occupational health and safety should be emphasized. In order to reduce work accidents and occupational diseases, the institution should adopt a common occupational health and safety culture and be implemented by all employees.

In our study, it is also aimed to evaluate the risks faced by healthcare professionals against ever-increasing chemicals, to contribute to the planning of the health sector, and to develop existing regulations by determining the relationship between the level of knowledge of employees about the risks and hazards of dangerous chemicals in hospitals with very dangerous workplaces and occupational safety culture.

## **MATERIALS AND METHODS**

### **Type of Research**

This research is of a cross-sectional type.

### **Place and Time of Research**

The research was carried out at the Yozgat Bozok University Research and Application Center. Data were collected in January-March 2022.

### **Population and Sample of the Research**

The universe of the research consists of the employees at the Yozgat Bozok University Research and Application Center.

All employees who agreed to participate in the study were included in the study, as a sample was not selected for the study. The minimum sample size for the research was calculated with the GPower 3.1 program. Before filling out the questionnaire forms, the subjects were informed about the subject and purpose of the study and their verbal consent was obtained.



### **Data Collection Methods**

The data were collected by means of a questionnaire for hospital staff. Before applying the data forms, the employees were informed about the purpose and importance of the research. The data were completed from 252 people who agreed to participate in the study by reaching everyone working in the hospital.

### **Data Collection Tools**

#### ***Socio-demographic Questionnaire Form***

The questionnaire created by the researchers to find out whether the participants received occupational health and safety training and whether they had a work accident, in addition to their age, gender, marital status, consists of 9 questions.

#### **Chemical Knowledge Level Questionnaire Form**

In order to determine the level of chemical knowledge, a 20-item questionnaire was created by literature review by the researchers (16).

#### **Occupational Safety and Employee Health Culture Scale (OSEHCS)**

OSEHCS was developed by Güven and İşcan in 2014 and consists of 30 items. Responses to each item in OSEH were scored on a 5-point Likert type, and the degree of agreement was: (5) "Strongly Agree", (4) "Agree", (3) "I am undecided", (2) "I do not agree" and (1) "Strongly Disagree" has been determined. As a result of the internal reliability analysis of the scale, the Cronbach Alpha value was found to be 0.963. Accordingly, it was concluded that the scale was highly reliable. In this study, Cronbach's  $\alpha$  coefficient of internal reliability of OSEHCS was found to be 0.969.

#### **Analysis of Data**

The data were evaluated in the SPSS program. In the comparison of the arithmetic means of the scores obtained from the scales according to the independent variables, t-test and Anova, Regression were used in independent groups. As the dependent variable; OSEHCS score, socio-demographic and chemical substance knowledge level questionnaire characteristics were taken as independent variables. Occupational health and employee culture level was charted according to socio-demographic and chemical substance knowledge characteristics and analyzed with the chi-square test. A p-value of

<0.05 was considered statistically significant in all tests.

#### **Ethic**

Institutional permission for the research was obtained from Yozgat Bozok University Research and Practice Chief Physician, and ethics committee approval was obtained from Yozgat Bozok University Ethics Committee. The research was conducted in accordance with the principles of the Declaration of Helsinki.

## **RESULTS**

When the OSEHCS score distribution was examined according to the socio-demographic characteristics of the participants working in the hospital; while there is a statistically significant difference between education level, occupation, place of work and exposure to chemical substances and OSEHCS, there was no significant relationship between gender, age group, years of work and occupational health and safety education (Table 1).

According to the results of the analysis with linear regression (backward) analysis of the factors affecting the OSEHCS score of the hospital staff; It has been concluded that there is a statistically significant difference between those who work in the profession, those who work as doctors and support staff, those who work in the intensive care and operating room as the place of work, and those who are not exposed to chemicals. A significant relationship was not found in those working in the laboratory (Table 2).

When the relationship between the level of OSEH according to the socio-demographic characteristics of the hospital staff is examined; It has been concluded that there is a statistically significant relationship between the place of duty and occupation of the employees and the level of OSEH. The level of OSEH was examined in 3 groups. Examined as insufficient, partially inadequate and adequate level. Those who are male (20.5%), In the 20-29 age range (19.0%), Post-graduate degree (16.8%), Doctors (41.4%), Operating room (25.0%) employees and 1-4 years in the profession It was concluded that (22.8%)

those with a working year had insufficient OSEH level. OSEH A sufficient level of female (24.2%) and male (25.2%) ratios has been reached. The level of proficiency was found to be high in the

30-39 age range. It has been concluded that while an associate degree (32.5%) is high at the education level, it is the lowest level of proficiency in postgraduate (14.3%).

