# GEORGIAN MEDICAL NEWS

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ЕЖЕМЕСЯЧНЫЙ НАУЧНЫЙ ЖУРНАЛ

Медицинские новости Грузии
საქართველოს სამედიცინო სიახლენი

# GEORGIAN MEDICAL NEWS

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**ЕЖЕМЕСЯЧНЫЙ НАУЧНЫЙ ЖУРНАЛ**

**ТБИЛИСИ - НЬЮ-ЙОРК**

**GMN: Georgian Medical News** is peer-reviewed, published monthly journal committed to promoting the science and art of medicine and the betterment of public health, published by the GMN Editorial Board and The International Academy of Sciences, Education, Industry and Arts (U.S.A.) since 1994. **GMN** carries original scientific articles on medicine, biology and pharmacy, which are of experimental, theoretical and practical character; publishes original research, reviews, commentaries, editorials, essays, medical news, and correspondence in English and Russian.

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3. Submitted material must include a coverage of a topical subject, research methods, results, and review.

Authors of the scientific-research works must indicate the number of experimental biological species drawn in, list the employed methods of anesthetization and soporific means used during acute tests.

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3. სტატიაში საჭიროა გაშუქდეს: საკითხის აქტუალობა; კვლევის მიზანი; საკვლევი მასალა და გამოყენებული მეთოდები; მიღებული შედეგები და მათი განსჯა. ექსპერიმენტული ხასიათის სტატიების წარმოდგენისას ავტორებმა უნდა მიუთითონ საექსპერიმენტო ცხოველების სახეობა და რაოდენობა; გაუტკივარებისა და დაძინების მეთოდები (მწვავე ცდების პირობებში).

4. სტატიას თან უნდა ახლდეს რეზიუმე ინგლისურ, რუსულ და ქართულ ენებზე არანაკლებ ნახევარი გვერდის მოცულობისა (სათაურის, ავტორების, დაწესებულების მითითებით და უნდა შეიცავდეს შემდეგ განყოფილებებს: მიზანი, მასალა და მეთოდები, შედეგები და დასკვნები; ტექსტუალური ნაწილი არ უნდა იყოს 15 სტრიქონზე ნაკლები) და საკვანძო სიტყვების ჩამონათვალი (key words).

5. ცხრილები საჭიროა წარმოადგინოთ ნაბეჭდი სახით. ყველა ციფრული, შემაჯამებელი და პროცენტული მონაცემები უნდა შეესაბამებოდეს ტექსტში მოყვანილს.

6. ფოტოსურათები უნდა იყოს კონტრასტული; სურათები, ნახაზები, დიაგრამები - დასათაურებული, დანომრილი და სათანადო ადგილას ჩასმული. რენტგენოგრამების ფოტოასლები წარმოადგინეთ პოზიტიური გამოსახულებით **tiff** ფორმატში. მიკროფოტოსურათების წარწერებში საჭიროა მიუთითოთ ოკულარის ან ობიექტივის საშუალებით გადიდების ხარისხი, ანათალების შეღებვის ან იმპრეგნაციის მეთოდი და აღნიშნოთ სურათის ზედა და ქვედა ნაწილები.

7. სამამულო ავტორების გვარები სტატიაში აღინიშნება ინიციალების თანდართვით, უცხოურისა – უცხოური ტრანსკრიპციით.

8. სტატიას თან უნდა ახლდეს ავტორის მიერ გამოყენებული სამამულო და უცხოური შრომების ბიბლიოგრაფიული სია (ბოლო 5-8 წლის სიღრმით). ანბანური წყობით წარმოდგენილ ბიბლიოგრაფიულ სიაში მიუთითეთ ჯერ სამამულო, შემდეგ უცხოელი ავტორები (გვარი, ინიციალები, სტატიის სათაური, ჟურნალის დასახელება, გამოცემის ადგილი, წელი, ჟურნალის №, პირველი და ბოლო გვერდები). მონოგრაფიის შემთხვევაში მიუთითეთ გამოცემის წელი, ადგილი და გვერდების საერთო რაოდენობა. ტექსტში კვადრატულ ფჩხილებში უნდა მიუთითოთ ავტორის შესაბამისი N ლიტერატურის სიის მიხედვით. მიზანშეწონილია, რომ ციტირებული წყაროების უმეტესი ნაწილი იყოს 5-6 წლის სიღრმის.

9. სტატიას თან უნდა ახლდეს: ა) დაწესებულების ან სამეცნიერო ხელმძღვანელის წარდგინება, დამოწმებული ხელმოწერითა და ბეჭდით; ბ) დარგის სპეციალისტის დამოწმებული რეცენზია, რომელშიც მითითებული იქნება საკითხის აქტუალობა, მასალის საკმაობა, მეთოდის სანდოობა, შედეგების სამეცნიერო-პრაქტიკული მნიშვნელობა.

10. სტატიის ბოლოს საჭიროა ყველა ავტორის ხელმოწერა, რომელთა რაოდენობა არ უნდა აღემატებოდეს 5-ს.

11. რედაქცია იტოვებს უფლებას შეასწოროს სტატია. ტექსტზე მუშაობა და შეჯერება ხდება საავტორო ორიგინალის მიხედვით.

12. დაუშვებელია რედაქციაში ისეთი სტატიის წარდგენა, რომელიც დასაბეჭდად წარდგენილი იყო სხვა რედაქციაში ან გამოქვეყნებული იყო სხვა გამოცემებში.