**Table 1.** OSEHCS score averages according to various characteristics of hospital staff

	Count	Column %	Mean	Standard deviation	t/F	p
Gender	Woman	124	49.4	100.2	18.6	1.35
	Male	127	50.6	96.5	24.9	0.177
Age groups	20-29	121	48.2	95.0	22.2	2.87
	30-39	89	35.5	100.8	22.8	0.059
	40+	41	16.3	102.9	18.8	
Education level	High school and below	74	29.5	102.2	23.9	2.92
	Associate degree	40	15.9	102.0	17.0	<b>0.035</b>
	License	95	37.8	97.1	22.3	
	Postgraduate	42	16.7	90.8	21.0	
Occupation	Doctor	29	11.6	85.2	23.0	5.95
	Nurse/Midwife/ATT	121	48.2	99.6	19.7	<b>0.001</b>
	Support personnel	51	20.3	105.5	24.6	
	Other Health Personnel	50	19.9	95.6	21.1	
Place of duty	Intensive care	62	24.7	92.5	23.0	4.85
	Operating room	28	11.2	89.0	24.7	<b>0.001</b>
	Laboratory	16	6.4	93.8	16.7	
	Clinic	61	24.3	100.4	19.4	
	Other units	84	33.5	105.1	21.2	
Working time in the profession (year)	1-4	79	31.5	93.9	21.0	2.08
	5-9	84	33.5	99.1	23.8	0.104
	10-14	44	17.5	99.1	20.0	
	15+	44	17.5	104.0	21.7	
Getting OHS training	Yes	231	92.0	99.1	22.0	1.94
	No	20	8.0	89.2	21.2	0.054
Exposure to chemicals	Yes	94	37.5	93.7	21.9	2.60
	No	157	62.5	101.1	21.8	<b>0.010</b>
	<b>Total</b>	<b>251</b>	<b>100.0</b>	<b>98.3</b>	<b>22.1</b>	

OSEHCS: Occupational Safety and Employee Health Culture Scale

**Table 2.** Linear regression (backward) analysis of the factors affecting the OSEHCS score of hospital staff.

Dependent Variable: OSEHCS	Unstandardized coefficients		Standardized coefficients	t	Sig.	95,0% Confidence Interval for B	
	B	Std. error	Beta			Lower bound	Upper bound
(Constant)	94.811	3.427		27.665	<b>0.001</b>	88.061	101.562
Working time in the profession	0.441	0.223	0.123	1.979	<b>0.049</b>	0.002	0.880
Occupation=Doctor	-11.424	4.349	-0.166	-2.627	<b>0.009</b>	-19.990	-2.858
Occupation = Support personnel	6.660	3.370	0.122	1.976	<b>0.049</b>	0.021	13.299
Place of duty = Intensive care	-8.046	3.274	-0.158	-2.458	<b>0.015</b>	-14.495	-1.598
Place of duty = Operating room	-9.660	4.538	-0.138	-2.128	<b>0.034</b>	-18.599	-0.720
Place of duty = Laboratory	-10.365	5.577	-0.115	-1.859	<b>0.064</b>	-21.351	0.620
Exposure to chemicals =No	5.615	2.823	0.123	1.989	<b>0.048</b>	0.055	11.176

Independent variables: Age, gender, education level, occupation, place of duty, working time in the profession, receiving OHS training, exposure to chemical substances.

Adj.R<sup>2</sup>: 0,119 OSEHCS: Occupational Safety and Employee Health Culture Scale

**Table 3.** OSEH level according to various characteristics of hospital staff

		OSEH Percent Group				X <sup>2</sup> P
		Insufficient (0-39)	Partly insufficient (40-59)	Partly enough (60-69)	Sufficient (70+)	
		n %	n %	%	%	
Gender	Women	14 (11.3)	52 (41.9)	28 (22.6)	30 (24.2)	4.487
	Male	26 (20.5)	46 (36.2)	23 (18.1)	32 (25.2)	0.213
Age groups	20-29	23 (19.0)	49 (40.5)	27 (22.3)	22 (18.2)	7.506
	30-39	14 (15.7)	32 (36.0)	16 (18.0)	27 (30.3)	0.277
	40+	3 (7.3)	17 (41.5)	8 (19.5)	13 (31.7)	
Education level	High school and below	8 (10.8)	30 (40.5)	14 (18.9)	22 (29.7)	15.783
	Associate degree	4 (10.0)	12 (30.0)	11 (27.5)	13 (32.5)	0.072
	License	16 (16.8)	36 (37.9)	22 (23.2)	21 (22.1)	
	Postgraduate	12 (28.6)	20 (47.6)	4 (9.5)	6 (14.3)	
Occupation	Doctor	13 (44.8)	12 (41.4)	1 (3.4)	3 (10.3)	32.996
	Nurse/Midwife/ATT	13 (10.7)	49 (40.5)	28 (23.1)	31 (25.6)	0.000
	Support personnel	7 (13.7)	15 (29.4)	9 (17.6)	20 (39.2)	
	Other Health Personnel	7 (14.0)	22 (44.0)	13 (26.0)	8 (16.0)	
Place of duty	Intensive care	13 (21.0)	27 (43.5)	13 (21.0)	9 (14.5)	21.817
	Operating room	7 (25.0)	13 (46.4)	4 (14.3)	4 (14.3)	0.040
	Laboratory	3 (18.8)	7 (43.8)	6 (37.5)	0 (0.0)	
	Clinic	8 (13.1)	23 (37.7)	10 (16.4)	20 (32.8)	
	Other units	9 (10.7)	28 (33.3)	18 (21.4)	29 (34.5)	
Working time in the profession (year)	1-4	18 (22.8)	32 (40.5)	14 (17.7)	15 (19.0)	10.289
	5-9	11 (13.1)	34 (40.5)	20 (23.8)	19 (22.6)	0.328
	10-14	6 (13.6)	19 (43.2)	8 (18.2)	11 (25.0)	
	15+	5 (11.4)	13 (29.5)	9 (20.5)	17 (38.6)	
	<b>Total</b>	<b>40 (15.9)</b>	<b>98 (39.0)</b>	<b>51 (20.3)</b>	<b>62 (24.7)</b>	

Independent variables: Age, gender, education level, occupation, place of duty, duration of employment, OSH training, exposure to chemicals. OSEH: Occupational Safety and Employee Health

**Table 4.** Multinomial logistic regression (backward) analysis of the factors affecting the OSEH culture level of hospital staff

OSEH culture level		B	Sig.	Exp (B)	95% CI for Exp (B)	
Reference group: Insufficient (0-39).					Lower Bound	Upper Bound
Partly insufficient (40-59)	Intercept	1.356	<b>0.001</b>			
	Place of duty = Intensive care	-0.484	0.267	0.617	0.263	1.448
	Place of duty = Laboratory	-0.360	0.632	0.698	0.160	3.049
	Occupation =Doctor	-1.351	<b>0.004</b>	0.259	0.103	0.654
Partly enough (60-69)	Intercept	0.809	<b>0.010</b>			
	Place of duty = Intensive care	-0.621	0.217	0.537	0.201	1.439
	Place of duty = Laboratory	0.081	0.918	1.085	0.230	5.116
	Occupation =Doctor	-3.292	<b>0.002</b>	0.037	0.005	0.305
Sufficient (70+)	Intercept	1.285	<b>0.001</b>			
	Place of duty = Laboratory	-1.474	0.005	0.229	0.082	0.638
	Place of duty = Laboratory	-21.059	0.000	7.146	7.146	7.146
	Occupation =Doctor	-2.553	<b>0.001</b>	0.078	0.020	0.307

OSEH: Occupational Safety and Employee Health

Professional support staff (39.2%) was high, while doctors (3.4%) had a low level of competence. When the units he works in are examined; While it was higher in other units (34.5%) and clinics (32.8%), it was concluded that the lowest level was in laboratories. Examining the working year, 15 years and above (38.6%) were found to be sufficient at the highest rate, while 1-4 years (19.0%) was found to be sufficient at the lowest level (Table 3).

When the factors affecting the OSEH culture

level of hospital staff are analyzed with multinomial logistic regression (backward); Compared to those with insufficient OSEH culture level, the state of being partially inadequate and partially competent is lower in doctors than in other occupational groups, and the level of proficiency was found to be higher in those who are not doctors, those who do not work in the intensive care unit and laboratory, compared to those with insufficient OSEH culture level (Table 4).

While the proportion of hospital workers who stated that they were at risk of being exposed to chemicals with partially adequate and adequate IGIS culture levels was 74.5% and 66.1%, respectively, Those who stated that they were exposed to chemicals were found to be 41.2% and 25.8%, respectively. The rate of those who stated that they were exposed to formaldehyde, latex, anesthetics, drugs, soda lime, Sevoflurane, bleach and radiation was found to be very low (0.0-10.0%) with a partially sufficient and adequate OSEH level (Table 5).

When the behavior of the participants towards chemical substances is examined; those who said yes (79.3%) to the toxicity of chlorine-based cleaning products were found to be high. They evaluated their chemical knowledge level as sufficient (56.2%). In the event that a chemical substance gets on their clothes, the rate of changing immediately (64.1%) was found to be high. The rate of using PPE during radiation extraction is not related to me (53.0%), while the rate of I usually use (12.7%) and I do not (11.6%) was found to be close to each other. While this

question is not about me (59.4%) the level of leakage control of the anesthesia device is the highest, I have no idea (14.7%) with the answer yes (19.9%).