აღნიშნული წესების დარღვევის შემთხვევაში სტატიები არ განიხილება.

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**РЕЗЮМЕ**

**ФИЗИЧЕСКОЕ РАЗВИТИЕ КАК ЗАЛОГ УСПЕШНОГО ФОРМИРОВАНИЯ РЕПРОДУКТИВНОГО ПОТЕНЦИАЛА**

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Цель исследования - определение особенностей физического и полового развития пациенток с нарушениями менструального цикла при мониторировании.

Оценивалось физическое и половое развитие 497 пациенток с аномальными маточными кровотечениями (АМК ПП) и 677 - с гипоменструальным синдромом (ГМС), в возрасте 10-18 лет, находящихся на лечении в период 1997-2002 гг. и 2010-2016 гг. Данные сравнивались с результатами эпидемиологических исследований популяции школьниц Харьковского региона.

Мониторинг физического развития больных с нарушениями менструальной функции (НМФ) показал значительное его ухудшение за последние годы. У современных девочек с НМФ дисгармоничность физического развития регистрировалась практически у каждой второй, что достоверно чаще, чем в популяции и по отношению к пациенткам, обследованным 15-20 лет назад (p<0,001-0,0001).

Отклонения в половом развитии регистрировались более чем у половины пациенток с НМФ. Причем его опережение значительно чаще регистрировалось при АМК ПП, а отставание - при гипоменструальном синдроме. Для АМК ПП более характерным было раннее менархе, при ГМС – позднее.

Физическое развитие девочек можно рассматривать как «маркер» состояния их здоровья, индикатор прогноза становления репродуктивного потенциала и фертильности в будущем.

რეზიუმე

ფიზიკური განვითარება, როგორც რეპროდუქციული პოტენციალის ფორმირების წარმატების წინაპირობა

[1]ვ.დინიკი, [2]ა.დინიკი, [1]ა.გავენკო, [1]ო.ვერხოშანოვა

[1]უკრაინის მედიცინის მეცნიერებათა ეროვნული აკადემიის ბავშვთა და მოზარდთა ჯანმრთელობის დაცვის ინსტიტუტი; [2]ხარკოვის ეროვნული სამედიცინო უნივერსიტეტი, უკრაინა

კვლევის მიზანს წარმოადგენდა მენსტრუალური ციკლის დარღვევების მქონე პაციენტების ფიზიკური და სქესობრივი მომწიფების თავისებურებების განსაზღვრა. შეფასებულია საშვილოსნოდან ანომალური სისხლდენების მქონე 497 პაციენტის (მკურნალობის პერიოდი – 1997-2002 წწ.) და ჰიპომენსტრუალური სინდრომის მქონე 10-18 წლის ასაკის 677 პაციენტის (მკურნალობის პერიოდი – 2010-2016 წწ.) ფიზიკური და სქესობრივი განვითარება. მონაცემები შედარდა ხარკოვის რეგიონის სკოლის მოსწავლე გოგონათა პოპულაციის ეპიდემიოლოგიური კვლევის შედეგებს.

მენსტრუალური ფუნქციის დარღვევების მქონე პაციენტების ფიზიკური განვითარების მონიტორინგით გამოვლინდა მისი მნიშვნელოვანი გაუარესება ბოლო წლების განმავლობაში. ფიზიკური განვითარების დისჰარმონიულობა აღენიშნებოდა მენსტრუალური ფუნქციის დარღვევების მქონე ყოველ მეორე გოგონას, რაც სარწმუნოდ უფრო ხშირია, ვიდრე პოპულაციაში და ვიდრე 15-20 წლის წინ გამოკვლეულ პაციენტებში (p<0,001-0,0001). გადახრები სქესობრივ განვითარებაში დარეგისტრირდა მენსტრუალური ფუნქციის დარღვევების მქონე პაციენტების ნახევარზე მეტში; ამასთან,მისი ნაადრეობა უფრო ხშირი იყო საშვილოსნოდან ანომალური სისხლდენების მქონე პაციენტებში, ხოლო ჩამორჩენა – ჰიპომენსტრუალური სინდრომის დროს. საშვილოსნოდან ანომალური სისხლდენების მქონე პაციენტებისათვის უფრო დამახასიათებელია ადრეული მენარხე, ჰიპომენსტრუალური სინდრომისთვის კი – გვიანი. გოგონების ფიზიკური განვითარება შეიძლება განხილულ იქნას, როგორც მათი ჯანმრთელობის "მარკერი" და ინდიკატორი რეპროდუქციული პოტენციალის ჩამოყალიბების და ფერტილობის პროგნოზისათვის მომავალში.

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# OPPORTUNITIES AND PROSPECTS OF MICROBIOLOGICAL DIAGNOSIS OF ENT MYCOSIS (REVIEW)

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The problem of increasing the number of patients with opportunistic mycotic diseases of various localizations, ENT organs in particular, has become acute in recent decades. According to Kunelskaya V.Ya. (2019) the share of fungal lesions of the ear and upper respiratory tract (URT) in the structure of chronic inflammation of these biotopes is currently 22.1%. Among them, the share of pharyngomycoses is 50%, otomycoses – 36%, laryngomycoses – 7%, fungal lesions of the nose and nasal cavities – 7% [13,14].