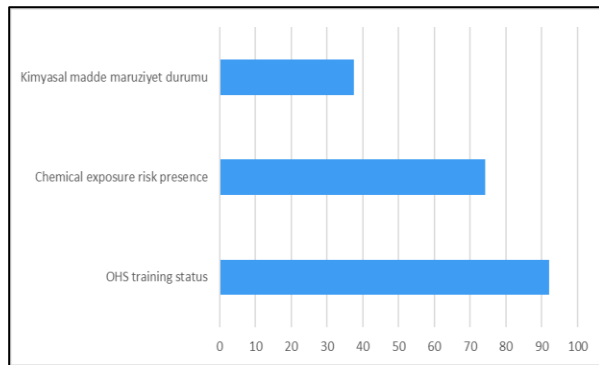
According to the evaluation of the responses to the behaviors towards chemical substances; Proper removal of unnecessary chemicals from the environment (71.3%), presence of clear labels of waste collection containers in the section (74.1%), presence of locked waste collection containers in the section (70.9%), detection status of dangerous substances in marking (56.2%), presence of warning labels in chemicals (82.1%), presence of chemical instructions for use (58.6%), presence of safety data sheet (43.8%) were found to be high. The rate of completion by transferring the decreasing chemicals was found to be high (80.5%).

When the knowledge levels for chemical substances are examined; It was found that the rate of those who participated in the study who received OHS training was high (92.0%), the risk of exposure to chemicals (74.1%) was high, and the rate of those who were exposed to chemicals (37.5%) was high (Fig. 1).

**Table 5.** OSEH culture level according to the exposure of hospital staff to chemical substance

		N=251	OSEH culture level			
			Insufficient (0-39) N=40 Col.%	Partly insufficient (40-59) N=98 Col.%	Partly enough (60-69) N=51 Col.%	Sufficient (≥ 0) N=62 Col.%
Chemical Exposure: Risk Existence	Yes	74.1	85.0	74.5	74.5	66.1
	No	25.9	15.0	25.5	25.5	33.9
Chemical Exposure: Status	Yes	37.5	47.5	38.8	41.2	25.8
	No	62.5	52.5	61.2	58.8	74.2
Chemical Exposure: Formaldehyde	No exposure	61.8	52.5	60.2	56.9	74.2
	Yes	3.2	10.0	2.0	3.9	0.0
	No	35.1	37.5	37.8	39.2	25.8
Chemical Exposure: Latex	No exposure	60.2	50.0	59.2	58.8	69.4
	Yes	1.6	0.0	4.1	0.0	0.0
	No	38.2	50.0	36.7	41.2	30.6
Chemical Exposure: Anesthetics	No exposure	61.8	52.5	60.2	56.9	74.2
	Yes	1.6	5.0	2.0	0.0	0.0
	No	36.7	42.5	37.8	43.1	25.8
Chemical Exposure: Drugs	No exposure	61.8	52.5	60.2	56.9	74.2
	Yes	1.6	0.0	1.0	3.9	1.6
	No	36.7	47.5	38.8	39.2	24.2
Chemical Exposure: Soda lime Sevoflurane	No exposure	61.8	52.5	60.2	56.9	74.2
	Yes	1.6	5.0	1.0	2.0	0.0
	No	36.7	42.5	38.8	41.2	25.8
Chemical Exposure: Bleach	No exposure	61.8	52.5	60.2	56.9	74.2
	Yes	9.2	10.0	11.2	9.8	4.8
	No	29.1	37.5	28.6	33.3	21.0
Chemical Exposure: Radiation	No exposure	61.8	52.5	60.2	56.9	74.2
	Yes	1.6	2.5	2.0	0.0	1.6
	No	36.7	45.0	37.8	43.1	24.2

OSEH: Occupational Safety and Employee Health



**Fig. 1.** Knowledge level of hospital staff about chemical substances.

When the distribution of chemical substances in hospital departments is examined; hand (83.3%), surface (82.9%) and cleaning chemicals (79.7%) and latex (58.2%), followed by radiation (31.5%) from high to low, respectively. anesthetic gases (17.1%), fluoroscopy radiation (15.5%), methylene blue (15.1%), mercury-containing instruments (14.3%), surgical smoke (10.8%), laboratory solvents (10.8%), ethyleneoxide (8.8%), methacrylate (7.6%), formaldehyde (3.6%), disinfection materials (0.8%), oxygen-based acidic (0.4%), neutral acidic solution. It was found that 0.4% (Fig. 2).