The increase in mycoses is due to the increase in the number and influence of exogenous and endogenous risk factors for their development, which lead to the suppression of the mechanisms of specific and nonspecific antifungal protection of the macroorganism. Decreased activity of the colonization resistance components of the ENT organs leads to the pathogenic potential realization in the main mycoses pathogens of the upper respiratory tract and ear. The dominant pathogens in the development of fungal lesions of the ENT organs are: opportunistic fungi of

the genera *Aspergillus*, *Penicillium, Mucor* and yeast-like fungi of the genus *Candida*, which are characterized by low levels of pathogenicity. In addition, they are part of the indigenous microflora of the macroorganism. Mycelial micromycetes are widespread in nature, but only under certain conditions can acquire pathogenic properties and cause damage to human organs and systems. The increase in the frequency and significance of opportunistic fungal diseases of the ENT organs is primarily associated with an increase in the number of immunocompromised patients. This is facilitated by long-term use of drugs (antibiotics, steroid hormones, cytostatics, etc.), which leads to a decrease in the immune resources of the macroorganism, as well as an increase in the number of diseases resulting in secondary immune deficiency (endocrinopathy, cancer, AIDS, gastrointestinal tract pathology, dysbiosis, etc.) In addition, the increase in the percentage of patients with opportunistic mycoses is associated with the widespread introduction of invasive methods of diagnosis and treatment [4,11,13,24,26,28,34,39].

The diagnostic problem of opportunistic fungal infections in otorhinolaryngology is becoming increasingly important and has some difficulties, as their clinical manifestations and signs don't have absolutely specific features, especially in immunocompromised patients [13,14]. The presence of the main pathogens of mycoses of the ENT organs in the test material taken from the patient may not always be grounds for diagnosis, because these microorganisms are components of autochthonous and allochthonous normoflora and can contaminate the mucous membranes of the upper airways and skin of the external acoustic meatus [18,22]. Under these conditions, the quantitative determination of the pathogen is considered as one of the diagnostic criteria. Thus, for fungi of the genus *Candida* the main diagnostic criteria are the presence of cells in micropreparations that are actively vegetating or their filamentous forms – true mycelium or pseudomycelium, as well as the number of isolated pathogens not less than $10^4$ colony-forming units (CFU) / ml. For mycelial fungi (genera *Aspergillus, Penicillium, Mucor*) the main diagnostic criterion is the presence of the pathogen in cultural, cytological and histological studies [10,15,18].

An important feature of inflammatory diseases of the upper respiratory tract and ear is the growing number of lesions caused by several pathogens that cause the development of mixed infections. Mixed infection is a fundamentally new form of infection, which does not involve the summation of different monoinfections, but is determined depending on the numerical ratio of pathogenic and opportunistic microorganisms, their interaction and activation of some against others. Mixed infection is characterized as a complex process of interaction between two or more pathogens and the human body. Complex interactions of associate microbes can lead to changes in their biological properties. Thus, the products of life that synthesize microorganisms of one species can stimulate the growth and reproduction of microorganisms of other species – members of the microbial community, thereby demonstrating the synergistic nature of the interaction. In addition to mutual reinforcement, the interaction of microorganisms may be accompanied by the opposite phenomenon – mutual inhibition (antagonism) of growth and reproduction. Complex processes of interaction between members of microbial communities depend on the conditions of existence of pathogens, the characteristics of the habitat and may remain constant or change under certain circumstances. Associates are able to change not only the biological properties of each other, but also their impact on the macroorganism due to its additional sensibilities, increased virulence of the pathogen and the formation of new factors that complicate the course of the disease. The formation of such microbial communities can cause a long course of inflammation [1,9,15,16].

In the structure of mixed infections of the ENT organs, a significant role belongs to fungal-bacterial associations, which according to various authors, are confirmed microbiologically in 26-70% of cases, depending on the habitat and the nature of the inflammatory process. [6,16,19,21,25]. More often, associate bacteria are identified by *Staphylococcus aureus, Staphylococcus epidermidis* and *Pseudomonas aeruginosa* [21,25]. The frequency of fungal-fungal associations is from 4 to 6% [19,25].

Due to the fact that clinical manifestations and signs of fungal lesions and mixed infections of ENT organs are not absolutely specific, their diagnosis is based on a set of clinical and laboratory examinations, which include patient complaints, anamnesis, objective examination, additional research methods and results of microscopic, mycological, serological, molecular genetic and histological methods [12,29].

Material for the study of ear and upper respiratory tract mycoses may be pathological secretions from the surface of the mucous membrane of the pharynx, nasal cavity, larynx, pathological discharge from the ear (crusts, scales on the walls and lumen of the external acoustic meatus and postoperative cavity), and pathological contents of the nasal cavities. The material is collected with a sterile swab, then placed in a test tube with sterile Sabouraud transport medium. Pathological material taken from the patient must be delivered to the laboratory as soon as possible. Delayed transportation can lead to the death of pathogens sensitive to environmental conditions, changes in the number of these microorganisms or contamination with secondary microflora [10,22,29].

Laboratory diagnosis to determine the etiology of the disease should begin with a microscopic method, which is based on the study of morphological features of micromycetes in the test material using different types of microscopy. To determine the structural elements of fungi, pathological material is studied in native (unpainted) and in stained preparations. At microscopic research of native preparations, it is necessary to carry out their preliminary enlightenment in a solution of 10% KOH or NaOH. In the preparation of stained preparations, the dried smear is fixed with a mixture of Nikiforov or Carnoua, 5% solution of chromic acid and others. The fixed smear is painted over by various methods: Gram-Welch, Romanovsky-Giemsa, McManus, Araviysky, Ziehl-Neelsen, calcofluor white, etc. [10, 14]. Insufficient informativeness of the microscopic method, which may be caused by low sensitivity and inability to identify the pathogen due to changes in the characteristic morphology of the pathogen under the influence of environmental factors and antimicrobials agents, necessitates the use of the mycological method.

The purpose of the mycological method of diagnosis is to isolate a pure culture of the pathogen and its identification. To solve these problems, it is initially conducted the microscopy of pathological material with subsequent seeding on nutrient media: Sabouraud agar, beer wort, meat-peptone glucose broth, Sabouraud broth, Czapek and Francis medium, wort agar, corn, rice, potato agar, chromogen CHROM agar and others. It is done to study the biological (morphological, tinctorial and cultural) properties of microorganisms, as well as to determine the sensitivity to antifungal medicines. Adding to the media of antibacterial antibiotics – gentamicin, chloramphenicol, rarely streptomycin and penicillin makes these media selective and protects primary crops from bacterial germination [10,22]. Incubation of crops is carried out in a thermostat at a temperature of 22-25 °C,

which is optimal for the growth of most species of fungi. The incubation period lasts from 1 to 4 weeks. Macroscopic study of the obtained colonies of fungi is carried out on the following grounds: evaluate the nature of the surface, margins, their shape and consistency, the color of the substrate and air part of the colonies. During the growth of the primary culture, it is necessary to conduct its microscopic examination every three days from the beginning of incubation. Microscopy of selected cultures allows to establish the genus of the pathogen by morphological features. Microscopic examination of mycelial fungi determines the structure of the mycelium: the color of the hyphae and the absence or presence of septa, the nature and frequency of their location. The results of macro- and microscopic study of the isolated pure culture are the main in the process of identification of molds. In some cases, to establish the type of pathogen (often yeast-like fungi) requires the use of special media, as well as the study of enzymatic activity of pure culture. The ability of representatives of this genus to assimilate and ferment carbohydrates is assessed (study results of auxonogram and zymogram). The results of such studies are reliable and highly informative, but their implementation takes some time, which leads to a delay in the start of rational treatment. [22,41].

The appearance of identification tablet test systems for biochemical detection of API RapID, CrystalTM in the late twentieth century has simplified and accelerated the process of identifying bacteria and fungi of the genus Candida [5]. API test systems consist of standard biochemical test strips (rows) and comprehensive identification databases. Each strip is made of plastic, in the holes of which are dry substrates and an indicator. The suspension of microorganisms is introduced into each well of the strip; after incubation, the results of the study are given concerning the change in color of the medium in the well [17]. When using these systems, the identification time of microorganisms is reduced to 48 hours, which gives them a significant advantage. With increasing frequency of fungal-bacterial associations, the use of these systems is appropriate and convenient.

Particular attention should be paid to determine the sensitivity of fungal pathogens to chemotherapeutic drugs, in cases of mixed infection in particular, on which the effectiveness of treatment depends. Extensive and uncontrolled use of antimicrobials leads to the formation of microorganisms resistance to these drugs. Therefore, it is advisable to introduce into clinical practice methods of rapid diagnosis of pathogens to determine the sensitivity of microorganisms to antimicrobial drugs, as well as the search for alternative treatments that reduce the development of resistance to pathogens (probiotics, bacterial lysates, phytopreparations, essential oils and their derivatives, etc.) [32, 33].

The main traditional methods for determining the antifungal susceptibility of fungal infections are disc-diffusion and serial dilutions. Disc-diffusion method is widely used to determine the sensitivity or resistance of the selected culture of micromycetes to antifungals of different chemical groups. The method of serial dilutions allows establishing the minimum inhibitory concentration (MIC) and the minimum fungicidal concentration (MFC) of the antifungal agent, which are quantitative indicators of drug activity. In recent years in order to determine the sensitivity of fungal and bacterial microorganisms to antifungals and antibiotics it was used the quantitative diffusion method of E-tests (epsilometric) [17]. E-tests are strips of inert plastic on which the antimicrobial drug is applied in concentrations that vary in a gradient from minimum to maximum. On the reverse side of the strip, there is a scale of the corresponding minimum inhibitory concentrations. During the cultivation of a pure culture of the pathogen in a Petri dish with a nutrient medium around the strip, an elliptical zone of growth retardation of microorganisms is formed, which crosses the strip in the place corresponding to the MIC. For reliability of results of research, it is recommended to use no more than two strips on one Petri dish. ATB strips, which are used to quantify the sensitivity of microorganisms to antimicrobial drugs (antibiotics and antifungals) in a semi-liquid medium, have been widely used. The results of the study are accounted by the presence or absence of microorganisms growth in the wells. The use of ATB strips makes it possible to simultaneously establish the susceptibility of the pathogen to 25 antifungal drugs and determine the MIC of each of them in 24 hours.

Today, the solution to the problem of rapid and reliable identification of fungi and determination of their sensitivity to antifungal drugs is provided by the use of semi-automatic and automatic microbiological analyzers VITEK, VITEK2, Walk Away, etc., which are presented in modern bacteriological and mycological laboratories in Ukraine. The use of automatic microbiological analyzers increases the accuracy of classical phenotypic identification up to 80% [5,17].