When the distribution of complaints after exposure to chemical substances in hospital departments is examined; Headache (18.3%), shortness of breath (13.1%) and skin disorders (12.0%) are in the top three, while the absence of symptoms (10.4%), allergic reactions (8.0%), Blurred vision (1.6%), impaired consciousness (1.6%) and intoxication (1.6%), facial swelling (0.4%) and forgetfulness (0.4%) were found to be the lowest (Fig. 3).

When the distribution of chemicals to which the workers are exposed is examined; Exposure to unknown chemicals (10.0%), cleaning chemicals (9.2%) and hand disinfectants (4.0%) are in the top three, Ranking from high to low formaldehyde (3.2%), caudex (2.8%), latex (1.6%), anesthetics (1.6%), drugs (1.6%), Sodalimesevoflurane (1.6%), radiation (1.6%), Hydrogen peroxide gas (1.6%), anesthetic gas (1.2%), descaler (1.2%), instrument disinfectant (1.2%), colodium (2.8%), dialysis solutions (0.8%), opaque (0.4%), glutaraldehyde exposure (0.4%) was found (Fig. 4).

When the distribution of the causes of

exposure of the employees to chemical substances is examined; inadequate precautions (10.8%), high workload (10.4%) and lack of PPE (8.0%) were found to be in the first three places. Then, from high rate to low rate, unknown reason (7.2%), carelessness (6.4%), acting in a hurry (3.6%), not using PPE (3.2%), necessity (2.8%) and the job is not suitable for the person (2.4%) (Fig. 5).

## DISCUSSION

Hospital services are considered as very dangerous workplaces according to the risks they involve and the level of danger. In this context, it is very important to create an organizational occupational health and safety culture in order to protect the health of employees and prevent them from being harmed by risks. In our study, the relationship between the knowledge levels of health workers working in a university hospital about the risks and hazards of hazardous chemicals and occupational safety culture was determined.

When the OSEHCS score distribution was examined according to the socio-demographic characteristics of the participants working in the hospital; while there is a statistically significant difference between education level, occupation, place of work and exposure to chemical substances and OSEHCS, there was no significant relationship between gender, age group, years of work and OHS education (Table 1). In the studies, it was determined that the occupational health and culture of the employees who had a work accident were lower than those who did not have a work accident (Lee et al., 1998). In our study, the OSEHCS score was found to be low in people exposed to chemicals. In other studies, evaluating the relationship between occupational accident status and occupational health and culture, it has been concluded that the formation of occupational health and culture reduces occupational accidents. In our study, a significant relationship was found between the level of learning and the OSEHCS score.

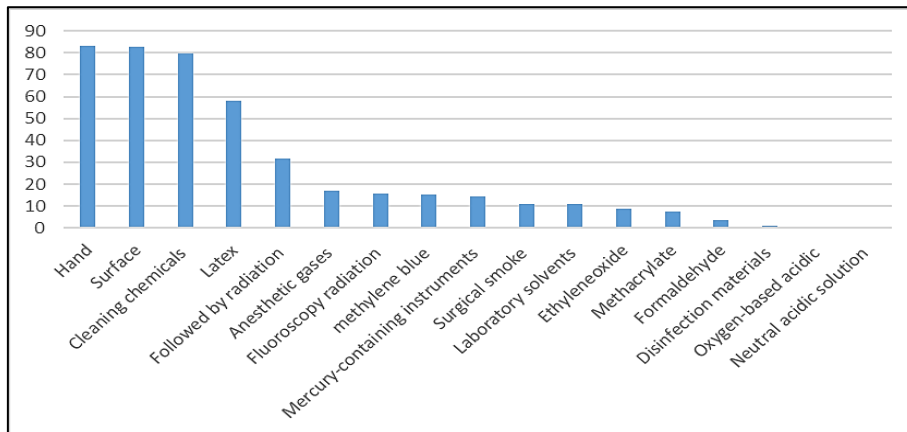


Fig. 2. Distribution of chemical substances in hospital departments.