Bacteriological analyzer VITEK with computer software, containing a special program, consists of two blocks – a device for filling and sealing cards, as well as an incubator and reader. The tests are performed in plastic cards. After preparing the solution from the selected culture, the turbidity of which (the number of microorganisms per unit liquid) must be assessed on a nephelometer, which is part of the device. The card is filled automatically with a vacuum to prevent contamination and uneven filling of holes, and then the card is hermetically sealed. Cards for pathogen identification contain 30 wells with lyophilized biochemical substrates and the necessary reagents. The result of the interaction of the microorganism with the reagents is recorded by the device and evaluated by a computer program. Cards for determining the sensitivity to antimicrobial drugs consist of 45 wells, which are filled with these drugs of different concentrations. The time of identification and determination of sensitivity to antimicrobial drugs does not exceed 4-6 hours. More advanced is VITEK2, in which the quantity of operations is automated [27]. The device contains up to 60 cards, each of which has 64 holes, which allows to speed up identification by using more biochemical substrates. The device uses a fluorescent indicator, and in the case of enzymatic tests – labeled substrate [17,31].

Another system of automated microbiological testing is a modern automatic microbiological analyzer Walk Away (Siemens Healthcare Diagnostics), which allows to identify microorganisms in 4 hours and determine their sensitivity to more than 50 antimicrobial drugs in 4-24 hours. The range of microorganisms that can be identified using this analyzer is more than 360 species. This analyzer uses a fluorescent detection method. Identification is based on a system of biotyping of microorganisms by more than 30 reactions simultaneously, which allows to ensure a high level of specificity and accuracy of the results.

However, despite a number of advantages listed above, there are some disadvantages of automated microorganism identification systems. Thus, their use requires mandatory pre-selection of pure culture, which lasts 18-24 hours and thus increases the duration of the study. Also, when using them, the frequency of detection of resistant strains can be reduced due to the slow growth of resistant isolates. The results of the studies in most of the analyzer data are recorded by comparing the growth of microorganisms (or its absence) in the presence of antibiotics with control, where there are only microorganisms. Therefore,

it is quite difficult to differentiate cells that die from those that multiply slowly. In addition, their widespread introduction is limited by the impossibility of identifying isolates with atypical biochemical properties and high cost of equipment and expenditures on materials.

Practical health care requires the introduction of more reliable, express and accurate methods of detection and identification of pathogens of fungal infections, which include the method of mass spectrometry [20]. This is a physical method of measuring the ratio of the mass of charged particles of matter (ions) to their charge. Instruments that implement this method are called mass spectrometers. Examples of mass spectrometers are VITEK MS, MALDI-TOF MS, MALDI Biotyper. The greatest clinical significance of mass spectrometry is achieved in the analysis of nucleic acids (DNA/RNA) and peptides (proteins). Identification of microorganisms is based on the definition of a unique set of proteins for each species of microorganisms – a kind of «fingerprint» (fingerprinting) of the microorganism, «proteomic fingerprinting» [30,35,44]. Identification is carried out mainly by ribosomal proteins, which are present in all microorganisms. The essence of the method is to convert (using laser pulses) organic matter of microorganisms into charged particles – ions. This process is called ionization. In this case, the molecules of the additional substance – the matrix (α-cyano-4-hydroxycinnamic acid) and the proteins of the microorganism under study pass into the gas phase, and the matrix molecules interact with the proteins and transfer a positive charge to them. The ions obtained as a result of ionization are transferred to the gas phase of the vacuum part of the mass spectrometer by means of an electric field. In the deep vacuum of the analyzer, the ionized proteins under the influence of an electric field move from the ionization source to the detector with accelerations inversely proportional to their atomic masses. This is how charged ions are sorted by mass (more precisely, in relation to mass to charge, m / z) and the time of their flight by a certain distance. After the ions hit the detector and subsequent digitization of the result, the mass analyzer program estimates the time of flight of particles and builds a mass spectrum – a graph on the abscissa that is the ratio m / z and on the ordinate – the number of ions registered by the detector at a particular time. The obtained mass spectrum is compared with the spectra from the database of the mass analyzer, and based on information about the masses of characteristic proteins is the identification of microorganisms. MALDI-TOF MS pathogen identification requires a mass spectrometer, an appropriate program and a database. The process of identifying one microorganism takes no more than 2 minutes. The main advantages of the MALDI-TOF MS methods are: fast and reliable identification of microorganisms, low requirements for staff qualifications, no need for special material expenditures, high sensitivity ($10^4$ – $10^5$ microbial cells), possibility of identification of microorganisms of different taxonomic groups (mycelial and yeast-like fungi, gram-positive and gram-negative bacteria), high identification speed – 1 min/sample, high accuracy of species identification (exceeding 98%), reduction of the cost of each study by 12-96 times compared to phenotypic (manual and automated) methods [3,5,17,20,31,35].

Directly, the method of mass spectrometry can not be used to directly determine the sensitivity of microorganisms to antimicrobial agents. Identification of the pathogen, determination of its sensitivity to antimicrobial medicines (AMM) and their MIC is carried out with the participation of hybrid equipment, combined with a special program two separate analyzers: mass spectrometer (for identification) and automatic bacteriological analyzer (to determine the sensitivity of microorganisms to AMM and MIC) [5,8,38].

In cases where the mycological method can only detect micromycetes, but not to establish their etiological role in the development of the inflammatory process, the application of serological reactions of immunological testing becomes relevant. Thus, immunological diagnosis of mycoses of the ENT organs in combination with microscopic, mycological and histological methods of research can confirm the fungal etiology of the disease. The reaction of the immunological method is used in two directions for the purpose of: detection of antibodies in patient's serum by means of specific diagnostic antigen; determination of pathogen antigens using standard diagnostic serum.

To determine the presence of antibodies to fungal antigens in the serum of the subjects, traditional and modern serological reactions are used: agglutination test (AT), complement fixation test (CFT), immunofluorescence assay (IFA), enzyme-linked immunosorbent assay (ELISA) and others. For serological confirmation of fungal diseases, it is especially important not only to detect specific antibodies, but also to determine their quantitative dynamics during the disease. For this purpose, the serum of the subject is studied several times: the first time - at the request of the patient, the second time - after 7-10 days. Diagnostically significant in serological reactions is an increase in antibody titer in paired serums by 4 times or more [10,22].

Immunological diagnostic methods used to identify pathogen antigens are represented by latex agglutination test, indirect hemagglutination test, immunofluorescence assay, enzyme-linked immunosorbent assay, immunoelectrophoresis test, etc.

Both traditional and modern immunological reactions in mycology have their drawbacks: low sensitivity and specificity. This is due to the presence of antigens common to certain species of fungi, a decrease in the level of antibody production in certain categories of patients (immunodeficiency, diabetes, chronic diseases, etc.). To increase the informativeness of the serological method in the diagnosis of mycoses of the upper respiratory tract and ear, it is recommended to use several reactions simultaneously. Methods of immunological diagnosis are constantly being improved, which is due to the emergence of new information about the structure of fungal antigens.

New highly effective molecular genetic methods that do not require prior isolation of pure culture simplify and improve the quality of laboratory diagnosis of mycoses, including ENT organs. The most popular methods include polymerase chain reaction (PCR) and sequencing, which can be attributed to express-methods [7,42,43].

The PCR method is used to detect an infectious agent, if the nucleotide sequence of the gene or its fragment, specific only to this pathogen, is known. The principle of PCR is based on multiple copying (amplification) of the studied DNA by the enzyme DNA polymerase in vitro, which leads to the rapid accumulation of a certain DNA sequence of interest to the researcher, in sufficient quantities necessary for detection. The polymerase chain reaction occurs in three stages. The first stage – denaturation of double-stranded DNA of the pathogen – takes place at a temperature of 95 °C for 30-40 seconds, resulting in the formation of two separate DNA strands. In the second stage (renaturation) at a temperature of 50-65 °C hybridization of primers is carried out, that is the formation of double-stranded primer-matrix complexes, which initiate DNA synthesis. Primers are synthetic oligonucleotides that are complementary to DNA sequences and serve as a starting point for its replication. This stage of the reaction lasts 20-60 seconds. The final (third) stage of this reaction

- elongation - is characterized by the elongation of complementary strands of DNA with the participation of the enzyme DNA polymerase. This reaction step takes place at a temperature of 72°C for 20-40 seconds. Copied sections of DNA must be identified by electrophoresis or other methods [5,17,43]. PCR has a high diagnostic sensitivity and is performed quickly. However, the high sensitivity of this method may limit its widespread use in the diagnosis of mycoses of the ENT organs, as the presence of a minimum amount of DNA of the fungus, as a representative of microbiocenoses of mucous membranes and human skin, causes false-positive results. It is also impossible to exclude the possibility of contamination of samples during the preparation and conduct of the study. A significant disadvantage of this method is the inability to determine the sensitivity of microorganisms to antimicrobial agents and the level of MIC, as well as to assess the viability of the detected pathogen.

The problem of quantification of the pathogen helps to solve PCR in real time. This is quantitative real-time PCR, a laboratory method based on standard PCR used to simultaneously amplify and quantify a given DNA molecule. The main difference from typical PCR is that the amount of amplified DNA is measured in real time after each amplification cycle. Fluorescent dyes and modified oligonucleotides that fluoresce after hybridization with complementary DNA regions are used for quantification. Devices equipped with a fluorescent detector are produced for real-time PCR. The detection procedure is similar to classical PCR, but the identification of the PCR product occurs as it accumulates. Thus, the fluorescence is amplified with each new cycle [8,36,37,40,43].

Sequencing is a promising method of identifying different types of microorganisms, including new ones. Sanger F. proposed the first concept of sequencing in 1977. The method is based on determining the nucleotide sequence of DNA, known as the «chain break method». Sanger was awarded the Nobel Prize in 1980 for developing this method. Also in 1977, Maxam AM and Gilbert WA developed an alternative method called the “chemical degradation method.” The need for mass quality and rapid sequencing has stimulated the emergence of numerous modifications of these methods [8, 42]. The development and implementation of PCR, as well as the automation of the main stages of «reading» DNA has led to the development of a new generation sequencing method (NGS). Its main advantage is the ability to repeatedly «read» each DNA molecule, which significantly increases the accuracy of genetic changes identification. However, this method allows for one start of the device to collect several genomes of microorganisms in the case of isolation of their DNA from pure cultures, as well as to determine the species composition of the microbiota of a particular biotope. The sequencing process on NGS platforms consists of several stages. In the first stage, the process of preparing a “library” of DNA is performed, which includes fragmentation of DNA by enzymes or ultrasound, followed by attachment to these fragments of universal oligonucleotide adapters of known sequence and indices by PCR. Adapters are needed for further amplification of fragments. The second stage is the amplification of each DNA fragment by PCR. The DNA fragment is hybridized to one or two primers using an adapter sequence, which are immobilized on a solid surface (microsphere or glass chip) and participate in PCR. A reaction mixture containing a set of fragments for sequencing is passed through the chip. Subsequently, there is an automatic step-by-step reading of each nucleotide type and detection of results. Today, the global market presents various NGS platforms from Roche (Switzerland), Illumina (USA), Life technologies (USA), which use different technological approaches: sequencing by synthesis, sequencing by ligation (Sequencing by Oligonucleotide Ligation and Detection) [2, 5, 8].