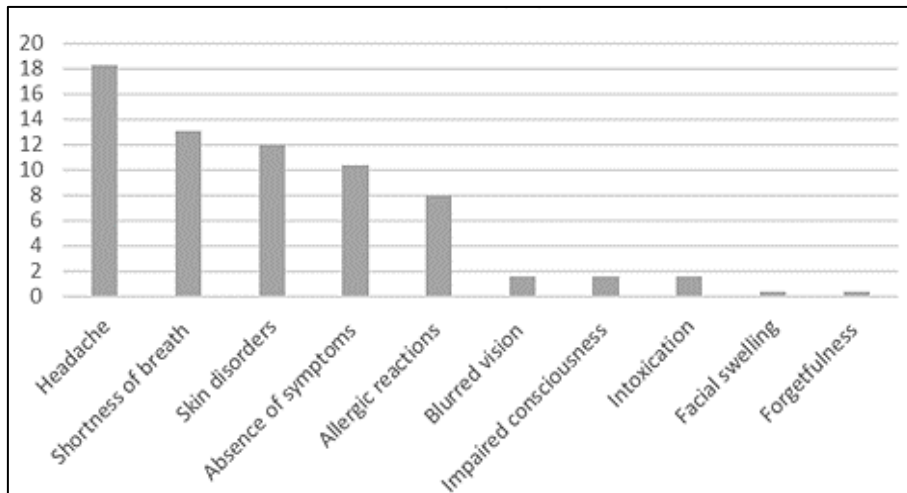


Fig. 3. Distribution of complaints after exposure to chemical substances in hospital departments.

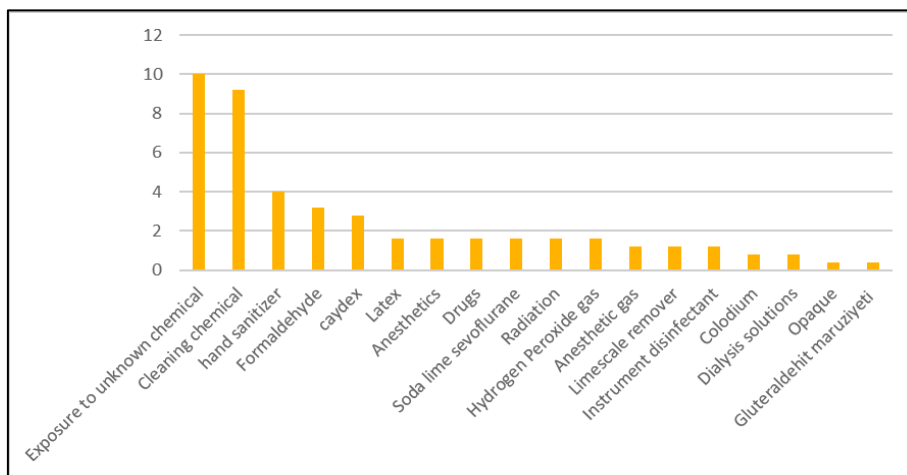
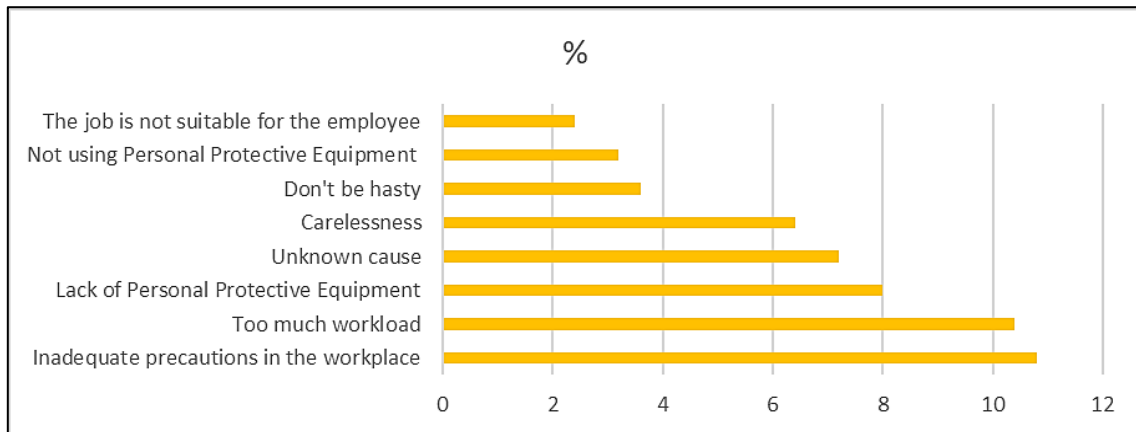


Fig. 4. Distribution of chemicals exposure of employees.



**Fig. 5.** Distribution of employees' reasons for exposure to chemical substances.

When the studies in the literature are examined, there are studies that support our study finding that there is a relationship between educational status and occupational health culture. Similar to our study result, according to previous study; there is a highly significant difference in the rate of health worker safety according to the occupational group and there is no difference according to gender has been concluded (Tozkoparan et al., 2011). Contrary to our study, there are studies that have concluded that there is a difference between gender and occupational health and safety culture.