Histological methods remain relevant in the diagnosis of mycoses of the ENT organs, along with high technologies for pathogen identification. With the help of histological examination of granulation tissue and removed polyps it is possible to detect the pathogen in the tissues of the macroorganism, to study the features of their structure and growth. The sections are stained with hematoxylin-eosin, according to the method of Van Gizon, Gram-Weigert, Grokott- Gomori, Gridley, Shabadash, and others. [17, 22, 23, 41]. When stained by the Gram-Weigert method, gentian violet and resorcinol-fuchsin or Orth carmine are used. Gram-positive microorganisms and fungi of the genus *Candida* are painted in dark blue. Shabadash and Gridley histological staining methods are considered more informative because they allow to detect polysaccharides of the cell wall of fungi. However, when using these methods it is necessary to consider the possibility of staining of bacterial microorganisms, because polysaccharides are a component of the cell wall of bacteria. In addition, they are contained in the cell structure of the macroorganism. In order to exclude mistaken results and to carry out differential diagnosis of fungal pathogens with cellular elements of the macroorganism and bacteria, sections stained using these methods are compared with sections stained with hematoxylin-eosin [23]. Coloring by the method of Groccot-Gomori is the impregnation of sections with silver. Fungal structures are painted in brown and black. With the help of this method, it is possible to detect mycelial fungi of the genera *Aspergillus, Penicillium, Mucor* and yeast-like fungi of the genus *Candida*.

Thus, in the diagnosis of fungal lesions of the ENT organs there is a large arsenal of methods nowadays to identify the pathogen, quantify it, determine the sensitivity of the pathogen to antimicrobial drugs and their MIC, establish the species composition of microbial associations, and decipher the genomes of new species. The introduction into clinical practice of a set of classical and modern diagnostic technologies should become the standard for the diagnosis of fungal diseases not only the upper respiratory tract and ear, but also other biotopes of the human macroorganism.

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**SUMMARY**

**OPPORTUNITIES AND PROSPECTS OF MICROBIOLOGICAL DIAGNOSIS OF ENT ORGANS MYCOSES (REVIEW)**

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The review considers one of the most important problems of modern otorhinolaryngology - the diagnosis of opportunistic mycoses. Data on the species spectrum of pathogens of opportunistic mycoses of the ENT organs and their epidemiology are presented, the analysis of existing methods of laboratory diagnosis of this pathology is given. The solution to the problem of rapid and reliable identification of fungi and determination of their sensitivity to antifungals is provided by the use of semiautomatic and automatic microbiological analyzers, as well as the use of mass spectrometry. Particular attention is paid to modern molecular genetic methods, such as polymerase chain reaction, sequencing of a new generation, which in combination with classical microbiological methods, make it possible, with the highest level of reliability, to identify microorganisms by species based on decoding their genomes. It also gives the possibility to assess the sensitivity of the isolated strains to chemotherapeutic drugs using the disco-diffusion method, the method of serial dilutions, epsilometric and the use of ATB strips. The introduction into clinical practice a set of classical and modern diagnostic technologies should become the standard for the diagnosis of fungal diseases not only upper respiratory tract and ear, but also other biotopes of the macroorganism.

**Key words:** opportunistic mycoses, ENT organs, diagnosis of mycoses.

**РЕЗЮМЕ**

**ВОЗМОЖНОСТИ И ПЕРСПЕКТИВЫ МИКРОБИОЛОГИЧЕСКОЙ ДИАГНОСТИКИ МИКОЗОВ ЛОР-ОРГАНОВ (ОБЗОР)**

**Безшапочный С.Б., Подовжний А.Г., Полянская В.П., Зачепило С.В., Федорченко В.И.**

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В обзоре рассматривается одна из значимых проблем современной оториноларингологии – диагностика оппортунистических микозов. Указаны данные о видовом спектре возбудителей оппортунистических микозов ЛОР-органов и их эпидемиологии, изложен анализ существующих методов лабораторной диагностики данной патологии. Решение проблемы быстрой и достоверной идентификации грибов и определение их чувствительности к антимикотикам обеспечивается применением полуавтоматических и автоматических микробиологических анализаторов, а также использованием метода масс-спектрометрии. Особое внимание уделено современным молекулярно-генетическим методам, таким как полимеразная цепная реакция, секвенирование нового поколения, которые в совокупности с классическими микробиологическими методами, дают возможность с самым высоким уровнем достоверности идентифицировать микроорганизмы до вида на основании расшифровки их геномов. Оценка чувствительности выделенных штаммов к химиотерапевтическим препаратам происходит с помощью диско-диффузионного, эпсилометрического и метода серийных разведений, а также использования АТВ стрипов. Внедрение в клиническую практику комплекса классических и современных диагностических технологий должно стать стандартом диагностики грибковых заболеваний не только верхних дыхательных путей и уха, но и других биотопов макроорганизма.