According to the results of the analysis, the linear regression (backward) analysis of the factors affecting the OSEHCS score of the hospital staff; It has been concluded that there is a statistically significant difference between those who work in the profession, those who work as doctors and support staff, those who work in the intensive care and operating room as the place of work, and those who are not exposed to chemicals. No significant relationship was found among those working in the laboratory (Table 2). According to the results of the study conducted by Listyowardojo et al. (2012), OSEHCS score was found to be higher and more significant in physicians compared to other employees. As a result of Tüzüner's (2011) study, it was observed that the other OSEHCS scores were found to be high in doctors and support staff, which supports our study (Listyowardojo et al., 2012; Tüzüner et al., 2011). Tozkoparan (2021) has reached that as the working year increases, employees have a greater sense of responsibility in the field of

occupational health safety. There are many studies that conclude that the OSEHCS score of employees who have had a work accident is low. It was concluded that the OSEHCS score affected the departments studied. This is because exposure is thought to vary according to risks and hazards.

When the relationship between the OSEH level according to the socio-demographic characteristics of the hospital staff is examined; It was concluded that there is a statistically significant relationship between the place of duty and occupation of the employees and the level of OSEH (Table 3). There are studies that have found a significant relationship between OSH and their place of work. In the study, it is thought that the OSEH level of the employees varies according to the clinics, the variability of the chemicals in the clinics, the risk and danger situations of the clinics. In our study, the OSEH score of doctors was found to be significantly lower than that of other employees,

Males (20.5%), aged 20-29 (19.0%), Graduate (16.8%), Doctors (41.4%), Operating room workers (25.0%) and professionals. It was concluded that there were insufficient OSEH level in those with 1-4 years (22.8%) working years (Table 3). As the working year increases, the increase in clinical experience and the identification of dangers and risks are thought to be effective in increasing the OSEH score. It is thought that the OSEH score may have been determined as low due to the inability of newly recruited employees to identify risks and dangers in an unfamiliar environment or to be unaware of the problems that may arise as a result of the



danger or risks.

As the education level increases, the OSEHCS score decreases. It was concluded that the OSEHCS score was the highest in the support staff and the lowest among the doctors. The highest OSEH score was found in those working in clinics and the lowest in laboratory workers (Table 3). The lowest OSEHCS score was found in those who worked for 1-4 years, and the highest in those who worked for 15 years or more (Table 3). In our study, the OSEHCS score of the physicians was found to be low. OSEHCS score of doctors was found to be lower and significant compared to other occupational groups. The fact is that the education of doctors is postgraduate and therefore as the education increases, the OSEHCS score decreases. From here, it can be counted among the reasons why doctors' OSEHCS score is insufficient. While all of the nurses received compulsory occupational health and safety training in our institution, it was observed that this rate was low for doctors.

The adequacy of OSEH culture level of hospital staff was found to be higher in non-physician, intensive care and laboratory staff (Table 4).

While the proportion of hospital workers who stated that they were at risk of being exposed to chemicals with partially adequate and adequate OSEH culture levels was 74.5% and 66.1%, respectively, On the contrary, these rates were found to be very low in those who stated that they were exposed to chemicals (41.2% and 25.8%, respectively). The rate of those who stated that they were exposed to formaldehyde, latex, anesthetics, drugs, soda lime, Sevoflurane, bleach and radiation was found to be much lower (0.0-10.0%) of those whose iGIS culture level was partially sufficient and adequate (Table 5). It is seen that the reasons for exposure to chemicals arise due to the low level of iGIS culture. It is seen that those who have sufficient or partially sufficient OSEH culture level have developed chemical substance exposure risk awareness, while those who have low OSEH culture level are those who have been exposed to chemicals. According to the results of the study, the increase in the OSEH culture level decreased the exposure rate to the chemical substance. It is thought that exposure to chemicals is caused by the inability of

the employees to identify the risks and dangers in the institution where they work or not to show the necessary care. As a result, it is due to the fact that the OSEH culture level is very weak or not formed at all.

When the behavior of the participants in the study towards chemical substances is examined; In general, they evaluated the chemical substance knowledge level as sufficient. The respondents working in the radiation unit, who gave the answer that they usually use PPE during radiation exposure, were found to be high. The number of employees working in the anesthesia unit who checked for anesthesia device leakage was found to be higher than those who did not control. It was concluded that the chemical management and knowledge level of the employees in the university hospital where the research was conducted were generally sufficient. It can be thought that the reason for this is the necessary inspections by the Ministry of Health, compulsory in-service training and controls. It is thought that the quality controls are carried out by the Ministry of Health, the management constantly monitors their follow-ups in this regard, and by making the necessary attempts to avoid penal action, they provide the necessary training to the personnel and provide the necessary inspections in the institution.

According to the evaluation of responses to behaviors towards chemical substances; The questions of the appropriate removal of unnecessary chemicals from the environment in the institution where the research was conducted, the presence of a clear label in the waste collection container, the presence of locked waste collection containers in the section, the detection status of the marking of dangerous substances, the presence of warning labels in chemicals, the presence of chemical instructions for use, the presence of safety data sheet. "yes" answer was found high. When the distribution of chemical substances in hospital departments is examined; hand, surface and cleaning chemicals and latex are high, Then, it was found that from high to low, there was radiation, anesthetic gases, scopy radiation, methylene blue, mercury-containing instruments, surgical smoke, laboratory solvents, ethylene oxide, methacrylate, formaldehyde, disinfection materials, oxygen-based acidic,

neutral acidic solution (Graphic 3). There are 299 different chemical components that can interfere with human health in hospitals,

When the distribution of complaints after exposure to chemical substances in hospital departments is examined; Headache, shortness of breath and skin disorders are in the top three, It was found that the lowest rate was found in the absence of symptoms, allergic reactions, blurred vision, impaired consciousness and poisoning, facial swelling and forgetfulness (Figure 2). According to the evaluation report of NIOSH, it was stated that the most common diseases among the health workers of 2600 hospitals are infection, dermatitis, drug and treatment reactions, especially respiratory problems. These results are similar to our results. When we look at the chemicals we were exposed to in the institution where our study was conducted, it is seen that there are anesthetic substances and anesthetic gases in general (Figure 3). The fact that there are health problems related to this in the symptoms shows that it is in parallel with the studies in this field.

When the distribution of chemicals to which the workers are exposed is examined; Exposure to unknown chemicals, cleaning and hand sanitizer are in the top three, It was found that the ranking from high to low is formaldehyde, caudex, latex, anesthetics, drugs, soda lime sevoflurane, radiation, hydrogen peroxide gas, anesthetic gas, lime remover, instrument disinfectant, collodion, dialysis solutions, opaque, glutaraldehyde exposure (Figure 4). According to these rates, the rate in our study was lower than the world average. It is thought that these variations vary according to the restriction of the purchase of gloves containing latex, and the use of gloves by the personnel. It is thought that exposure to chlorine-based hand disinfectant was high in our study since the period in which the study was conducted was the pandemic period, the use of hand sanitizer was a lot and it was found all over the hospital.

When the distribution of the causes of exposure of the employees to chemical substances is examined; It has been found that taking insufficient precautions in the workplace, having a high workload and not having PPE are in the top three ranks. Then from high rate to low rate;

unknown reasons, carelessness, hasty behavior, not using PPE, necessity and unsuitability for the job were found (Figure 4).

## **CONCLUSION AND RECOMMENDATIONS**

In our study to determine the relationship between the knowledge levels and behaviors of hospital employees about chemical risks and occupational safety and employee health, it was concluded that there is a relationship between the OSEHCS score and the place of duty, occupation, working year, exposure to chemical substances and education status. It has been concluded that the OSEHCS score is insufficient for doctors and postgraduate education. It can be concluded that the practice of giving compulsory training to other health workers annually according to the danger class of the Ministry of Health is partially effective. In many studies, it is seen that doctors do not receive this training and their OSEHCS score is lower than other occupational groups. As a result of our study, it is understood that the situation of exposure to chemicals is the chemicals in the operating room and latex, which is mainly used in surgical clinics. It is recommended that more comprehensive research be conducted to measure awareness to chemicals in the operating room. In addition, as a result of our study, it has been determined that headache, skin disorders and allergies are among the complaints experienced after exposure. It is thought that the effects of the exposed substance can be reduced by removing the employees from the environment at certain periods. Studies on the effects of ventilation systems in risky units should be increased. It is recommended that the study be done more comprehensively with other universes.

### **Limitations of the Study**

Only one university hospital employee was included in our study, so it creates a limitation in terms of generalizing the study to the Ministry of Health and private hospitals. The fact that the willingness to participate in the survey application is low among health workers is a limitation.

## REPRESENTATIONS

**Ethical Approval:** Employees were informed about the research and their consent was obtained. Before starting the survey, it was stated that their participation in the research was on a voluntary basis. The research was conducted in accordance with the rules and ethical codes specified in the Declaration of Helsinki.

**Conflict of Interest:** The authors declare that there is no conflict of interest in this study.

**Financial support:** No financial support was received from any person or institution for the research.

**Authorship Contributions:** GU, MK, VAT and SO. Planning, implementation, statistical analysis of the research, writing and reviewing the article.

### Declaration of competing interest

The authors declare no financial interests or personal relationships.

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