რეზიუმე

ლორ-ორგანოთა მიკოზების მიკრობიოლოგიური დიაგნოსტიკის შესაძლებლობები და პერსპექტივები (მიმოხილვა)

ს.ბეზშაპოჩნი, ა.პოდოვჟნი, ვ. პოლიანსკაია, ს.ზაჩეპილო, ვ. ფედორჩენკო

უკრაინის სამედიცინო სტომატოლოგიური აკადემია, პოლტავა, უკრაინა

მიმოხილვაში განხილულია თანამედროვე ოტორინოლარინგოლოგიის ერთ-ერთი უმნიშვნელოვა-

ნესი პრობლემა - ოპორტუნისტული მიკოზების დიაგნოსტიკა. მითითებულია ლორ-ორგანოების ოპორტუნისტული მიკოზების გამომწვევების სახეობათა სპექტრის მონაცემები და მათი ეპიდემიოლოგია, წარმოდგენილია აღნიშნული პათოლოგიის ლაბორატორიული დიაგნოსტიკის არსებული მეთოდების ანალიზი. სოკოების სწრაფი და სარწმუნო იდენტიფიკაციის პრობლემის გადაწყვეტა და ანტიმიკოტიკებისადმი მათი მგრძნობელობის განსაზღვრა უზრუნველყოფილია ნახევრად ავტომატური და ავტომატური მიკრობიოლოგიური ანალიზატორების გამოყენებით, აგრეთვე მას-სპექტრომეტრიის მეთოდის გამოყენებით. განსაკუთრებული ყურადღება ეთმობა თანამედროვე მოლეკულურ-გენეტიკურ მეთოდებს: პოლიმერაზულ ჯაჭვურ რეაქციას და ახალი თაობის სეკვენირებას, რომლებიც, კლასიკურ მიკრობიოლოგიურ მეთოდებთან ერთობლიობაში მაღალი სარწმუნოობით იძლევიან მიკროორგანიზმების სახეობის იდენტიფიცირების შესაძლებლობას, მათი გენომების გაშიფვრის საფუძველზე. გამოყოფილი შტამების ქიმიოთერაპიული პრეპარატების მიმართ მგრძნობელობის შეფასება შესაძლებელია დისკო-დიფუზური, სერიული განზავების, ეპსილომეტრიული მეთოდების და ATB სტრიპების გამოყენებით. კლინიკურ პრაქტიკაში კლასიკური და თანამედროვე სადიაგნოსტიკო ტექნოლოგიების კომპლექსის დანერგვა უნდა გახდეს სოკოვანი დაავადებების დიაგნოსტიკის სტანდარტი არა მხოლოდ ზედა სასუნთქი გზების და ყურის დაავადებებისათვის, არამედ მაკროორგანიზმის სხვა ბიოტოპებისაც.

# EVALUATION OF THE UKRAINIAN VERSION OF SNOT-22 QUESTIONNAIRE VALIDITY FOR ASSESSING THE QUALITY OF LIFE IN PATIENTS WITH CHRONIC RHINOSINUSITIS AND NASAL SEPTUM DEVIATION

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An important criterion for assessing the condition of patients with various types of pathology and for determining the effectiveness of treatment is a change in their quality of life (QoL). One of the components of QoL is health related quality of life of a person (HRQoL). Particular attention has been recently paid to the above-mentioned criterion for assessing the treatment effectiveness. That is why there were developed several questionnaires aimed at integral assessment of the quality of a patient's life and the impact of a particular pathological process on this patient.

One of the most common pathological conditions is chronic inflammatory disease of the paranasal sinuses, which is called chronic rhinosinusitis. A special urgency of this pathology is due to the significant prevalence of chronic rhinosinusitis, which reaches 4-15% of the entire adult population [1-3]. This pathology is also characterized by a significant impact on the quality of life of patients and a significant annual cost of treatment.

The Quality of Life (QoL) structure includes a health-related quality of life index that depends on health state and may change as a result of the treatment. This index is defined as the difference between patient's expectations and experience [4]. The QoL system for assessing the quality of life of patients with various pathological conditions acquired special development more than 20 years ago. At this period there appeared specific questionnaires which took into account the symptoms of diseases.

There are many variants of questionnaires evaluating the quality of life of patients with diseases of the paranasal sinuses, but it is impossible to say with certainty that the results of the survey completely match the actual state of quality of life of a person [5]. One of the problematic issues is the assessment of the patient's quality of life using sociological tools that allow comparing the data obtained in different countries in different languages. That happens because language peculiarities can affect the patient's understanding of the essence of the question and thus influence the results of the survey. The development of the Ukrainian version of questionnaires for assessing the quality of life in various nosology is an urgent task for modern medicine.

One of the world's most widely recognized and widely used questionnaires for assessing the impact of nasal diseases on the quality of life is SNOT-22 (sinonasal outcome test 22) [6], which is an improved version of SNOT-20. The questions in SNOT-22 are aimed at assessing the degree of nasal obstruction and detecting disorders in smell and taste function as important components of the quality of life of a person [7]. There are some studies aimed at assessing the impact of chronic and acute rhinosinusitis and endoscopic rhinosurgery on the quality of life of patients using this questionnaire [8,9]. In order to compare scientific data of domestic and foreign researchers, there is a need to develop and verify the sensitivity of the Ukrainian version of SNOT-22 using the methodology applied by other authors [10], i.e. suggest a bilateral translation (English-Ukrainian and Ukrainian-English) of the questionnaire to patients with diseases of the paranasal sinuses and compare the obtained results with already published ones.

The objective of the study was to develop the Ukrainian version of SNOT-22 questionnaire and to study its effectiveness while evaluating the impact of nasal diseases on quality of life of patients.

**Material and methods.** We developed the Ukrainian version of SNOT-22 questionnaire using its bilateral translation (English-Ukrainian and Ukrainian-English) by different translators. Then we identified the original (English) version of the questionnaire and the one obtained as a result of the bilateral translation [11].